ALBA TRILLA CISA

PHYSIOLOGICAL RESPONSES TO EXTREME EVENTS OF DIFFERENT MEAN INTENSITY AND VARIABILITY IN A FOUNDATION MACROALGA AT ITS DISTRIBUTIONAL LIMIT

Dissertação de Candidatura ao grau de Mestre em Ciências do Mar – Recursos Marinhos submetida ao Instituto de Ciências Biomédicas de Abel Salazar da Universidade do Porto.

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ABSTRACT

Changes on the mean intensity or temporal variability of climatic extreme events have been reported all over the globe. Interactive effects within those factors driving community response to several types of disturbances on benthic systems have been studied but research in the context of climate change and specifically about organisms physiological response to those changes is nearly absent.

We carried out two types of manipulative experiments simulating different mean intensity and temporal variance of extreme events based on seawater and air temperature on the intertidal area, using a common intertidal species in the area of study, namely *Fucus serratus.* The selection of temperatures was done based on the results obtained on a selection of stress level experiment, where *F. serratus* was maintained at 12 different temperatures within the interval between 8 and 30ºC during 6 weeks.

Both experiments showed statistically significant or near to significant effects of the interaction within intensity and temporal variance of extreme events on the growth and physiological response *of F. serratus* individuals. The trends observed were similar in seawater and air temperature experiments; it seemed that high temporal variance may be buffering the negative effects of high intensity. The idea discussed by other authors of temporal variance being a determinant key factor on high intensity rather than in low intensity events was supported by our results, although it was not in all response variables.

Our results revealed interactive effects of mean intensity and temporal variance of seawater and air temperature stress events on *F. serratus* growth and physiological response and provided empirical support to Jensen's inequality. The mechanisms driving these responses remains still unknown, thus future work may aim on the plasticity of species, regarding acclimation and recovery responses under climate change scenarios.

RESUMO

Alterações de intensidade e variabilidade temporal de eventos climáticos extremos tem sido registados globalmente. Muitos estudos mostram que os efeitos interativos entre estes dois fatores podem determinar a resposta das comunidades ecológicas que se deparam com vários tipos de perturbações presentes no intertidal rochoso. No entanto, existe uma falta de estudos no contexto das alterações climáticas e mais especificamente na resposta fisiológica dos organismos frente a essas mudanças.

Foram realizadas dois tipos de experiências manipulativas para simular diferentes intensidades médias e variabilidades temporais de eventos climáticos extremos, baseados na temperatura de água do mar e do ar no intertidal rochoso. A espécie usada foi *Fucus serratus*, uma espécie de alga muito comum na área intertidal de estudo.

Nas duas experiências observaram-se efeitos estatisticamente significativos ou perto de significativos para a interação entre a intensidade e a variabilidade temporal no crescimento e na resposta fisiológica de *F. serratus*. As tendências observadas nas duas experiências foram muito semelhantes sugerindo que a alta variabilidade temporal pode reverter os efeitos negativos de alta intensidade de eventos extremos. Em concordância com os nossos resultados, na maioria das variáveis de resposta, outros estudos também demonstraram que a variabilidade temporal tem um papel mais importante nas situações de alta intensidade do que nas de baixa intensidade.

Os nossos resultados revelaram efeitos interativos da intensidade média e a variabilidade temporal dos eventos extremos relacionados com a temperatura da água do mar e do ar no crescimento e na resposta fisiológica de *F. serratus* e ofereceram suporte empírico à desigualdade de Jensen (Jensen’s inequality). Os mecanismos que controlam essas respostas ainda são desconhecidos. Assim, futuros estudos devem ser direcionados para avaliar a plasticidade das espécies no que se refere às respostas de aclimatação e recuperação em cénarios de alterações climáticas.

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1. INTRODUCTION

When exploring causality behind species distribution or abundance, ecologists have usually focused on studying the variation of species response to changes on the mean intensity of predictor variables, e.g. environmental factors, while few attention has been paid to the variability of predictor variables as causal explanation for the species response. Temporal and spatial variability of environmental factors is a wide recognized trait of natural systems (Horne et al. 1995), thus designing experiments including variability in the predictor variables may in fact lead to more realistic results rather than those experiments using mean constant values of the predictor variables (Benedetti-Cecchi 2003; Benedetti-Cecchi et al. 2006; Bertocci et al. 2005).

Recent interest on how natural systems are affected by changes in temporal variability and mean intensity of extreme events is linked to the current concerns on climate change impacts (Beniston et al. 2007). Changes on the mean intensity or temporal variability of extreme events have been reported worldwide since 1950 (see International Panel on Climate Change IPCC 2012). Occurrence of extreme events, like the combination of warm summers followed by cold winters or the occurrence of record-breaking temperatures and precipitations (Alexander et al. 2006), offer scientist a very interesting opportunity to test biogeographic hypotheses (Wethey et al. 2011). For example, the winter of 1962-1963, one of the coldest in Europe since 1740, lead to mass mortality of several species and contractions of ranges of hundreds of kilometers (Crisp 1964). Some species and their associated communities took almost 40 years to reoccupy to their previous range limits (Mieszkowska et al. 2007). Likewise, warm winters can collapse cold species such as *Semibalanus sp*., in this late case the species needed two cold winters to recolonize. Researchers suggest that these fluctuations between presence/absence maybe the precursor of local extinctions (Drake et al. 2010; Oborny et al. 2005; Svensson et al. 2005; Wethey et al. 2011).

Despite the wide knowledge on the ecological importance of extreme events, there is not much agreement on its definition and attempting to formulate a universal definition of extreme may not be even appropriate (McGregor et al. 2005). McGregor et al. (2005) considered that an event may be considered extreme if any of its properties (e.g. extension, duration or intensity) present values outside of a determined range, regardless of whether the event is frequent or occasional. The IPCC Special Report (IPCC 2012) defined an extreme event as “the occurrence of a value of a weather or climate variable above (or below) a threshold value near the upper (or lower) ends of the range of observed values of the variable”. The IPCC report also remarked that absolute functional thresholds can be used to identify extreme events (e.g. specific critical temperatures for health impacts) and the fact that impacts of extreme weather or climate events, do not necessarily have to be extreme. While McGregor et al. (2005) definition of extreme event does not include the temporal variance, it just takes into account the intensity of the event, IPCC (2012) definition does include both parameters, intensity and temporal variability. Besides the definition, there are several proposed ways to classify extreme events, based on the existing disparity of types of extreme events and their differentiation by traits including time scale or spatial scale. For example and regarding time scale, tornados and thunderstorms may last less than one day, contrarily hot summers or cold winters that last weeks to months. The variation of the spatial scale resides on being concentrated in a single geographic area such as tornados or diffused events, affecting more extended geographic area such as droughts (McGregor et al. 2005). Some of the most extreme events are the result of the Earth’s climate variability like tropical cyclones, mid-latitude winter storms, droughts and dust storms, floods, monsoons, heat waves and cold waves among others (IPCC 2007; WMO). Also some of them tend to occur every year and others have decadal or multi-decadal variations which are part of the Earth’s climate temporal variability. Thus, even with the lack of the current anthropogenic pressure leading to climate change, extreme events would still occur at various temporal and spatial scales. However current climate change seems to modify the nature of extreme events. Those modifications include changes in the intensity of the event, the temporal variance (i.e. probability of occurrence) or the shape of the distribution (i.e. both intensity and temporal variance) (see Figure SPM3, pag.7 from the Summary of Policymakers from IPCC (IPCC 2012)). It is also important to remark that some weather or climatic events, when occur separately are not extreme, but when acting together (accumulative effect) they can lead to extreme events such as droughts (IPCC 2007). Likewise extreme events may result from single or multiple environmental drivers acting simultaneously; thus Easterling et al. (2000b) classify extreme events as (i) simple extremes based on climate statistics (i.e. just one climate variable is responsible for the extreme event) including very low or very high daily temperatures or monthly precipitations or (ii) as complex event-driven extreme events (i.e. two or more climate variables are involved) such as droughts (i.e. high temperatures and low precipitations) or hurricanes (high precipitations and strong winds). Interestingly, Gutschick et al. (2003) propose that extreme events also need to be defined in terms of organismal responses of acclimation or hysteresis (a term adapted from physical sciences, it means that a system does not retrace its path as a driving variable varies cyclically) and not just based in the mean values of the climatic or environmental variables.

Early experiments on temporal variability manipulated frequency of extreme events (McCabe et al. 2000; Navarrete 1996) but were unable to disentangle frequency and intensity. Frequency is the number of events per unit of time, and the higher is the frequency the higher will also be the overall intensity i.e. higher frequency means more stress events in the same period of time (Benedetti-Cecchi 2003). To avoid this problem Benedetti-Cecchi (2003) proposed a new esperimental design in which intensity and variability are ortogonal factors. This design was applied by several studies to examine the interactive effects of mean intensity and temporal variability of some types of disturbance on rocky shore habitats, such as grazing on algal cover (Benedetti-Cecchi et al. 2005), aerial exposure (Bertocci et al. 2007), the effect of disturbance on the invasion of *Caulerpa racemosa* (Incera et al. 2010), the effect of sediment scouring on assemblages (Vaselli et al. 2008), or the effect of disturbance in fouling assemblages (Atalah et al. 2007; Cifuentes et al. 2007). Most of them found significant interactive effects between mean intensity and temporal variability (but see Atalah et al. 2007; Cifuentes et al. 2007). Vaselli et al. (2008) found significant differences within assemblages exposed to low intensity and high temporal variance of disturbance and other treatments. Benedetti-Cecchi et al. (2006) suggested that high temporal variance may mitigate the impacts of predicted climate change, i.e. higher mean intensity.

Most of the studies regarding spatial or temporal variability cited above have focused on processes such as competition, predation or disturbance, and there is not much research in the context of climate change (Benedetti-Cecchi et al. 2006). Similarly, only community level responses to changes on mean intensity and temporal variance have been studied and few or no information is available about the physiological response of individual species. Since assemblage's dynamic is ultimately driven by the dynamic of the species, studying the response of species to the predicted changes in mean intensity and temporal variance of extreme events will help to understand the mechanisms behind community level responses.

In our study we used the algae *Fucus serratus* as model species. *F. serratus* is an intertidal cold-temperate fucoid with a restricted distribution along the Atlantic coast of the Iberian Peninsula (Fig. 1a) (Araujo et al. 2011; Arrontes 1993; Arrontes 2002; Lima et al. 2007; Martínez et al. 2012a; Pearson et al. 2009; Viejo et al. 2011). *F. serratus* southernmost European populations occurs in North Portugal and is absent in the inner part of the Gulf of Biscay. Thus in the Iberian Peninsula the species presents two distributional limits, mid-Cantabrian and north Portugal (Lüning 1990). These two distributional limits cannot be explained by the traditional biogeographic models (Martínez et al. 2012a) which link the southern distributional limit of those cold-temperate fucoids to the August oceanic isotherm (Fig. 1b) (reviewed in Lüning 1990).The mid-Cantabrian limit, could possibly be explained by the warmer sea surface temperatures (Arrontes 1993; Martínez et al. 2012a; Viejo et al. 2011), but on the North Portugal limit, summer upwelling events prevent August sea temperatures to reach mid-Cantabrian values (Gomez-Gesteira et al. 2008). Although low tide stress (i.e. air temperature and other physical factors) have also been suggested as driving factors on the setting of this distributional limits (Martínez et al. 2012a; Viejo et al. 2011).

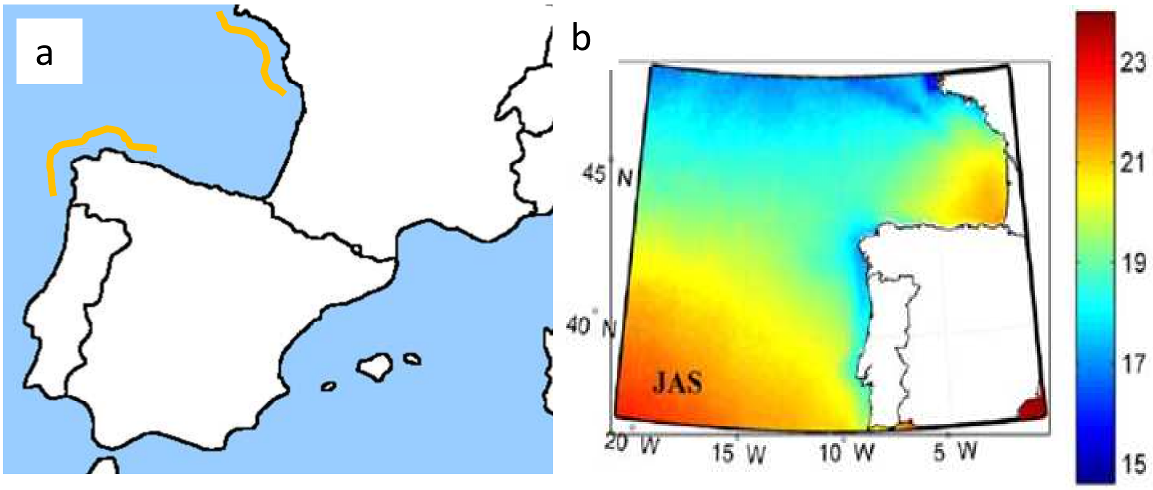

Figure 1. a) *Fucus serratus* distribution along the Atlantic coast of the Iberian Peninsula. b) Mean sea surface temperature (SST) (ºC) for summer season (July, August and September) on the Atlantic coast of the Iberian Peninsula (Gomez-Gesteira et al. 2008).

Its singular distribution patterns, the east- and west-wards displacements of its distributional limit on the mid-Cantabrian during the last century (Lima et al. 2007), and the possibility of using *F. serratus* as a model species for other cold-temperate macroalgae that also seem to be declining in North Iberian Peninsula (Martínez et al. 2012a), incited us to select *F. serratus* as our target species. Thus, we assumed that temporal variance may have a more determinant role under high intensity treatments than under low intensity treatments as our initial hypothesis and we tested it through a manipulative experiment with *F. serratus*, simulating high temporal variability or increased mean intensity of extreme events, i.e. seawater and air temperature heat waves and studying the possible interactive effects of those two factors using a factorial design (Underwood 1997).

2. MATERIAL AND METHODS

2.1 Manipulative seawater stress experiment

2.1.1 Algae collection and acclimation

Individual fronds of *Fucus serratus* (n=288) (Fig. 2a) were collected during low tide on the 17th of October 2012 in Amorosa 41º 38' N, 8º 49' W (Northern Portugal) (Fig. 2b). Algae were transported to the laboratory at CIIMAR (Centro Interdisciplinar de Investigação Marinha e Ambiental, Porto University, Portugal) within the following 2 hours in a portable plastic refrigerator box in darkness. Once at the laboratory, algae were rinsed with freshwater to remove grazers and submersed in a 300 l seawater outdoor sheltered tank set at 14ºC, for one day acclimation and a further 5 days acclimation at the experimental tanks, set at 16 ºC. Individual fronds of *F. serratus* weighting on average 1.29 ± 0.2 g (mean±SE, n=288) were picked up from the 300 l tank to run the experiment. Each replicate was individually labeled (Fig. 2c) and hold using plastic pegs marked with numbers from lines attached to plastic frames (Fig. 2d).

Figure 2. a) *Fucus serratus*, b) View of Amorosa beach, Northern Portugal, c) Each replicate was individually labeled using pegs marked with numbers, d) Replicates were individually labeled and hold using pegs from lines attached to plastic frames.

2.1.2 Experimental design

The experiment was performed in an outdoor area (Fig. 4a), from October to December 2012, lasting 45 days. During the experiment, solar irradiance (PAR) reached maximum irradiance values of about 2000 µmol photons m^{-2} s^{-1}. Average daily irradiance per hour during the whole experiment is represented in Fig. 3.

The aim of the experiment was to assess the effects of mean intensity and temporal variance of water temperature extreme events on *F. serratus* physiological and growth responses. Experimental design consisted of four factors: (1) mean intensity of stress (water temperature) with two levels (low temperature: 22ºC and high temperature: 26 ºC), fixed factor; (2) temporal variance, two levels (low and high), fixed and crossed with mean intensity; (3) sequence (different stress events distribution, in order to detect possible effects of distribution of stress events instead of temporal variance effects), 2 levels, random and nested with mean intensity and temporal variance; (4) chamber, 2 levels (each treatment was replicated in two different chambers), random and nested with the other three factors. Eight chambers set at 16ºC and with no changes on mean intensity or temporal variance were used as un-manipulated controls. Each treatment combination included 12 replicated fronds.

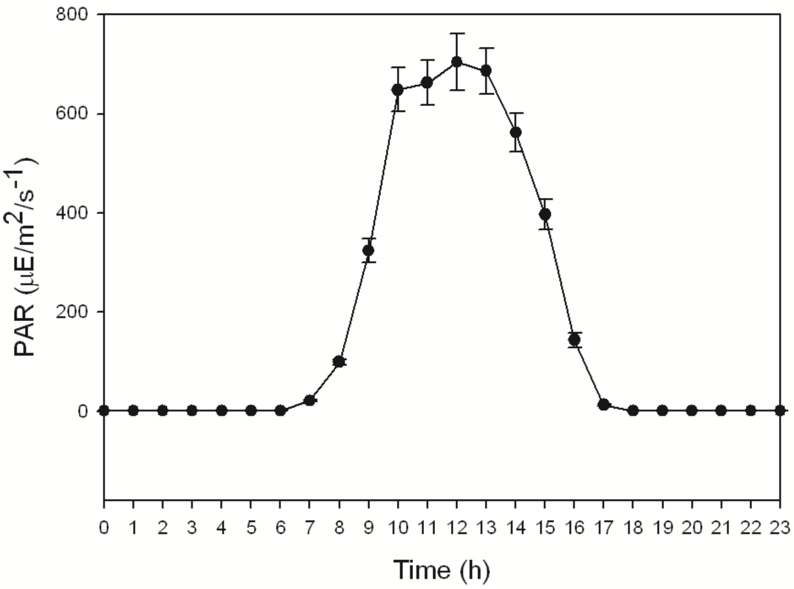

Figure 3. Average daily irradiances per hour during the whole experiment.

Seawater temperature was controlled using titanium heaters (Fig. 4b) regulated by digital controllers and individual temperature probes (Aqua Medic ® AT Control System controllers, GmbH, Bissendorf, Germany). This system allowed a continuous control and record of seawater temperature with a programmed error of 0.2ºC. Individual 20 l seawater tanks were submersed in a fresh water bath system (Fig. 4c), permanently set at 16ºC, in order to ameliorate the effects of changes in air temperature. Tanks were covered with a neutral fiberglass mesh (Fig. 4d), reducing 33% of the incident light with no changes on light quality, in order to avoid light excess due to the setting and nature of the experimental tanks (i.e. unsheltered outdoor area and white tanks). To avoid nutrient limitation, seawater was enriched every two days by adding inorganic N ($NaNO_3$) and P (NaH_3PO_4) to a final concentration of 50µM N and 5µM P. Salinity was regularly monitored and tanks were regularly refilled with freshwater, mainly the tanks with higher temperatures every few days, to compensate for water evaporation. Water inside the tanks was aerated and agitated by bubbling air systems.

Figure 4. a) General view of the outdoor seawater tanks system used for the seawater stress experiments, b) Detailed view of each chamber, containing the titanium heater and the temperature probes, c) Seawater 20 l tanks submersed in the water bath system, d) General view of the outdoor system covered with the fiberglass mesh, reducing 33% of the incident light.

2.1.3 Selection of stress levels

We used information gathered from literature and results from a previous experiment to decide the intensity of our stress treatments. The previous experiment was performed in a sheltered outdoor area during May and June 2012. Individual fronds of *Fucus serratus* were maintained at 12 different temperatures within the interval between 8 and 30ºC (Fig. 5) during 6 weeks in order to determine the lethal, sub-lethal and optimal water temperatures for *F. serratus* performance, through the increase or decrease of species growth rates.

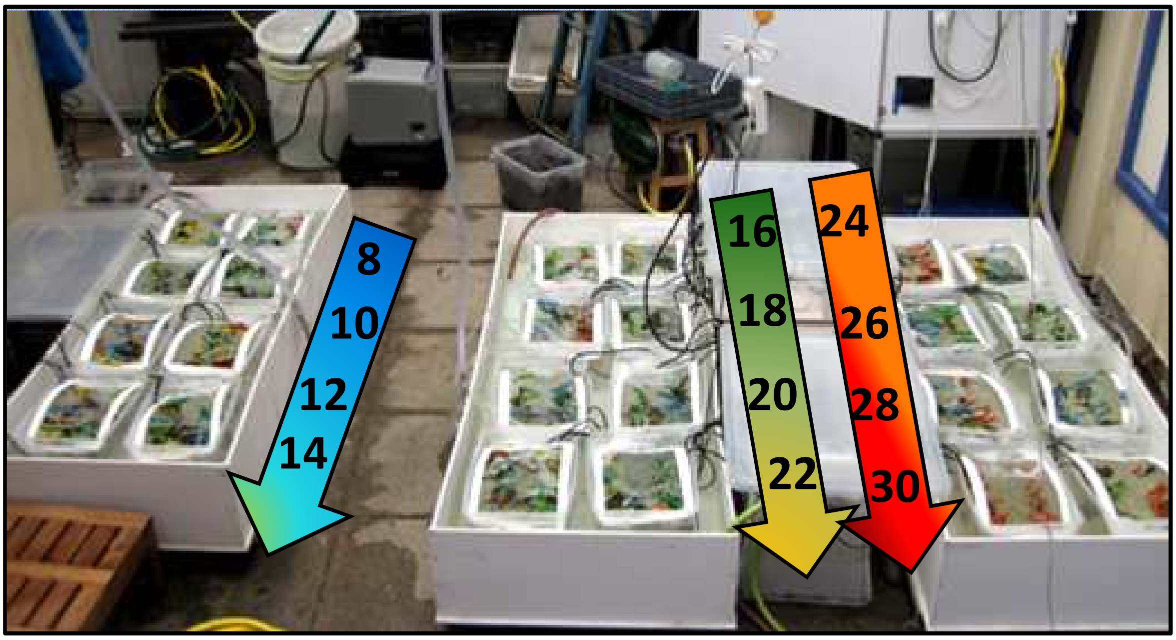

Figure 5. Experimental setup used for the selection of stress levels experiment. Individuals of *F. serratus* were maintained at 12 different temperatures within 8 and 30ºC.

2.1.4 Treatments application

Stress events were simulated as 3 days heat waves (Table 1) (Fig. 6). During no stress events simulations, seawater was set at 16ºC (optimal) and for the heat waves simulations, each chamber was manually set at the corresponding intensity (sea water temperature) level (low or high) within the following 2 hours. Average temperature values of the intensity treatments were, for low intensity 19.47±0.08 ºC (mean±SE, n=8) and for high it corresponded to 22.04±0.13 ºC (mean±SE, n=8) (Table 2.). Temporal variance treatments, low (0.25±0.04 days, mean±SE, n=8) and high (4.11±0.33 days, mean±SE, n=8) were applied calculating the variance of the number of days among consecutive heat wave simulations. The precise values of variance have no specific reason, they were obtained trying to create different sequence of events in order to get the lowest or highest values of variance, but maintaining similar values within temporal variance levels.

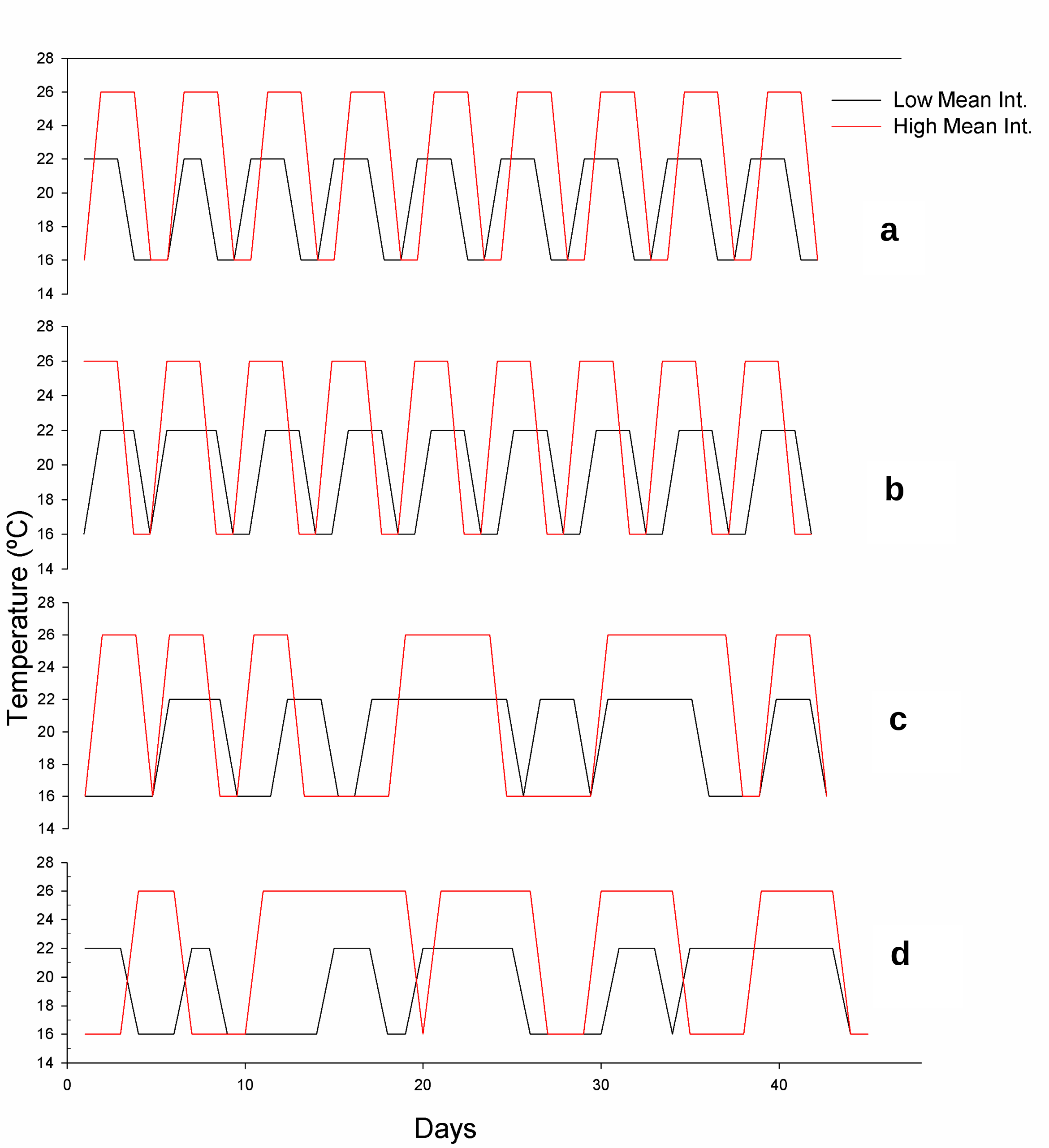

Figure 6. Water temperature experiment treatments. a) Low temporal variance, sequence 1, b) Low temporal variance, sequence 2, c) High temporal variance, sequence 1, d) High temporal variance, sequence 2. Black line corresponds to low intensity treatments and red line corresponds to high intensity treatments.

Table 1. Distribution of stress events (seawater temperature) through the totality of the experiment (45 days). Real (not theoretical) distribution, including errors due to logistical constraints. First four lines correspond to low level of intensity (22ºC) and second four to the high level of intensity (26ºC). The first two lines of each level of mean intensity correspond to the temporal variance treatments, low (stress events distributed in a homogeneous way) and high (stress events distributed differently). Each combination of mean intensity and temporal variance had two different sequences (1 and 2) in order to dismiss a possible effect of the distribution sequence of stress events.

			Day																																												
Int.	TV	Seq.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
Low	Low	1	22	22	22	16	16	16	22	22	16	16	22	22	22	16	16	22	22	22	16	16	22	22	22	16	16	22	22	22	16	16	22	22	22	16	16	22	22	22	16	16	22	22	22	16	16
Low	Low	2	16	22	22	22	16	22	22	22	22	16	16	22	22	22	16	16	22	22	22	16	16	22	22	22	16	16	22	22	22	16	16	22	22	22	16	16	22	22	22	16	16	22	22	22	16
Low	High	1	16	16	16	16	16	22	22	22	22	16	16	16	22	22	22	16	16	22	22	22	22	22	22	22	22	22	16	22	22	22	16	22	22	22	22	22	22	16	16	16	16	22	22	22	16
Low	High	2	22	22	22	16	16	16	22	22	16	16	16	16	16	16	22	22	22	16	16	22	22	22	22	22	22	16	16	16	16	16	22	22	22	16	22	22	22	22	22	22	22	22	22	16	16
High	Low	1	16	26	26	26	16	16	26	26	26	16	16	26	26	26	16	16	26	26	26	16	16	26	26	26	16	16	26	26	26	16	16	26	26	26	16	16	26	26	26	16	16	26	26	26	16
High	Low	2	26	26	26	16	16	26	26	26	16	16	26	26	26	16	16	26	26	26	16	16	26	26	26	16	16	26	26	26	16	16	26	26	26	16	16	26	26	26	16	16	26	26	26	16	16
High	High	1	16	26	26	26	16	26	26	26	16	16	26	26	26	16	16	16	16	16	16	26	26	26	26	26	26	16	16	16	16	16	26	26	26	26	26	26	26	26	26	16	16	26	26	26	16
High	High	2	16	16	16	26	26	26	16	16	16	16	26	26	26	26	26	26	26	26	26	16	26	26	26	26	26	26	16	16	16	26	26	26	26	26	16	16	16	16	26	26	26	26	26	16	16

Table 2. Treatment values (mean ± SE, n=8) of intensity (seawater temperature, ºC) and temporal variance (variance of number of days between stress events).

	Mean intensity		Temporal variance	
Level	Low	High	Low	High
Mean	19.47	22.04	0.25	4.11
SE	0.08	0.13	0.04	0.33

2.2 Manipulative air temperature (low tide) stress experiment

In order to confirm the patterns detected on the first experiment and to clarify potential interactive effects of the intensity and the temporal variance of stress events on the physiological performance of *Fucus serratus*, a manipulative experiment based on low tide stress (air temperature) was realized.

2.2.1 Algae collection and acclimation

Individual fronds of *Fucus serratus* (n=160) were collected during low tide on the 25th of February 2013 in Amorosa 41º 38' N, 8º 49' O (Northern Portugal). Individuals of *Fucus serratus* were maintained in a 300 l seawater tank set at 16ºC (optimal temperature) (Fig. 7a).

2.2.2 Experimental design and treatments application

Sixteen indoor air incubators (Fig. 7b) were set at 2 different air temperatures, 24 and 34 ºC , those temperatures correspond to ameliorated and harsh summer temperatures used in previous low tide experiments with the same species from the same geographical area (Martínez et al. 2012a). Ten replicated fronds were used for each combination of temperature, temporal variance and sequence treatment. Tanks were allocated inside a CT chamber set at 24ºC, working as an air bath system. To regulate and monitor the air temperature in each single tank, a system of sensors and terrarium heater cables (Fig. 7b) controlled by computer (AT Control System, AB Aqua Medic GmbH, Bissendorf, Germany) was used.

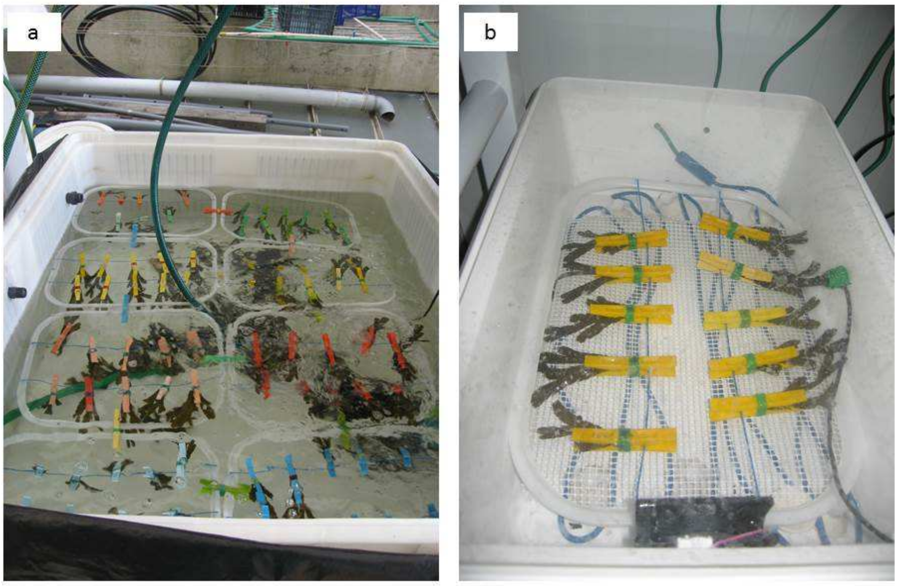

Figure 7. a) 300 l seawater tank were *Fucus serratus* individuals were maintained at optimal conditions (16ºC). b) Indoor air tanks set with the thermic cable.

Table 3. Characterization (mean ±SE, n=12) of intensity treatments (air temperature, ºC) of each combination of mean intensity, temporal variance (TV) and sequence (S) levels. Each value corresponds to the average of 6 measurements from chamber 1 and 6 of chamber 2 of each treatment.

	Intensity Low				Intensity High			
	TV Low		TV High		TV Low		TV High	
	S1	S2	S1	S2	S1	S2	S1	S2
Average	22.48	22.92	22.73	23.38	32.36	32.20	32.28	32.90
SE	0.71	0.45	0.31	0.38	0.50	0.55	0.33	0.17

Treatments were applied transferring individuals from the 300 l seawater tank (Fig. 7a) set at 16ºC to the air tanks (Fig. 7b) set at two different temperatures (Table 3) and following the designed sequences of temporal variance of stress (Table 4) (Fig. 8) in order to achieve the low and high temporal variance treatments (Table 5). In those situations were two stress events had to be applied one after the other (e.g. Table 4, line 3, corresponding to low intensity, high temporal variance, sequence 1, hours 2, 3, and 4) algae were briefly submersed in a seawater tank set at 16ºC, between each stress event, in order to buffer the possible effect of accumulation of stress due to consecutive stress events. The experimental design was almost the same as in the water temperature experiments, i.e. chamber factor was nested in the interaction of mean intensity, temporal variability and sequence. In this case sequence was orthogonal with mean intensity and temporal variance, instead of nested, because sequence 1 and 2 were the same through the different combinations of mean intensity and temporal variance treatments.

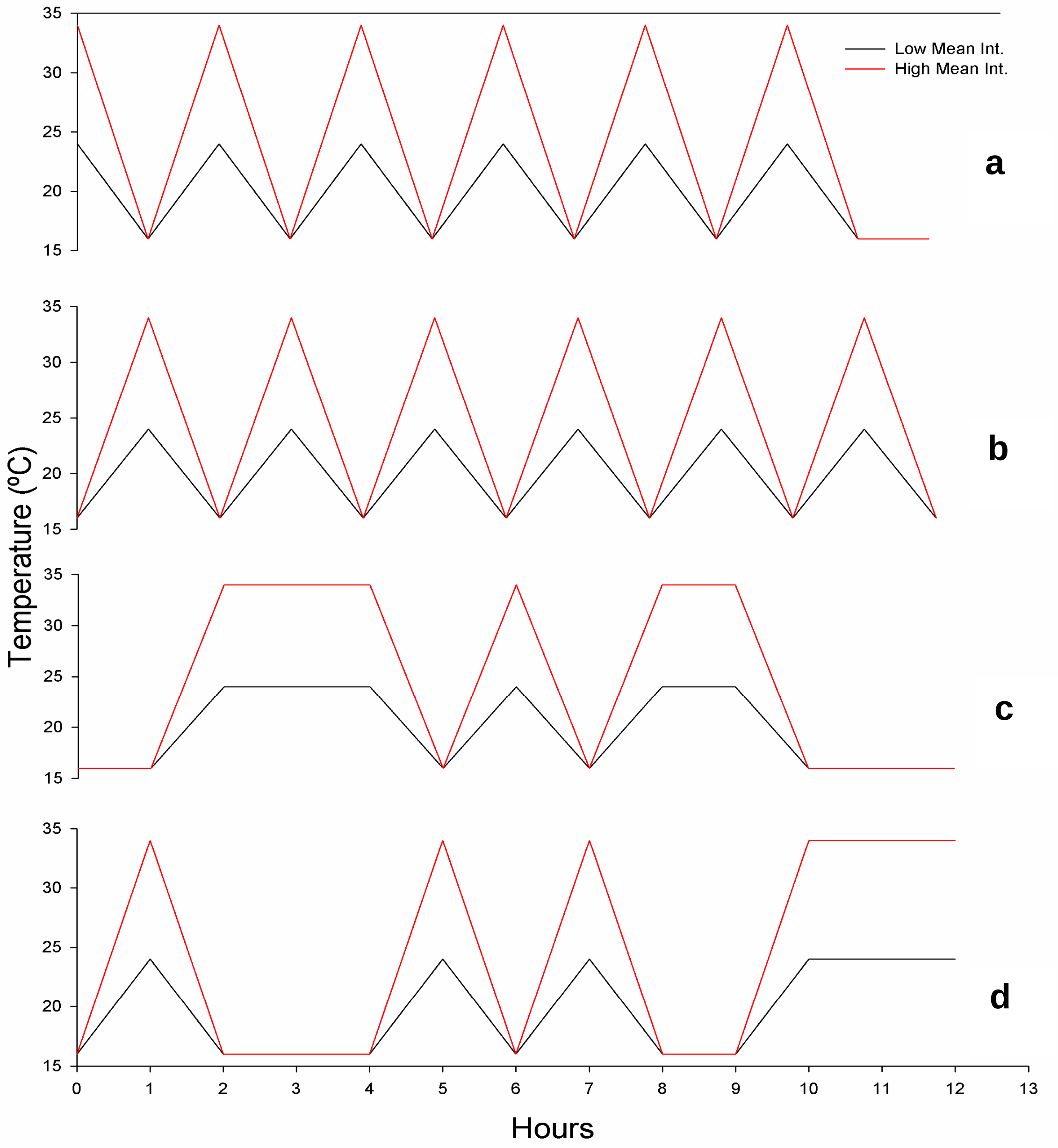

Figure 8. Graphic representation of all air temperature experiment treatments. a) Low temporal variance, sequence 1, b) Low temporal variance, sequence 2, c) High temporal variance, sequence 1, d) High temporal variance, sequence 2. Black line corresponds to low intensity treatments and red line corresponds to high intensity

Table 4. Distribution of stress events (seawater temperature), in grey, through the totality of the experiment (12 hours). First four lines correspond to low level of mean intensity (Int.) (24 ºC) and second four to the high level of mean intensity (34 ºC). The first two lines of each level of mean intensity correspond to temporal variance (TV) treatments, low (stress events distributed in a homogeneous way) and high (stress events distributed differently). Each combination of mean intensity and temporal variance had two different sequences (1 and 2) in order to dismiss a possible effect of the distribution sequence of stress events.

			Hour												
Int.	**TV**	**Seq.**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Low	**Low**	**1**	24	16	24	16	24	16	24	16	24	16	24	16	16
Low	**Low**	**2**	16	24	16	24	16	24	16	24	16	24	16	24	16
Low	**High**	**1**	16	16	24	24	24	16	24	16	24	24	16	16	16
Low	**High**	**2**	16	24	16	16	16	24	16	24	16	16	24	24	24
High	**Low**	**1**	34	16	34	16	34	16	34	16	34	16	34	16	16
High	**Low**	**2**	16	34	16	34	16	34	16	34	16	34	16	34	16
High	**High**	**1**	16	16	34	34	34	16	34	16	34	34	16	16	16
High	**High**	**2**	16	34	16	16	16	34	16	34	16	16	34	34	34

Table 5. Theoretical treatment values (mean ± SE, n=4) of mean intensity (air temperature, ºC) and temporal variance (variance of number of days between stress events).

	Mean intensity		Temporal variance	
Level	Low	High	Low	High
Mean	19.62	24.31	0.08	0.92
SE	0.00	0.00	0.05	0.00

2.3 Functional responses

2.3.1 Growth response (seawater temperature experiment)

Fresh individual weight of each frond was weighted to the nearest 0.1g at the beginning of the experiment and then at the end, after 45 days. Fronds were also weighted during the experiment, approximately every 10 days, in order to supervise the growth of the fronds and to make sure that treatments were replicating as expected. Growth was calculated as: $(FW_f - FW_0) / T$, being FW_f the final fresh weight (g) at the end, FW_0 the initial fresh weight (g) and T, the time (days).

2.3.2 In vivo physiological parameters (seawater and air temperature)

The physiological status of the algae was measured through the photosynthetic capacity of their photosystem II or Maximal Quantum Yield of Photosynthesis (Fv/Fm) and the Electronic transport rate (ETR) as an indicative of their stress in response to the treatments. MINIPAM (Heinz Walz GmbH, Effeltrich, Germany) was used for the measurements.

The Fv/Fm ratio determines the maximum efficiency on photochemical energy converting processes of a darkness adapted plant (Maxwell et al. 2000). Electron transport rate (ETR) was determined by using rapid light curve (RLC) technique taking 15 s as time of incubation in each irradiance of actinic light (red light). ETR is calculated as: $Y(II) x\ E\ x\ A\ x\ F_{II}$, being Y(II) the Fv/Fm, E the incident irradiance, A the absorptance and FII is the fraction of chlorophyll associated to PSII being in brown algae 0.8 according to (Grzymski, Johnsen et al. 1997). Absorptance was determined as A=1-Et/Eo according to Figueroa et al. (2003) being Eo the incident irradiance of a lamp (located 90^oC related to the algal thallus) and Et irradiance transmitted with the thallus located on the light sensor (Quantum Photo Radiometer Thermometer HD9021, Delta Ohm S.r.L, Padova Italy).

ETR parameters as maximal ETR (ETRmax), efficiency as the initial slope of the function ETR versus irradiance (alpha) and the strength of the inhibition as the final slope of the function ETR versus irradiance (beta) was determined by equation 1 and equation 2 according to Platt et al. (1980). Regressions were fitted using the method of Least Squares, implemented with the R program, version 2.14.2.

$$ETR = Ps\left(1 - e^{\left(-\frac{\alpha E}{Ps}\right)}\right) \times e^{\left(-\frac{\beta E}{Ps}\right)} \qquad (1)$$

$$ETRmax = Ps\left(\frac{\alpha}{\alpha+\beta}\right) \times \left(\frac{\beta}{\beta+\alpha}\right)^{\beta/\alpha} \qquad (2)$$

For the seawater stress experiment, Fv/Fm and ETR parameters were measured after treatments were completed (i.e. 45 days). For the air temperature experiments, these physiological variables were also measured after treatments were completed (i.e. 12 hours) but fronds had a 90 minutes recovery period submersed in seawater at 16 ºC since the last stress event occurred and then measured again the following day, after 18 hours of recovery.

2.4 Statistical analyses

Growth rates from stress level selection experiment were fitted to a piecewise regression (segmented package for R from V. Muggeo, 2012). Piecewise regressions are models where two or more lines are joined at unknown point, called breakpoint, their use for identifying ecological thresholds has been demonstrated to be a useful tool (Toms et al. 2003). In our case the breakpoint represents the optimal temperature for growth, minimum and maximal thresholds may be identified from the temperature values were the regression finds the X axis at the right and left sides, respectively, of the breakpoint.

Significant differences in the growth and in vivo physiological parameters in response to the different intensity and temporal variance treatments, were explored using analysis of variance ANOVA,(Underwood 1997). Analyses were done with Statistica 5.0 (StatSoft Inc., Tulsa, OK, USA). Homogeneity of variance was tested by Cochran's Test (Underwood 1997).

3. RESULTS

3.1 Manipulative seawater stress experiment

3.1.1 Stress level selection

Stress level selection experiment allowed to characterize precisely the functional response of the species to temperature (Fig. 9). From this functional response we decided to select low level of intensity in our mean-variability seawater experiment as 22 ºC because it seemed to be the first temperature of the right side (negative) of the curve. Not surprisingly 22 ºC is the maximum summer SST in the mid-Cantabrian sea, where *Fucus serratus* seems to be retracting (Viejo, Martínez et al. 2011). Similarly high level of intensity was selected at 26 ºC because it appeared to be the lethal sea water temperature in the stress level selection experiment (Fig. 9).

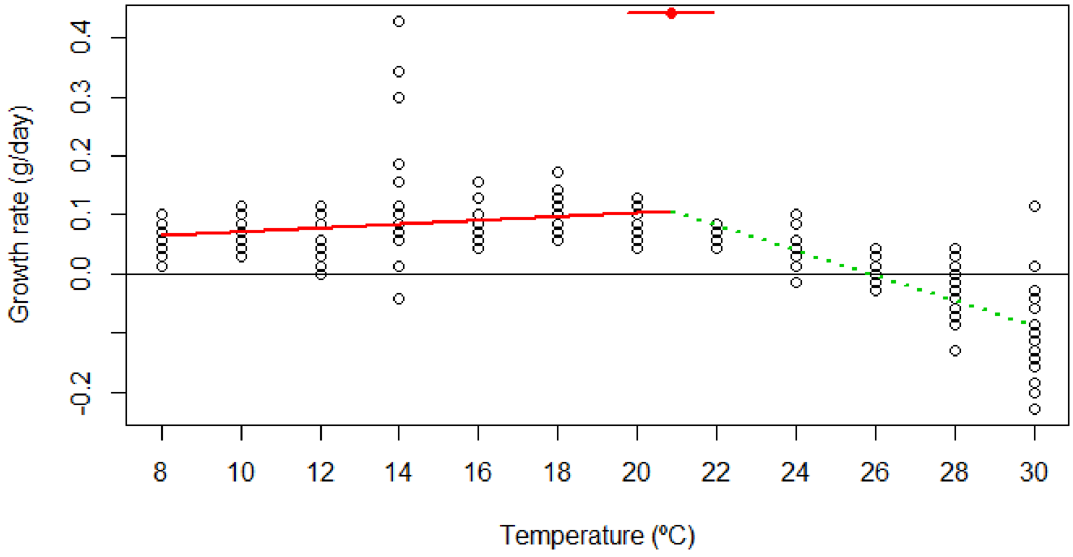

Figure 9. Piece-wise regression (segmented package for R from V. Muggeo, 2012) of the growth response of *Fucus serratus* after 14 days of laboratory culture at different temperatures, from 8 to 30ºC. Red line represents the positive slope of the curve and the green dotted line represents the negative slope. The red point is the breakpoint with SD.

3.1.2 *Changed intensity and temporal variance experiment*

Figure 10 shows the differences between the growth rate values of the control treatment and all the other treatments, clearly demonstrating that there was an effect of the intensity treatments applied and suggesting some temporal variance effects.

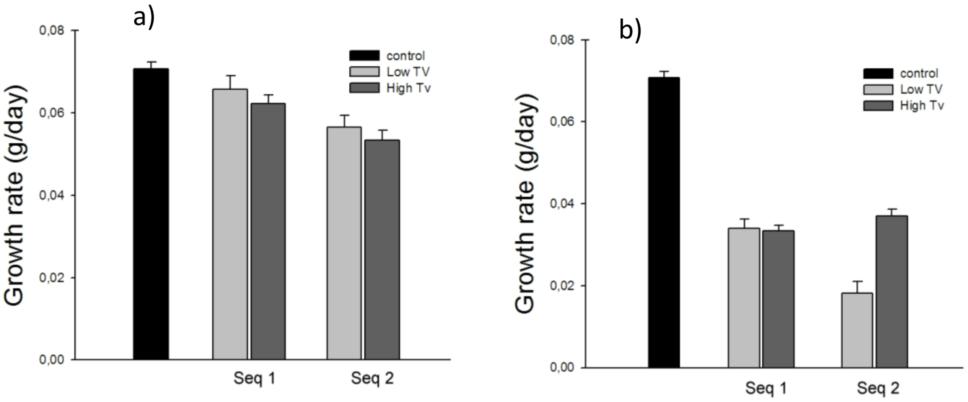

Figure 10. Growth rates (mean±SE, n=96 (control), n=24 (other treatments)) of the seawater stress experiment after accomplishment of treatments (i.e. 45 days). a) Low intensity treatments (i.e. 22ºC), b) High intensity treatments (i.e. 26ºC)

The analyses of variance of the functional measurements taken at the end of the seawater experiment (Table 6) indicated significant effects of the intensity treatment, but only for beta, ETRmax and growth rate response. For the other response variables, there were no significant effects, except for the chamber treatment on the beta, Fv/Fm and growth response.

Despite this general lack of significant effects some trends emerged when observing the plot of means of the growth data (Fig. 11). In the case of alpha, i.e. the initial slope of the rapid light curves (RLC), efficiency seems to be higher when alpha is higher. We could observed (Fig. 11a) that at high intensity treatments the mean alpha values were placed higher at high temporal variance (TV) and low TV treatments, contrarily to low intensity treatments where no visual differences were observed. A similar pattern was found in the case of maximum electronic transport rate (ETRmax) and growth rate. In both cases the plot showed again what seems to be not significant but consistent signals of a positive effect of high temporal variability in the treatments of high intensity. Furthermore in the case of growth rate (Fig. 11d), there was a clear and significant effect of intensity of stress ($p<0.01$) (Table 6.). Finally, beta controls the strength of photoinhibition, when beta is higher the photoinhibition occurs in a faster way. In this response variable there is no effect of temporal variance (Fig.11b), in fact the photoinhibition was very strong in both high intensity treatments and also on the low intensity treatments.

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Table 6. ANOVAs of the effect of intensity, temporal variance, sequence and chamber treatments on alpha, beta, ETRmax, Fv/Fm (n=3), and growth rate, responses of *Fucus serratus,* on the manipulative seawater stress experiments. Significant differences at α<0.05 are shown in bold.

	df	alpha			beta			ETRmax		
		MS	F	p	MS	F	p	MS	F	p
Intensity (I)	1	16.782	0.487	0.524	93.031	8.117	**<0.05**	0.015	9.397	**<0.05**
Temporal variance (TV)	1	3.882	0.113	0.754	29.595	2.582	0.183	0.002	1.037	0.366
Sequence (S)	4	34.451	0.442	0.776	11.461	0.141	0.962	0.002	1.067	0.432
Chamber C (I x TV x S)	8	77.906	0.915	0.508	81.487	2.243	**<0.05**	0.001	1.683	0.115
I x TV	1	12.367	0.359	0.581	28.323	2.471	0.191	0.008	5.149	0.086

	df	Fv/Fm			growth rate (g/day)		
		MS	F	p	MS	F	p
Intensity (I)	1	0.002	2.811	0.169	0.040	31.117	**<0.01**
Temporal variance (TV)	1	0.001	1.993	0.231	0.000	0.320	0.602
Sequence (S)	4	0.001	0.137	0.964	0.001	1.639	0.256
Chamber C (I x TV x S)	8	0.004	11.986	**<0.01**	0.001	6.762	**<0.01**
I x TV	1	0.001	1.097	0.354	0.002	1.447	0.295

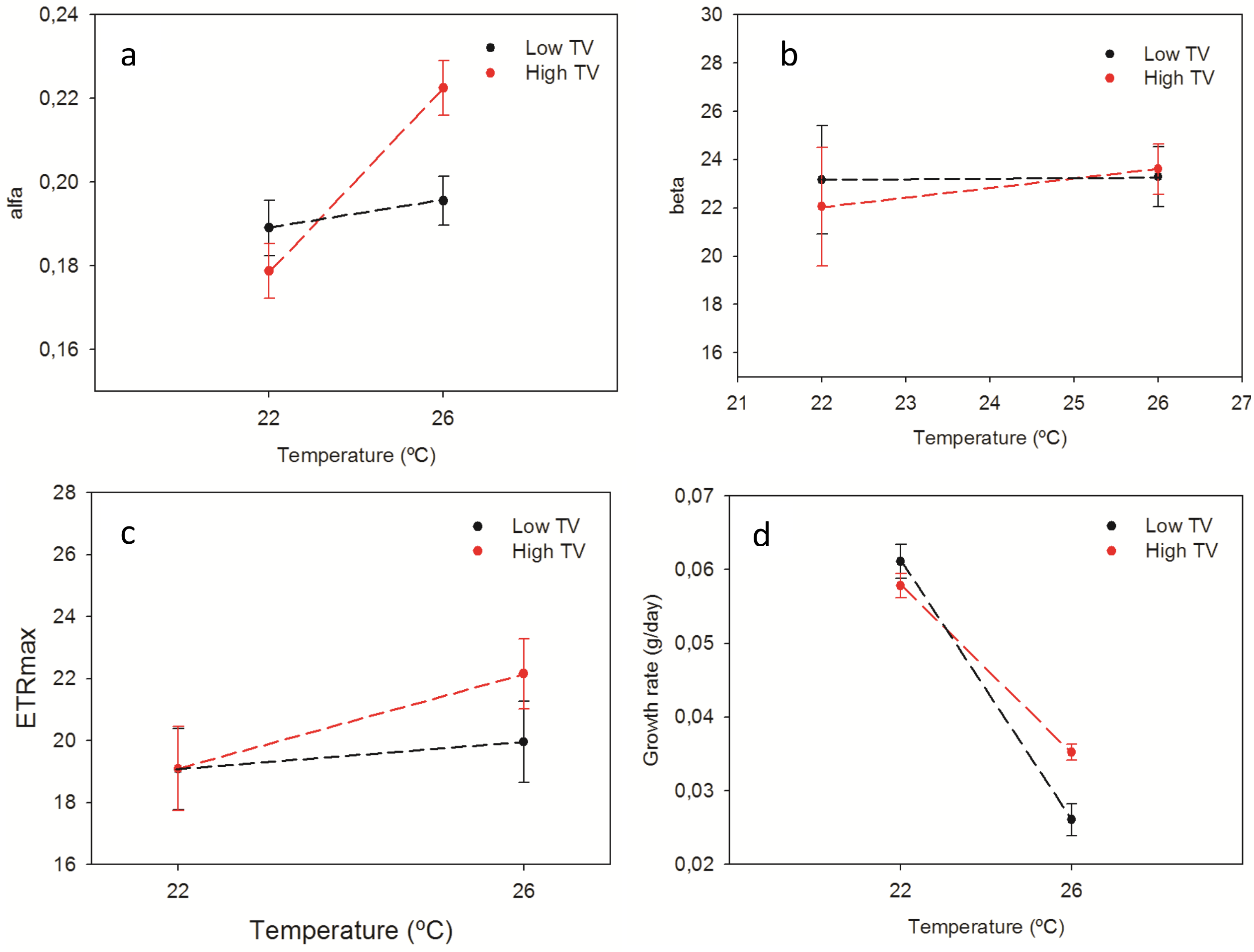

Figure 11. Plot of means of a) alpha, b) beta, c) ETRmax (mean±SE, n=48) and d) growth rate (g/day), (mean ±SE, n=96) of F. serratus exposed to the manipulative seawater temperature stress experiment treatments. On the X axis is represented the intensity treatment (22 or26ºC) and on the Y axis is represented the mean and the SE values. Black and red represent low temporal variance (TV) and high TV treatments, respectively.

3.2 Manipulative air temperature (low tide) stress experiment

In the air experiments, response differences among treatment were broader in comparison to the water temperature experiments. In this case, the previously suggested synergic effect between intensity and temporal variance was statistically significant for most of the parameters measured, confirming that high TV may buffer the negative effects of high intensity levels of stress.

Table 7 shows the results from the analyses of variance performed for all the functional parameters measured in this experiment. The significant interaction I x TV found in almost all cases, except for beta and Fv/Fm 90 min recovery. The last was marginally not significant ($p=0.067$), very noticeable on the plots (Figure 11).

Table 7. ANOVAs of the effect of intensity, temporal variance and sequence treatments on the alpha, beta, ETRmax, Fv/Fm after 90 minutes recovery ($n=3$) and Fv/Fm after 18hours recovery ($n=10$) of *Fucus serratus* exposed to the manipulative air temperature (low tide) stress experiments. Significant differences at $\alpha<0.05$ are shown in bold.

	df		alpha			beta			ETRmax	
		MS	F	p	MS	F	p	MS	F	p
Intensity (I)	1	0.986	193.678	**<0.05**	43.784.801	0.940	0.510	3.936.288	98.756.414	**<0.01**
Temporal variance (TV)	1	0.097	14.006	0.166	136.412.531	3.714	0.305	42.252	1.095	0.486
Sequence (S)	1	0.017	0.761	0.408	26.721.861	0.481	0.508	33.042	0.260	0.624
Chamber C (I x TV x S)	8	0.022	2.663	**<0.05**	55.602.438	4.460	**<0.01**	126.916	9.725	**<0.01**
I x TV	1	0.134	216.202	**<0.05**	147.273.063	4.184	0.289	87.980	199.776	**<0.05**
I x S	1	0.005	0.230	0.644	46.569.645	0.838	0.387	0.040	0.000	0.986
TV x S	1	0.007	0.314	0.591	36.733.746	0.661	0.440	38.592	0.304	0.596
I x TV x S	1	0.001	0.028	0.871	35.196.660	0.633	0.449	0.440	0.003	0.954

	df	Fv/Fm 90' recovery			Fv/Fm 18h recovery		
		MS	F	p	MS	F	p
Intensity (I)	1	2.891	23.409.000	**<0.01**	16.435	2.812.070	**<0.05**
Temporal variance (TV)	1	0.534	126.126	0.057	0.083	124.524	0.057
Sequence (S)	1	0.001	0.043	0.842	0.015	0.158	0.701
Chamber C (I x TV x S)	8	0.017	2.776	**<0.05**	0.097	7.636	**<0.01**
I x TV	1	0.597	90.334	0.067	0.108	1.036.441	**<0.05**
I x S	1	0.000	0.007	0.935	0.006	0.060	0.812
TV x S	1	0.004	0.243	0.635	0.001	0.007	0.936
I x TV x S	1	0.007	0.378	0.556	0.000	0.001	0.975

The trends observed are on the same way as water temperature experiments. High temporal variance has a buffering effect on the negative effects of high intensity in alpha (Fig. 12a), ETRmax (Fig.12b), and Fv/Fm (Fig.12d), being this fact supported by the analyses of variance results (Table 7). For the case of Fv/Fm, it is necessary to remark that the n was different for 90 minutes (n = 3) and 18h (n = 10) of recovery, due to logistical constraints. Giving non-significant effects for I x TV in Fv/Fm 90 minutes recovery, when the plot of means shows apparently significant different means for low and high TV in high intensity. For beta (Fig.12b) the results are not much reliable due to the high values of the error bars.

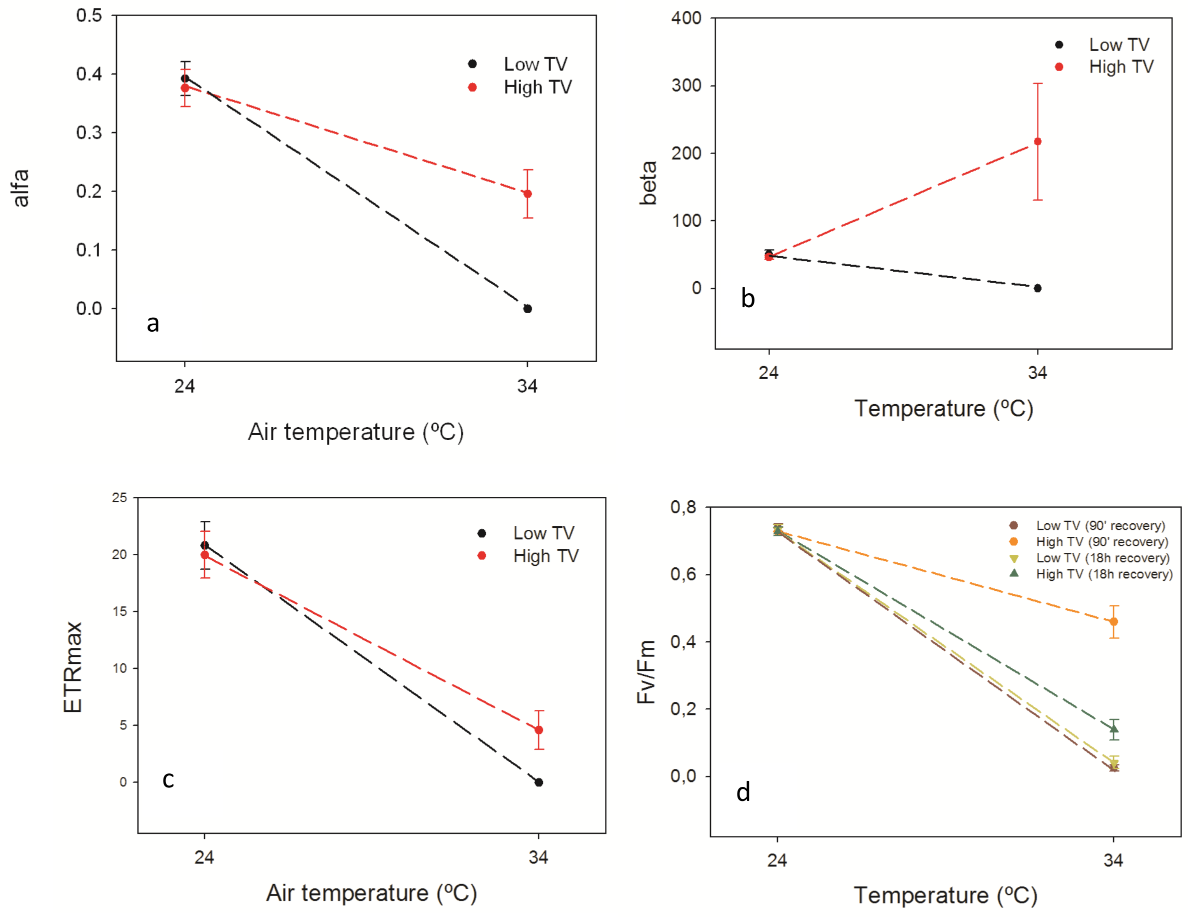

Figure 12. Plot of means of the a) alpha, b) beta, c) ETRmax and d) Fv/Fm after 90 min recovery vs. Fv/Fm after 18 hours recovery (mean ±SE) of algae exposed to the manipulative air temperature stress experiment. On the X axis is represented the intensity factor (24 or 34ºC) and on the Y axis is represented the mean and the SE value. Black and red correspond to low and high temporal variance (TV) treatments respectively. Except for d), where brown and light green correspond to low TV and orange and green to high TV.

4. DISCUSSION AND CONCLUSIONS

This study revealed interactive effects of intensity and temporal variance of seawater and air temperature stress (i.e. low tide stress) events on *Fucus serratus* growth and physiological response. On the study of seawater stress, no statistically significant interactions (i.e. p-value < 0.05) appeared but trends on the way of interactive effects were found. Results were more obvious in the air temperature experiments, where statistically significant interactions between intensity and temporal variance appeared for some response variables. On overall, the results from both set of experiments suggested that high temporal variance buffers the negative physiological effects of high intensity of stress on *Fucus serratus*. Thus, our initial hypothesis of temporal variance being a determinant key factor on high intensity rather than in low intensity treatments, based on what is reported in Benedetti-Cecchi et al. (2006) was mostly supported by our results. Seawater stress experiment results showed trends on the way of high temporal variance buffering negative effects of high intensity mainly for the growth rate and for alpha. For the ETRmax occurred the same but the effect of intensity was nearly absent, still showing healthier values in high intensity treatments than in low intensity, which is an unexpected response. On the air temperature stress experiment, as previously described in the results, effects were much clearer. There was a strong negative effect of high intensity and a buffering effect of high temporal variance on high intensity treatments for alpha, ETRmax, Fv/Fm after 90 minutes and 18 hours of recovery but not for beta. We found also an unexpected response on Fv/Fm, showing better physiological status after 90 minutes of recovery than after 18 hours of recovery (Fig.6d). It is important to consider that the goal of this study was not to simulate real field extreme event conditions but to disentangle the possible interactive effects between intensity and temporal variance of stress events on physiological responses at the organism level, namely *Fucus serratus*.

Unlike Bertocci et al. (2005) that due to logistical constraints did not test the effects of the specific sequence of events chosen for each level of temporal variance, we included sequence in our design as an orthogonal factor with two levels, sequence 1 and sequence 2 (but see Atalah et al. 2007). One of the adventages of this approach was the possiblity to test for potential effects of different distributions of stress events within the same level of temporal variance. None of the analyses showed any statistically significant effect of the factor sequence, meaning that most of the differences generated by the treatments was due mainly to intensity and temporal variance treatments. These lack of significant effects are similar to those reported on experiments where sequence was explicitly included in the design (Atalah et al. 2007; Cifuentes et al. 2007). Significant chamber effects (factor with two levels and nested to the interaction of intensity, temporal variance and sequence) found in most of the cases were result of differences among some of the mesocosms of the same treatment and are usual results in these kind of experiments.

Most studies regarding species physiological or population level response are based on the idea of changes on the mean intensity of predictor variables such as water or air temperature, UV radiation, nutrients availability, etc. Martínez et al. (2012b) developed several manipulative experiments in order to disentangle the significance or the possible interactions within the physical and climatic factors driving the physiological stress of *Fucus serratus* of a marginal vs. central population. Thermal variability, instead of the mean intensity or the temporal variability which are the studying variables in our case, has also been of interest within ecological studies regarding metabolic or physiological responses, mainly on ectothermic animals (Lalouette et al. 2007; Terblanche et al. 2010; Williams et al. 2012). Williams et al. (2012) studied the effects of thermal variability on the metabolic rate response of an overwintering butterfly. Their results provided empirical support to Jensen's inequality prediction, which stays that the mean value of metabolic rate over the accelerating portion of the curve will increase with increasing variance in temperature (Ruel et al. 1999), i.e. increases in thermal variability elevates metabolic rates. Larvae at variable temperatures decreased thermal sensitivity of metabolic rate and were larger than those at stable temperatures. But, even with depressed thermal sensitivity, the variable environment was more energy-demanding, limiting the successful development of the larvae exposed to high thermal variability after the dormant period. In this context, contrarily to our results, environmental variability has negative effects on the species success. Other similar studies, regarding plastic responses and climatic stress resistance in an invasive pest (Terblanche et al. 2010) found that large temperature fluctuations could limit plastic responses, reducing insects fitness, although not in all traits. Also were found significant effects of acclimation temperature (i.e. mean temperature) and variation of acclimation temperature (i.e. thermal variability), in the direction of increased mean temperature together with increased thermal variability having stronger negative effects on the agricultural pest than high temperatures alone, which in fact means that the species performs better. This was the case of the egg production or the critical thermal maximal, which were significantly higher at high variability levels, contrarily to the critical thermal minimal which was also higher (i.e. worse) at high variability levels. So, the combination of changes in mean temperature together with increased thermal variability may alter the direction of thermal tolerance responses, being advantageous at high temperatures in the target species of these studies. Authors suggest that this response may be related to heat shock protein production (Kalosaka et al. 2009), enhanced by the heat shock response found in all living organisms, which offers an effective defense against exposure to adverse environments (Lindquist 1986). This results have some similarities with ours, since significant effects of mean intensity and temporal variability were found for some response variables and in the majority of the cases on the way of high mean intensity and high temporal variability being more beneficial for *F. serratus* response than high mean intensity and low temporal variability, which may also be related to heat shock protein production (Ireland et al. 2004), although this was not analyzed in our work.

Mean growth rates found in the seawater stress experiments were similar to those suggested from the functional response to temperature under similar averaged conditions. Thus, if we consider the average temperatures of our intensity treatments (19 ºC and 22 ºC for low and high intensity respectively) and compare the averaged growth values for the whole experiment with the growth rates obtained at similar temperatures from the experiment on selection of stress level, we could see that values were similar. This suggests certain predictability of species performance in environmentally variable systems from average conditions. However despite this predictability, our results also suggest that temporal variance has a significant effect on our target species response, at several levels (i.e. physiological performance and growth rate). In most cases, the trends observed were on the way of high temporal variance buffering the negative effects of high mean intensity. In the growth rate response to different seawater temperature treatments (Stress level selection experiment), algae maintained at 26ºC seawater (corresponding to the high intensity treatment of changed intensity and temporal variance experiment) showed a decrease of the growth rate after the second week of experiment (measurements were only done once a week) and ended having a negative growth rate after 28 consecutive days at 26ºC. In the intensity-variability experiment, the longest period of high intensity was 9 days, having a total of 27 days at 26ºC and the growth rate differences between algae exposed to low or high temporal variability under high intensity of stress was relatively small, but it still showed a trend, being the positive effect of high temporal variability a possible explanation to this growth rate differences.

Nevertheless, the precise mechanism remains unknown. Benedetti-Cecchi et al. (2006) highlighted that variance may increase the probabilities that some environmental variable exceeds the physiological thresholds for some organisms. Thus even existing the physiological threshold it also seems to have a temporal component, meaning that it may be necessary to exceed this threshold for a determined period of time to observe negative effects such as reduction of growth.

Furthermore in the intensity-variability experiments, the treatment with high variance did have a positive effect by buffering the intensity of the high stress treatment. Several mechanisms have been proposed to explain positive effects of temporal variance on other studies at community level. Thus, Vaselli et al. (2008) suggested several processes explaining the interactive effects of intensity and variability of disturbance on the recruitment of benthic assemblages: (i) at low intensity of disturbance by allowing higher colonization only to the more resistant species e.g. algal turfs and (ii) at high intensity by exceeding the threshold of physiological tolerance of the algae. Bertocci et al. (2005) suggested that high temporal variance my affect assemblages in two mechanisms: (i) preventing recovery by several events occurring in a short time and (ii) by the specific sequence of events occurring during reproduction and recruitment periods. In our experiments the positive effects of high temporal variance could result from the longer periods with no events occurring on those treatments. These longer stress free periods would eventually allow the fronds to acclimatize to certain conditions, and as results these fronds will perform more successfully than those fronds rapidly changing from extreme/no extreme situations (i.e. low temporal variability treatments). A similar case was described in *Fucus vesiculosus* in a study of increased freezing tolerance and reduced reactive oxygen production after freezing (Collen et al. 2001). Thus, our result raises an interesting question. What is more determinant, the fact of having long periods of recovery or the ability of acclimation during long extreme events periods? Gutschick et al. (2003) refer that the fitness effects of extreme events occur during recovery. They examined this idea through the comparison of the recovery response to a drought event of two co-occurring plants of the chaparral (Courtesy of D. Sims, California State University, Los Angeles, CA, USA). One of it behaves as if drought is an extreme, acclimating late and experiencing delayed recovery. The other behaves as stressed but not as being an extreme; it acclimates continuously and recovers without hysteresis. With our experimental design it is not possible to discern this question. We measure the response variables at the end of the experiment in order to get the treatments completed, and the effects of recovery and acclimation are accumulated and confounded. It is needed research in this area and we propose an experimental design to assess this question (see Annex).

Changes in mean intensity, thermal variability and frequency of extreme events are within the present and forecasted climate change scenarios (Alexander et al. 2006; Easterling et al. 2000a; McGregor et al. 2005) and it is important to understand the possible species responses to these changes and the demographic consequences of them for forecasting the impacts (reviewed in Chown et al. 2010). Several authors have worked on the demographic and evolutionary significance of extreme events and combination of events that increase physiological stress (Gutschick et al. 2003; Parmesan et al. 2000; Wethey et al. 2011). In order to forecast the biological impacts of those environmental changes, it is important to understand the phenotypic plasticity (i.e. the capacity of individuals to respond over short term) and the speed at which the population can evolve. Plasticity is often seen as a suitable way for organisms to deal with climate change, but the effectiveness of this strategy depends on the capability of organisms predicting stressful conditions. In areas where climate change involves a decline in predictability of extremes, those species currently presenting plasticity may suffer high costs due to inappropriate responses (reviewed in Chown et al. 2010).

Even more, this variability expected under future climates could produce plastic changes in the opposite direction to those considered beneficial (Chown et al. 2010) or limit plastic responses such as beneficial acclimation (Wilson et al. 2002). The beneficial acclimation assumption stays that phenotypic acclimation confers an advantage in the environment that stimulates the response, although several authors have rejected this assumption (Leroi et al. 1994 and references therein; Wilson et al. 2002). Regarding our results, it may seem that increased variability could lead to beneficial acclimation responses, thus individuals from high intensity treatments (i.e. fluctuating from 16ºC to 26ºC) showed higher growth rates than those from constant temperature treatments (Selection of stress levels experiment). But here exists a confounding effect of several factors such as mean intensity of stress, which in any case is the same although the number of days at 26ºC was the same in both experiments. Thermal variability is also confounded with intensity of the stress, since low intensity treatments were fluctuating from 16ºC to 22ºC and high intensity treatments fluctuated from 16ºC to 26ºC. So, this type of manipulative experiments used in our case is not appropriate for studying plastic changes in response to thermal variability. It would be of high interest to perform similar manipulative experiments but including the thermal variability in the experimental design.

For example, the experimental design proposed by Terblanche et al. (2010) could be interesting to apply with our target species. It consists of an orthogonal design with three mean temperatures and three variability levels (delta). A possible experimental design could be a combination of both, an orthogonal design with n levels of mean intensity, n levels of thermal variability and n levels of temporal variance, leading to a n^3 different treatments experiment.

In conclusion, our results revealed interactive effects of mean intensity and temporal variance of seawater and air temperature stress events on *F. serratus* growth and physiological response under laboratory conditions and provided empirical support to Jensen's inequality, thus *F. serratus* growth rates and some physiological parameters showed increased values at high temporal variability, under high mean intensity conditions. The mechanisms driving these responses remains still unknown, thus future work may aim on the plasticity of species, regarding acclimation and recovery responses. As previous studies (Terblanche et al. 2010; Thompson et al. 2013) our results suggest that mean temperature variation experiments, may be inappropriate for predicting species response under climate change scenarios.

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ANNEX

Acclimation vs. recovery experimental design

To assess whether recovery or acclimation have a more determinant role in species growth and physiological performance, after prolonged extreme event situations, we would propose the following experimental design.

The response variable used to assess this would be the growth rate (g/day). All treatments need to be initially exposed to a long extreme event period (i.e. 5 days at 26ºC seawater), as the aim is to assess what is more determinant on the algae performance after a long extreme event, whether the fact of having a long recovery period or the ability to acclimatize to extreme conditions.

If there is an acclimation to the extreme condition, it is possible that algae perform better if conditions continue extreme (possible situation in our high temporal variance treatment) than if they rapidly change from extreme no extreme conditions (our low temporal variance treatment). On the other side, if the fact of having a long recovery is more determinant, those algae being exposed to something similar to our high temporal variance treatments (i.e. having some probabilities of the benefits from a long recovery period) would perform better than those exposed to something similar to our low temporal variance treatment.

Two types of “a priori” contrasts (Table 1) within different treatments (Table 2) are proposed in order to answer the following questions: (i) “is there an acclimation during a long extreme event?” and (ii) “does a long recovery period have a determinant paper on algae’s performance after a long extreme event?” Samplings (i.e. weighting each individual) would be done: (i) after day 5 for all treatments, in order to check that all treatments are at similar points, (ii) after day 8 for treatment 4, (iii) after day 11 for treatment 3, (iv) after day 14 for treatment 1, (v) after day 17 for all treatments.

Table 1. Contrasts and questions proposed in order to assess the effects of acclimation and recovery after long extreme event situations.

Contrast	Question
Treatment 3 (day 11) vs. treatment 4 (day 8) & Treatment 1 (day 17) vs. treatment 2 (day 14)	(i) *Is there an acclimation during a long extreme event?* Which treatment performs better? Those rapidly changing to extreme/no extreme (1 or 3) or being exposed to a continued extreme event situation (4 or 2).
Treatment 1 vs. treatment 3 & Treatment 2 vs. treatment 4 (Completed treatments)	(ii) *Does a long recovery period have a determinant paper on algae's performance after a long extreme event?* Did the long recovery period have any positive effect compared to the treatments with no recovery period just after the extreme event?

Table 2. Distribution of stress events (seawater temperature, ºC) through the totality of the experiment (i.e. 17 days).

	Day																
Treatment	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	26	26	26	26	26	16	16	16	16	16	16	26	16	26	16	26	16
2	26	26	26	26	26	16	16	16	16	16	16	26	26	26	16	16	16
3	26	26	26	26	26	26	16	26	16	26	16	16	16	16	16	16	16
4	26	26	26	26	26	26	26	26	16	16	16	16	16	16	16	16	16