Resistance and resilience of macroalgae to thermal disturbance

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Todas as correções determinadas pelo júri, e só essas, foram efetuadas.

O Presidente do Júri,

Porto, ______/______/_________

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Abstract

Disturbances can shape the structure, function, and biodiversity of ecosystems, and vary in intensity, duration, frequency, and predictability across ecosystems. The disturbance might cause effects in the resistance (the changes in response to a disturbance) and resilience (the response of the biota to disturbance) of species. A major factor that affects the distribution of species is the temperature, thus this is a potential major driver of changes in the ecosystems and an important disturbance factor for organisms.

The intertidal zone of rocky shores is an interesting area to study the changes in species' distribution, since species found in this zone can function as early indicators of the impacts of climate change, as they are frequently living on the limits of their physiological tolerance, reciving influences from terrestrial and marine environments. For this study, we used three intertidal macroalgae: *Ascophyllum nodosum*, *Fucus serratus* and *Himanthalia elongata*.

The resistance and resilience of these species was tested at different intensities, frequencies and durations of thermal disturbance using as response variable the vegetative growth, photosynthetic efficiency, gross primary production (GPP), maximum production rate and respiration.

Resistance and resilience to disturbance varied with species identity, with an increase in tolerance of organisms with the tidal level that species occupy. All the factors tested (intensity, frequency and duration of disturbance) significantly affected the response of the different species acting, in many cases, in an interactive way.

Key words: Resistance, resilience, disturbance, intertidal, growth, photosynthetic efficiency.

Resumo

As perturbações são capazes de modificar a estrutura, a função e a biodiversidade dos ecossistemas, podendo variar em termos de intensidade, duração, frequência e previsibilidade consoante o ecossistema. A perturbação pode afetar a resistência (alterações em resposta a uma perturbação) e a resiliência (a resposta da biota a perturbações) das espécies. Um importante fator que afeta a distribuição das espécies é a temperatura, pelo que este é um potencial fator de alterações nos ecossistemas e um importante fator de perturbação para os organismos.

A zona intertidal de praias rochosas é uma área interessante para estudar alterações ao nível da distribuição das espécies, dado que as espécies que aqui se encontram podem funcionar como indicadoras precoces de impactes das alterações climáticas uma vez que estas estão constantemente expostas aos limites das suas tolerâncias fisiológicas, recebendo influências de ambientes terrestres e marinhas. No presente trabalho de investigação, foram utilizadas três macroalgas que estão tipicamente presentes na zona intertidal: *Ascophyllum nodosum*, *Fucuc serratus* e *Himanthalia elongata*.

A resistência e a resiliência destas três espécies foi testada com diferentes intensidades, frequências e durações de perturbação térmica, usando como variável de resposta o crescimento vegetativo, a eficiência fotossintética, a produção primária bruta (GPP), a taxa de produção máxima e a respiração.

A resistência e resiliência a perturbações variou com a identidade das espécies, com um aumento da tolerância dos organismos com o nível de maré que as espécies ocupam. Todos os fatores testados (intensidade, frequência e duração da perturbação) afetaram significativamente a resposta das diferentes espécies atuando, em muitos casos, de maneira interativa.

Palavras-chave: resistência, resiliência, intertidal, crescimento, eficiência fotossintética

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Full name	Abbreviation
Gross primary production	GPP
Pulse amplitude-modulated fluorescence	PAM
Sea surface temperature	SST

Introduction

The Portuguese coast is situated in the warm-temperate Atlantic region and is characterized by a latitudinal gradient of temperature. This area is a transitional biogeographic region where a large number of cold and warm water species have their southern and northern boundaries of distribution, respectively. It is a region with particular habitat conditions because it receives climatic influence from the Atlantic Ocean and the Mediterranean Sea, and therefore the communities are composed by a unique combination of species (Araújo et al., 2009; Lima et al., 2007).

In recent years, there is an ongoing documented trend of climatic change with a general warming of seawater temperature. At the end of the twentieth century warmer temperatures have affected, in only 30 years, the phenology of organisms, the range and distribution of species, and the composition and dynamics of communities (Walther et al., 2002). The main consequences of this temperature increase is the polewards migration of cold water species and an increase in the number of introduced and warm water species (Byers, 2002; Lima et al., 2007; Walther et al., 2002).

Mobile organisms are able to respond to some extent to these changes by tracking their favorable environment across space, while sessile organisms, like algae, might adapt morphologically or genetically to environmental changes or change their geographical distribution (Guo et al., 2005).

Species' responses to environmental parameters may depend on the region that a population occupies, resulting in different responses of the same species in different ecoregions (Tapolczai et al., 2016). An example of this are the populations inhabiting at the edges of species distribution that are assumed to have lower density and to be more fragmented and suffer higher extinction risks than populations from the center of species distribution (Guo et al., 2005).

Disturbances can shape the structure, function, and biodiversity of ecosystems, and vary in intensity, duration, frequency, and predictability across ecosystems (Lake, 2000).

A disturbance is defined as a discrete event that can kill, displace or damage one or more individuals (or colony) in a particular place (Sousa, 1984). These disturbances, both natural and anthropogenic, are a structuring factor in intertidal habitats, performing an extremely important role influencing the distribution, abundance and diversity of organisms. These events can remove organisms, increasing their mortality, and can also provide resources such as space, nutrients and light that can increase the emergence of new species, not previously occurring in the site (Byers, 2002; Lake, 2000). Climate change may be a significant disruption in the structure of the distribution of different taxa, for example, by facilitating biological invasions or increasing the competitive capacity of introduced species (Ruiz et al., 1999; Sorte et al., 2010).

The response of organisms to disturbance might involve different degrees of resistance and resilience of species.

Resistance is defined as the capacity of a system to persist after a disturbance (the changes in response to a disturbance event) (Fritz and Dodds, 2004; Hershkovitz and Gasith, 2013). The resistance of algae to disturbances, a measure of their capacity to survive a disturbance, depends on the physical properties of the disturbance and the composition, morphology and physiology of algal assemblages (Lake, 2000).

Resilience is the capacity of a system to recover from disturbance and depends on factors that control benthic algae, such as resource availability (nutrients, light), hydraulic conditions, stressors, such as pollutants, and the strength of biotic interactions, such as grazing and competition (Hershkovitz and Gasith, 2013; Lake, 2000).

A major factor that presents important spatial variations and affects the distribution of species is the temperature. Over the last decades, an increase in sea temperature throughout the coast was observed (Lima et al., 2007; Walther et al., 2002) and changes in the geographical distribution of some macroalgal species have also been recorded. However, a clear link between the recorded temperature changes and species distribution shifts is difficult to establish since these geographical changes were not consistent across species (Araújo et al., 2009; Lima et al., 2007). Additionally, others factors can influence the geographical species distribution and there is still lack a clear model linking physiology, climate and macroecology (Beaugrand et al., 2008; Sagarin et al., 2006). Globally, the changes in species abundance might also be associated with warming temperatures since changes in abundance have been related with the geographic range of species, since range shifts in southern warm and northern cold species distribution and abundance have been recorded in the last decades (Barry et al., 1995; Holbrook et al., 1997; Parmesan et al., 2005; Sagarin et al., 1999). In addition, with the climate change, non- native species from adjacent areas may cross borders and become new elements of the biota. Temperature changes have however documented effects on the distribution of species and the composition and dynamics of communities (Walther et al., 2002).

The most effective method in predicting the decline of species and expansion of non-native species is through the monitoring of the border conditions or marginal populations structure and dynamics (Guo et al., 2005).

Marginal populations frequently experience extreme conditions and to deal with these conditions may develop unique adaptive traits which give them high conservation value and persistence capacity to meet future environmental changes. The persistence of these populations depends on adaptations both at the genetic and phenotypic level (Guo et al., 2005).

The intertidal zone of rocky shores is an adequate site to study the changes in species' distribution (Duarte et al., 2013), since intertidal species are early indicators of the impacts of climate change, as they are frequently living on the limit of their physiological tolerance, having terrestrial and marine influences (Helmuth et al., 2006). Additionally, canopy-forming algae are important components of intertidal rocky shores, as these species are ecosystem engineers that provide a diversity of services to the ecosystem like food, shelter, habitat and nursery to several associated species from different trophic levels (Graiff et al., 2015; Jones et al., 1997; Kautsky et al., 1992) and variations in their distribution may cause changes in the structure and functioning of the entire ecosystem. Additionally they are recognized as foundation species that form canopies, providing important ecological services: they are involved in nutrient cycling; nitrogen and carbon storage; add energy to food webs; provide essential habitat and shelter for invertebrate, vertebrate and algal communities (Schmidt et al., 2011; Thompson et al., 2002; Tuya et al., 2011; Wernberg et al., 2011; Worm et al., 2000). Thus, they have an important effect on abiotic conditions, community assembly and ecosystem functioning (Benedetti-Cecchi et al., 2001; Schmidt et al., 2011; Tait and Schiel, 2011; Worm et al., 2000). Variations in their distribution may cause changes in the whole system, such as shifts in community composition or changes in the height and structure of the canopy itself.

Recent distributional retractions have been reported for *Himanthalia elongata, Fucus serratus* (Duarte et al., 2013) and *Ascophyllum nodosum* in the northern coast of Spain (Viana et al., 2014). These retreats follow the sea surface temperature (SST) warming trend in the study area (Duarte et al., 2013). These three species find their Southern limit of distribution in Portugal, where *H. elongata* and *F. serratus* retreated their distributional range, but there were no changes in the distribution of *A. nodosum*. (Lima et al., 2007).

H. elongata is a biennial species (Russell, 1990) that growths usually between September and May, with a highest growth rate during spring (Stengel et al., 1999). The time of reproduction is generally from June to December, and is strongly site dependent, probably due to water temperature.

The species is able to disperse over long distances through detached and floating receptacles.

Due to these characteristics this specie is likely to follow environmental changes very fast (Duarte et al., 2013).

This alga has a two-stage morphology: first, the 'button-like' stage is initially club-shaped, becoming peltate ('mushroom-shaped') when mature, about 2-3 cm in height and 2-4 cm in diameter, slightly dimpled in the middle, and attached to the substratum by a short stipe and discoid holdfast; second, the mature peltate stage typically produces two long (up to 3m) thong-like reproductive receptacles (Stengel et al., 1999).

H. elongata form dense stands on temperate rocky shores (Creed, 1995) and is limited to semi-exposed shores of the north-eastern Atlantic from Faeroe Islands and northern Norway to northern Portuguese cost (Yarish and Kirkman, 1990) and usually occurs near the low water mark of spring tides, but in some locations it also grows subtidally.

In addition to its ecological role, *H. elongata* is also of commercial importance. It was traditionally harvested for centuries along the eastern Atlantic in Norway, France, Spain, Scotland, and Ireland for fertilizer, human food, and alginate extracts. *H. elongata* is currently harvested mainly as edible seaweed for human consumption in France, Ireland and Spain (Plaza et al., 2008).

Dispersal is limited in *Fucus serratus* because: gametes are released only during calm water conditions, thereby achieving a nearly 100% fertilization success (Berndt et al., 2002; Pearson and Brawley, 1996); sperm are attracted to egg-produced pheromones that are effective only at millimeter distances (Serrao et al., 1996); and fertilization success decreases rapidly with increasing water motion (Denny and Shibata, 1989; Levitan et al., 1992). Additionally, it's a dioecious specie and thus, for efficient dispersal, it is necessary that 2 propagules (zygotes) of different sex arrive at the same suitable site and become fertile at the same time (Arrontes, 2002). Moreover, this specie cannot float, which decreases its dispersion capability.

F. serratus is a specie with a life-span of up to 5 years (Arrontes, 2002) and dominates the low and intermediate intertidal zone. Within its normal range of distribution*, F.*

vesiculosus and *B. bifurcate* occur above and below, respectively, the tidal level occupied by *F. serratus* (Arrontes, 1993). Competition of *F. serratus* with *F. vesiculosus* and perhaps *Bifurcaria bifurcata* and *Mastocarpus stellatus* may occur in the transition zone and beyond the distributional boundary (Arrontes, 1993).

F. serratus is a specie widely distributed on the Atlantic shores of Europe and is locally abundant in Nova Scotia (Canada). Its southern distributional boundary on the European shores lies in northern Portugal (Coyer et al., 2003; Pazo and Niell, 1977; Yarish and Kirkman, 1990).

The limits of distribution of *F. serratus* are often associated with the effect of critical temperatures on survival, reproduction and growth, but temperature cannot, alone, explain the changes in the limit of distribution (Arrontes, 1993). If the temperature is a factor that control the distribution of *Fucus serratus,* its influence is most likely to occur during summer, because high temperatures in summer have frequently a lethal effect (Arrontes, 1993).

Variation in the environmental conditions, low dispersal range and competition with other macroalgae probably act in conjunction with physical factors in shaping the present boundaries of *F. serratus* (Arrontes, 1993).

Ascophyllum nodosum can reach up to 2 m long as, even though it shows slow growth rate, it is one of the most long-lived macroalgae. Populations older than 15 years old have been described worldwide (Soneira and Niell, 1975; Viana et al., 2014). Fronds have large egg-shaped gas bladders at intervals along the leaf that enable them to float during high tides. It presents apical growth and gas bladders are formed at the tip prior to the dichotomously branching that generally occurs once a year (MacFarlane, 1932; Moss, 1970). Receptacles are formed in lateral branches that could also be vegetative (MacFarlane, 1932).

A. nodosum populations show poor sexual reproduction success. Zygotes disperse short distances and suffer high mortality before and after settlement (Dudgeon and Petraitis, 2001).

This specie is present from sheltered to moderately exposed sites. Populations usually cover moderately large areas of the rocky meso-littoral zone (Aberg, 1992; Soneira and Niell, 1975).

A. nodosum is found along rocky shores of the temperate North Atlantic Ocean as well as parts of the Arctic Ocean. In the northwest Atlantic, it ranges from New Jersey to

Baffin Island in the Canadian Arctic; in the northeast Atlantic, it extends from northern Portugal to the White Sea in the Russian Arctic (Araújo et al., 2009; Viana et al., 2014).

The aim of this work is to, using as study models three intertidal species occupying different intertidal levels and with documented distinct patterns of changes in distributional range, test the resistance and resilience of different species to different levels of intensity, frequency and duration of thermal disturbance.

Material and Methods

Sampling and accommodation in the laboratory

Sampling was done at two locations along the limit of distribution of each of the studied species, in order to compare different populations. For *Himanthalia elongata* the sites were Ria de Arousa (42°30′00″N; 8°56′00″W) and Vigo (42°14'10"N; 8°43'36"W); and for *Fucus serratus* and for *Ascophyllum nodosum* the sampling sites were Viana do Castelo (41º42'N; 8º49'W) and Ria de Arousa (42°30′00″N; 8°56′00″W).

The fronds were collected randomly from different plants and from different zones of the beach in order to increase the diversity.

In the laboratory, the fronds were divided in different groups according to the different treatments with each replicate of each treatment containing three fronds. Each frond was marked with glassbeads with different color combinations in order to distinguish the fronds.

Samples were placed in a tank at 16ºC (this temperature was kept with the help of a refrigerator) with constant aeration, and maintained in these conditions during a week before the experiment for acclimation to laboratory conditions.

In this experiment the following factors were tested: intensity, frequency and duration of disturbance.

For the factor Intensity of disturbance three levels were tested by manipulating the water temperatures: 24ºC, 28ºC, 32ºC and control (16ºC); for the specie *H. elongata* the temperature 32ºC was excluded because our results showed a mortality of 100% at this temperature and an additional temperature level was included, 20ºC. To maintain these temperatures, 3 tanks were filled with seawater and kept at the correspondent temperatures with the help of heaters connected to temperature sensors. The two tanks with the highest temperatures were also placed in a bigger tank filled with water to help to maintain the temperatures constant (Fig. 1).

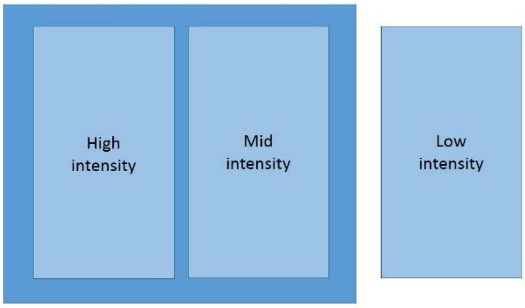

Fig.1 – Scheme of the organization of the tanks and different temperatures in ºC. The dark blue area represents the bigger tank filled with water to help to maintain the temperatures constant in the smaller thanks.

For the factor frequency of disturbance three levels were tested, corresponding to the periods of time spent at each temperature until the total duration of disturbance of the treatment: 6 hours (corresponding to successive 6 hours periods, every 24h at the different disturbance intensities and until the full duration of disturbance was reached), 12 hours and full.

For the factor duration of disturbance three levels of disturbance were tested, corresponding to the total amount of time the organisms were subjected to the disturbance source. Three levels of duration of disturbance were considered: 24 hours, 48 hours and 72 hours. For each of these treatments 3 replicates were used (Fig. 2).

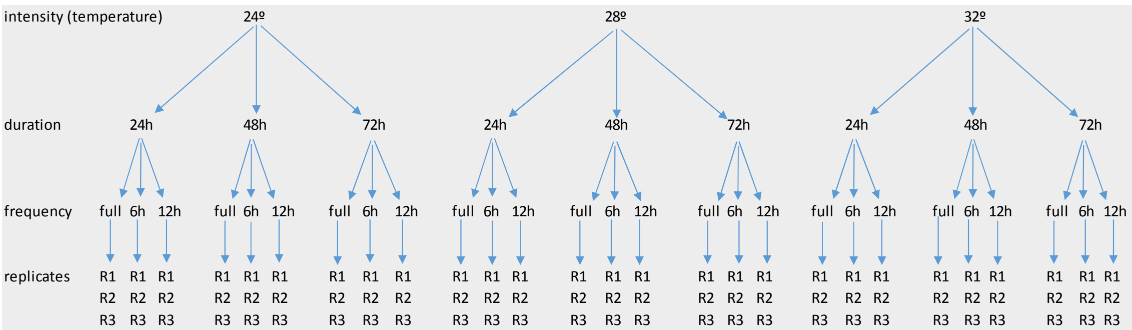

Fig. 2 - Experimental design of the experiment with the different levels of each of the factors tested: intensity, duration and frequency of disturbance

Growth

The samples were weighted before the beginning and in the end (after 2 weeks of the first measurement) of the experiment, in order to evaluate the growth rate (Growth rate (g/day) = (weight t2-weight t1)/ number of the days).

Pulse Amplitude-Modulated fluorescence (PAM)

The pulse amplitude-modulated fluorescence (PAM) measurements of the fronds were registered before the beginning of the experiment (t0), immediately after the treatment (t1) and one week after the end of the experiment (t2), to estimate the recover capability of the individuals.

The PAM monitors the maximum quantum yield of Photosynthetic System II (Fv/Fm). The standard values of Fv/Fm in brown algae are usually between 0.7 and 0.8, and a decrease in these values is indicative of photoinhibitory damage to photosystem II (Maxwell and Johnson, 2000; Ritchie, 2006).

The algae were measured by using a chlorophyll fluorometer (Junior PAM, Heinz Walz). Fv/Fm measurements were taken, for each replicate treatment, in 3 fronds (Fv/Fm was measured twice in each frond and the two measures averaged).

To perform the PAM measures, each replicate was placed in a small box, and these small boxes were maintained in obscurity during 10 minutes before measurements (Fig. 3).

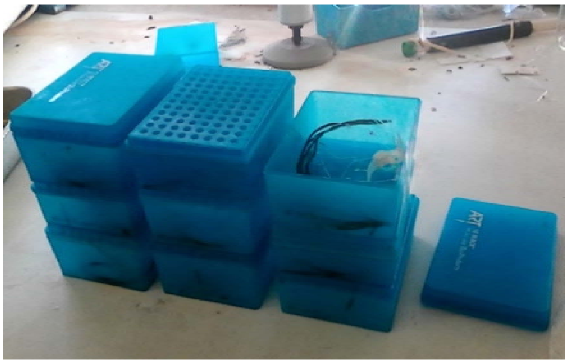

Fig.3 – The small boxes used to place each replicate separately and to maintain the replicates in the dark.

Incubations

Immediately after each treatment incubations were performed to estimate additional ecophysiological parameters indicative of the alga fitness.

Respiration and productivity were measured inside an experimental chamber at 16ºC (temperature kept with a refrigerator). Each incubation had a total duration of 2 hours: 30 minutes dark (0 Imol photons/m^{2}s^1 irradiance), 15 min light 1 (40 Imol photons/m^{2}s^1 irradiance), 15 min light 2 (114 Imol photons/m^{2}s^1 irradiance), 15 min light 3 (191 Imol photons/m^{2}s^1 irradiance), 15 min light 4 (267 Imol photons/m^{2}s^1 irradiance), 15 min light

5 (342 Imol photons/m^2s^1 irradiance) and 15 min full light (1510 Imol photons/m^2s^1 irradiance). The irradiance inside the experimental chamber was measured using a spherical scalar quantum sensor connected to a computer (Biospherical QSL 2000, San Diego, CA, USA).

The incubation chambers consisted of 2L sealed transparent glass cylinders that were almost fully submersed in a larger white Plexiglass chamber used as a cooling bath to assure constant temperature during incubations (fig. 4).The samples were placed in these cylinders with 2L of filtered saltwater, and with small submersible aquarium pumps in order to maintain the water movement inside the incubation cylinders. The water of the containers was changed in every 2 incubations. Dissolved oxygen concentration and temperature inside the incubation chambers were measured every 30 s using a luminescent dissolved oxygen (LDO) probe connected to a portable oxygen meter (Hach HQ40, Dusseldorf, Germany).

Fig.4 – Incubator with six containers, each one with an O2 sensor.

The photosynthetic efficiency, the gross primary production (GPP), the maximum production rate and the respiration were estimated through oxygen fluxes by regressing oxygen amount produced or consumed (Imol) through time (s^1) during dark and light periods of increasing intensities. Estimations were normalized by sample biomass (dry weight) and volume of incubation chamber.

To do that, the production of oxygen was calculated for each light intensity in each replicate through the slope of the line that show the oxygen concentration (mg/L) per hour (h) and multiplying this value for the volume of the incubation chambers (2L) and the dividing by the dry weight (g) of the replicate. Then, with the slope of the values of production of the first four light intensities the photosynthetic efficiency was calculated. The GPP was calculated for each replicate adding the maximum production rate (the maximum value of production) with the module of respiration (the value of production in the dark).

Statistical analysis

To test the effects of the experimental factors, intensity, duration and frequency of disturbance, on the growth and ecophysiological indicators of performance of the different species univariate analysis was performed. Analysis included the factors Intensity (3 fixed levels), Frequency (3 fixed levels) and Duration (3 fixed levels) of disturbance. For the PAM analysis was also included the factor time (t) with 3 fixed levels.

The analysis was performed using the software Statistica. Post Hoc Tukey HSD test were used for *a posteriori* comparison of means.

Results

Himanthalia elongata

Growth

In general, the growth of *H. elongata* varied with population identity. The population of Vigo showed a higher growth rate than the population of Ria de Arousa for all the temperatures tested except in control condition. For both populations, there was a general decreasing trend in growth from lower to higher temperatures with negative values of growth for most of the conditions tested (Fig. 5).

The general pattern for both populations was a loss of biomass with the increase of frequency of disturbance. Despite this, in population of Vigo the loss of biomass at higher frequency of disturbance was sight lower than at mid frequency of disturbance (Fig. 5).

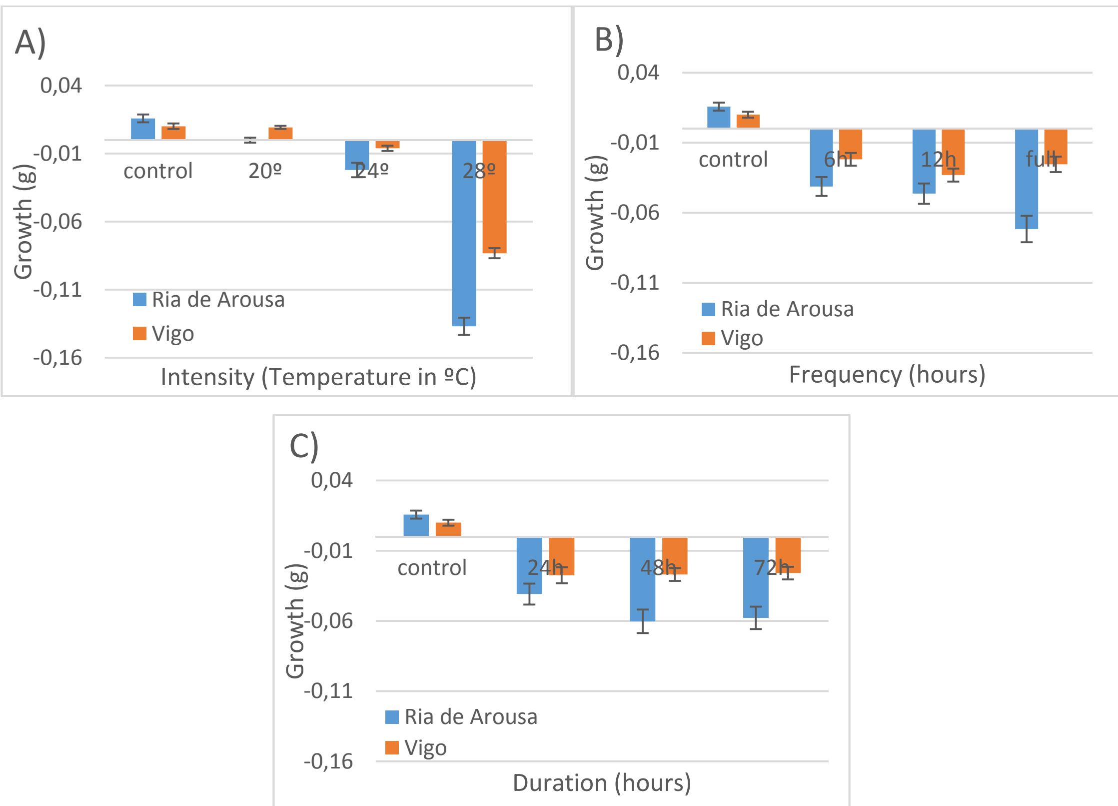

Fig. 5 - Growth of *Himanthalia elongata* of Ria de Arousa (blue) and Vigo (orange) at different A) intensities, B) frequencies and C) durations of disturbance.

In general there was a loss of biomass in all durations of disturbance, except in the control. In the population of Ria de Arousa the loss of biomass was lower at low duration of disturbance than in the others durations of disturbance and in population of Vigo the loss of biomass was similar in all durations (Fig. 5).

The ANOVA tests showed for both populations of *H. elongata* effects of the intensity of disturbance in the growth of individuals. In the population of Ria de Arousa there was a significant interaction between the intensity and the frequency of disturbance, while for the population of Vigo there was a significant interaction between the frequency and the duration of the disturbance. In the population of Ria de Arousa, at the highest frequency of disturbance, there was a significant decrease of growth with increasing temperature and, at low and mid frequencies, the growth was significantly lower at the highest intensity of disturbance. For the population of Vigo the same pattern (decrease of growth with temperature) was found for the mid frequency of disturbance but not for the low and high levels of frequency. For this population, growth decreased significantly with temperature only at the highest duration of disturbance, while for the low and mid duration growth significantly decreased only at the highest intensity of disturbance (Table 1).

In the population of Ria de Arousa the effects of the duration of disturbance significantly varied with the frequency tested, with a significantly decrease of growth at the highest frequency of disturbance only for the mid level of duration of disturbance (Table 1).

The temperature 32ºC was used for the experiment with *H. elongata* of Ria de Arousa but, as no growth was observed, this temperature was excluded for the population of Vigo. Besides that, at 28ºC there was no growth too, for both populations.

Table 1 – Results of ANOVA testing the effects of intensity, frequency and duration of disturbance in the growth of *H. elongata*. *p < 0.05, **p < 0.01

	Ria de Arousa			**Vigo**		
Factors	**df**	**MS**	**F**	**df**	**MS**	**F**
Intensity	2	0,484930	282,9906**	2	0,220941	452,2224**
Frequency	2	0,023773	13,8732**	2	0,002985	6,1102**
Duration	2	0,010322	6,0233**	2	0,000058	0,1189
Intensity*Frequency	4	0,008515	4,9692**	4	0,002490	5,0958**
Intensity*Duration	4	0,003919	2,2869	4	0,001727	3,5347**
Frequency*Duration	4	0,005709	3,3315**	4	0,001041	2,1303
Intensity*Frequency*Duration	8	0,002175	1,2693	8	0,000435	0,8898
Error	243	0,001714		243	0,000489	
	Duration*Frequency 24h: 6h=12h=full 48h: 6h=12h>full 72h: 6h=12h=full **Frequency*Intensity** 6h: 20º=24º>28º 12h: 20º=24º>28º Full: 20º>24º>28º			**Duration*Intensity** 24h: 20º=24º>28º 48h: 20º=24º>28º 72h:20º>24º>28º **Frequency*Intensity** 6h: 20º=24º>28º 12h: 20º>24º>28º Full: 20º=24º>28º		

PAM

In both populations the PAM at t0 wasn't significantly different between the replicates.

In general, the Fv/Fm decreased at the highest intensities of disturbance tested, 28ºC and 32ºC (when tested), for both populations (Fig.6).

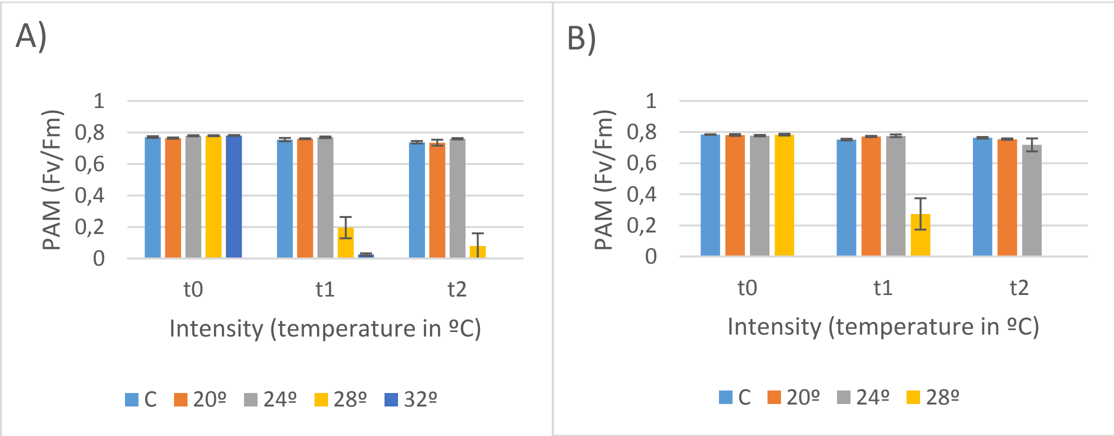

Fig. 6 - PAM of *Himanthalia elongata* of A) Ria de Arousa and B) Vigo at different intensities of disturbance.

In general, there were no differences in the Fv/Fm between the end of the experiment (t1) and after the recovery period (t2), when considering the frequency and duration of disturbance. However, Fv/Fm decreased when compared to control conditions (Fig.7 and 8).

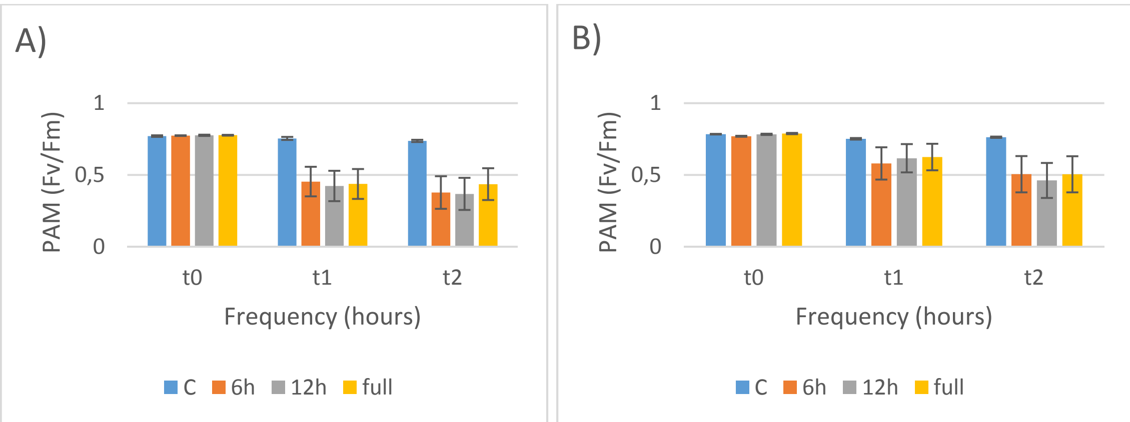

Fig. 7 - PAM of *Himanthalia elongata* of A) Ria de Arousa and B) Vigo at different frequencies of disturbance.

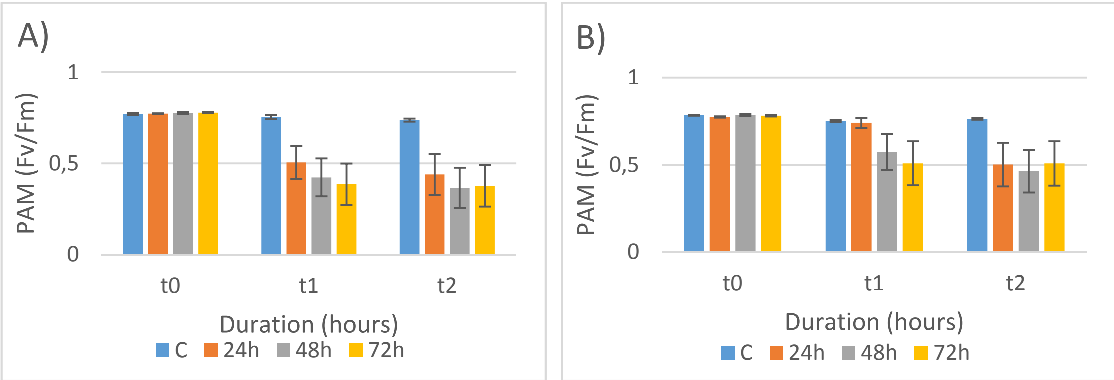

Fig. 8 - PAM of *Himanthalia elongata* of A) Ria de Arousa and B) Vigo at different durations of disturbance.

The ANOVA tests showed for both populations of *Himanthalia elongata* a significant interaction between the 4 factors tested: t, duration, frequency and intensity of disturbance.

In the population of Ria de Arousa, at t1, the effects of duration of disturbance varied with intensity of disturbance, with a significant decrease of the PAM values with the 2 highest intensities of disturbance. Additionally, at the lowest duration of disturbance, the PAM values were significantly lower at 32ºC than at 28ºC. At t2, although the same pattern was found at the mid and high duration of disturbance, at the lowest duration of disturbance, the effects varied with disturbance frequency. While for the two lowest frequencies of disturbance (6h and 12h), the two lowest intensities significantly differ from the two highest intensities, for the highest frequency of disturbance (full) differences were found only for the temperature of 32ºC (Table 2).

In the population of Vigo, at t1, at the highest duration of disturbance (72h) PAM values significantly decreased at the highest temperature tested (28ºC). This pattern was also found for the mid duration of disturbance (48h) except when considering the highest frequency of disturbance where significant differences were found for all the intensities of disturbance tested. For the lowest duration of disturbance, significant differences were only found for the lowest frequency (6h) with a significant decrease in PAM values at the highest intensity of disturbance (28ºC). At t2, it was possible to see a pattern of decrease of PAM with the increase of intensity of disturbance but just at high frequency of disturbance (full) when the duration was mid (48h) and at mid frequency (12h) when the duration is high (72h). At low duration of disturbance (24h), the PAM values significantly decreased at high intensity of disturbance (28ºC). This pattern was also found at mid duration of disturbance (48h) when the frequency was low (6h) and at high duration (72h), except when the frequency was mid. At mid duration of disturbance (48h), when the frequency of disturbance was mid (12h), the PAM was significantly higher at low intensity of disturbance (20ºC) (Table 2).

Table 2 – Results of ANOVA testing the effects of intensity, frequency and duration of disturbance in the PAM of *H. elongata*. *p < 0.05, **p < 0.01

	Ria de Arousa			**Vigo**		
Factors	**df**	**MS**	**F**	**df**	**MS**	**F**
intensity	3	17,1510	10346,3**	2	12,2865	1156,44**
frequency	2	0,0595	35,9**	2	0,0293	2,76
duration	2	0,3521	212,4**	2	0,7212	67,88**
t	2	14,2385	8589,3**	2	7,0999	668,27**
intensity*frequency	6	0,0520	31,4**	4	0,1520	14,31**
intensity*duration	6	0,3014	181,8**	4	0,3349	31,53**
frequency*duration	4	0,0504	30,4**	4	0,0357	3,36**
intensity*t	6	4,4768	2700,6**	4	3,4455	324,30**
frequency*t	4	0,0363	21,9**	4	0,1227	11,55**
duration*t	4	0,1263	76,2**	4	0,4151	39,07**
intensity*frequency*duration	12	0,0537	32,4**	8	0,0642	6,05**
intensity*frequency*t	12	0,0440	26,5**	8	0,1052	9,90**
intensity*duration*t	12	0,0927	55,9**	8	0,4159	39,15**
frequency*duration*t	8	0,0456	27,5**	8	0,0492	4,63**
intensity*frequency*duration*t	24	0,0417	25,1**	16	0,0348	3,27**
Error	864	0,0017		648	0,0106	
	t*Duration*Frequency*Intensity t0: no differences t1: 24h: 20º=24º>28º>32º 48h: 20º=24º>28º=32º 72h: 20º=24º>28º=32º t2: 24h: 6h: 20º=24º>28º=32º 12h: 20º=24º>28º=32º Full: 20º=24º=28º>32º 48h: 20º=24º>28º=32º 72h: 20º=24º>28º=32º			**t*Duration*Frequency*Intensity** t0: no differences t1: 24h: 6h: 20º=24º>28º 12h:20º=24º=28º Full: 20º=24º=28º 48h: 6h: 20º=24º>28º 12h: 20º=24º>28º Full: 20º>24º>28º 72h: 20º=24º>28º t2: 24h: 20º=24º>28º 48h: 6h: 20º=24º>28º 12h: 20º>24º=28º Full: 20º> 24º> 28º 72h: 6h: 20º=24º>28º 12h: 20º>24º>28º Full: 20º=24º>28º		

Photosynthetic efficiency

Generally, there was a decrease in the photosynthetic efficiency of the *H. elongata* fronds with the intensity of disturbance while no clear changes were observed with the frequency of disturbance. A pattern of increase in photosynthetic efficiency was observed with the increase in the duration of disturbance, but the photosynthetic efficiency of the control of Ria de Arousa was higher than in the others durations of disturbance (Fig. 9).

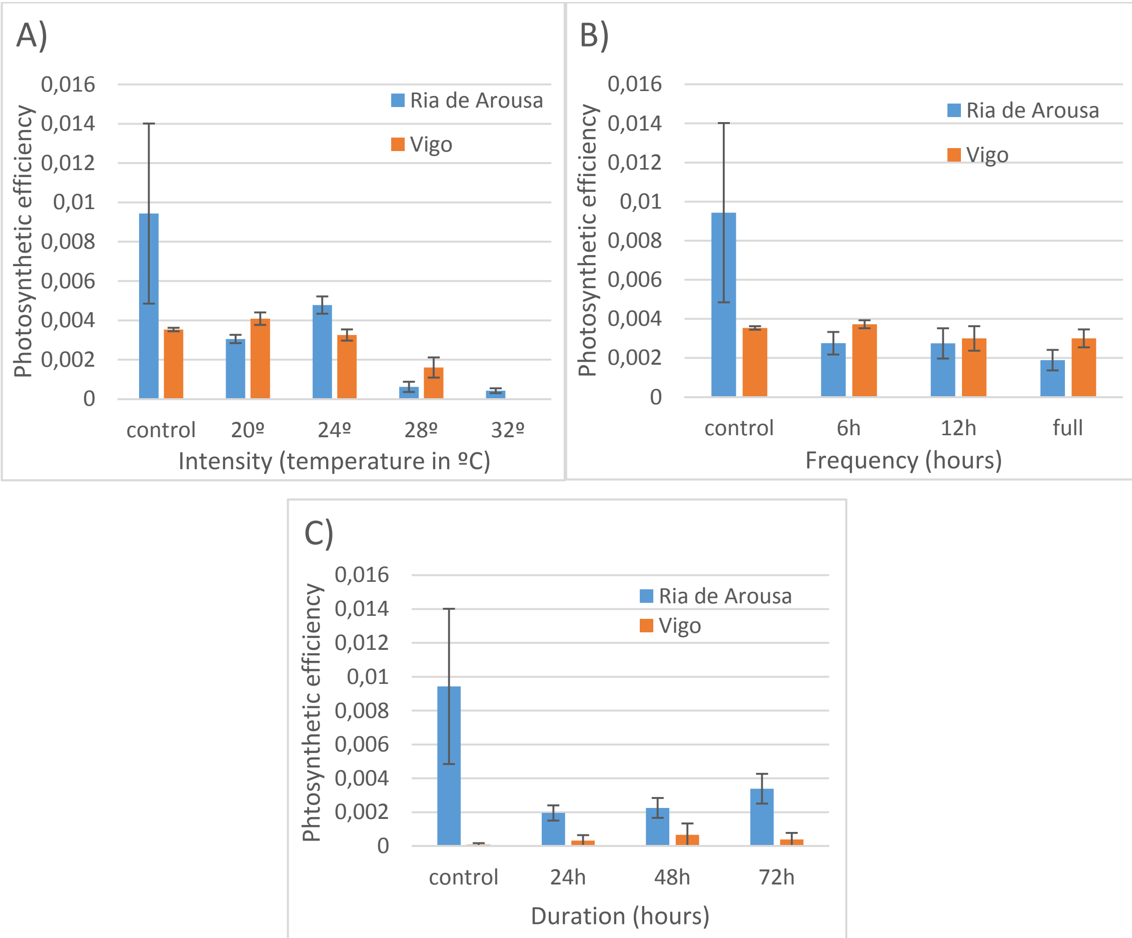

Fig. 9 – Photosynthetic efficiency of *Himanthalia elongata* of Ria de Arousa (blue) and Vigo (orange) at different A) intensities, B) frequencies and C) durations of disturbance.

In both populations the effects of the intensity of disturbance significantly varied with the frequency and duration of disturbance (Table 3).

For the population of Ria de Arousa, photosynthetic efficiency significantly increased with intensity of disturbance except for the lowest duration of disturbance (24h) and the highest frequency of disturbance (72h), where there were only significant differences between the 2 highest and the 2 lowest intensities of disturbance (Table 3).

For the population of Vigo, the photosynthetic efficiency significantly decreased at the highest intensity of disturbance for the two highest durations (48h, 72h) and the two lowest frequencies (6h, 12h) of disturbance. At the lowest duration of disturbance the different levels of intensity of disturbance did not cause significant effects on the photosynthetic efficiency. On the other hand, at the highest frequency level (full) the photosynthetic efficiency significantly decreased at the two highest intensities of disturbance (Table 3).

Table 3 – Results of ANOVA testing the effects of intensity, frequency and duration of disturbance in the photosynthetic efficiency of *H. elongata*. *p < 0.05, **p < 0.01

	Ria de Arousa			Vigo		
Factors	**df**	**MS**	**F**	**df**	**MS**	**F**
intensity	3	0,000125	120,2133**	2	0,000072	70,7666**
frequency	2	0,000002	1,8530	2	0,000000	0,3840
duration	2	0,000001	1,0424	2	0,000003	2,5149
intensity*frequency	6	0,000004	3,6013**	4	0,000004	3,7575**
intensity*duration	6	0,000004	3,9197**	4	0,000007	6,5884**
frequency*duration	4	0,000001	1,0463	4	0,000002	2,4498
intensity*frequency*duration	12	0,000001	0,6495	8	0,000002	1,5267
Error	72	0,000001		54	0,000001	
	Duration*Intensity 24h: 20º=24º>28º=32º 48h: 24º>20º>28º=32º 72h: 24º>20º>28º=32º **Frequency*Intensity** 6h: 24º>20º>28º=32º 12h: 24º>20º>28º=32º Full: 20º=24º>28º=32º			**Duration*Intensity** 24h: no differences 48h: 20º=24º>28º 72h: 20º=24º>28º **Frequency*Intensity** 6h: 20º=24º>28º 12h: 20º=24º>28º Full: 20º>24º=28º		

GPP

In general, in both populations there was a decrease in GGP values with the intensity of disturbance while the frequency did not importantly affected the GGP values. An increase in these values was observed, for both populations, with the increase of duration of the disturbance, nevertheless these values were lower than in the control (Fig.10).

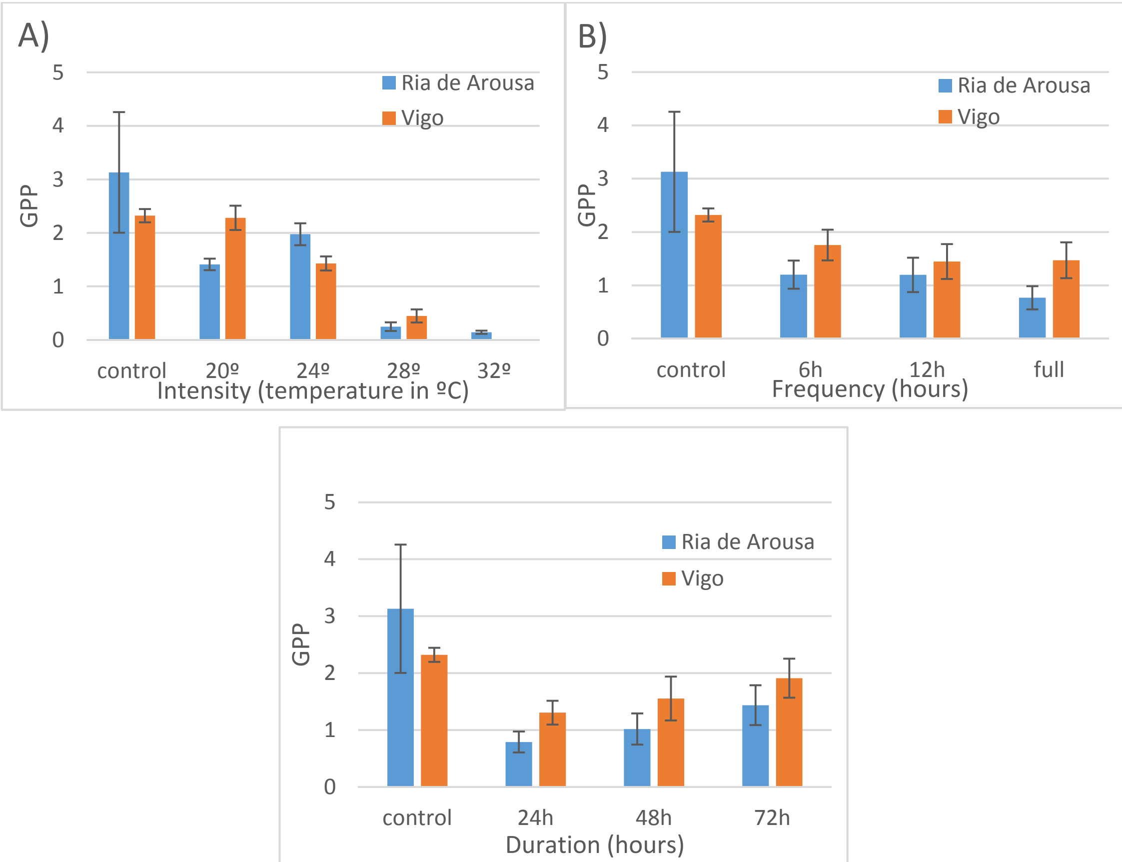

Fig. 10 –GPP of *Himanthalia elongata* of Ria de Arousa (blue) and Vigo (orange) at different A) intensities, B) frequencies and C) durations of disturbance.

In both populations intensity of disturbance caused significant differences in the values of GPP and was correlated with the frequency and duration of disturbance.

In population of Ria de Arousa the highest values of GPP were at mid intensity of disturbance (24ºC), but it was only possible to see at high duration and at mid frequency of duration. In the others frequencies and durations of disturbance, the GPP at 20ºC and 24ºC was significantly higher than in 28ºC and 32ºC (Table 4).

In the population of Vigo was possible to see a significantly decrease in GPP with the increase of intensity of disturbance, but just at mid and high duration of disturbance. Except at mid frequency (12h) when the duration was high and at high frequency (full) when the duration was mid. In the high duration at mid frequency, the GPP at high intensity (28ºC) was significantly lower than in the low and mid intensity of disturbance. The same happened at low duration of disturbance, but just at high frequency (full). At mid duration and high frequency the GPP at low intensity was significantly higher (Table 4).

Table 4 – Results of ANOVA testing the effects of intensity, frequency and duration of disturbance in the GPP of *H. elongata*. *p < 0.05, **p < 0.01

	Ria de Arousa			Vigo		
Factors	**df**	**MS**	**F**	**df**	**MS**	**F**
intensity	3	22,83170	108,9940**	2	27,7350	219,055**
frequency	2	0,64005	3,0555	2	0,0438	0,346
duration	2	0,50051	2,3894	2	0,0668	0,528
intensity*frequency	6	0,63656	3,0388*	4	1,0965	8,660**
intensity*duration	6	0,67774	3,2354**	4	1,7627	13,922**
frequency*duration	4	0,09245	0,4413	4	0,7159	5,654**
intensity*frequency*duration	12	0,19256	0,9192	8	0,3206	2,532*
Error	72	0,20948		54	0,1266	
	Duration*Intensity 24h: 20º=24º>28º=32º 48h: 20º=24º>28º=32º 72h: 24º>20º>28º=32º **Frequency*Intensity** 6h: 20º=24º>28º=32º 12h: 24º>20º>28º=32º Full: 20º=24º>28º=32º			**Duration*Frequency*Intensity** 24h: 6h: 20º=24º=28º 12h: 20º=24º=28º Full: 20º=24º>28º 48h: 6h: 20>24º>28º 12h: 20>24º>28º Full: 20º>24º=28º 72h: 6h: 20º>24º>28º 12h: 20º=24º>28º Full: 20º>24º>28º		

Maximum production rate

In the population of Ria de Arousa the maximum production rate was highest in mid intensity of disturbance (24ºC) and above this temperature the maximum production rate decrease, while for the population of Vigo the highest maximum production rate was at low intensity of disturbance (20ºC) and decrease with the increase of intensity (Fig. 11).

Frequency of disturbance affected the maximum production rate of both populations of *H. elongata*: in all frequencies of disturbance the values were lower than the values in the control, but the decrease were similar in all frequencies of disturbance.

The increase in the duration of disturbance caused an increase in maximum production rate of *H. elongata*, however, in all durations of disturbance, the values of the maximum production rate were lower than in the control (Fig. 11).

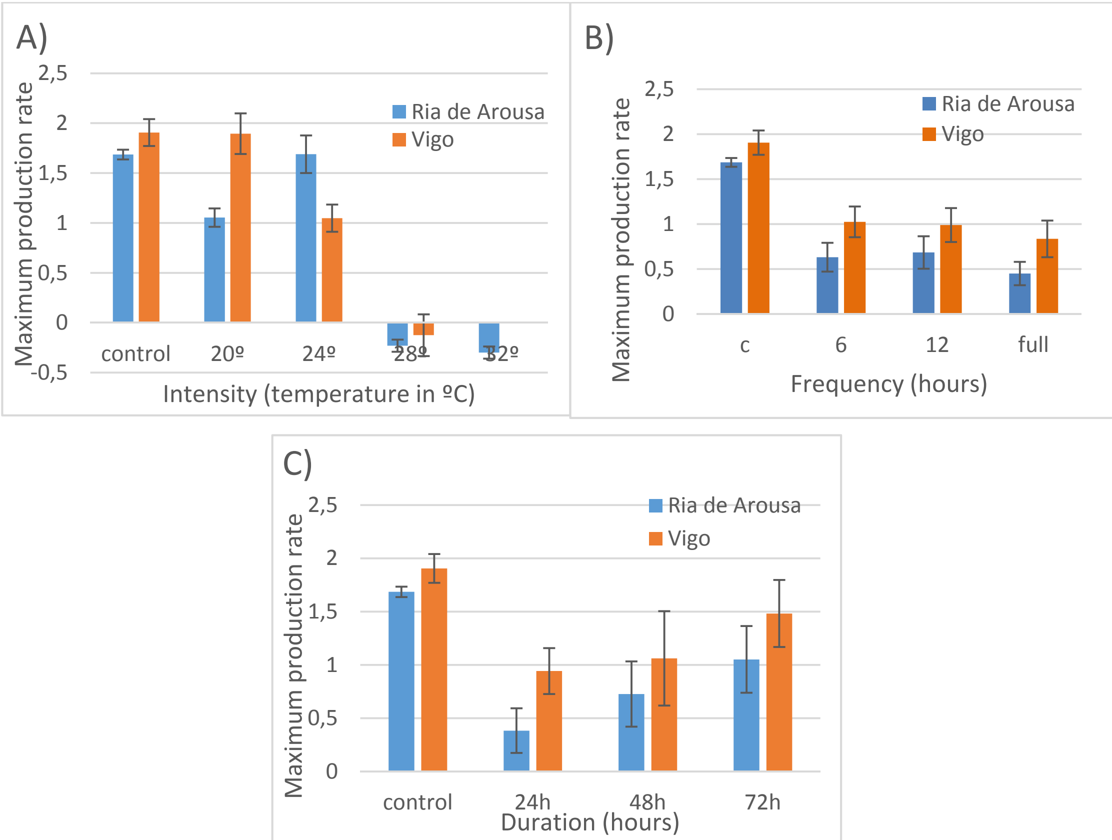

Fig. 11 – Maximum production rate of *Himanthalia elongata* of Ria de Arousa (blue) and Vigo (orange) at different A) intensities, B) frequencies and C) durations of disturbance.

The intensity of disturbance caused significant differences in the maximum production rate of *H. elongata*, and was correlated with the duration and frequency of disturbance.

In the population of Ria de Arousa, the maximum production rate was highest at mid intensity of disturbance, but just at high duration and at low and mid frequency of disturbance. At low and mid duration and at high frequency (full), the maximum production rate was significantly higher at 20ºC and 24ºC than in 28ºC and 32ºC (Table 5).

In the population of Vigo, it was possible to see a significant decrease in maximum production rate with the increase of the intensity but just at mid and high duration of disturbance, except at low frequency (6h) and mid frequency (when the durations was high). At low frequency (6h), the maximum production rate was significantly higher at low intensities of disturbance. At mid frequency, when the duration was high, the maximum production rate was significantly lower at high intensity of disturbance (Table 5).

Table 5 – Results of ANOVA testing the effects of intensity, frequency and duration of disturbance in the maximum production rate of *H. elongata*. *p < 0.05, **p < 0.01

	Ria de Arousa			**Vigo**		
Factors	**df**	**MS**	**F**	**df**	**MS**	**F**
intensity	3	24,21761	144,1307**	2	26,88921	262,2027**
frequency	2	0,52036	3,0969	2	0,27370	2,6690
duration	2	1,24267	7,3957**	2	0,00412	0,0402
intensity*frequency	6	0,46508	2,7679*	4	0,89422	8,7198**
intensity*duration	6	0,43552	2,5920*	4	1,49537	14,5817**
frequency*duration	4	0,23706	1,4108	4	0,83636	8,1556**
intensity*frequency*duration	12	0,10341	0,6155	8	0,29213	2,8486*
Error	72	0,16803		54	0,10255	
	Duration*Intensity 24h: 20º=24º>28º=32º 48h: 20º=24º>28º=32º 72h: 24º>20º>28º=32º **Frequency*Intensity** 6h: 24º>20º>28º=32º 12h: 24º>20º>28º=32º Full: 20º=24º>28=32º			**Duration*Frequency*Intensity** 24h: no differences 48h: 6h: 20º>24º=28º 12h: 20º>24º>28º ……..full: 20º>24º>28º 72h: 6h: 20º>24º=28º 12h: 20º=24º>28º Full: 20º>24º>28º		

Respiration

In general the values of respiration were similar for both populations at all intensities, frequencies and durations of disturbance (Fig.12).

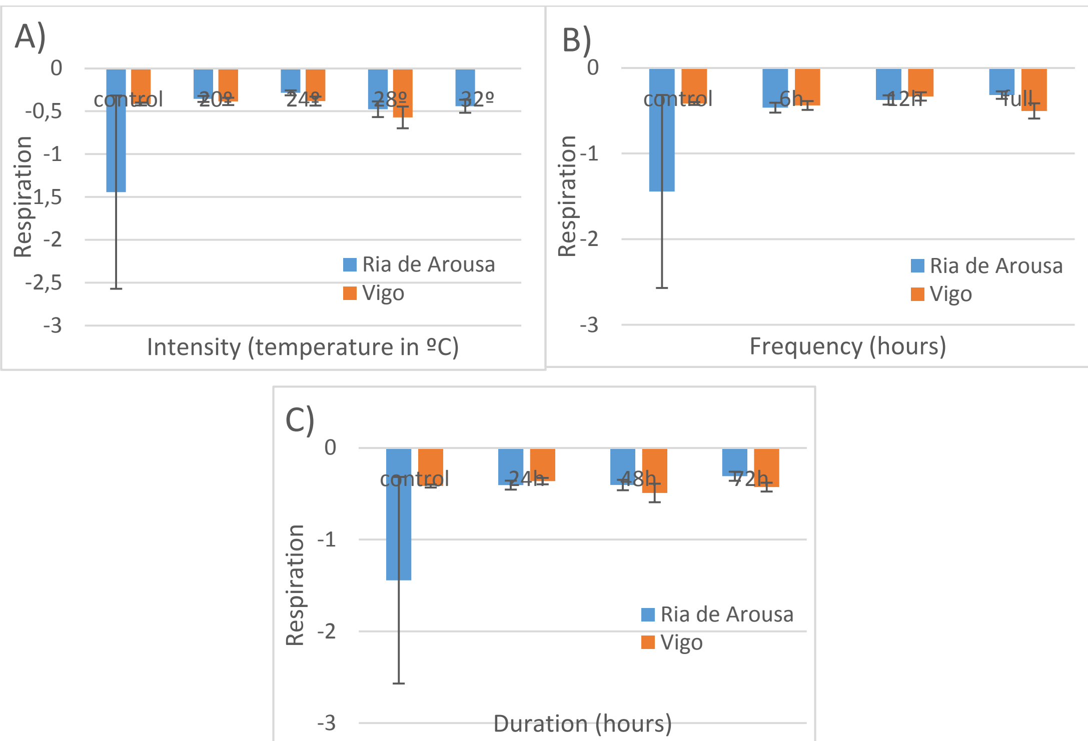

Fig. 12 – Respiration of *Himanthalia elongata* of Ria de Arousa (blue) and Vigo (orange) at different A) intensities, B) frequencies and C) duration of disturbance.

In both populations the effect of the different factors in the respiration were few.

In population of Ria de Arousa the respiration had significant effects at mid and high duration of disturbance. At mid duration and low frequency of disturbance, the respiration was significantly higher at 28ºC than in the others intensities of disturbance. And at high duration of disturbance and low and mid frequencies, the respiration was higher at 28ºC and 32ºC than in the others intensities of disturbance (Table 6).

In population of Vigo at mid duration of disturbance and high frequency of disturbance, the respiration was significantly high at 28ºC (Table 6).

Table 6 – Results of ANOVA testing the effects of intensity, frequency and duration of disturbance in the respiration of *H. elongata*. $^{*}p < 0.05$, $^{**}p < 0.01$

	Ria de Arousa			**Vigo**		
Factors	**df**	**MS**	**F**	**df**	**MS**	**F**
intensity	3	0,03821	1,9246	2	0,00731	0,1934
frequency	2	0,06271	3,1589*	2	0,25947	6,8667**
duration	2	0,47896	24,1263**	2	0,09179	2,4291
intensity*frequency	6	0,05059	2,5484*	4	0,23995	6,3500**
intensity*duration	6	0,33668	16,9591**	4	0,19485	5,1564**
frequency*duration	4	0,06292	3,1693*	4	0,02936	0,7769
intensity*frequency*duration	12	0,04789	2,4123*	8	0,15750	4,1680**
Error	72	0,01985		54	0,03779	
	Duration*Frequency*Intensity 24h: no differences 48h: 6h: 28º>20º=24º=32º 12h: no diferences full: no diferences 72h: 6h: 28º=32º>20º=24º 12h: 28º=32º>20º=24º Full: no differences			**Duration*Frequency*Intensity** 24h: no differences 48h: 6h: no differences 12h: no differences Full: 28º>20º=24º 72h: no differences		

Fucus serratus

Growth

Generally, both populations showed a decrease in the vegetative growth with the increase of the intensity, frequency and duration of disturbance (Fig.13).

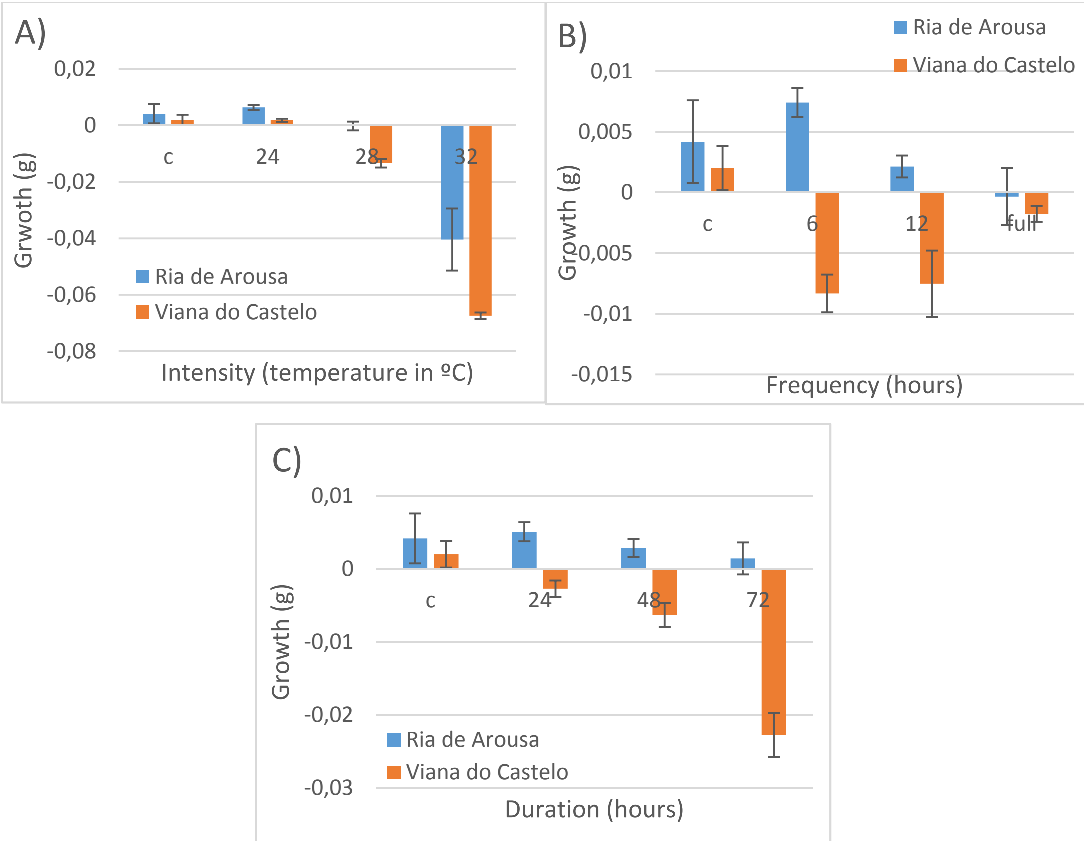

Fig.13 - Growth of *Fucus serratus* of Ria de Arousa (blue) and Viana do Castelo (orange) at different A) intensities, B) frequencies and C) durations of disturbance.

Both population of *F. serratus* showed a significant influence of intensity in the growth that was correlated with the frequency and duration of disturbance. (Table 7). In population of Ria de Arousa was possible to see a pattern of decrease in the growth with the increase of intensity of disturbance but just at high duration and high frequency of disturbance. At the others durations and frequencies there was no differences between low and mid intensity of disturbance, but these vegetative growth at these intensities was significantly higher than in high intensity (32ºC). At low duration of disturbance there was no differences in the growth at low and mid frequencies of disturbance (Table 7).

Generally, in population of Viana do Castelo was possible to see a decrease in the growth with the increase of intensity of disturbance, with some variations depending on the duration and frequency of disturbance. At low duration of disturbance (24h) this pattern only occurred at low (6h) and mid (12h) frequencies of disturbance and at high frequency of disturbance (full) the growth was significantly lower at 32ºC than in the others intensities of disturbance. At mid duration of disturbance (48h) this pattern only occurred at low frequency of disturbance (6h); and at mid frequency (12h) the growth was significantly higher at 24ºC than in the others intensities of disturbance, and at high frequency (full) the growth was significantly lower at 32ºC than in the others intensities of disturbance. At high duration of disturbance (72h), at low frequency, the growth at 32ºC was significantly lower than in the others intensities of disturbance, and at mid (12h) and high (full) frequencies of disturbance, the growth at 24ºC was significantly higher than in the others intensities of disturbance (Table 7).

Table 7 – Results of ANOVA testing the effects of intensity, frequency and duration of disturbance in the growth of *F. serratus* of Ria de Arousa. *p < 0.05, **p < 0.01

	Ria de Arousa			**Viana do Castelo**		
Factors	**df**	**MS**	**F**	**df**	**MS**	**F**
intensity	2	0,048268	277,0655**	2	0,128483	634,134**
frequency	2	0,001719	9,8672**	2	0,001614	7,968**
duration	2	0,005474	31,4238**	2	0,005617	27,724**
intensity*frequency	4	0,001066	6,1206**	4	0,003458	17,068**
intensity*duration	4	0,003381	19,4078**	4	0,003376	16,661**
frequency*duration	4	0,000208	1,1919	4	0,001062	5,242**
intensity*frequency*duration	8	0,000603	3,4599**	8	0,003621	17,874**
Error	189	0,000174		297	0,000203	
	Duration*Frequency*Intensity 24h: 6h: no differences 12h: no differences Full: 24º=28º>32º 48h: 24º=28º>32º 72h: 6h: 24º=28º>32º 12h: 24º=28º>32º Full: 24º>28º>32º			**Duration*Frequency*Intensity** 24h: 6h: 24º>28º>32º 12h: 24º>28º>32º Full: 24º=28>32º 48h: 6h: 24º>28º>32º 12h: 24º>28º=32º Full: 24º=28º>32º 72h: 6h: 24º=28º>32º 12h: 24º>28º=32º Full: 24º>28º=32º		

PAM

At the beginning of the experiment (t0) no significant differences were recorded for the PAM measured in the individuals of both populations.

Generally, in population of Ria de Arousa the PAM (Fv/Fm) after the treatment (t1) and after the recovery time (t2) was lower at high intensity of disturbance (Fig. 14).

In population of Viana do Castelo, it was possible to see a general decrease in the PAM (Fv/Fm), after the treatment (t1) and after the recovery time (t2), with the increase of intensity of disturbance (Fig. 14).

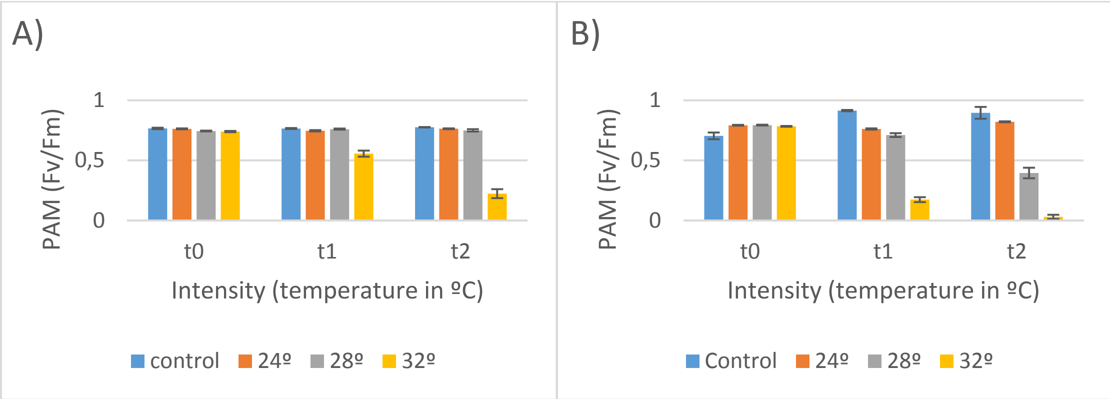

Fig.14 - PAM of *Fucus serratus* of A) Ria de Arousa and B) Viana do Castelo at different intensities of disturbance.

In general, in both populations there was a decrease in the Fv/Fm with increase of intensity, frequency and duration of disturbance but this decrease was more pronounced for the population of Viana do Castelo (Fig 15 and 16).

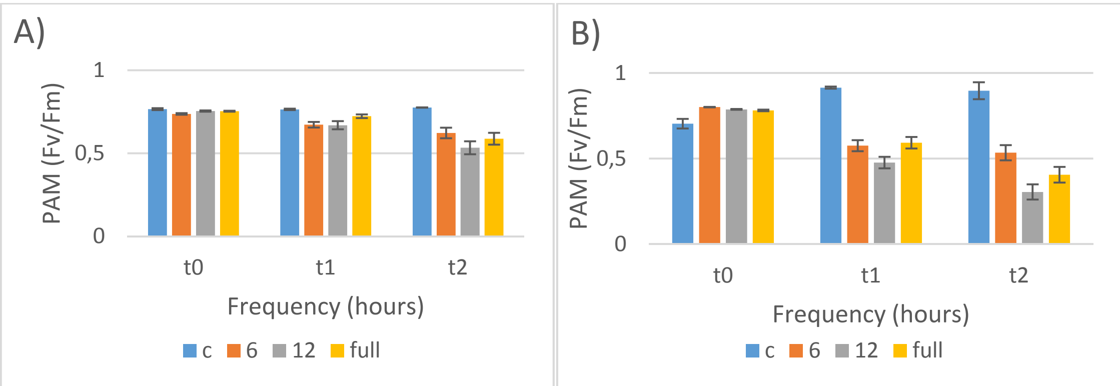

Fig.15 - PAM of *Fucus serratus* of A) Ria de Arousa and B) Viana do Castelo at different frequencies of disturbance.

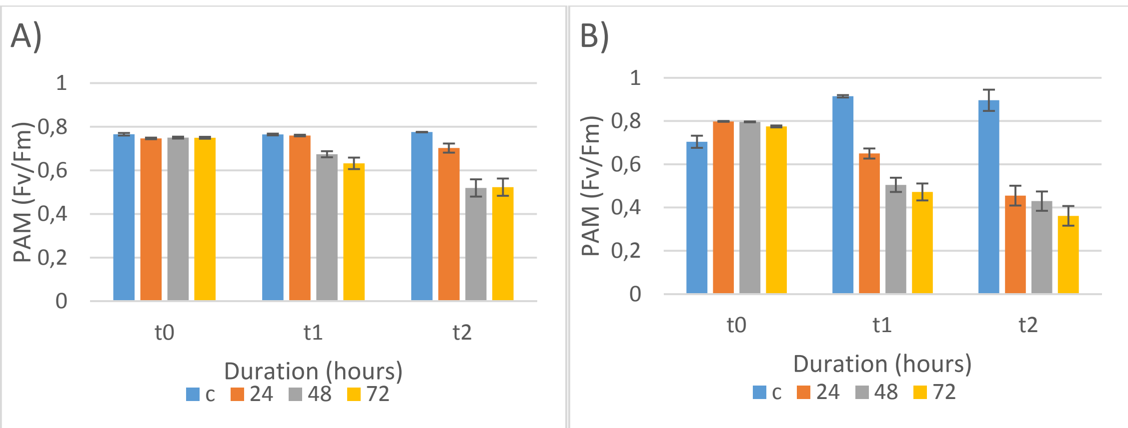

Fig.16 - PAM of *Fucus serratus* of A) Ria de Arousa and B) Viana do Castelo at different durations of disturbance.

In both populations the PAM (Fv/Fm) was influenced by all the factors and the factors were significantly correlated.

In the population of Ria de Aroura, the PAM was significantly lower at high intensity of disturbance, but only at mid and high durations of disturbance, except at high frequency when the duration was mid at t1. This also happened at mid frequency, when the duration was low (24h), at t2 (Table 8).

Table 8 – Results of ANOVA testing the effects of intensity, frequency and duration of disturbance in the PAM of *F. serratus*. *p < 0.05, **p < 0.01

	Ria de Arousa			Viana do Castelo		
Factors	**df**	**MS**	**F**	**df**	**MS**	**F**
intensity	2	4,7136	691,12**	2	13,4418	1467,61**
frequency	2	0,0903	13,24**	2	0,8016	87,52**
duration	2	0,7600	111,43**	2	0,5864	64,02**
t	2	1,6746	245,54**	2	8,7583	956,25**
intensity*frequency	4	0,0973	14,26**	4	0,5240	57,21**
intensity*duration	4	0,7081	103,82**	4	0,1480	16,16**
frequency*duration	4	0,0133	1,95	4	0,0667	7,28**
intensity*t	4	1,7481	256,31**	4	3,9396	430,14**
frequency*t	4	0,0776	11,38**	4	0,2959	32,30**
duration*t	4	0,2441	35,79**	4	0,1481	16,17**
intensity*frequency*duration	8	0,0176	2,58**	8	0,1003	10,95**
intensity*frequency*t	8	0,0568	8,33**	8	0,4272	46,65**
intensity*duration*t	8	0,2454	35,98**	8	0,0894	9,76**
frequency*duration*t	8	0,0643	9,42**	8	0,0344	3,75**
intensity*frequency*duration*t	16	0,0471	6,90**	16	0,0611	6,67**
Error	624	0,0068		648	0,0092	
	t*Duration*Frequency*Intensity t0: no differences t1: 24h: no differences 48h: 6h: 24º=28º>32º 12h: 24º=28º>32º Full: no differences 72h: 24º=28º>32º t2: 24h: 6h: no differences 12h: 24º=28º>32º Full: no differences 48h: 24º=28º>32º 72h: 24º=28º>32º			**t*Duration*Frequency*Intensity** t0: no differences t1: 24h: 24º=28º>32º 48h: 24º=28º>32º 72h: 6h: 24º=28º>32º 12h: 24º>28º>32º Full: 24º=28º>32º t2: 24h: 6h: 24º=28º>32º 12h: 24º>28º>32º Full: 24º>28º=32º 48h: 6h: 24º=28º>32º 12h: 24º>28º=32º Full: 24º>28º>32º 72h: 6h: 24º=28º>32º 12h: 24º>28º=32º Full: 24º>28º=32º		

In the population of Viana do Castelo, after the treatments (t1), it was possible to see a pattern of decrease of PAM with the increase of intensity of disturbance, but just at mid frequency and high duration of disturbance. In the others frequencies and intensities of disturbance the PAM was significantly lower at high intensity of disturbance, but there was no difference between the PAM at low and mid intensity of disturbance. After the recovery time (t2), the decrease of PAM with the increase of intensity of disturbance was observed at low duration and mid frequency of disturbance and at mid duration and high frequency of disturbance. At low frequency of disturbance the PAM was significantly lower at high intensity (32ºC), but there was no differences in the PAM at low and mid intensities of disturbance. At mid frequency and mid and high duration of disturbance and at high frequency and low and high duration of disturbance, the PAM was significantly higher at low intensity of disturbance than in the others intensities (Table 8).

In the population of Viana do Castelo, after the treatments (t1), it was possible to see a pattern of decrease of PAM with the increase of intensity of disturbance, but just at mid frequency and high duration of disturbance. In the others frequencies and intensities of disturbance the PAM was significantly lower at high intensity of disturbance, but there was no difference between the PAM at low and mid intensity of disturbance. After the recovery time (t2), the decrease of PAM with the increase of intensity of disturbance was observed at low duration and mid frequency of disturbance and at mid duration and high frequency of disturbance. At low frequency of disturbance the PAM was significantly lower at high intensity (32ºC), but there was no differences in the PAM at low and mid intensities of disturbance. At mid frequency and mid and high duration of disturbance and at high frequency and low and high duration of disturbance, the PAM was significantly higher at low intensity of disturbance than in the others intensities (Table 8).

Photosynthetic efficiency

Generally, there was a decrease in the photosynthetic efficiency with intensity, frequency and duration of disturbance for both populations. There was a high variability in the photosynthetic efficiency at the highest levels of disturbance for all the factors tested (Fig. 17).

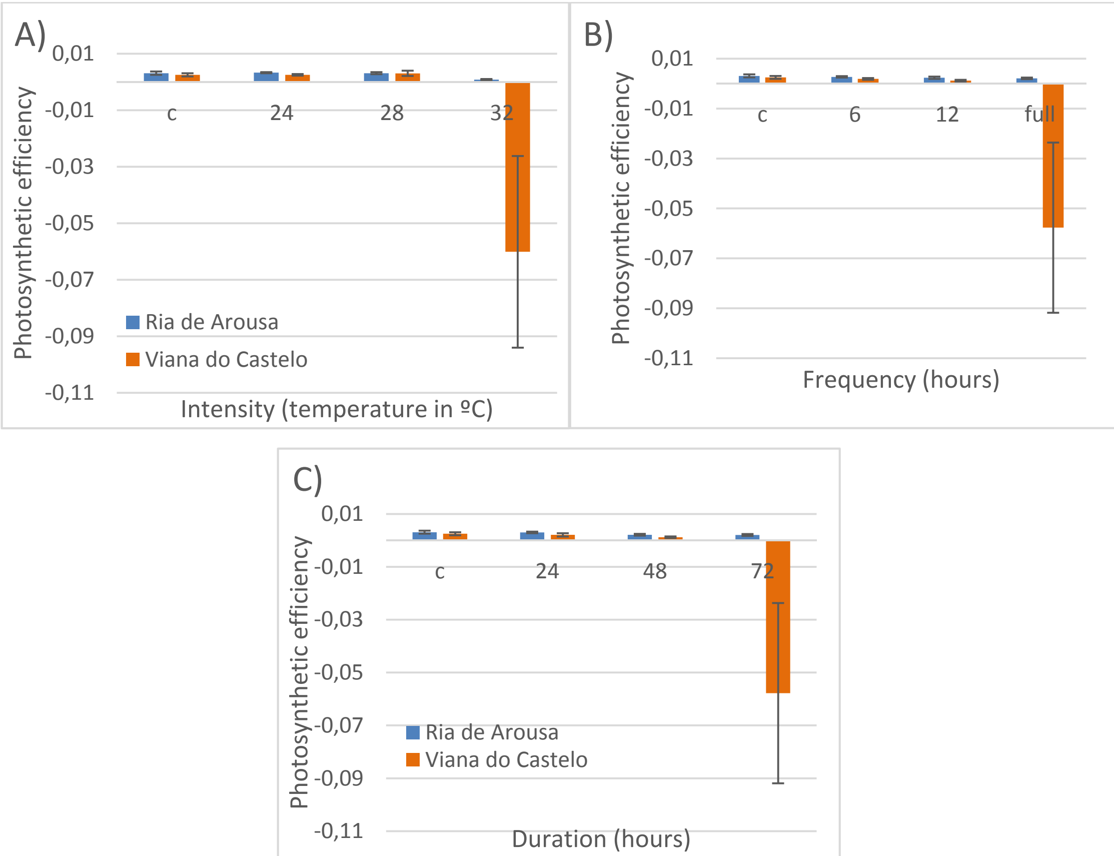

Fig.17 – Photosynthetic efficiency of *Fucus serratus* from Ria de Arousa (blue) and Viana do Castelo (orange) at different A) intensities, B) frequencies and C) durations of disturbance.

In the population of Ria de Arousa, the intensity of disturbance influenced the photosynthetic efficiency, but just at high duration of disturbance. Thus, in this duration, the photosynthetic efficiency was significantly lower at high intensity than in the others intensities of disturbance (Table 9).

Table 9 – Results of ANOVA testing the effects of intensity, frequency and duration of disturbance in the photosynthetic efficiency of *F. serratus*. *p < 0.05, **p < 0.01

	Ria de Arousa			**Viana do Castelo**		
Factors	**df**	**MS**	**F**	**df**	**MS**	**F**
intensity	2	0,000052	33,6810**	2	0,035595	187,3427**
frequency	2	0,000002	1,5917	2	0,031587	166,2514**
duration	2	0,000008	5,3471**	2	0,031788	167,3095**
intensity*frequency	4	0,000003	1,9687	4	0,033024	173,8129**
intensity*duration	4	0,000005	3,3999*	4	0,034288	180,4644**
frequency*duration	4	0,000001	0,7505	4	0,031626	166,4545**
intensity*frequency*duration	8	0,000003	1,8867	8	0,034379	180,9456**
Error	54	0,000002		54	0,000190	
	Duration*Intensity 24h: no differences 48h: no differences 72h: 24º=28º>32º			**Duration*Frequency*Intensity** 24h: no differences 48h: no differences 72h: 6h: no differences 12h: no differences Full: 24º=28º>32º		

In the population of Viana do Castelo, the intensity only affects the photosynthetic efficiency at high duration and high frequency of disturbance. In these conditions, the photosynthetic efficiency was significantly lower at high intensity of disturbance (Table 9).

GPP

In general, in both populations, the GPP decreased with the increase of intensity of disturbance (Fig. 18).

In the population of Ria de Arousa, the GPP was similar in all frequencies of disturbance. In the population of Viana do Castelo the highest GPP was at high frequency and the lowest was at mid frequency of disturbance (Fig. 18).

Generally, in the population of Ria de Arousa the GPP slightly decreased with the increase of the duration of disturbance. In the population of Viana do Castelo the highest GPP was at high duration (72h) and the lowest was at mid and low durations (Fig. 18).

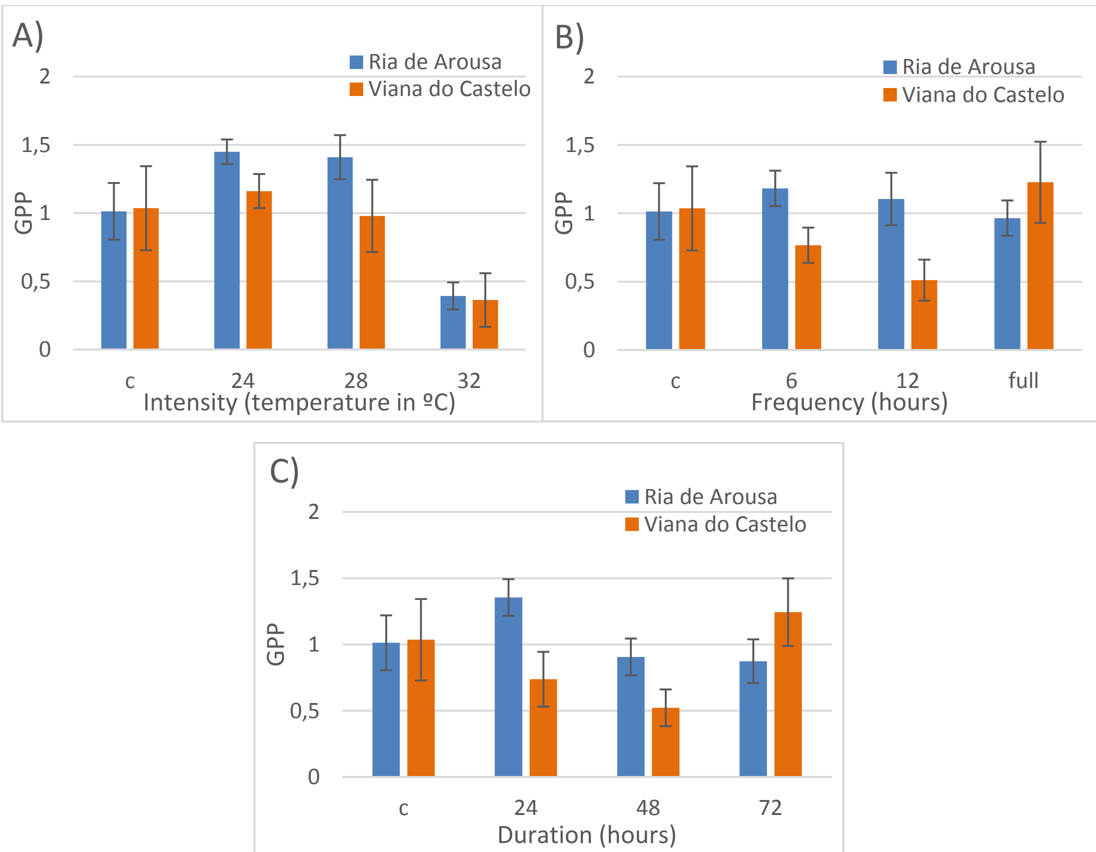

Fig.18 – GPP of *Fucus serratus* of Ria de Arousa (blue) and Viana do Castelo (orange) at different A) intensities, B) frequencies and C) durations of disturbance.

In both populations, the intensity of disturbance influenced the GPP and was significantly correlated with the frequency and duration of disturbance.

In population of Ria de Arousa the pattern was a significantly lower GPP at high intensity of disturbance, but this pattern just happened at mid and high durations of disturbance and at mid frequency of disturbance (Table 10).

In the population of Viana do Castelo the differences were just seen at high duration and high frequency of disturbance, and in this conditions the GPP was significantly higher at mid intensities than in the others intensities of disturbance (Table 10).

Table 10 – Results of ANOVA testing the effects of intensity, frequency and duration of disturbance in the GPP of *F. serratus*. *p < 0.05, **p < 0.01

	Ria de Arousa			**Viana do Castelo**		
Factors	**df**	**MS**	**F**	**df**	**MS**	**F**
intensity	2	9,67938	37,4497**	2	4,72294	9,3890**
frequency	2	0,32773	1,2680	2	3,55874	7,0746**
duration	2	2,09537	8,1070**	2	3,70928	7,3739**
intensity*frequency	4	0,87918	3,4016*	4	3,17861	6,3189**
intensity*duration	4	1,19320	4,6165**	4	0,89085	1,7710
frequency*duration	4	0,14876	0,5755	4	3,43331	6,8252**
intensity*frequency*duration	8	0,40199	1,5553	8	1,90279	3,7827**
Error	54	0,25846		54	0,50303	
	Duration*Intensity 24h: no differences 48h: 24º=28º>32º 72h: 24º=28º>32º **Frequency*Intensity** 6h: no differences 12h: 24º=28º>32º Full: no differences			**Duration*Frequency*Intensity** 24h: no differences 48h: no differences 72h: 6h: no differences 12h: no differences Full: 28º>24º=32º		

Maximum production rate

Generally, in both populations, the maximum production rate decreased with the increase of the intensity, frequency and duration of disturbance, with exception of the population of Ria de Arousa where the values of maximum production rate were simililar in all frequencies of disturbance (Fig. 19)

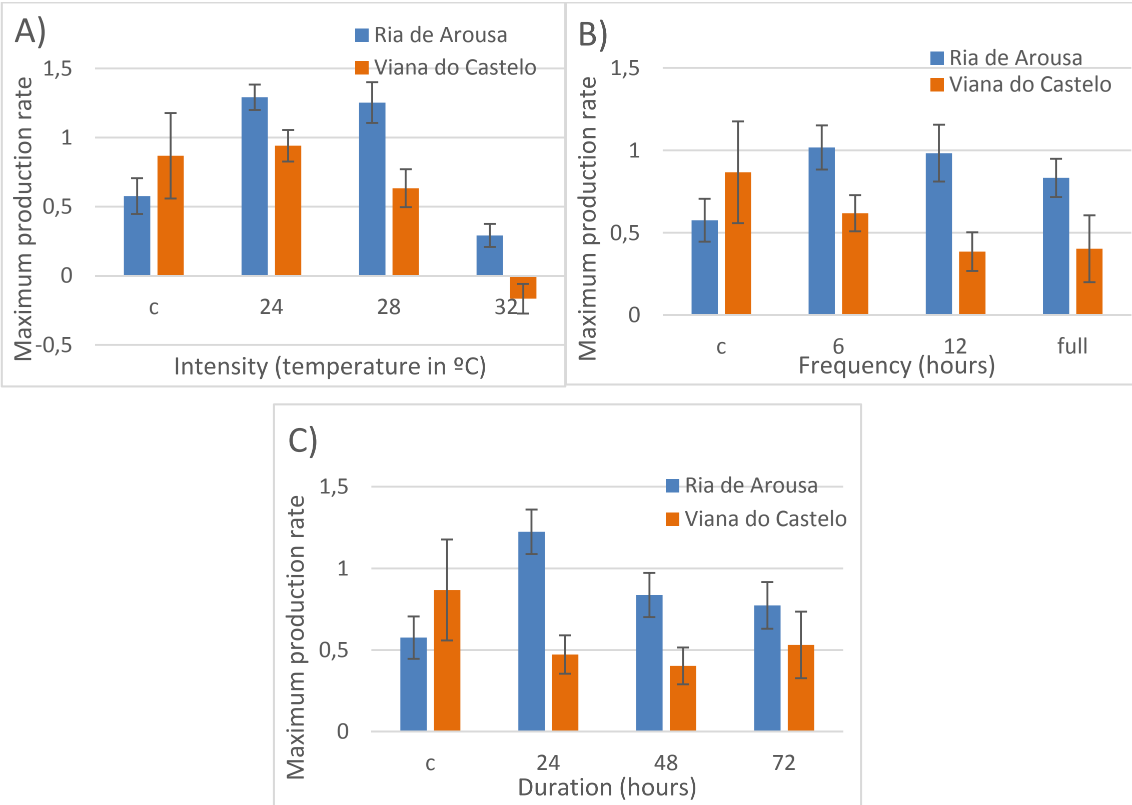

Fig.19 – Maximum production rate of *Fucus serratus* of Ria de Arousa (blue) and Viana do Castelo (orange) at different A) intensities, B) frequencies and C) of disturbance.

In the population of Ria de Arousa was possible to see a significantly lower maximum production rate at high intensity of disturbance, but just at mid and high durations of disturbance and at low and mid frequencies of disturbance (Table 11).

Table 11 – Results of ANOVA testing the effects of intensity, frequency and duration of disturbance in the maximum production rate of *F. serratus*. *p < 0.05, **p < 0.01

	Ria de Arousa			**Viana do Castelo**		
Factors	**df**	**MS**	**F**	**df**	**MS**	**F**
intensity	2	8,65789	36,6130**	2	8,80679	58,9517**
frequency	2	0,26033	1,1009	2	0,45562	3,0499
duration	2	1,60415	6,7837**	2	0,11167	0,7475
intensity*frequency	4	0,82256	3,4785*	4	1,39534	9,3402**
intensity*duration	4	0,89988	3,8055**	4	1,13896	7,6241**
frequency*duration	4	0,04897	0,2071	4	0,05850	0,3916
intensity*frequency*duration	8	0,31344	1,3255	8	1,34700	9,0167**
Error	54	0,23647		54	0,14939	
	Duration*Intensity 24h: no differences 48h: 24º=28º>32º 72h: 24º=28º>32º **Frequency*Intensity** 6h: 24º=28º>32º 12h: 24º=28º>32º Full: no differences			**Duration*Frequency*Intensity** 24h: no differences 48h: no differences 72h: 6h: no differences 12h: 24º>28º=32º Full: 24º=28º>32º		

In the population of Viana do Castelo at high duration and mid frequency of disturbance the maximum production rate was significantly higher at low intensity of disturbance. At high duration and high frequency of disturbance, the maximum production rate was significantly lower at high intensity of disturbance (Table 11).

Respiration

In general, in the population of Ria de Arousa, the respiration decreased with the increase of intensity of disturbance. In the population of Viana do Castelo, the respiration increased with the increase of intensity (Fig. 20).

Generally, in population of Ria de Arousa, the respiration was similar at all frequencies of disturbance. In population of Viana do Castelo, the respiration was higher at high frequency of disturbance (Fig. 20).

Generally, in the population of Ria de Arousa the respiration was similar in all durations of disturbance. In the population of Viana do Castelo, the respiration was higher at high duration of disturbance (Fig. 20).

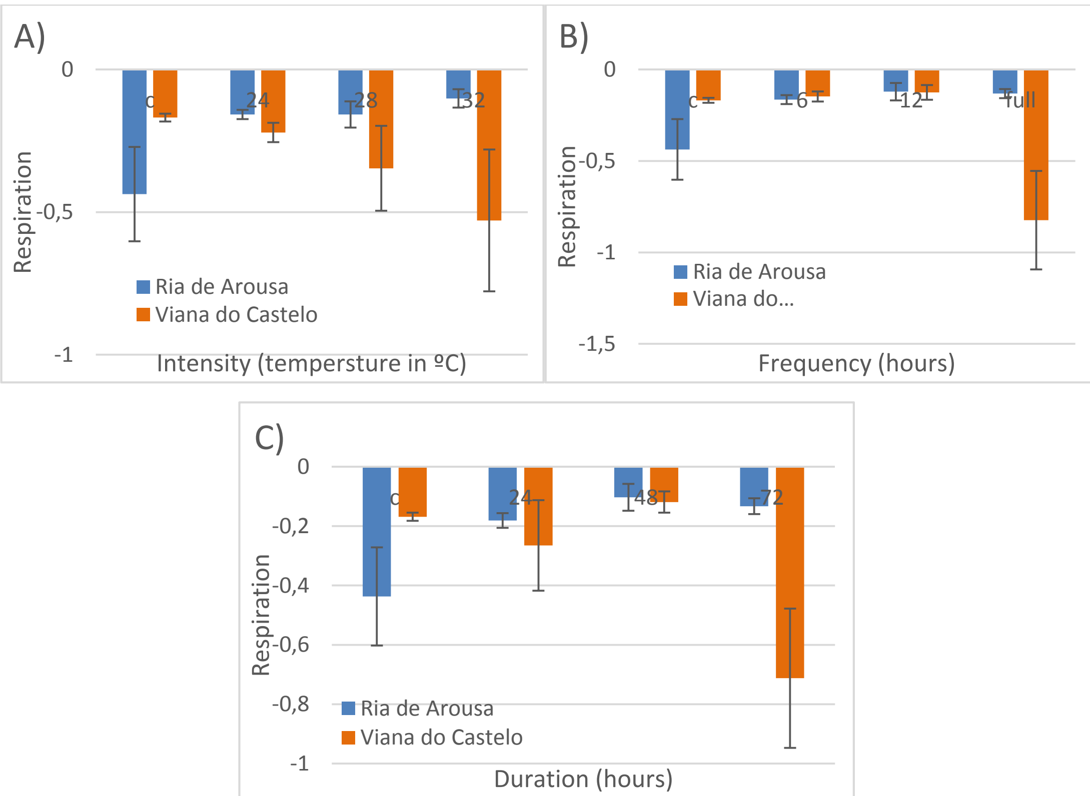

Fig.20 – Respiration of *Fucus serratus* of Ria de Arousa (blue) and Viana do Castelo (orange) at different A) intensities, B) frequencies and C) durations of disturbance.

In the population of Ria de Arousa the results of the ANOVA test showed effects of duration and frequency of disturbance in the respiration, but when a post-hoc test was done no significant differences were detected (Table 12).

In the population of Viana do Castelo there were differences only at high duration and high frequency of disturbance and, at these conditions, the respiration was significantly lower at low intensity than in the others intensities of disturbance (Table 12).

Table 12 – Results of ANOVA testing the effects of intensity, frequency and duration of disturbance in the respiration of *F. serratus*. *p < 0.05, **p < 0.01

	Ria de Arousa			Viana do Castelo		
Factors	**df**	**MS**	**F**	**df**	**MS**	**F**
intensity	2	0,028496	1,03527	2	0,64939	2,44199
frequency	2	0,013697	0,49763	2	4,26233	16,02836**
duration	2	0,041930	1,52335	2	2,58327	9,71430**
intensity*frequency	4	0,021575	0,78384	4	2,46869	9,28341**
intensity*duration	4	0,058550	2,12716	4	1,02957	3,87165**
frequency*duration	4	0,083608	3,03754*	4	2,74683	10,32936**
intensity*frequency*duration	8	0,019749	0,71749	8	0,83982	3,15811**
Error	54	0,027525		54	0,26592	
				Duration*Frequency*Intensity 24h: no differences 48h: no differences 72h: 6h: no differences 12h: no differences Full: 28º=32º>24º		

Ascophyllum nodosum

Growth

Both populations showed a decrease in the growth with the increase of the intensity frequency and duration of disturbance (Fig. 21).

In the population of *A. nodosum* of Ria de Arousa the intensity of disturbance significantly affected individuals growth, that significantly decreased at the highest intensity of disturbance tested (despite the values in low and mid intensities were lower than in the control) (Table 13).

In the population of Viana do Castelo, at high duration of disturbance it was possible to see a significantly lower growth at high frequency (full). It was also possible to see an increase of the growth with the decrease of intensity, but just visible high frequency of disturbance (full). The growth was significantly lower at high intensity of disturbance (32ºC), but just at mid and high durations and mid frequency of disturbance (Table 13).

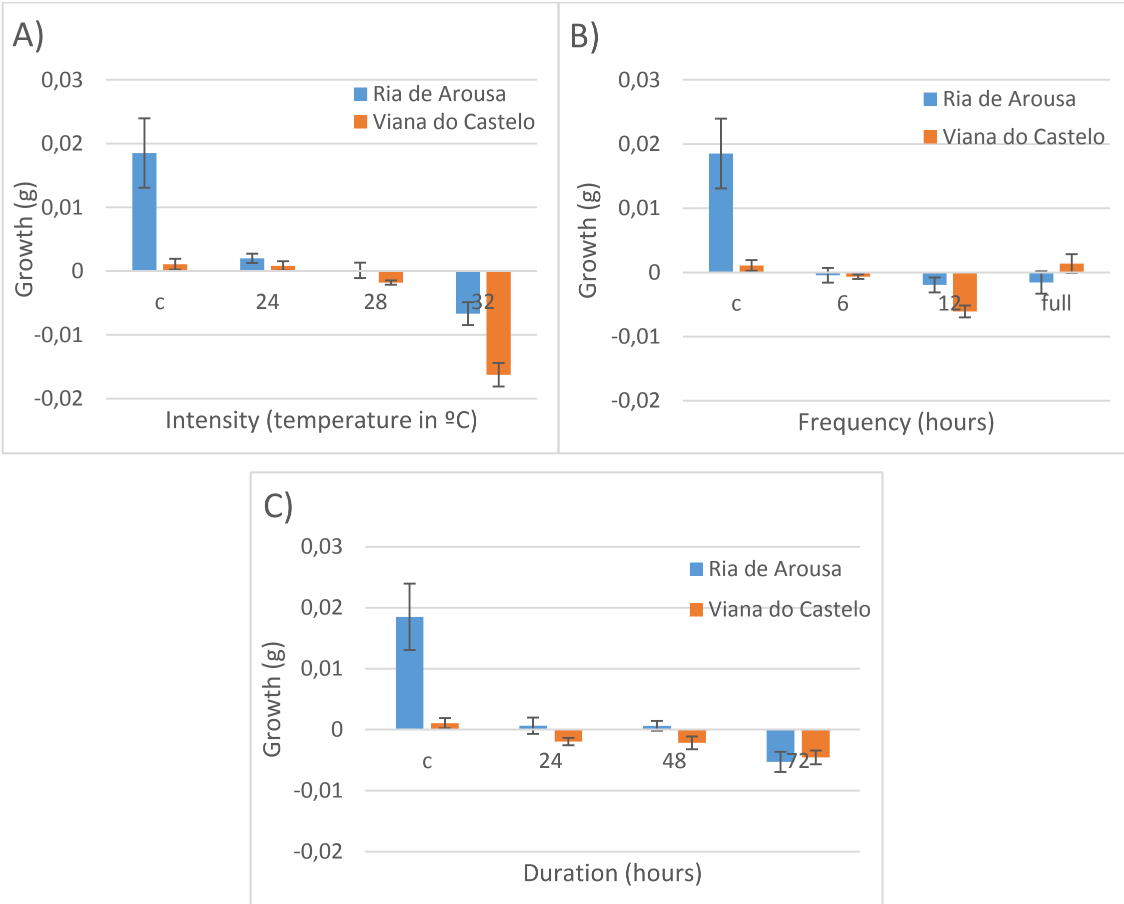

Fig.21 - Growth of *Ascophyllum nodosum* of Ria de Arousa (blue) and Viana do Castelo (orange) at different A) intensities, B) frequencies and C) durations of disturbance.

Table 13 – Results of ANOVA testing the effects of intensity, frequency and duration of disturbance in the growth of *A. nodosum*. *p < 0.05, **p < 0.01

	Ria de Arousa			**Viana do Castelo**		
Factors	**df**	**MS**	**F**	**df**	**MS**	**F**
intensity	2	0,001760	8,31327**	2	0,007974	74,1356**
frequency	2	0,000025	0,11882	2	0,002042	18,9871**
duration	2	0,000409	1,93361	2	0,001432	13,3135**
intensity*frequency	4	0,000312	1,47487	4	0,001240	11,5258**
intensity*duration	4	0,000159	0,74997	4	0,000710	6,6033**
frequency*duration	4	0,000210	0,99034	4	0,000300	2,7895*
intensity*frequency*duration	8	0,000166	0,78401	8	0,000189	1,7554
Error	135	0,000212		297	0,000108	
	Intensity 24º=28º>32º			**Duration*Frequency** 24h: no differences 48h: no differences 72h: 6h=12h>full **Duration*Intensity** 24h: no differences 48h: 24º=28º>32º 72h: 24º=28º>32º **Frequency*Intensity** 6h: no differences 12h: 24º=28º>32º Full: 24º>28º>32º		

PAM

In both populations the PAM at t0 wasn't significantly different between the replicates.

Generally, in the population of Ria de Arousa the PAM (Fv/Fm) was similar in all intensities, frequencies and durations of disturbance, while in the population of Viana do Castelo the Fv/Fm decreased with increasing levels of intensity, frequency and duration of disturbance (Fig. 22, 23 and 24).

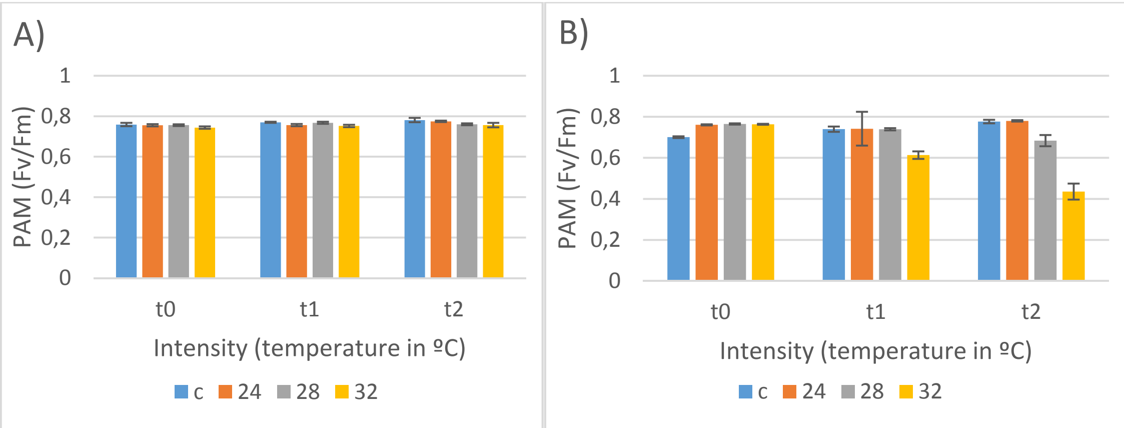

Fig.22 - PAM of *Ascophyllum nodosum* of A) Ria de Arousa and B) Viana do Castelo at different intensities of disturbance.

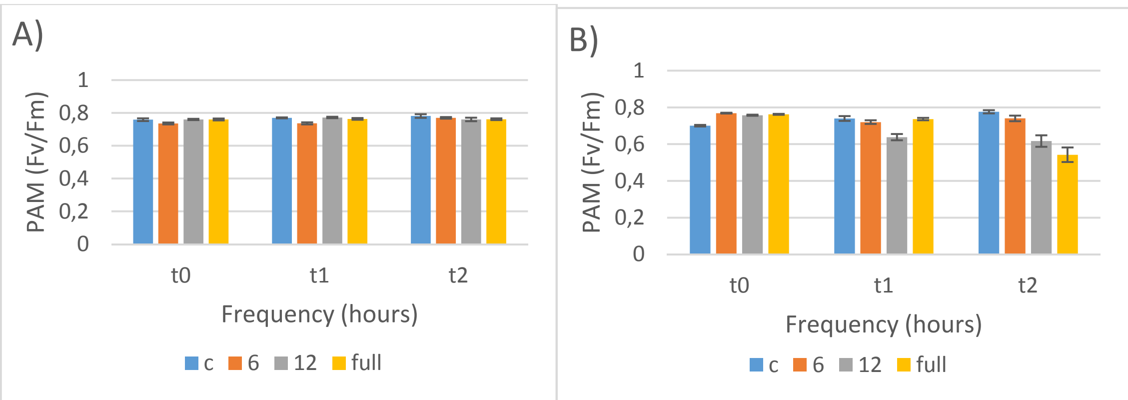

Fig.23 - PAM of *Ascophyllum nodosum* of A) Ria de Arousa and B) Viana do Castelo at different frequencies of disturbance.

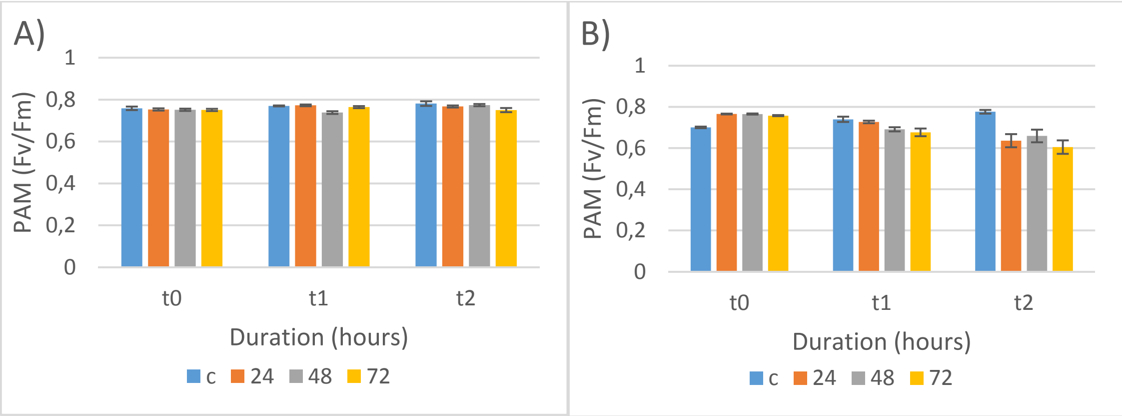

Fig.24 - PAM of *Ascophyllum nodosum* of A) Ria de Arousa and B) Viana do Castelo at different durations of disturbance.

In population of Ria de Arousa, after the treatment (t1), at mid duration and low and mid intensities of disturbance, it was possible to see a lower PAM at low frequency than in the others frequencies of disturbance. After the recovery time (t2), the only effects were at high duration and mid frequency of disturbance, in these conditions was possible to see a lower PAM at high intensity of disturbance (Table 14).

In the population of Viana do Castelo, after the treatment (t1) was possible to see a lower PAM at high intensity of disturbance, but just at mid and high durations and at mid frequency of disturbance. After the recovery time (t2) the pattern was the same only at mid and high durations and mid and high frequencies of disturbance. At low duration and mid frequency of disturbance the PAM at low intensity of disturbance was significantly higher than in the others intensities, and at high frequency it was possible to see a decrease in PAM with the increase of intensity of disturbance (Table 14).

Table 14 – Results of ANOVA testing the effects of intensity, frequency and duration of disturbance in the PAM of *A. nodosum*. *p < 0.05, **p < 0.01

	Ria de Arousa			Viana do Castelo		
Factors	**df**	**MS**	**F**	**df**	**MS**	**F**
intensity	2	0,0065	4,7**	2	1,6737	124,55**
frequency	2	0,0113	8,2**	2	0,3733	27,78**
duration	2	0,0052	3,8*	2	0,0627	4,67**
t	2	0,0059	4,3*	2	1,0213	76,00**
intensity*frequency	4	0,0148	10,8**	4	0,1610	11,99**
intensity*duration	4	0,0039	2,8*	4	0,2998	22,31**
frequency*duration	4	0,0046	3,4*	4	0,0242	1,80
intensity*t	4	0,0022	1,6	4	0,6617	49,24**
frequency*t	4	0,0079	5,8**	4	0,3322	24,72**
duration*t	4	0,0103	7,5**	4	0,0266	1,98
intensity*frequency*duration	8	0,0018	1,3	8	0,0521	3,88**
intensity*frequency*t	8	0,0029	2,1*	8	0,1412	10,50**
intensity*duration*t	8	0,0026	1,9	8	0,1914	14,25**
frequency*duration*t	8	0,0110	8,0**	8	0,0412	3,07**
intensity*frequency*duration*t	16	0,0030	2,2**	16	0,0558	4,15**
Error	405	0,0014		648	0,0134	
	t*Duration*Frequency*Intensity t0: no differences t1: 24h: no differences 48h: 24º: 12h=full>6h 28º: 12h=full>6h 32º: no diferences 72h: no differences t2: 24h: no differences 48h: no differences 72h: 6h: no differences 12h: 24º=28º>32º Full: no differences			**t*Duration*Frequency*Intensity** t0: no differences t1: 24h: no differences 48h: 6h: no differences 12h: 24º=28º>32º Full: no differences 72h: 6h: no differences 12h: 24º=28º>32º Full: no differences t2: 24h: 6h: no differences 12h: 24º>28º=32º Full: 24º>32º>28º 48h: 6h: no differences 12h: 24º=28º>32º Full: 24º=28º>32º 72h: 24º=28º>32º		

Photosynthetic efficiency, GPP and maximum production rate

Neither the intensity, nor the frequency, nor the duration of disturbance had any significant effect in the photosynthetic efficiency (Fig. 25), GPP (Fig. 26) nor maximum production rate (Fig. 27) of both populations of *A. nodosum*.

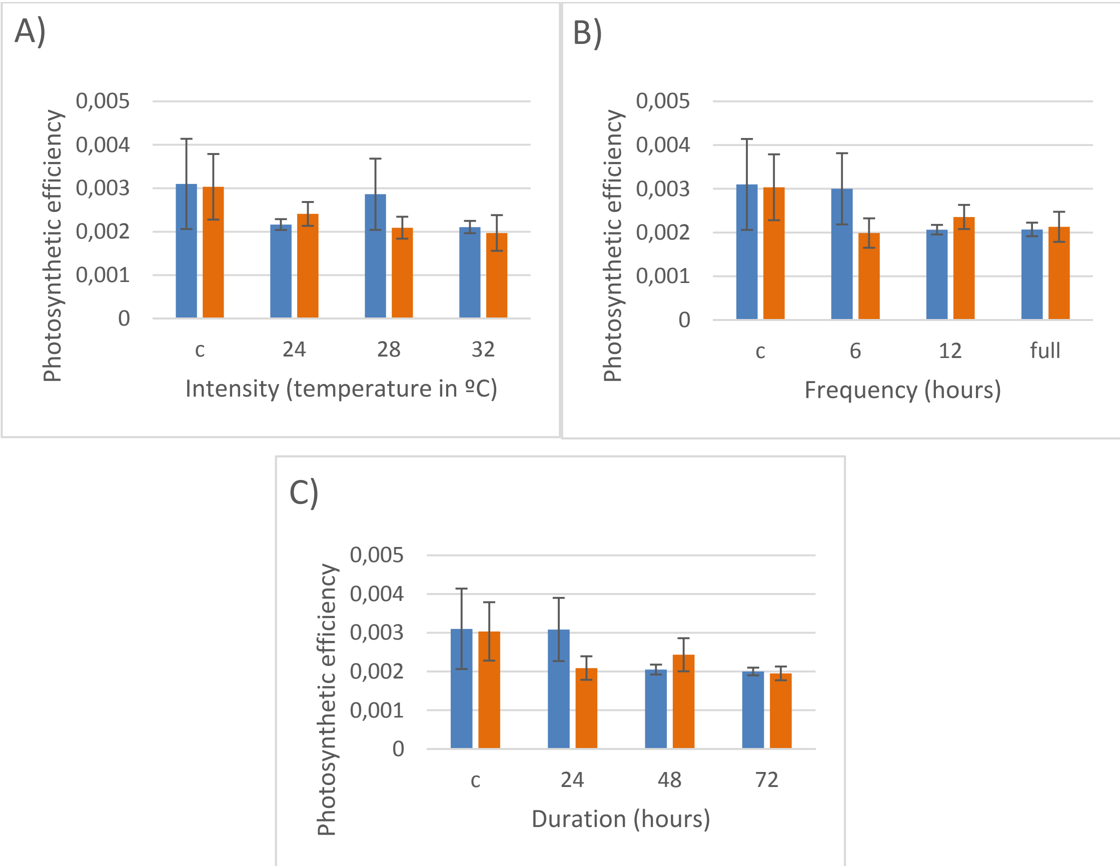

Fig.25 – Photosynthetic efficiency of *Ascophyllum nodosum* of Ria de Arousa (blue) and Viana do Castelo (orange) at different A) intensities, B) frequencies and C) durations of disturbance.

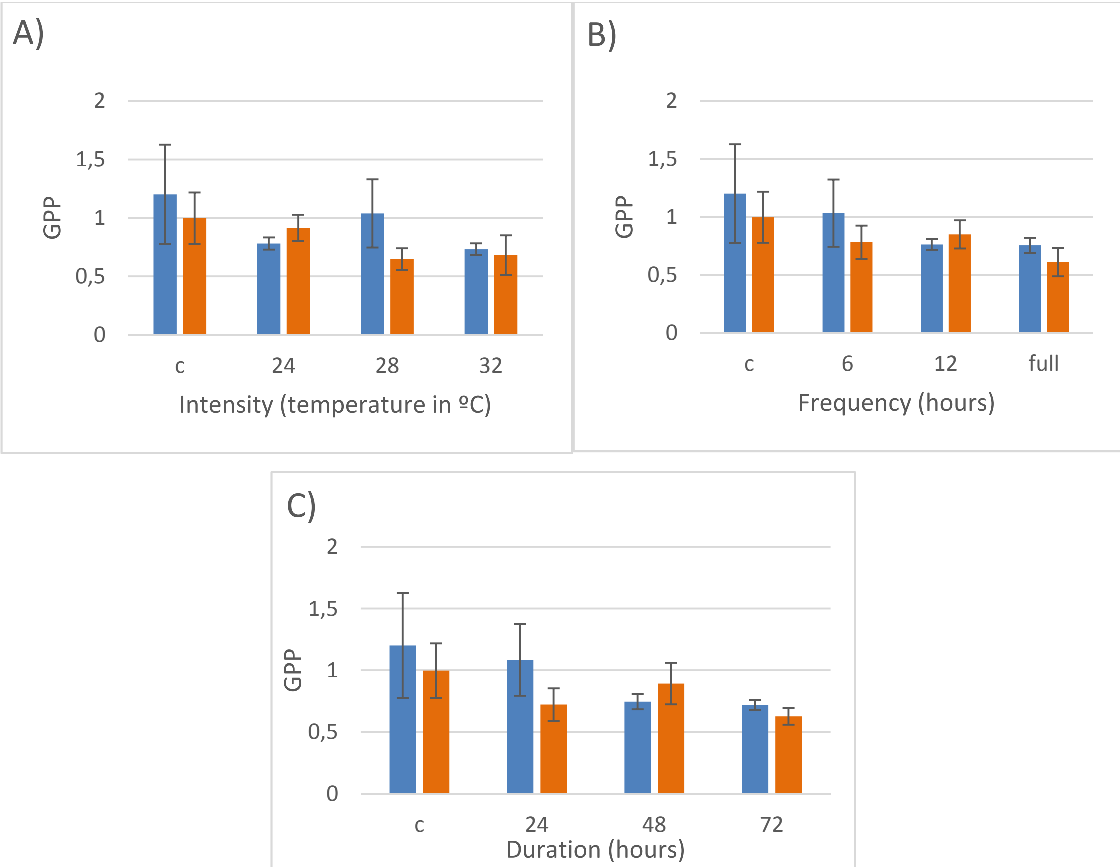

Fig.26 - GPP of *Ascophyllum nodosum* of Ria de Arousa (blue) and Viana do Castelo (orange) at different A) intensities and B) frequencies of disturbance.

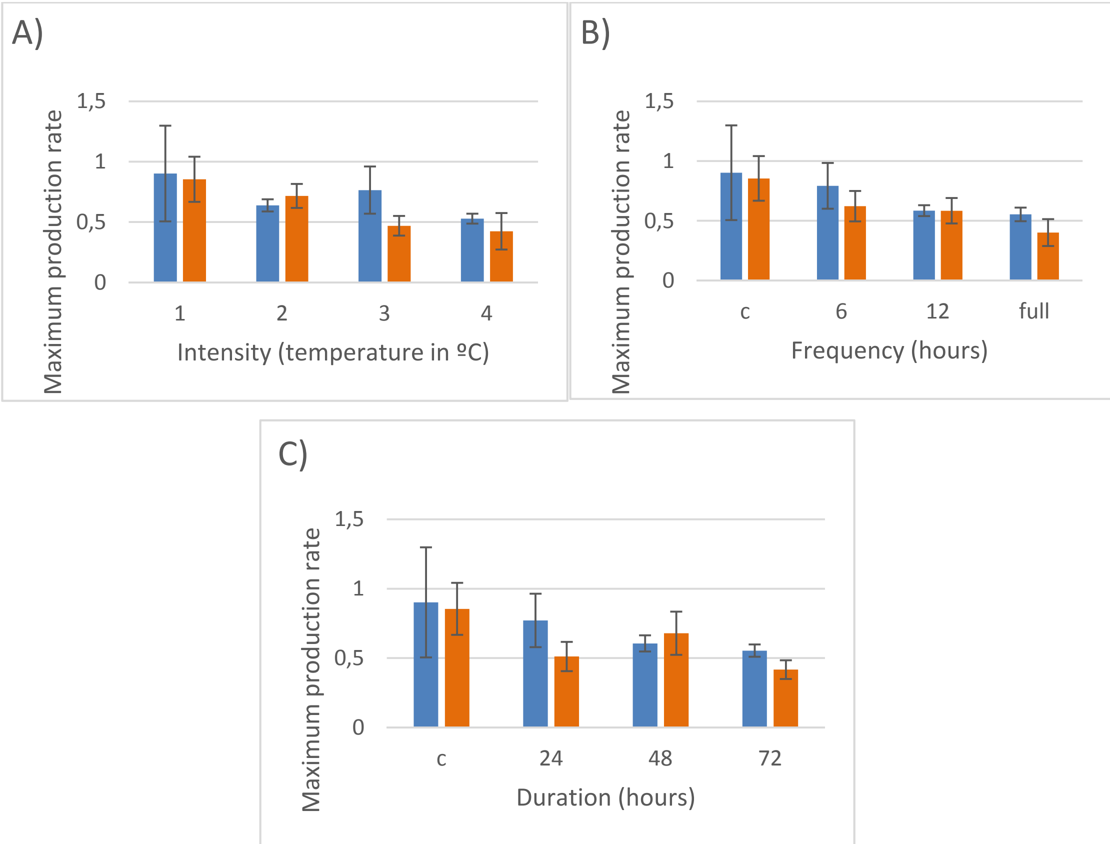

Fig.27 – Maximum production rate of *Ascophyllum nodosum* of Ria de Arousa (blue) and Viana do Castelo (orange) at different A) intensities, B) frequencies C) durations of disturbance.

Respiration

The factors of disturbance hadn't significant effects in the respiration of population of Ria de Arousa (Fig. 28). In population of Viana do Castelo the respiration was influenced by these factors and the factors were correlated, but when a post-hoc test was done no significant differences were seen (Table 15).

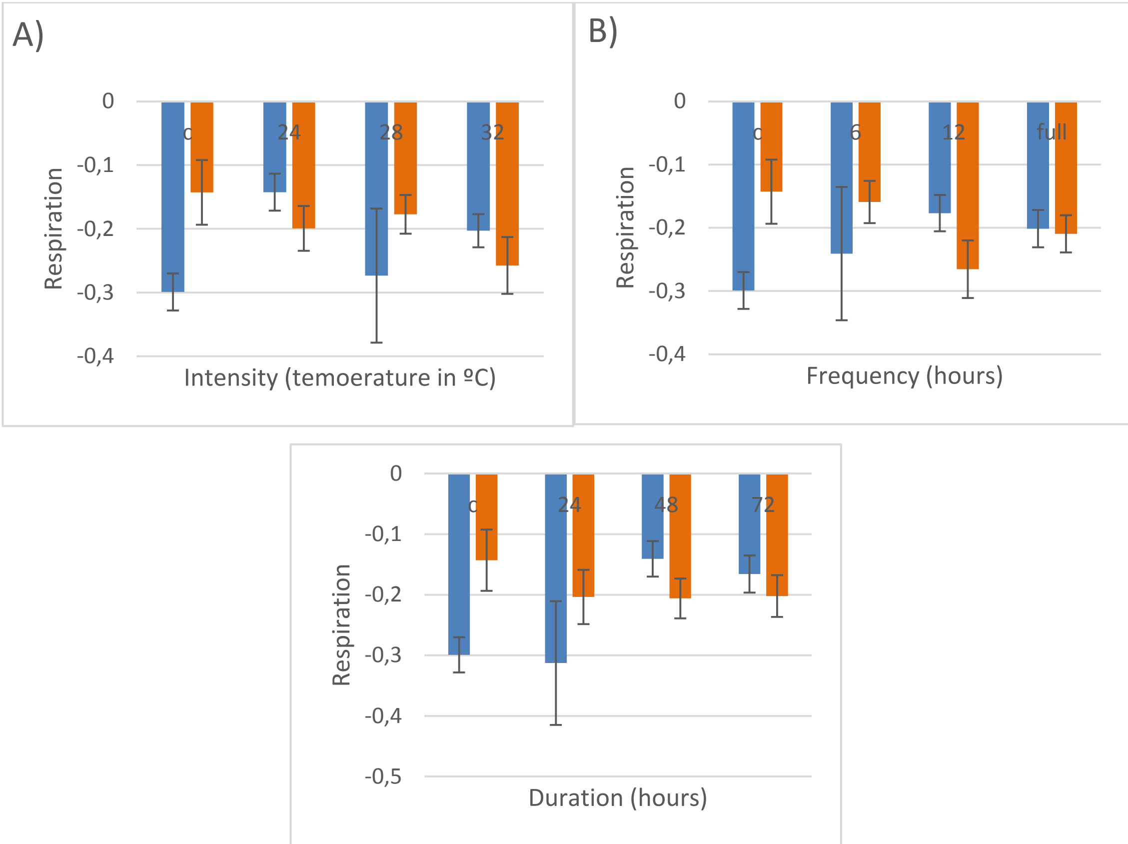

Fig.28 – Respiration of *Ascophyllum nodosum* of Ria de Arousa (blue) and Viana do Castelo (orange) at different A) intensities, B) frequencies and C) durations of disturbance.

Table 15 – Results of ANOVA testing the effects of intensity, frequency and duration of disturbance in the respiration of *A. nodosum*. *p < 0.05, **p < 0.01

	Ria de Arousa			Viana do Castelo		
Factors	**df**	**MS**	**F**	**df**	**MS**	**F**
intensity	2	0,115829	1,14122	2	0,046296	1,5900
frequency	2	0,028169	0,27754	2	0,076202	2,6171
duration	2	0,233427	2,29988	2	0,000112	0,0039
intensity*frequency	4	0,063669	0,62731	4	0,067548	2,3199
intensity*duration	4	0,105695	1,04138	4	0,050600	1,7379
frequency*duration	4	0,216890	2,13694	4	0,025965	0,8918
intensity*frequency*duration	8	0,159711	1,57358	8	0,075703	2,6000*
Error	54	0,101495		54	0,029116	

Discussion

A. nodosum and *F. serratus* showed higher tolerances to thermal disturbance than *H. elongata* (*A. nodosum* and *F. serratus* registered a positive growth at 24ºC while *H. elongata* showed a decrease in weight at this temperature). For *A. nodosum* and *F. serratus*, the highest growth rate under thermal disturbance treatments was registered at 24ºC while for *H. elongata* was 20ºC. These results are in accordance with previous findings such as a recent laboratory experiment that found higher growth tolerance of *F. serratus* to higher sea temperature (around 24ºC for adult plants of *F. serratus*) than *H. elongata* (around 18ºC for reproductive individuals of *H. elongata*) (Martinez et al., 2012).

Although with a similar growth rate tolerances as *F. serratus*, *A. nodosum* showed a higher tolerance when considering productivity and photosynthetic efficiency.

The increase in the tolerance to thermal disturbance showed by the different species corresponds to the level that the studied species occupy in the intertidal area suggesting that species occupying higher intertidal levels, with correspondent higher degrees of variation of environmental parameters, might have developed higher plasticity to deal with disturbance events (Helmuth et al., 2006). In this study a better resistance and resilience to disturbance was found for *A. nodosum* which occupies a higher level in the intertidal and a lower resistance and resilience was found for the specie from lower levels in the intertidal (*H. elongata*). These results are in accordance with other studies showing different tolerance degree, according to the species position in the intertidal (Ferreira et al., 2014) and that the tolerance to climatic variability and the capacity to tolerate desiccation is greater in *F. spiralis* than in fucoid species living on lower areas of the shore (Dring and Brown, 1982; Schonbeck and Norton, 1978).

The susceptibility of the studied species to undergo geographical shifts in their distribution range is also in accordance with the results obtained in this study. The specie that registered a biggest shift in its geographical distribution over the last decades was *H. elongata*, showing a distributional retraction northwards of 219 km when comparing its geographical position in 2007 with historical data from the 1970s (Lima et al., 2007). Moreover, during the sampling done for this study in 2016, it was possible to register a further retraction with the actual distributional limit of the species in Mindelo where the *H. elongata* population has a small number of individuals of very small size comparing with populations further north like the ones of Vigo and Ria de Arousa. *F. serratus* also changed its distribution range, with a shift of 9 km from Forte da Vigia (historical limit) to Amorosa and *A. nodosum* didn't show changes in the distribution (Lima et al., 2007). However, others studies showed that there are some evidences of maladaptation of *F. serratus*: a reduced fitness and lower adaptive capacity of the southern edge populations relative to central populations and a narrower vertical range at border locations. For the specie *A. nodosum* there are no evidences of maladaptation (Araújo et al., 2011; Araújo et al., 2014; Pearson et al., 2009).

The higher range shift observed for *Himanthalia elongata* than *F. serratus* may reflect a higher resistance of the latter to higher temperatures (Duarte et al., 2013). *F. serratus* is found higher on the shore than *Himanthalia elongata* (Anadón, 1983) and thus it is probably more influenced by climatic factors related to emersion times.

The populations further south (Viana do Castelo) of *F. serratus* and *A. nodosum* showed a higher susceptibility to thermal disturbance. The populations of *H. elongata* studied showed a similar tolerance to disturbance, however, the population further north (Ria de Arousa) showed a better tolerance to mid intensities of disturbance (24ºC), while the population of Vigo showed a better tolerance to low intensities of disturbance (20ºC). These populations of *H. elongata* are not the ones located further south, because we didn't want to disturb the southernmost available population due to the low number of individuals available and its expected susceptibility, thus the populations studied of *H. elongata* probably are not as stressed as the southernmost ones of *F. serratus* and *A. nodosum*. The differences between the populations of *F. serratus* and *A. nodosum* might be related with its southernmost situation which increases their exposure to disturbance in consequence of being the populations of the last point of distribution and, consequently, being in their limit of physiological tolerance. This is supported by other studies that show a more variable and lower growth rates in populations near the marginal areas of distribution (Angert, 2009; Viana et al., 2014).

In Europe, the general trend is the decrease in abundance of some native cold-water species at their southern distributional range limits and the increase in abundance in other parts of their distribution. Multiple possible drivers of these shifts were identified, including global warming, sea urchin grazing, harvesting, pollution and fishing pressure, and their impact varied between geographical areas. In the Iberian Peninsula, the major factor that affects the distributional range of species is the global warming (Araújo et al., 2016; Diez et al., 2012; Fernandez, 2011). The present study is in accordance with this as the specie that showed more intolerance to thermal disturbance is the specie that had a higher regression on the distributional limit (Lima et al., 2007).

In general, all the species showed a decrease in tolerance to disturbance with the increase of intensity, frequency and duration of disturbance and all of these factors tested showed interaction between them. It's important to study these factors simultaneously because one factor may not cause by itself any effect. For example, in a study done in the north of Spain the shifts in the distribution cold-water species of macroalgae may be caused not only by the increase in temperature (intensity of disturbance) but also by the increase in the duration of disturbance, as the number of summer days with temperatures above 22.1ºC has increased from less than 10 to more than 35 from 1980 to 2008 (Diez et al., 2012; Fernandez, 2011).

The frequency of disturbance is also an important factor to be tested as the frequency of events of disturbance is increasing, like in the case of the storms, a factor of disturbance that affects the distribution of species particularly in the Iberian Peninsula (Araújo et al., 2016).

Conclusions

All species presented a better physiology at low intensity of disturbance, with and evident relation between the better temperature for each specie with the level that the specie appears in the intertidal. Thus, species from higher levels of intertidal are, normally, more tolerant to disturbance, because these species are accustomed to stress during low tides, which can take them close to their physiological tolerance limit.

The level that the species occupy in the intertidal is also related to the changes in the species distribution, as species from low intertidal levels are more likely to change the distribution because are more sensitive to disturbances.

Despite the differences between populations haven't been tested in this work, it's possible to see some differences. Thus, in future studies it's important to include this factor in the statistical analysis.

This study is important to predict future changes of distribution and physiology of the species in response to disturbance

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