The challenge of *Cystoseira crinita*: Combined effect of temperature and radiation

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“Only if we can we .
Only if we care will we .
Only if we help shall they be .”
– Jane Goodall, 2010.

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ABSTRACT

The Mediterranean Sea is a hotspot of marine species biodiversity where most of the submerged forests are dominated by fucoid species of the genus *Cystoseira*. Benthic assemblages subjected to a low or moderate hydrodynamics between zero and one meter deep and restricted to rocky subtracts are occasionally dominated by *Cystoseira crinita*, that because of the shallow habitat is particularly vulnerable and finds refuge in rock pools and in very shallow waters of sheltered coves.

The most important consequences of climate change on aquatic ecosystems are probably the temperature and UV radiation increases. These rocks pools, due to the low deep and hydrodynamics may experience high temperatures and radiations that, especially considering the present climate warming framework, may impair these populations which are actually vulnerable.

The main objective of this study is to determine if positive thermic anomalies may be a driver of *C. crinita* decline, at least in the north-western Mediterranean Sea. For this, our specific aims are: determine if the temperature and ultraviolet radiation increases affects physiological state of adults individuals and elucidate if temperature and radiation have a synergistic, cumulative or antagonistic effect on this physiological state.

The experiment consisted on the application of six treatments combining two different environmental parameters, radiation (PAR and PAR + UV) and temperature (21, 24 and 28 °C) during 30 days. At 21 and 24 °C, in both radiations, total height and maximum quantum yields of photosynthesis, F_v/F_m, remained constant during experiment, but humid weight began to decrease after 30 days and the F_v/F_m after 12. At 28 °C effects were differentially caused by radiation exposure, so that under PAR began to lose humid weight after day 12 and reduced its total height, but under PAR + UV began to lose humid weight after day 18. In addition, at 21 °C under PAR + UV radiation individuals increased its apex height.

These results suggest that *C. crinita* has efficient protective mechanisms against UV radiation and its thermic tolerance is adapted to resist a long time under the current normal summer temperatures. However, our results also show that although *C. crinita* is able to resist short and punctual periods at high temperatures, it is not able to resist mid-long periods at temperatures up to 28°C (thermal anomalies). Also, the interaction between temperature and radiation has an antagonistic effect.

RESUMEN

El mar Mediterráneo es un punto caliente de biodiversidad de especies marinas donde la mayoría de los bosques sumergidos están dominados por especies de fucoides del género *Cystoseira*. Las comunidades de macroalgas sometidas a una hidrodinámica baja o moderada entre cero y un metro de profundidad y restringida a substratos rocosos están dominadas ocasionalmente por *Cystoseira crinita*, que debido al hábitat poco profundo es particularmente vulnerable y encuentra refugio en los charcos excavados en las rocas y en aguas muy poco profundas de calas protegidas.

Las consecuencias más importantes del cambio climático en los ecosistemas acuáticos son probablemente los aumentos de la radiación UV y la temperatura. Estos charcos, debido a la baja profundidad e hidrodinámica, pueden experimentar altas temperaturas y radiaciones que, especialmente considerando el presente marco de calentamiento climático, pueden afectar a estas poblaciones que son realmente vulnerables.

El objetivo principal de este estudio es determinar si las anomalías térmicas positivas pueden ser un factor de disminución de *C. crinita*, al menos en el área mediterránea del noroeste. Para ello, nuestros objetivos específicos son: determinar si el aumento de la temperatura y radiación ultravioleta afectan a el estado fisiológico de los individuos adultos y elucidar si la temperatura y la radiación tienen un efecto sinérgico, acumulativo o antagonista sobre este estado fisiológico.

El experimento consistió en la aplicación de seis tratamientos combinando dos parámetros ambientales, la radiación (PAR y PAR + UV) y la temperatura (21, 24 y 28 °C) durante 30 días. A 21 y 24 °C, en ambas radiaciones, la altura total y el rendimiento cuántico máximo de fotosíntesis, F_v/F_m, permanecieron constantes durante el experimento, pero el peso húmedo comenzó a disminuir a partir del día 30 y el F_v/F_m después de 12. A 28 °C los efectos fueron causados diferencialmente según la exposición a la radiación, de manera que bajo PAR comenzaron a perder peso húmedo después del día 12 y redujeron su altura total, pero bajo PAR + UV comenzaron a perder peso húmedo después del día 18. Además, a 21 °C bajo radiación PAR + UV los individuos incrementaron la altura del ápice.

Estos resultados sugieren que *C. crinita* tiene mecanismos protectores eficientes contra la radiación UV y su tolerancia térmica está adaptada a resistir mucho tiempo bajo las temperaturas normales de verano actuales. Sin embargo, nuestros resultados también muestran que aunque *C. crinita* es capaz de resistir periodos cortos y puntuales a altas temperaturas, no es capaz de resistir períodos medios-largos a temperaturas de hasta 28°C (anomalías térmicas). Además, la interacción entre temperatura y radiación tiene un efecto antagónico.

RESUM

El mar Mediterrani és un punt calent de biodiversitat d'espècies marines, on la majoria dels boscos submergits estan dominats per espècies de fucoides del gènere *Cystoseira*. Les comunitats bentòniques dominades per macroalgues sotmeses a una hidrodinàmica baixa o moderada entre zero i un metre de profunditat i restringits a substrats rocosos estan ocasionalment dominades per *Cystoseira crinita*, que a causa de l'hàbitat poc profund és particularment vulnerable i troba refugi en les basses excavades a les roques i en aigües molt poc profundes de cales protegides.

Les conseqüències més importants del canvi climàtic en els ecosistemes aquàtics és probablement l'augment de la radiació UV i la temperatura. Aquestes basses excavades, a causa de la baixa hidrodinàmica, poden experimentar altes temperatures i radiacions que, especialment considerant l'actual marc d'escalfament climàtic, poden afectar a aquestes poblacions que són realment vulnerables.

L'objectiu principal d'aquest estudi és determinar si les anomalies tèrmiques positives poden ser un factor de disminució de *C. crinita*, almenys en l'àrea mediterrània del nord-oest. Per això, els nostres objectius específics són: determinar si l'augment de la temperatura i radiació ultraviolada afecten l'estat fisiològic dels individus adults i esbrinar si la temperatura i la radiació tenen un efecte sinèrgic, acumulatiu o antagonista sobre aquest estat fisiològic.

L'experiment va consistir en l'aplicació de sis tractaments combinant dos paràmetres ambientals, la radiació (PAR i PAR + UV) i la temperatura (21, 24 i 28 °C) durant 30 dies. A 21 i 24 °C l'alçada total i el rendiment quàntic màxim de fotosíntesi, F_v/F_m, van romandre constants durant l'experiment, però el pes humit va començar a disminuir a partir del dia 30 i el F_v/F_m després de 12. A 28 °C els efectes van ser causats diferencialment segons l'exposició a la radiació, de manera que sota PAR van començar a perdre pes humit després del dia 12 i van reduir la seva alçada total, però sota PAR + UV van començar a perdre pes humit després del dia 18. A més, a 21 °C sota radiació PAR + UV els individus van incrementar la alçada de l'àpex.

Aquests resultats suggereixen que *C. crinita* té mecanismes protectors eficients contra la radiació UV i la seva tolerància tèrmica està adaptada a resistir molt de temps sota les temperatures normals d'estiu actuals. No obstant això, els nostres resultats també mostren que tot i que *C. crinita* és capaç de resistir períodes curts i puntuals a altes temperatures, no és capaç de resistir períodes mitjos-llargs a temperatures de fins a 28°C (anomalies tèrmiques). A més, la interacció entre temperatura i radiació té un efecte antagònic.

1. INTRODUCTION

Along temperate rocky coasts worldwide, the large canopy-forming kelps and fucoids represent the dominant species in pristine environments. These communities are both at structure and function level, very similar to the terrestrial forests because, like trees, kelps and fucoids are foundational species that provide shelter, food, nursery and habitat for many other associated species (Fredersdorf et al., 2009; Thibaut et al., 2015; Blanfuné et al., 2016; Marine forest, 2017). Furthermore, seaweeds forest are also important primary producers involved in the maintenance of diversified trophic levels, and basically the larger species can attenuate wave action (Thibaut et al., 2015; Blanfuné et al., 2016). Therefore, they are the miniature analogues of terrestrial forests and for this reason can be considered as submerged forests (Fredersdorf et al., 2009; Thibaut et al., 2015; Blanfuné et al., 2016; Marine forest, 2017).

The regression of these submerged forests is a worldwide phenomenon mainly due to the cumulative impact of increasing human pressures like destruction of habitats, pollution, species introduction, overfishing, coastal aquaculture and global warming that cause a regression of large and long-lived species and consequently a loss of biodiversity (Fredersdorf et al., 2009; Sales & Ballesteros, 2009). In littoral habitats such changes have been observed when engineering species such as kelps or fucoids disappear mainly due to environmental degradation because different pressures act over time and in unison, with possible synergistic effects, affecting species, ecosystems and their ability to deliver ecosystem services. These impacts are leading to shifts in habitat structure from a state with canopy forming species to alternative states, in the worst case to barren grounds composed of filamentous and encrusting species with flow-on effects on adjacent communities (Fredersdorf et al., 2009; Sales & Ballesteros, 2009; Thibaut et al., 2015; Blanfuné et al., 2016).

The Mediterranean Sea is a hotspot of marine species biodiversity where most of these submerged forests are dominated by fucoid species of the genus *Cystoseira*, which is characterized by being a genus of canopy-forming brown algae, dominating several benthic assemblages from the littoral fringe down to the lower sublittoral zone (Sales & Ballesteros, 2009; Gianni et al., 2013; Thibaut et al., 2015; Blanfuné et al., 2016; Marine forest, 2017). Several *Cystoseira* species are endemic of the Mediterranean Sea, and most of them have specific ecological constraints that limit their development to specific habitats (Thibaut et al., 2005; Sales & Ballesteros, 2009; Thibaut et al., 2015; Blanfuné et al., 2016).

As other seaweeds, Mediterranean *Cystoseira* populations are currently experiencing a severe decline and even disappearing in many regions, affected by the environmental degradation of several areas caused by habitat destruction, eutrophication and overgrazing by herbivores, leading to a shift from highly structured ecosystems to poorly organised benthic assemblages such as turf-forming, filamentous or other ephemeral seaweeds or urchin barren grounds where urchin density is a driver of habitat homogenization (Thibaut et al., 2005; Sales & Ballesteros, 2009; Sales et al., 2011; Gianni et al., 2013; Marine forest, 2017). However, although the impact of warming has been described for other kelp species, no evidence has been reported for *Cystoseira* species yet.

The rocky substrate areas of the upper part of the infralitoral Mediterranean coast usually is dominated by the community defined by *Cystoseira mediterranea* specie, although in most calmed areas this community can be replaced by communities dominated by *Cystoseira caespitosa*. More exceptionally, when these areas are sheltered and well illuminated, *Cystoseira mediterranea* communities can be replaced by *Cystoseira crinita* or by *Cystoseira barbata* communities (Ballesteros, 1992; Ballesteros et al., 2014). In brief, *C. crinita* and *C. barbata* species are representative of those species characteristic of very restricted areas.

Benthic assemblages dominated by *C. crinita* are restricted rocky subtracts subjected to a low or moderate hydrodynamics between zero and one metre deep, and may host 234 species of flora and fauna in only few square meters (Blanfuné et al., 2016). Because of the shallow habitat, *C. crinita* and the other subsurface *Cystoseira* species are particularly vulnerable to sea surface pollution, habitat destruction, trampling and overgrazing by large herbivores like the sea urchins *Paracentrotus lividus* and *Arbacia lixula*, and the teleosts of the genus *Sarpa*, and recently *Siganus*. Because of that, *C. crinita* finds refuge in rocks pools and in very shallow waters of sheltered coves, where are no large herbivorous and the area is relatively protected from human activities (Ballesteros et al., 2014; Blanfuné et al., 2016). However, this rocks pools, due to the low hydrodynamics may experience high temperatures that, especially considering the present climate warming framework, may impair these populations which are in fact, actually vulnerable to many other impacts.

Although, probably the major consequence of climate change effects on aquatic ecosystems is the temperature increase. Climate change will also have among others, an important effect on the underwater UV radiation through a variety of mechanisms. In fact, it must be borne in mind that climate models predict an increase in UV radiation levels, especially UV-A radiation, reaching Mediterranean ecosystems in the coming decades due to a decrease in cloud cover. Besides, the UV-B radiation levels (the most energetic part of the daylight spectrum) have increased in recent decades due to the reduction of the stratospheric ozone layer. Solar UV radiation can damage aquatic organisms and decrease the productivity of aquatic ecosystems. Although for vegetal life UV-A and UV-B radiation have a great importance to its normally development due to photomorphogenesis, an excess of UV radiation can generate reactive oxygen species resulting in cellular damage, unless vegetals have effective protective mechanisms against this radiation, which may also be adversely affected by this excess radiation (Häder et al., 2011; Verdaguer et al., 2012; Nenadis et al., 2015).

2. OBJECTIVES

In the year 2015 a severe mortality was observed in the populations of *Cystoseira crinita* located in the Badia de Port de la Selva (Figure 1), in the province of Alt Empordà. This phenomenon of high mortality coincided with an abnormally hot summer, where extreme and anomalous values of temperature were recorded during summer (Agencia Estatal de Meteorología, 2017).

Figure 1. *Cystoseira crinita communities located on the Bay of Port de la Selva in the summers of 2012, 2013 and 2015, respectively. Pictures taken from Marine forest, 2017.*

The main objective of this study is to determine if positive temperature anomalies may be a driver of *Cystoseira crinita* decline, at least in the NW Mediterranean Area. For this, our specific aims are:

I) Determine if the temperature increase affects physiological state of adults individuals.

II) Determine if the ultraviolet radiation also affects physiological state of adults individuals.

III) Elucidate if temperature and radiation have a synergistic, cumulative or antagonistic effect on this physiological state.

3. MATERIAL AND METHODS

3.1. TARGET SPECIE

The *Cystoseira crinita* specie was first described in 1830 by Duby (Figure 2) (AlgaeBase, 2017). This kind of *Cystoseira* has a talus over 40 cm and has a smooth stem and branches. The stem approximately with a high of 2 mm is short and stretched. The main branches are 5-10 cm long and 1 mm thick, which branch out alternatively. The branches are cylindrical shaped with a large number of crystostoma coming out on the surface in tuber shapes visible to the naked eye. It has a reduced number of airbladders distributed at a considerable distance from one another. These airbladders are large, ellipsoidal and the top of the last bladders are blunded. Cylindrical or spareshaded receptacles are placed on the top of the branches, often with bladders and sometimes with some small thorns on the surface, without sterile shoots on the top (UN environmental, n.d.).

Kingdom: Chromista
Phylum: Ochrophyta
Class: Phaeophyceae
Order: Fucales
Family: Sargassaceae
Genus: *Cystoseira*
Species: *Cystoseira crinita*

Figure 2. *Detailed images and taxonomic classification of Cystoseira crinita. Pictures extracted from Ballesteros, 2014. Taxonomic classification extracted from World Register of Marine Species, n.d & AlgaeBase, 2017.*

At present, the distribution of the habitats formed by *Cystoseira crinita* within the Catalan coasts is limited only to small well-sheltered coves, lowlands generated on the rock quite protected from the waves with an abundant presence of sand. Thus, the distribution of this species is limited to certain points of the coasts of La Selva, el Baix Empordà and l'Alt Empordà, such as the Bay of Port de la Selva and Cala Montjoi in Cap de Creus, Cala Estreta in Palamós and Cala Bona in Tossa de Mar (Ballesteros et al., 2014).

3.2. STUDY SITE

The experiment was carried out at the greenhouse of the Faculty of Sciences of the University of Girona (41° 57' 44'' N, 2° 49' 44'' E, Girona, Spain) during a period of 30 days, which covered from the 24th of April to the 22th of May, 2017. The field site which was the source of *C. crinita* plants for greenhouse experiment was located in Cala Estreta (41° 51' 55'' N, 3° 10' 22'' E, Palamós, Spain) a cove included in the Site of natural interest (PEIN) of Castell-Cap Roig in Palamós (Baix Empordà).

3.3. ALGAL MATERIAL

Figure 3. *Collection and cleaning of samples.*

Given that the specie of study is part of the List of Wild Species in Special Protection Regime (Agencia Estatal Boletín Oficial del Estado, 2011), the collection of the samples was carefully carried out in order to not damage the rest of the population. *C. crinita* specimens were collected with a knife from the base, and when necessary we used a chisel and a hammer. Then, individuals were carefully cleaned in order to eliminate the presence of epiphytic organisms or species that inhabit on the shelter of these algae (Figure 3). Finally, Individuals were transport them form the cove to the greenhouse without water in a cooler (Appendix 1).

3.4. INFRASTRUCTURE AND CULTURE CONDITIONS

The culture was carried out in glass tanks of 20 litters of volume that were filled only to 75% of its capacity so that, the individuals of *Cystoseira* excelled from the water by the upper part in order to reproduce *in situ* conditions in the aquariums.

The studied specie inhabits in battered zones, that is, in areas where hydrodynamics oxygenates water and provides them with nutrients. For this reason, every day 10% of the water of each tank was renewed with new seawater. In addition, in order to maintain high levels of dissolved oxygen in the water, each aquarium was connected to a water pumping system and an aerator *Aquarium air-pump ACO-5501 of Hailea*.

The experiment consisted on the application of six different treatments that combined two different environmental parameters, radiation and temperature (Figure 4). Each of these treatments was applied over a period of one month to three different water tanks, so that the system included a total of 18 aquariums.

PAR	**PAR**	**PAR**	**PAR + UV**	**PAR + UV**	**PAR + UV**
21 °C	**24 °C**	**28 °C**	**21 °C**	**24 °C**	**28 °C**
Aquarium	Aquarium	Aquarium	Aquarium	Aquarium	Aquarium
1, 2, 3	4, 5, 6	7, 8, 9	10, 11, 12	13, 14, 15	16, 17, 18

Figure 4. *Scheme of the different treatments applied on Cystoseira crinita.*

On the one hand, two irradiance treatments were performed. The fist one consists in the illumination with two PAR radiation fluorescents emitting a total of 74 watt, one *Master TL-D 36W/386 of Philips* with an emission of 38 watt, and one *Biolux L 36W/965 of Osram* with an emission of 36 watt. The second one consists, as in the other case, in the illumination with two PAR radiation fluorescents, and the addition of three more fluorescents, two UV-A radiation florescent *Actinic BL TL-K 40W/10-R of Philips* with an emission of 7,4 watts and one UV-B radiation florescent *Ultraviolet-B 40W/12 RS SLV/25 of*

Philips with an emission of 4,5 watts, so this treatment was submitted to a total of 93,3 watts.

On the other hand, three different thermal treatments were applied, at 21±1 °C, 24±1 °C and 28±1 °C. To maintain these temperatures water heaters with programmable thermostat *Aquarium heather thermostat 75W of Sera* and water refrigerators *Hailea Chiller HC 500 A of Hailea* were used.

3.5. MEASUREMENTS AND STATISTICAL ANALYSIS

Experiments were monitored at 9, 12, 18, 26 and 30 days of exposure. Humid weight, total height and apex height were recorded at each time for all specimens using a *Serie DM-1100 of Gram* and a ruler. Physiological status of samples was also monitored after 5, 12, 15, 19 and 26 days of exposure by using an underwater chlorophyll fluorometer *Diving PAM of Walz* . The maximal quantum yields of photosynthesis as an indicator of this physiological status was calculated as $F_v/F_m=(F_m-F_o)/F_m$ being F_o the initial fluorescence in the dark- adapted state, when all PS II reaction centres are oxydized. In order to obtain this parameter the upper branch of each sample was covered to submit it in darkness over a period of 10 minutes prior to the measurement (Viñegla et al., 2006; Fredersdorf et al., 2009). If any of these branches were lost, the measure was not taken and its value was assumed as zero.

Weight, height and yield were analysed by means of generalized linear mixed model incorporating radiation, temperature and time as fixed factors and aquarium and individuals as random effects in a linear predictor, via maximum likelihood, and the response variables were morphological and physiological data. Also a post-hoc Tukey test were performed for the significant factors and interactions . In this type of statistical analysis, the linear predictor is related to the conditional mean of the response through the inverse link function defined in the GLM family, and the expression for the likelihood of a mixed-effects model is an integral over the random effects space (Rdocumentation, 2017).

3.6. ETHICAL AND SUSTAINABILITY CRITERIA

Target specie is within the List of Wild Species in Special Protection Regime (Agencia Estatal Boletín Oficial del Estado, 2011), because of that only 36 individuals were taken, the number of feet necessary for the realization of the experiment. Also, to perform the samplig all permits of the Generalitat de Catalunya were in order and the samples collection samples were carefully carried out in order to not damage the rest of the population. Moreover, during the experiment the waste generated was correctly recycled.

4. RESULTS

4.1. HUMID WEIGHT

Temperature, radiation, time, and all the possible interactions between these three factors generated significantly effects on humid weight (Table 1).

Table 1. *Generalized linear mixed model test for relationships between temperature (Temp), radiation (Rad) and time (Day) on humid weight. Significant values are highlighted in bold.*

Variable	DF	denDF	F-value	P-value
Temp	2	175	81,1337	**< 0,0001**
Rad	1	175	4.7094	**0.0313**
Day	5	175	72.3524	**< 0.0001**
Temp:Rad	2	175	22.0831	**< 0.0001**
Temp:Day	10	175	10.3826	**< 0.0001**
Rad:Day	5	175	2.7640	**0.0198**
Temp:Rad:Day	10	175	3.3484	**0.0005**

Table 2. *Post-hoc tukey tests of the generalized linear mixed model test for relationships between temperature (Temp), radiation (Rad) and time (Day) on humid weight: (I) Temperature x Radiation contrast; (II) Temperature x Time contrast; (III) Radiation x Time contrast. Significant values are highlighted in bold.*

Contrast	P-value	Contrast	P-value	Contrast	P-value
(I) Temperature x Radiation contrast					
21, PAR – 24, PAR	1,0000	21, UV – 24, UV	0,9989	21, PAR – 21, UV	0,8181
21, PAR – 28, PAR	**< 0,0001**	21, UV – 28, UV	**0,0017**	24, PAR – 24, UV	0,5485
24, PAR – 28, PAR	**< 0,0001**	24, UV – 28, UV	**0,0066**	28, PAR – 28, UV	**< 0,0001**
(II) Temperature x Time contrast					
21, 1 – 24, 1	1,0000	24, 1 – 28,1	1,0000	28, 1 – 21, 1	1,0000
21, 1 – 21, 9	0,9836	24, 1 – 24, 9	**0,0155**	28, 1 – 28, 9	0,3950
21, 1 – 21, 12	0,9968	24, 1 – 24, 12	1,0000	28, 1 – 28, 12	0,1695
21, 1 – 21, 18	0,9998	24, 1 – 24, 18	0,9987	28, 1 – 28, 18	**< 0,0001**
21, 1 – 21, 26	0,9987	24, 1 – 24, 26	0,4797	28, 1 – 28, 26	**< 0,0001**
21, 1 – 21, 30	**0,0043**	24, 1 – 24, 30	**0,0022**	28, 1 – 28, 30	**< 0,0001**
(III) Radiation x Time contrast					
PAR, 1 – UV, 1	1,0000	PAR, 1 – PAR, 9	0,0545	UV, 1 – UV, 9	0,0411
PAR, 9 – UV, 9	1,0000	PAR, 1 – PAR, 12	0,3907	UV, 1 – UV, 12	0,8878
PAR, 12 – UV, 12	**0,0038**	PAR, 1 – PAR, 18	**0,0005**	UV, 1 – UV, 18	**0,0398**
PAR, 18 – UV, 18	0,9877	PAR, 1 – PAR, 26	**< 0,0001**	UV, 1 – UV, 26	**< 0,0001**
PAR, 26 – UV, 26	1,0000	PAR, 1 – PAR, 30	**< 0,0001**	UV, 1 – UV, 30	**< 0,0001**
PAR, 30 – UV, 30	1,0000				

The effects generated by the treatments submitted to 21 °C and 24 °C were equivalent, although at the beginning of the experiment there were small differences (Table 2 and Figure 5). In both temperature treatments, time has significantly effects, but radiation not affects (Table 2). Although an increase was detected after 9 days in individuals kept at 24 °C, in both temperature treatments, after 30 days of exposition humid weight was lower than the initial day around 0,2708 ± 0,2148 grams (Table 2 and Figure 5).

Contrary to treatments subjected to lower temperatures, at 28 °C the radiation does have significant effects, and time too (Table 2). Cultures kept at this temperatures lost around 0,8203 ± 0,1232 grams, but did so differently between both radiation treatments (Table 2). While this weight loss was not detected until 18 days of culture in individuals irradiated with PAR + UV, in individuals who were only submitted to PAR was detected at 12 days (Table 2 and Figure 5). In addition, PAR + UV treatment only produced a 0,9095 ± 0,1147 grams decrease, meanwhile in PAR treatment 0,7312 ± 0,1317 grams were lost.

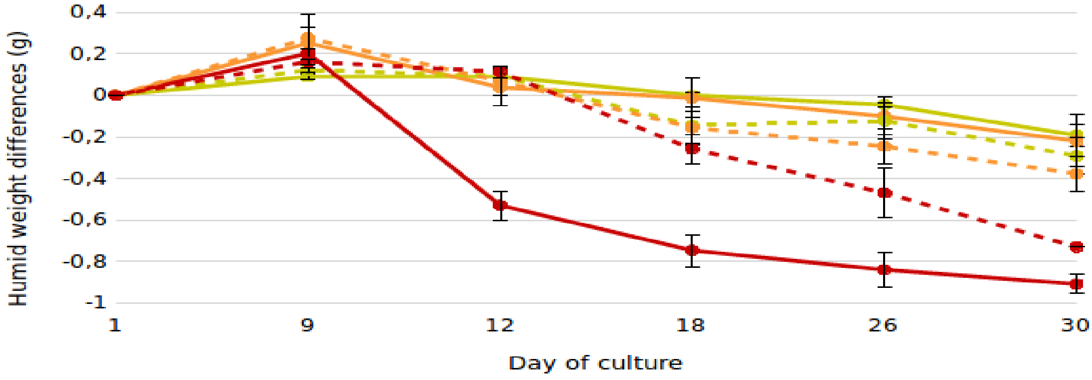

Figure 5. *Humid weight differences from the initial culture day of Cystoseira crinita at three temperatures (21 °C represented as yellow, 24 °C represented as orange and 28 °C represented as red) and two radiation conditions (PAR represented as a continuous line and PAR + UV represented as a dashed line) along 30 days of exposure. Standard error are represented by vertical bars (n=6).*

4.2. TOTAL HEIGHT

Temperature, radiation, time and also the interactions between these factors, except the temperature and radiation one, produced statistical detected changes on total height (Table 3).

Table 3. *Generalized linear mixed model test for relationships between temperature (Temp), radiation (Rad) and time (Day) on total height. Significant values are highlighted in bold.*

Variable	DF	denDF	F-value	P-value
Temp	2	175	18.7974	**< 0.0001**
Rad	1	175	6.2011	**0.0137**
Day	5	175	8.5228	**< 0.0001**
Temp:Rad	2	175	10.6637	**< 0.0001**
Temp:Day	10	175	3.5837	**0.0002**
Rad:Day	5	175	1.2433	0.2909
Temp:Rad:Day	10	175	2.2711	**0.0160**

Table 4. *Post-hoc tukey tests of the generalized linear mixed model test for the significant relationships between temperature (Temp), radiation (Rad) and time (Day) on total height: (I) Temperature x Radiation contrast; (II) Temperature x Time contrast. Significant values are highlighted in bold.*

Contrast	P-value	Contrast	P-value	Contrast	P-value
(I) Temperature x Radiation contrast					
21, PAR – 24, PAR	0,9982	21, UV – 24, UV	0,9958	21, PAR – 21, UV	1,0000
21, PAR – 28, PAR	**< 0,0001**	21, UV – 28, UV	0,8318	24, PAR – 24, UV	0,9472
24, PAR – 28, PAR	**< 0,0001**	24, UV – 28, UV	0,9815	28, PAR – 28, UV	**< 0,0001**
(II) Temperature x Time contrast					
21, 1 – 24, 1	1,0000	24, 1 – 28,1	1,0000	28, 1 – 21, 1	1,0000
21, 1 – 21, 9	1,0000	24, 1 – 24, 9	1,0000	28, 1 – 28, 9	1,0000
21, 1 – 21, 12	1,0000	24, 1 – 24, 12	1,0000	28, 1 – 28, 12	1,0000
21, 1 – 21, 18	1,0000	24, 1 – 24, 18	1,0000	28, 1 – 28, 18	0,9716
21, 1 – 21, 26	1,0000	24, 1 – 24, 26	1,0000	28, 1 – 28, 26	**0,0017**
21, 1 – 21, 30	1,0000	24, 1 – 24, 30	0,9517	28, 1 – 28, 30	**< 0,0001**

Radiation and time did not show significant effects on 21 °C and 24 °C treatments, and also these temperatures not affects total height (Table 4). As shown Figure 6, total height of individuals kept at 21 °C and 24 °C remained constant thought the experiment.

As the less temperatures treatments, individuals who were maintained at 28 °C and submitted to PAR + UV irradiance were not changing its total height during the culture period and were not affected by radiation or time (Table 4 and Figure 5). Instead, individuals only irradiated by PAR lost height after 26 days cultured (Table 4 and Figure 5). In this way, while in cultures irradiated with PAR + UV total height remained constant, in cultures irradiated only with PAR decrease 0,6343 ± 0,2864 centimeters.

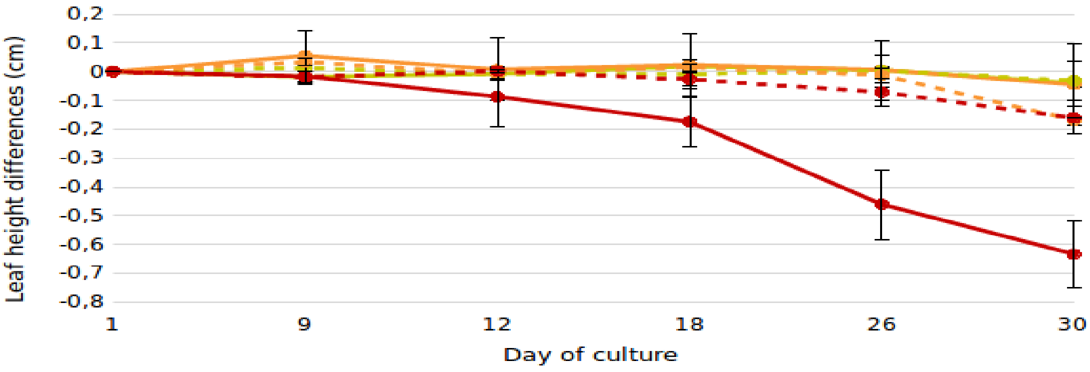

Figure 6. *Total height differences from the initial culture day of Cystoseira crinita at three temperatures (21 °C represented as yellow, 24 °C represented as orange and 28 °C represented as red) and two radiation conditions (PAR represented as a continuous line and PAR + UV represented as a dashed line) along 30 days of exposure. Standard error are represented by vertical bars (n=6).*

4.3. APEX HEIGHT

Significant changes on apex height were due to effects produced by temperature, radiation and its interaction (Table 5).

Table 5. *Generalized linear mixed model test for relationships between temperature (Temp), radiation (Rad) and time (Day) on apex height. Significant values are highlighted in bold.*

Variable	DF	denDF	F-value	P-value
Temp	2	175	5.0827	**0.0072**
Rad	1	175	6.9440	**0.0092**
Day	5	175	0.2281	0.9499
Temp:Rad	2	175	4.1889	**0.0167**
Temp:Day	10	175	0.2232	0.9938
Rad:Day	5	175	0.3034	0.9104
Temp:Rad:Day	10	175	0.1720	0.9979

Time did not affect apex height, since it remained constant over time (Table 6 and Figure 7). However, individuals cultured at 21 °C and submitted to PAR + UV radiation increased about 2,3245 ± 5,7203 (Table 6 and Figure 7).

Table 6. *Post-hoc tukey tests of the generalized linear mixed model test for relationships between temperature (Temp), radiation (Rad) and time (Day) on apex height: (I) Temperature x Radiation contrast. Significant values are highlighted in bold.*

Contrast	P-value	Contrast	P-value	Contrast	P-value
(I) Temperature x Radiation contrast					
21, PAR – 24, PAR	1,0000	21, UV – 24, UV	**0,0056**	21, PAR – 21, UV	**0,0020**
21, PAR – 28, PAR	0,9992	21, UV – 28, UV	**0,0025**	24, PAR – 24, UV	0,9998
24, PAR – 28, PAR	0,9989	24, UV – 28, UV	0,9999	28, PAR – 28, UV	0,9983

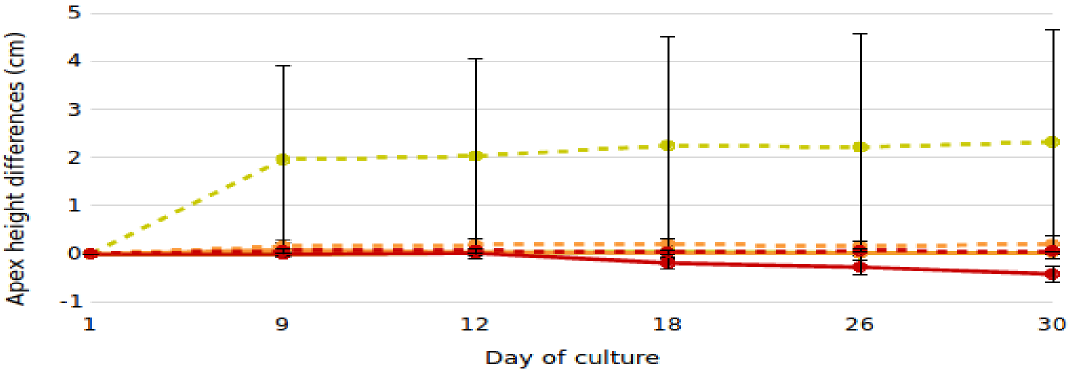

Figure 7. *Apex height differences from the initial culture day of Cystoseira crinita at three temperatures (21 °C represented as yellow, 24 °C represented as orange and 28 °C represented as red) and two radiation conditions (PAR represented as a continuous line and PAR + UV represented as a dashed line) along 30 days of exposure. Standard error are represented by vertical bars (n=6).*

4.4. MAXIMUM QUANTUM YIELDS OF PHOTOSYNTHESIS

In the case of the differences of F_v/F_m regarding the initial day the factors that affected were temperature, radiation and the interactions of the temperature and radiation, and the temperature and time (Table 7).

Table 7. *Generalized linear mixed model test for relationships between temperature (Temp), radiation (Rad) and time (Day) on maximum quantum yield of photosynthesis. Significant values are highlighted in bold.*

Variable	DF	denDF	F-value	P-value
Temp	2	85	72,9758	**< 0,0001**
Rad	1	85	3,9809	**0,0492**
Day	2	85	2,3783	0,0989
Temp:Rad	2	85	15,4707	**< 0,0001**
Temp:Day	4	85	4,6979	**0,0018**
Rad:Day	2	85	1,1012	0,3372
Temp:Rad:Day	4	85	0,7597	0,5544

The culture conditions at 21 °C and 24 °C did not generate changes in the yield during the 30 days of cultivation (Table 8 and Figure 8). Instead, individuals maintained at 28 °C showed a significant decrease of this measure over time, although this decrease occurred in a differential way between both radiations treatments (Table 8 and Figure 8).

This decrease in the cultures subjected to the highest temperature was detected after 12 days of exposure, but while PAR + UV conditions only produce a total decrease of 0,7057 ± 0,4030 of the F_v/F_m value, the exposure only to PAR generate a total decrease of 1,0000 ± 0,0000 (Table 8 and Figure 8).

Table 8. *Post-hoc tukey tests of the generalized linear mixed model test for relationships between temperature (Temp), radiation (Rad) and time (Day) on maximum quantum yield of photosynthesis: (I) Temperature x Radiation contrast; (II) Temperature x Time contrast. Significant values are highlighted in bold.*

Contrast	P-value	Contrast	P-value	Contrast	P-value
(I) Temperature x Radiation contrast					
21, PAR – 24, PAR	0,9991	21, UV – 24, UV	0,1263	21, PAR – 21, UV	0,9969
21, PAR – 28, PAR	**< 0,0001**	21, UV – 28, UV	**< 0,0001**	24, PAR – 24, UV	0,1615
24, PAR – 28, PAR	**< 0,0001**	24, UV – 28, UV	**0,0457**	28, PAR – 28, UV	**< 0,0001**
(II) Temperature x Time contrast					
21, 1 – 24, 1	1,0000	24, 1 – 28,1	1,0000	28, 1 – 21, 1	1,0000
21, 1 – 21, 5	1,0000	24, 1 – 24, 5	1,0000	28, 1 – 28, 5	0,2442
21, 1 – 21, 12	1,0000	24, 1 – 24, 12	1,0000	28, 1 – 28, 12	**0,0009**
21, 1 – 21, 15	0,9999	24, 1 – 24, 15	1,0000	28, 1 – 28, 15	**0,0001**
21, 1 – 21, 19	1,0000	24, 1 – 24, 19	1,0000	28, 1 – 28, 19	**0,0015**
21, 1 – 21, 26	0,9991	24, 1 – 24, 26	1,0000	28, 1 – 28, 26	**0,0139**

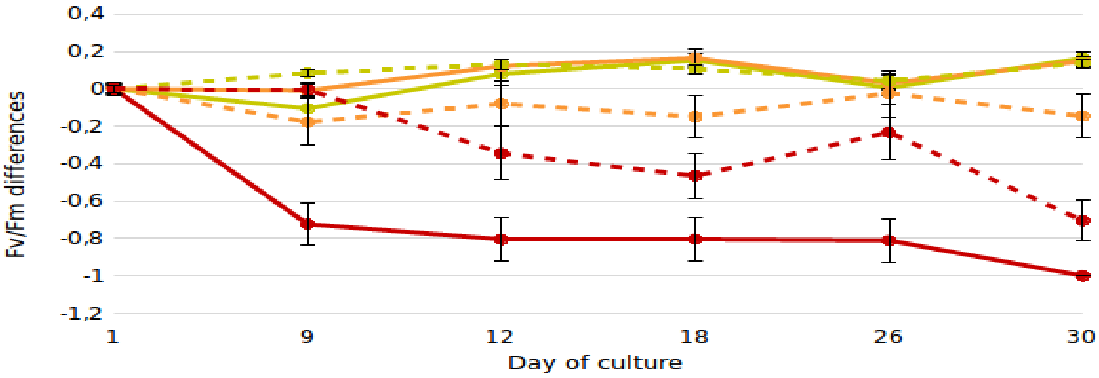

Figure 8. *Maximum quantum yield of photosynthesis differences from the initial culture day of Cystoseira crinita at three temperatures (21 °C represented as yellow, 24 °C represented as orange and 28 °C represented as red) and two radiation conditions (PAR represented as a continuous line and PAR + UV represented as a dashed line) along 30 days of exposure. Standard error are represented by vertical bars (n=6).*

5. DISCUSSION

In the year 2015 a severe mortality was observed in the populations of *Cystoseira crinita* coinciding with an abnormally hot summer, where extreme and anomalous values of temperature were recorded during summer (Agencia Estatal de Meteorología, 2017). Although this mortality event may be related to high temperatures, the primary cause remains controversial until now.

Previous massive benthic assemblages mortality events in the Mediterranean have been related to high temperatures because of exposure to high temperatures may cause partial or total death of specimens as a result of physiological stress, a decrease in the efficacy of defence mechanisms, and the development of pathogens (Cebrián et al., 2011). Likewise, there is a positive relationship between some of the Mediterranean benthic assemblages mortality rate and the time during which the populations experienced temperatures above a certain threshold (Cebrián et al., 2011).

Other potential stress factor is UV radiation because it has negative effects on the health of some species (Häder et al., 2011). These negative effects of UV radiation in several cases are enhanced by other environmental stressors because of a synergistically interaction (Häder et al., 2011).

Populations of *Cystoseira* conservation strongly relies on environmental conditions. Thus, studying relationships between ecological and biological factors is crucial to assess the current ecological status and to understand future changes derived from climate change (Sales & Ballesteros, 2009). Moreover, there is a critical importance of studying biological factors as a response to interactions between several stress factors, particularly with regard to ecological aspects and against the climate change framework (Fredersdorf, 2009).

5.1. TEMPERATURE EFFECTS

Temperature has a key role of in triggering mortality events; in fact there is a significant positive correlation between mortality and exposure time to elevated temperature conditions of several marine benthic species populations (Cebrián et al., 2011).

Often, the atmospheric temperature can reach up to 30,1 °C (Agencia Estatal de Meteorología, 2017). Similarly, water temperatures can peak up to 30 °C, although in NW Mediterranean Sea mean water temperature during summer is about 25 °C and usually it is not too many days above 27 °C or 28 °C (Agencia Estatal de Meteorología, 2017).

Consequently, temperatures between 21 °C and 24 °C are normally recorded during summer days and highest temperature is only reached punctually. So that, we show that *C. crinita* is adapted to resist a long periods under temperatures between 21 and 25 °C, but not to resist at the highest tested conditions. Our results shown that exposure time to elevated temperature conditions is a mortality driver (Appendix 1 and 2).

The thermal tolerance of *C. crinita* depends on both the exposure time and the temperature. In fact, at temperatures between 21 °C and 24 °C resists perfectly up to a month of continuous exposure while at 28 °C they are damaged in less than 15 days. This thermal tolerance over these periods of time is superior to that resisted by species that live in more stable environmental conditions, such as in the lower littoral levels (Cebrián et al., 2011). In summary, temperature increases affects negatively physiological state of adult individuals of *C. crinita*.

The final driver of *Cystoseira* mortality at highest temperatures is actually unknown, However, for other organisms, there are evidences of changes in the composition of the microbial community subjected to elevated temperatures, both at the level of the microbiota associated with the different species and at the level of the microbial community of water and the consequent establishment of alien microbial populations, including potential pathogens (Cebrián et al., 2011). So that, microbial studies on *Cystoseira* populations submitted to high temperatures, should be taken into account for future studies.

5.1. RADIATION EFFECTS

The irradiative tolerance of *C.crinita* is enough to support up to a whole month of continuous exposure underneath 74 watts of PAR and 93,3 watt of PAR + UV. Thus, at those levels of irradiance, ultraviolet radiation does not affect physiological state of adult individuals of *C. crinita*.

Total biomass and photosynthetic activity was not affected by UV-radiation. These results suggest that, like higher plants and other seaweeds adapted to Mediterranean clime, *C. crinita* have an efficient protective mechanisms against UV radiation because it was not affected by this ecological stressor factor (Appendix 1 and 2).

It's accepted that morphology and anatomy of leaves are relevant mechanisms against harmful effects of UV radiation on vegetal life, such as the inhibition of the photosystem II activity (Bernal et al., 2013). Maybe, as for terrestrial plants, certain photoinhibition and recovery patterns are a photoprotective mechanism against high radiation levels to avoid some reactive products (Viñegla et al., 2006). However, on some Mediterranean species is demonstrated that the increase of leaf mass area, the size of cells and the leaf carotenoids/chlorophyll a + b ratio to avoid UV radiation damage (Verdaguer et al., 2012; Bernal et al., 2013).

So, *C. crinita* radiation tolerance might not be due to an increase of leaves area because total biomass does not increase, but it could be due to pigmentation or to size cells changes such in other Mediterranean species (Verdaguer et al., 2012; Bernal et al., 2013). Further, there are also evidences of changes in the composition of the microbial community subjected to elevated UV exposures, specifically a mortality on microbial populations, including potential pathogens (Häder et al., 2011). So that, again, it should be taken into account for future studies the effect of temperature and radiation on the microbiota associated to the specie, the microbial community of water and also the pigmentation and the size of the cells to clarify the reason of the radiation tolerance of *C. crinita.*

5.1. TEMPERATURE AND RADIATION EFFECTS

Natural systems are almost always simultaneously subjected to multiple stressors. Yet, interactive impacts of multiple stressors are less frequent in research than the individual effects of these stressors, because is often assumed to be the additive accumulation of impacts associated with single stressors (Crain et al., 2008). However, there are three types of interaction describing the outcome of multiple stressors, depending on how the joint effect of various factors in relation to the effect of these independently (Crain et al., 2008). In this way, if the factor F1 produces the effect E1 and the factor F2 generates the effect E2, when the combined effect of F1 + F2 is equal to E1 + E2 the effect of the interaction is considered additive, when the combined effect of F1 + F2 is less than E1 + E2 the effect of the interaction is considered antagonistic and when the combined effect of F1 + F2 is greater than E1 + E2 the effect of the interaction is considered synergistic (Crain et al., 2008).

Contrary to what might be expected *a priori*, under the impact of combined temperature and radiation conditions, the highest temperature cultures were much more harmed when UV radiation was eliminated. Thus, the only changes for the benefit of the individuals were detected merely under 21 °C submitted to PAR + UV conditions. So that temperature and radiation did not have a cumulative or synergistic effect, it has an antagonistic effect on physiological state of individuals of *C. crinite* (Appendix 1 and 2).

This kind of interaction effect it could be due to that in trying to protect themselves from the harmful effects of UV radiation, both physically and chemically, individuals have a higher resistance to decomposition. However, it is also possible that this effect is not due to the direct impact on the individuals of *C. crinita*, but also on the microbial community. So that, radiation may be generating enough mortality on the microbial community to slow down the decomposition process although temperature increase promotes the appearance of normally pathogenic microorganisms. In this way, to clarify the reason why this type of interaction is generated should also consider variables such as the effect on pigmentation, size of cells and both microbial communities, the associated to the individuals and the one who inhabits in the water.

Future climatic projections for the Mediterranean region clearly indicate warming, with increases in extreme temperatures and heat waves, such as the one who has induced the recorded mortality in 2015 or even more severe (Cebrián et al., 2011). Because of that, new mass mortality events are extremely likely to occur during the next several decades. Given that *C. crinita* have been demonstrated to be very sensitive to global change perturbations, rich Mediterranean biodiversity may be threaten because of this seaweeds plays a structural role in benthic assemblages.

The results of this work should be taken into account for future conservation and restoration projects of this species since carrying out this kind of projects is costly and laborious. In this way it is advisable to carry out this type of actions taking into account the level of exposure of individuals to temperature and radiation with the aim that the probability of survival over time of the population is as high as possible. In addition, it is recommended to focus efforts on conserving and restoring those areas where these exposure boundaries are not going to be overcome.

6. CONCLUSION

In low and moderate temperature treatments (21 °C and 24 °C), total height and maximum quantum yields of photosynthesis remained constant during experiment. However, abnormal and constant high temperatures (up to 28°C) negatively affected physiological state of individuals. It means, that *C. crinita* thermal tolerance is adapted to resist a long time under the current normal summer temperatures *in situ*, but it is not able to resist long periods of abnormal high temperatures (reached only punctually: the so-called thermal anomalies).

Ultraviolet radiation does not affect physiological state of individuals. *C. crinita* dwells at the superior fringe of the littoral, and it' is probably adapted to very high irradiances, so irradiance itself does not affect physiological status of adult individuals.

The combined effect of temperature and radiation is antagonistic effect since individuals subjected at higher temperatures and UV radiations were significantly less impaired that the ones cultured at the same temperature and in PAR irradiance exclusively.

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8. REFERENCES

- Agencia Estatal de Meteorologia. (2017). Recovered from http://www.aemet.es/es/serviciosclimaticos
- AlgaeBase. (2017). Cystoseira crinta Duby. Recovered from http://www.algaebase.org/search/species/detail/?species_id=Bc5622901ffdb7574&sk=0&from=results
- Ballesteros, E. (1992). *Els vegetals i la zonació litoral: Espècies, comunitats i factors que influeixen en a seva distribució* (1st ed.). Barcelona: Institut d'Estudis Catalans.
- Ballesteros, E., Mariani, S., Cefalì, M. E., Chappuis, E. & Terradas, M. (2014). *Manual dels hàbitats Litorals de Catalunya* (1st ed.). Barcelona: Generalitat de Catalunya, Departament de Territori i Sostenibilitat.
- Bernal, M., Llorens, L., Badosa, J. & Verdaguer, D. (2013). Interactive effects of UV radiation and water availability on seedlings of six woody Mediterranean species. *Physiologia Plantarum, 147*(2), 234-247. Recovered from https://www.researchgate.net/publication/225277178_Interactive_effects_of_UV_radiation_and_water_availability_on_seedlings_of_six_woody_Mediterranean_species
- Blanufé, A., Boudouresque, C.F., Verlaque, M. & Thibaut, T. (2016). The fate of *Cystoseira crinita*, a forest-forming Fucale (Phaeophyceae, Stramenopiles), in France (North Western Mediterranean Sea). *Estuarine Coastal and Shelf Science, 181*(18), 196-208. Recovered from http://www.sciencedirect.com/science/journal/02727714/181/supp/C
- Cebrián, E., Uriz, M.J., Garrabou, J. & Ballesteros, E. (2011). Sponge Mass Mortalities in Warming Mediterranean Sea: Are Cyanobacteria-Harbouring Species Worse Off? *PLoS ONE,* 6(6): e20211. Recovered from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3105983/
- Crain, C.M., Kroeker, K. & Halpern, S. (2008). Interactive and cumulative effects of multiple human stressors in marine systems. *Ecology Letters, 11*(12), 1304-1315. Recovered from http://micheli.stanford.edu/pdf/crain%20et%20al.%202008%20ecol%20lett.pdf
- Fredersdorf, J., Müller, R., Becker, S., Wiencke, C. & Bischof, K. (2009). Interactive effects of radiation, temperature and salinity on different life history stages of the Arctic kelp *Alaria suculenta* (Phaeophyceae). *Oecologia, 160*(3), 483-492. Recovered from https://www.ncbi.nlm.nih.gov/pubmed/19330357
- Gianni, F., Bartolini, F., Airoldi, L., Ballesteros, E., Francour, P., Guidetti, P., Meinesz, A., Thibaut, T. & Mangialajo, L. (2013). Conservation and restoration of marine forests in the Mediterranean Sea and the potential role of Marine Protected Areas. *Advances in Oceanography and Limnology, 4*(2), 83-101. Recovered from http://www.tandfonline.com/toc/taol20/4/2?nav=tocList
- Häder, D.P., Helbling, E.W., Williamson, C.E. & Worrest, R.C. (2011). Effects of UV radiation on aquatic ecosystems and interactions with climate change. *Photochemical & Photobiological Sciences, 10*(2), 242-260. Recovered from https://www.ncbi.nlm.nih.gov/pubmed/21253662
- Marine forest. (2017). Forest: Cystoseira forest restoration. Recovered from http://www.marineforest.com/home.php
- Nenadis, N., Llorens, L., Koufogianni, A., Díaz, L., Font, J., Gonzalez, J.A. & Verdaguer, D. (2015). Interactive effects of UV radiation and reduced precipitation on the

seasonal leaf phenolic content/composition and the antioxidant activity of naturally growing *Arbutus unedo* plants. *Journal of Photochemistry & Photobiology, B: Biology, 153*(54), 435-444. Recovered from http://www.sciencedirect.com/science/article/pii/S1011134415003371

- RDocumentation. (2017). glmer function. R Documentation. Recovered from https://www.rdocumentation.org/packages/lme4/versions/1.1-13/topics/glmer
- Sales, M. & Ballesteros, E. (2009). Shallow Cystoseira (Fucales: Ochrophyta) assemblages thriving in sheltered areas from Menorca (NW Mediterranean): Relationships with environmental factors and anthropogenic pressures. *Estuarine Coastal and Shelf Science, 84*(4), 476-482. Recovered from http://www.sciencedirect.com/science/article/pii/S0272771409003382
- Sales, M., Cebrián, E., Tomas, F., & Ballesteros, E. (2011). Pollution impacts and recovery potential in three species of the genus Cystoseira (Fucales, Heterokontophyta). *Estuarine Coastal and Shelf Science,* 92(3), 347-357. Recovered from http://www.sciencedirect.com/science/article/pii/S0272771411000187
- Thibaut, T., Blanfuné, A., Boudouresque, C.F. & Verlaque, M. (2015). Decline and local extinction of Fucales in the French Riviera: the harbinger of future extinctions? *Mediterranean Marine Science, 16*(1), 206-224. Recovered from https://ejournals.epublishing.ekt.gr/index.php/hcmr-med-mar-sc/article/view/12875
- Thibaut, T., Pinedo, S., Torras, X. & Ballesteros, E. (2005). Long-term decline of the populations of Fucales (*Cystoseira* spp. and *Sargassum* spp.) in the Albères coast (France, North/western Mediterranean). *Marine Pollution Bulletin*, *50*(12), 1472-1489. Recovered from http://www.sciencedirect.com/science/article/pii/S0025326X05002602
- UN environament (n.d.). GRID-Geneva. Cystoseira crinita. Recovered from http://www.grid.unep.ch/bsein/redbook/txt/cystos-c.htm
- Verdaguer, D., Llorens, L., Bernal, M. & Badosa, J. (2012). Photomorphogenic effects of UVB and UVA radiation on leaves of six Mediterranean sclerophyllous woody species subjected to two different watering regimes at the seeding stage. *Environmental and Experimental Botany, 79*(9), 66-75. Recovered from http://www.sciencedirect.com/science/article/pii/S0098847212000263
- Viñegla, B., Segovia, M. & Figueroa, F.L. (2006). Effect of artificial UV radiation on carbon and nitrogen metabolism in the macroalgae *Fucus spiralis* L. and *Ulva olivascens* Dangeard. *Hydrobiologia, 560*(1), 31-42. Recovered from https://link.springer.com/article/10.1007/s10750-005-1097-
- World Register of Marine Species. (n.d.). WoRMS taxon details. Cystoseira crinita. Recovered from http://www.marinespecies.org/aphia.php?p=taxdetails&id=145514

APPENDIX

1. INITIAL DAY IMAGES

Table 9. *Representative images of the status of Cystoseira crinita at the beginning of the experiment for each treatment: (I) PAR 21 °C; (I) PAR 24 °C; (III) PAR 28 °C; (IV)* PAR + UV 21 °C; *(V)* PAR + UV 24 °C; *(VI)* PAR + UV 28 °C.

(I) PAR 21 °C	*(II) PAR 24 °C*	*(III) PAR 28 °C*
		
(IV) PAR + UV 21 °C	*(V) PAR + UV 24 °C*	*(VI) PAR + UV 28 °C*
		

2. FINAL DAY IMAGES

Table 10. *Representative images of the status of Cystoseira crinita at the ending of the experiment for each treatment: (I) PAR 21 °C; (II) PAR 24 °C; (III) PAR 28 °C; (IV)* PAR + UV 21 °C; *(V)* PAR + UV 24 °C; *(VI)* PAR + UV 28 °C.

(I) PAR 21 °C	*(II) PAR 24 °C*	*(III) PAR 28 °C*
		
(IV) PAR + UV 21 °C	*(V) PAR + UV 24 °C*	*(VI) PAR + UV 28 °C*