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Relationship between strains of *Coolia monotis* (Dinophyceae) from the Atlantic Iberian Peninsula and their sampling sites

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ABSTRACT

Strains of *Coolia monotis* from three locations (north, center and south) of the Atlantic Iberian Peninsula were studied to undercover possible geographic preferences in their photophysiology. Growth rates and photosynthetic parameters such as F_v/F_m, α, E_k and $rETR_{max}$ were analyzed with a Coulter counter, a Water-PAM and a FRRF. The photosynthetic properties were investigated in experiments using strains acclimated to Low Light (LL) and High Light (HL) during the course of a day. The same strains were also used for a HL stress experiment. The highest growth rate (0.29 d^{-1}) corresponded to Galé strain, from the south of the Peninsula, under 200 μmol photons m^{-2} s^{-1}, suggesting that it was adapted to higher irradiances than the other 2 strains. The rapid light curves taken during the course of a day showed no differences between the strains, but the ones acclimated to LL had higher $rETR_{max}$ and α, and lower E_k values than the ones acclimated to HL. The HL stress exposure induced photodamage to all strains and the recovery period was not sufficiently long for full recovery of F_v/F_m. Results demonstrated that strains responded differently to treatments, even though were genetically identical at the 28S/ITS-levels. These differences could be related to their original location.

1. Introduction

Epibenthic dinoflagellates of the potentially toxic genus *Coolia* have been widely studied throughout the world over the past 20 years (David et al., 2014; Fraga et al., 2008; Jeong et al., 2012; Leaw et al., 2016; Momigliano et al., 2013; Rhodes et al., 2000; Ten-Hage et al., 2000). Species in the genus can be found in assemblages with other potentially toxic epibenthic dinoflagellates of the genera *Prorocentrum*, *Ostreopsis* and *Gambierdiscus* (Aligizaki and Nikolaidis, 2006; Faust, 1995; Pagliara and Caroppo, 2012; Penna et al., 2005; Ten-Hage et al., 2000), which under certain environmental conditions can proliferate, causing a negative impact on the environment, industry and tourism (Armi et al., 2010; Ciminiello et al., 2006; Shears and Ross, 2009; Totti et al., 2010). Changes in temperatures, irradiation, salinity, and nutrients, among other environmental factors, are known to influence cell proliferations (Fraga et al., 2012; Heredia-Tapia et al., 2002; Zhang and Hu, 2011) as well as the amount of toxins, which can vary even in strains of the same species (Guerrini et al., 2009). All of these make it of paramount importance to know the response of strains to environmental factors triggering microalgae blooms.

Originally described by Meunier (1919) from Nieuport (Belgium), *Coolia monotis*, the type species of the genus is widely distributed in the Atlantic Iberian Peninsula coast (David et al., 2014; Laza-Martínez et al., 2011). After a detailed study verified that no morphological or phylogenetic differences existed throughout the study area (David et al., 2014), it was decided to investigate the response of this microalgae to varying light irradiances. The south of the Iberian Peninsula presents lower mean cloud coverage in relation to the center and north, according to a study performed from 1961 to 2004, whilst the north is characterized by higher annual mean cloud coverage (Sanchez-Lorenzo et al., 2009). Another temporal study from 1985 to 2009 revealed that the mean average sea surface temperature in the south of the Peninsula was 20.5–21 °C, in the center 17.5–18 °C, and in the north between 19 and 20 °C (David et al., 2012). The fact that waters are colder in the center of the Peninsula can be explained due to upwelling phenomena (Santos et al., 2011).

Most of the recent work on *Coolia* has been focused on the species taxonomy (David et al., 2014; Jeong et al., 2012; Karafas et al., 2015; Mohammad-Noor et al., 2013) or chemical and toxin analyses (Carnicer et al., 2015; Holmes et al., 1995; Rhodes and Thomas, 1997; Wakeman et al., 2015). Physiological analyses have been poorly investigated within benthic dinoflagellates with only a handful related to Ostreopsidaceae (Ben-Gharbia et al., 2016; Fraga et al., 2012; Parsons et al., 2012; Rhodes et al., 2000; Vidyarathna and Granéli, 2013). Epibenthic dinoflagellates are common in clear waters where high irradiances can be expected. As a mechanism of protection, it has been

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suggested that some dinoflagellates can migrate to shaded areas of macroalgae (Ballantine et al., 1988). Nevertheless, cell photophysiology is strongly dependent on the time of the day (Fraga et al., 2012; VanDolah and Leighfield, 1999) and microalgae possess an adaptive response system to highly variable light conditions (Ihnken et al., 2010). Even so, excessive irradiances can trigger different levels of photoinhibition (Franklin and Forster, 1997) that can be linked to changes in chlorophyll fluorescence parameters (Franklin et al., 1992; Genty et al., 1989; Jones and Hoegh-Guldberg, 2001). Prolonged exposure to stresses such as the excess of irradiances can lead to a P_{max} decline, as damage to the reaction centres occurs. Dynamic and chronic photoinhibition are mainly based on the typical kinetics of relaxation (recovery) of the biochemical and biophysical processes implicated in photoinhibition (Franklin and Forster, 1997). Photodamage is thought to be coupled with the PSII reaction centre damage, as revealed by the rapid turnover of the D1 protein (Aro et al., 1993). These can be assessed as changes in the chlorophyll fluorescence parameters, measured by a pulse amplitude modulation (PAM) apparatus (Schreiber et al., 1994).

The present study aims to investigate the interspecific variability in the ecophysiology of different isolates of *Coolia monotis* collected along the Atlantic Iberian Peninsula coast in order to know whether the geographical and climatic differences of the study area are reflected in the photosynthetic performance of different strains to varying light intensities.

2. Methods

2.1. Strain locations

Several locations in the Atlantic coast of the Iberian Peninsula were previously sampled and unialgal cultures were established (David et al., 2014). After morphological and molecular analysis demonstrated no differences at the LSU/ITS-level of the different isolates of *Coolia monotis*, it was decided to study the photophysiological requirements of cells from 3 different locations with different climatology. The three algal strains used for this study were: Dn73EHU from Galé (37.078863, −8.314001) to represent the south of the Iberian Peninsula; Dn178EHU from Vigo (42.223871, −8,767,948) to represent the western central Iberian Peninsula; and Dn89EHU from San Sebastian (43.321397, −1,986,706) to represent the north of the Peninsula (Fig.1). All cells were collected from macroalgae at about 1 m depth during low tides. During sampling, the San Sebastian location presented a sea surface temperature (SST) of 22.8 °C and relatively calm waters but the amount of organic matter present and cloud coverage made the water visibility limited. Vigo had 18.0 °C of SST, and less cloud coverage than in San Sebastian, giving a better visibility underwater. The south location in Galé, presented no cloud coverage and a SST of 20.5 °C. This site had calm and crystalline waters where irradiance penetrated further into the water column. Strains from lower latitudes are expected to be more adapted to high-light intensities.

2.2. Growth rate experiments

Cultures were maintained in NuclonTM culture flasks containing 20 mL of f/2 Guillard's marine water enrichment (Sigma) with a salinity of 35. This was the salinity of the filtered sea water, collected from coastal sampling sites. The three *Coolia monotis* strains were previously acclimated to a cold fluorescence light of about 90 μmol photons m^{-2} s^{-1} and a 12:12 light/dark hour cycle at 18 °C in a culture chamber. This species has a widespread distribution and can be found in a range of latitudes varying from cold (north of Scotland, U. K.; Dodge, 1981) to warm-temperate locations (south of the Iberian Peninsula and Mediterranean Sea; Aligizaki and Nikolaidis, 2006; David et al., 2014; Penna et al., 2005). Mean annual temperatures in the Iberian Peninsula vary

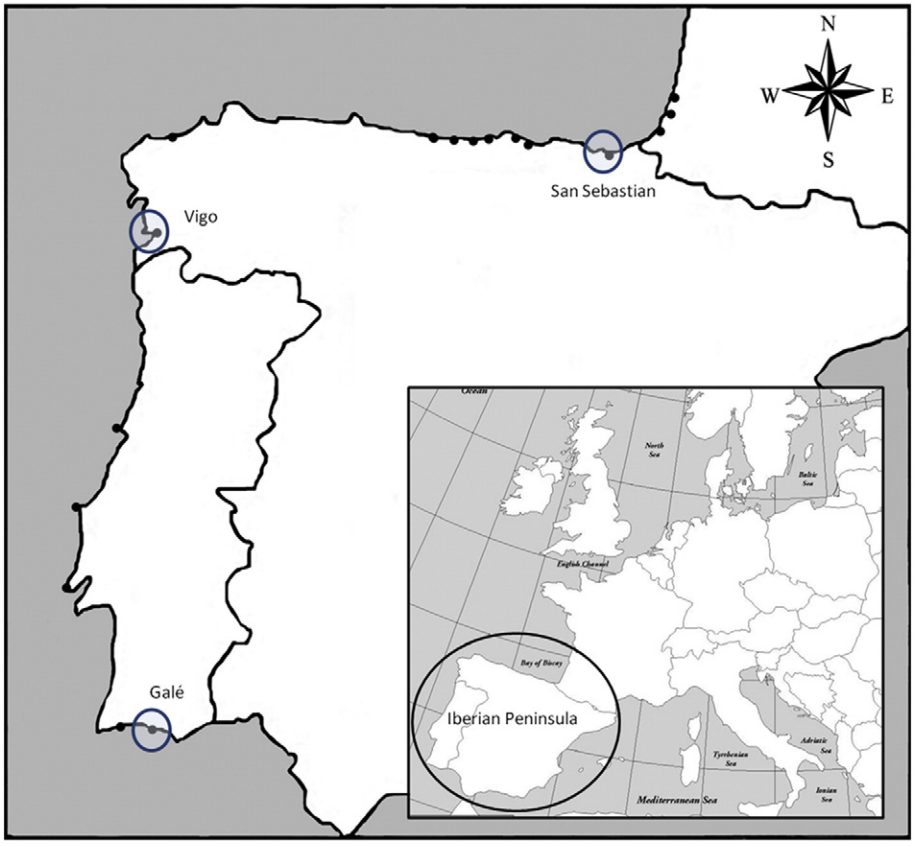

Fig. 1. Sampling sites: Dn73EHU from Galé to represent the south of the Iberian Peninsula; Dn178EHU from Vigo to represent the western central Iberian Peninsula; and Dn89EHU from San Sebastian to represent the north of the Peninsula.

from 16 to 19 degrees (David et al., 2012) and the temperature of 18 °C was chosen as it was the highest found in the location of Vigo.

To test for growth rates an aliquot was used to start fresh new cultures. Number of cells was measured every other day, in triplicate with a Coulter counter (Beckman coulter, Inc., Netherlands) during 20 days to compare with the fluorescence values given from the pulse amplitude modulated (PAM) fluorometer (Water-Pam, Walz, Germany). From the exponential phase, growth rates were obtained by fitting the data in SigmaPlot 13.0 (Systat Software, Inc. GmbH, Germany) according to the 2-variable exponential growth rate formula $N_t = N_2 \exp(\mu \times t)$ where N_2 is the cell concentration at day 2. During the same time frame, an aliquot of 1 mL from the freshly renewed cultures was placed in duplicate into a 24-multiwell culture plate that was left under cold fluorescent light, at a constant temperature of 18 °C and seven different light intensities (30, 60, 80, 100, 150, 200 and 300 μmol photons m^{-2} s^{-1}). Increases in biomass were measured every day in triplicate by a saturating light pulse from the water-PAM, provided with an optical sensor (water-EDF). Culture plates were left in the dark for 10 min, shaken to uniformly resuspend the cells, and the optical sensor was placed in the middle of each well for the measurements. Increases in the minimal fluorescence (F_o) were followed for 11 days. The residual fluorescence due to the empty well and culture medium without algae was subtracted before calculation of fluorescent yields. Growth rates, μ (day^{-1}), were calculated as $\mu = \ln(N_t/N_0)/t$, where N_t and N_0 are the cell number at time t and 0, respectively, and t = x days (Crow and Kimura, 1970). Generation times (G) were calculated with the formula $G = \ln 2/\mu$.

2.3. RLCs during the course of a day

Cell photophysiology is strongly dependent on the time of day. To verify if fluctuations existed in cultures and investigate how cells differed in their photosynthetic parameters during the course of a daytime, several rapid light curves (RLCs) were recorded using a Fast Repetition rate fluorometer (FRRF). The three strains were measured at 9:30 am, 11:30 am, 1:30 pm, 3:30 pm and 5:30 pm. Each RLC consisted of 10 irradiance steps between 0 and 1504 μmol photons m^{-2} s^{-1}, and each light step lasted 30 s.

2.4. High light stress experiment

For the High Light (HL) stress experiment, strains were previously acclimated in a 12:12 light/dark hour cycle for 3 months and changed regularly to maintain cultures in exponential phase. During that time, cultures were divided and exposed to different irradiances of low light - LL (40 μmol photons m^{-2} s^{-1}) and high light - HL (150 μmol photons m^{-2} s^{-1}) at a constant temperature of 18 °C before they were subjected to the HL stress experiment. All strains were measured at approximately the same time. Samples of cultures were first incubated for 10 min in the dark and then submitted to a photon flux density of 500 μmol photons m^{-2} s^{-1} for 20 min. Following this, their recovery was recorded in the dark for the next 30 min. The high light intensity chosen was about 3 times their E_k, which is the irradiance value where photosynthesis switches from light-limited to light-saturated rates. During the entire experiment, RLCs were measured with an FRRF (Fast^{Tracka}-II/FastAct) (Fast Repetition Rate Fluorometer, Chelsea Technologies Group Ltd) at crucial changing points in the experiment (point 0 in acclimated conditions, point 1 after dark acclimation, point 2 after light stress, point 3 in the middle of recovery, point 4 at the end of the experiment). Furthermore, several acquisitions points were measured in between the RLCs to measure the kinetics in inhibition and recovery in the PSII quantum efficiency. For a better comprehension, the data series was separated into a photoinhibition phase and a recovery phase, where the first was from minute 10 to 30 and the latter from minute 30 until the end. These data were fitted using the following equations (Hanelt, 1998):

$$\text{inhibition kinetics}: \frac{\Delta F}{F_m'} = P_1 \cdot e^{-k_1 \cdot t} + P_2 \cdot e^{-k_2 \cdot t}$$

$$\text{recovery}: \frac{\Delta F}{F_m'} = F_v/F_m - \left(P_1 \cdot e^{-k_1 \cdot t} + P_2 \cdot e^{-k_2 \cdot t}\right)$$

These equations, according to Hanelt (1998), assume that the kinetics of photoinhibition can be explained by combining two different processes for both the inhibition and recovery: one with a relative proportion (P_1) of PSII centres with a fast rate constant (k_1) and another with a relative proportion (P_2) with a slow rate constant (k_2) at a given time (t). F_v/F_m is the maximum PSII efficiency of the non-inhibited control at $t = 0$ and $P_1 + P_2 = F_v/F_m$. Recovery becomes faster when k_1 is larger or when k_2 becomes smaller.

2.5. Statistical data

All data were analyzed with the statistical program SigmaPlot 13. To find differences between strains and time on growth rate, the two-way analysis of variance (ANOVA) followed by the all pairwise multiple comparison Tukey's *t*-test was used to compare differences within groups. It was also used to compare strains and different light intensities on growth rates. To test if different light intensities presented relevant changes, a *t*-test was performed between groups. Whenever equal variance test failed, a Mann-Whitney Rank Sum test was performed.

3. Results

3.1. Growth rate experiments

Strains of *C. monotis* from different geographic areas (Galé from the south, Vigo from the western central and San Sebastian from the north) of the Atlantic Iberian Peninsula were used for photophysiological analyses. Cell concentration, measured with the Coulter counter under 90 μmol photons m^{-2} s^{-1}, increased in all cultures during the measuring time of 20 days (Fig. 2), except the one from Galé, which reached the stationary phase before the end of the experiment. For this strain, the last 3 data-points were omitted from the fit. A maximum cell density of 77,350 cells·mL^{-1} was found at day 14 with the strain from Galé. Growth rates, calculated over their exponential phase, demonstrated that strains from different localities had different growth rates, where

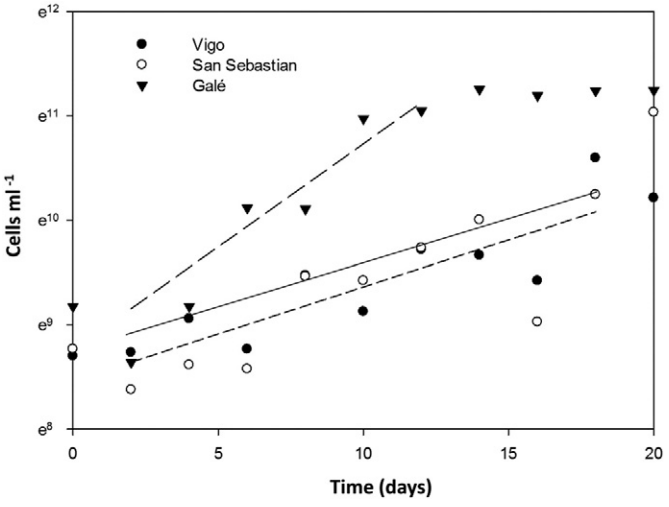

Fig. 2. Growth curves of different strains of *C. monotis* in stock culture flasks using a Coulter counter during 18 days under control conditions. (12:12 light:dark cycle, PAR = 90 μmol photons m^{-2} s^{-1}). Growth rates: Vigo 0.083 ± 0.0088 d^{-1}; Galé 0.208 ± 0.0076 d^{-1}; San Sebastian 0.096 ± 0.0082 d^{-1}.

the strains from San Sebastian and Vigo showed similar values (0.096 ± 0.0082 and 0.083 ± 0.0088 d^{-1} respectively), which were significantly lower than the one from Galé (0.208 ± 0.0076 d^{-1}; $P < 0.001$).

Data from the F_0 values were also used to calculate different growth rates under different light irradiances (30–300 μmol photons m^{-2} s^{-1}). Growth rates were usually very low at the lowest PAR (~0.08 day^{-1}; Table 1) and increased under moderate light intensities, with the strain from Galé growing faster than the ones from Vigo and San Sebastian (Fig. 3) at higher irradiances. Under a light intensity of 90 μmol photons m^{-2} s^{-1} measured by the Coulter counter, strains had growth rates in between the ones of 80 and 100 μmol photons m^{-2} s^{-1} measured with the water-PAM, with exception of the one from San Sebastian, where the Coulter counter data revealed lower growth rates than the ones expected. The strain from San Sebastian had its highest growth rate (0.18 d^{-1}) at 80 μmol photons m^{-2} s^{-1}. The one from Vigo increased in growth rates as irradiances got higher and had its maximum (0.24 d^{-1}) at 300 μmol photons m^{-2} s^{-1}. The strain from Galé showed the highest growth rate (0.29 d^{-1}) of this study at 200 μmol photons m^{-2} s^{-1}. The high standard deviations seen at the higher light intensities however, prevented the finding of significant differences between the irradiances.

3.2. RLCs during the course of a day

The RLCs parameters taken throughout the day were similar for the strains acclimated at the same conditions and only those corresponding to Vigo are represented here (Fig. 4). The fit parameters describing the RLCs are shown in Table 2. The differences of the photosynthetic parameters during the light period are small. Strains acclimated to low light (LL) showed higher $rETR_{max}$ values. Also, the alpha values of the LL acclimated strains of Vigo were higher than the ones acclimated to high light (HL). Due to this, the E_k values of the LL acclimated strains are lower than the HL acclimated cells. All differences in photosynthetic parameters between the LL and HL strains are highly significant (ANOVA, $p < 0.001$) whilst no significance was found between strains.

3.3. High light stress experiment

For the high light (HL) stress experiment, aliquots of strains acclimated to HL and LL were first left in the dark for 10 min, and then submitted to a high light stress with an irradiance of 500 μmol photons m^{-2} s^{-1} for 20 min. After this HL stress, the algae were placed again in the dark for the next 30 min to study their recovery. Strains in LL showed higher values of the maximum quantum efficiency (F_v/F_m) than cells in HL ($P < 0.001$; not shown). Because of the different starting of F_v/F_m values, the change in PSII quantum efficiency was normalized to $t = 0$ (Fig. 5). All three strains showed a very rapid decline in $\Delta F/F_m'$ upon exposure to HL where $\Delta F/F_m'$ decreased to values between 40 and 60% after 3 min exposure in the HL cultures, depending on the strain. In general the decrease in $\Delta F/F_m'$ was smaller in the LL strains. Between 13 and 20 min there was a further gradual linear decrease in $\Delta F/F_m'$, and the rate (slope) of the decrease was similar between the LL and HL cells. Interestingly, the Vigo strain showed a different behavior compared to the strains isolated from San Sebastian or Galé. Whereas the decrease in $\Delta F/F_m'$ in the latter two strains was significantly higher in the HL strains, the pattern for the HL and LL strain of Vigo did not differ. At the end of the high light stress $\Delta F/F_m'$ decreased to ~50% of the initial values.

Table 1
Growth rates under different light intensities of the different strains. In bold are the highest growth rates found for each strain.

Light intensity	Vigo	Galé	San Sebastian
300,00	**0.24 ± 0.12**	0.22 ± 0.05	0.17 ± 0.22
200,00	0.21 ± 0.06	**0.29 ± 0.02**	0.16 ± 0.02
150,00	0.17 ± 0.08	0.23 ± 0.03	0.16 ± 0.10
100,00	0.17 ± 0.05	0.25 ± 0.05	0.15 ± 0.09
80,00	0.09 ± 0.03	0.17 ± 0.03	**0.18 ± 0.09**
60,00	0.09 ± 0.03	0.08 ± 0.05	0.04 ± 0.05
30,00	0.09 ± 0.01	0.02 ± 0.01	0.14 ± 0.08

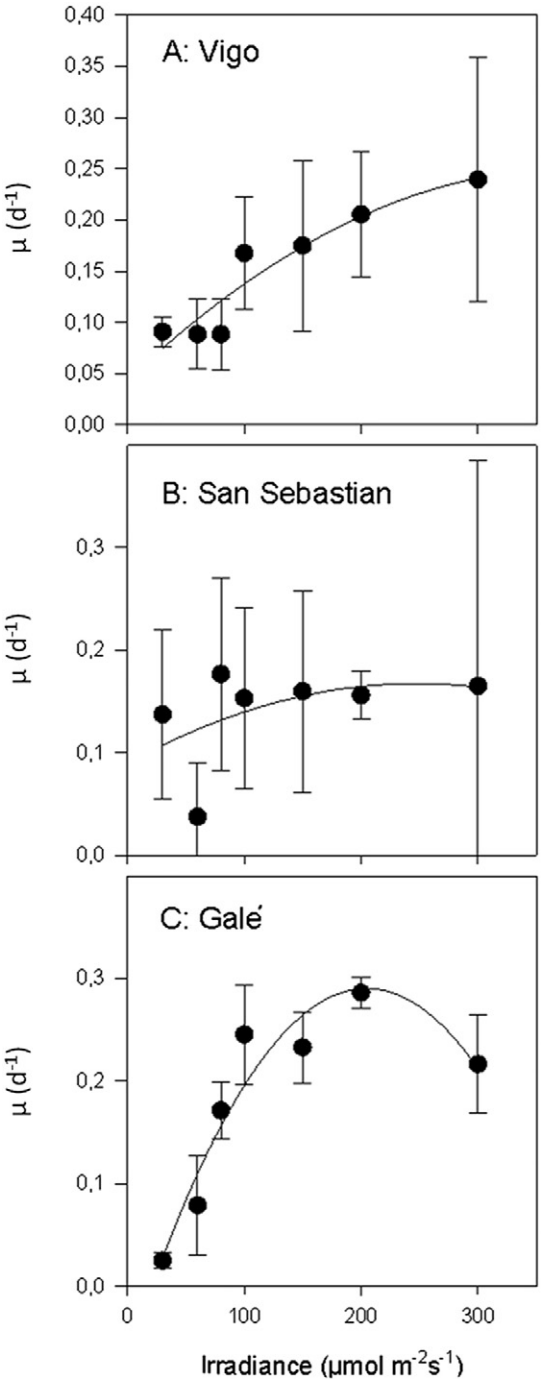

Fig. 3. Growth rates under different light intensities. A: Strain from Vigo, B: Strain from San Sebastian, C: Strain from Galé. A 2nd order polynomial was fitted through the data to make the pattern in the growth rate response more clear.

When cells were left to recover from the high light stress it became apparent that recovery was only partial. After 30 min recovery, both LL and HL strains of Vigo recovered to approximately 70% of the initial values. The LL strains of San Sebastian and Galé showed a slightly lower recovery reaching 63 and 67% of the initial values respectively. The acclimation patterns of the HL strains were quite different. All strains showed a biphasic recovery, which was least noticeable in Galé. After the initial rapid phase, the slow phase was most prominent

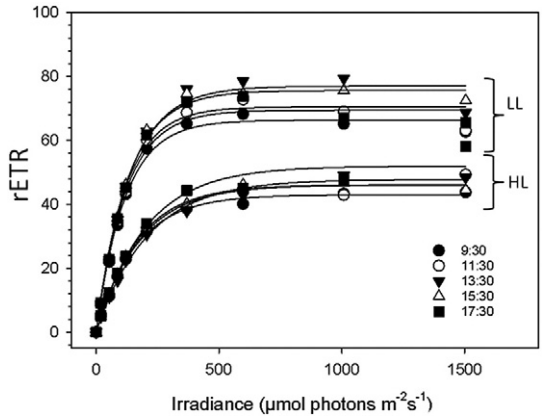

Fig. 4. Relationship between the photosynthetic parameter rETR of the RLCs taken every 2 h against irradiance, during the course of a day of Vigo strains acclimated to HL and LL.

where the recovery showed a linear pattern. The 2nd phase was very slow in San Sebastian and Galé strains, but these showed a faster recovery when compared to the other strains ($P < 0.05$; Fig. 5). The HL strains of San Sebastian and Galé showed a much lower recovery reaching only 42 and 53% of the initial values, respectively. Regarding localities, both strains from Galé, reached the lowest quantum efficiency values at the inhibition end and showed a faster recovery when comparing with the other localities ($P < 0.05$).

The RLCs taken at critical points of the experiment, suggested that cells start to deactivate themselves after 10 min of acclimation in the dark (RLC1 in Fig. 6). Especially the HL of Galé showed a strong (50%) reduction in $rETR_{max}$ after 10 min of darkness. After 20 min of exposure to HL (RLC2), $rETR_{max}$ decreased to approximately 50% of the initial values, and the relative differences between the LL and HL acclimated cells were not significant. In RLC3 and RLC4, after 15 min ($t = 45$ min) and 30 ($t = 60$ min) of recovery in the dark respectively, $rETR_{max}$ showed no signs of recovery: values were even lower than at the end of the HL period although the F_v/F_m was increasing, a possible sign of photoinhibition.

The original PSII efficiency data (not the normalized ones) were fitted into the previously mentioned equations, with the inhibition phase (Fig. 7) separated from the recovery phase (Fig. 8). During the inhibition phase, both the HL and the LL acclimated cells showed a very rapid decrease in the effective PSII quantum efficiency $\Delta F/F_m'$, and the rate constant for fast inhibition (k_1) equalled 9.05 min^{-1} for all of them (not shown). Most likely, the measuring frequency during the initial inhibition stage was too low to get a full resolution of k_1. Strains showed a biphasic decrease in the $\Delta F/F_m'$, with a value for the slow rate constant of inhibition (k_2) of 0.017 ± 0.005 min^{-1}. The proportion of fast reacting PSII centres (P_1) varied between 17% and 57% and was generally higher in HL than in the LL cells. P_2 was generally higher in the LL acclimated strains. Only the strain from Vigo showed nearly identical P_1 and P_2 at HL and LL (Fig. 7). During recovery, the proportion of P_1, the fast reacting PSII centres, was very similar for both the HL and LL acclimated cells and the fraction of P_1 varied between 55 and 77% whereas the fraction of P_2, the slow reacting PSII centres, varied between 23 and 45% (Fig. 8). The strain of Galé was the only one showing a larger difference between the HL and LL cells in P_1 and P_2. Interestingly, both k_1 and k_2 were higher for the LL acclimated cells, and whereas k_1 showed strain specific differences (highest for Galé and lowest for San Sebastian), there was no differences in k_2 between the different strains.

4. Discussion

Growth rates of *Coolia monotis* under the described conditions had a generation time of approximately 3 to 8 days. These results agree well with other data from benthic dinoflagellates of approximately the same size (Bravo et al., 2012; Faust, 1993; Tosteson et al., 1989). Our data supports the contention that both methodologies used, the Coulter counter and the Water-Pam, are useful to study growth rates in microalgae species as both gave similar results, in agreement with Lürling and Verschoor (2003). In this study, growth rates from 0.08 up to 0.21 d^{-1} were found at 90 µmol photons m^{-2} s^{-1}. Ben-Gharbia et al. (2016) reported higher growth rates (0.35 d^{-1}) from the Mediterranean Sea, using approximately the same light intensity and salinity but higher temperatures (25 °C). Increases in temperatures have been known to lead to an increase biomass in benthic dinoflagellates (Granéli et al., 2011). Unfortunately, studies characterizing *Coolia monotis* growth rates are very limited. Considering the whole light-intensity range, a maximum growth rate of 0.29 d^{-1} was observed with the strain from Galé under 200 µmol photons m^{-2} s^{-1}. Growth rates observed at low/medium light intensities were lower but more stable than the growth rates for the high light intensities as reported previously by Sada et al. (1989) and Raikar et al. (2001). When trying to associate the strain specific irradiance response to their geographical area, it seems that the Galé strain is adapted to a higher range of light intensities. The area of Galé, located in lower latitudes, is known to have a lesser mean annual cloud coverage than the localities of Vigo and San Sebastian, located at higher latitudes (Sanchez-Lorenzo et al., 2009). This, along with the frequent presence of calm waters, makes the location one in which irradiance enters deeper into the water column. It suggests that the strain isolated from Galé is genetically adapted to these higher light conditions, but more research is necessary to prove this. The strain of San Sebastian seems optimally adapted for medium light intensities, which correlates with the mean annual cloud coverage for that region, which is the highest for the Peninsula (Sanchez-Lorenzo et al., 2009). The strain from Vigo appears to be adapted to a higher range of light intensities, although the higher intensities elicited higher standard deviations. Other abiotic factors such as water turbulence, upwelling, salinity or temperatures might also influence how the cells grow. Regarding other species of the genus, different growth rates (0.20–0.66 d^{-1}) have been reported in *Coolia malayensis* using higher temperatures than in our study (25–30 °C) (Mohammad-Noor et al., 2013; Morton et al., 1992; Rhodes et al., 2000). Using different conditions, it was found that *C. tropicalis* showed no growth bellow a salinity of 20 and above 35, and a maximum growth rate of 0.38 d^{-1} was reported (Mohammad-Noor et al., 2013). This means that growth rates vary considerably depending on culturing conditions. Rhodes et al. (2000) reported optimal different preferences of temperature and salinity

Table 2
Fit parameters of $rETR_{max}$ (maximum rate of relative electron transport), α (initial slope of the light curve), E_k light saturation parameter (= $rETR_{max}/\alpha$) the r^2 of the fit of sigmaplot — the standard error of the fit is in $rETR_{max}$ generally <2–3% and the standard error in α < 6%, and are for these reason omitted from the table. Based on values fitted in Sigmaplot according to Webb et al., 1974. Adjusted r^2 are given.

	HL acclimated strains					LL acclimated strains				
Time	09:30	11:30	13:30	15:30	17:30	09:30	11:30	13:30	15:30	17:30
$rETR_{max}$	43.0	46.0	47.8	46.1	52.0	66.3	77.0	75.6	75.6	70.5
α	0.26	0.26	0.23	0.27	0.26	0.57	0.58	0.59	0.59	0.60
E_k	165	177	208	171	200	116	133	128	128	117
r^2	1.00	0.99	0.99	1.0	0.98	0.99	0.98	0.99	0.99	0.98

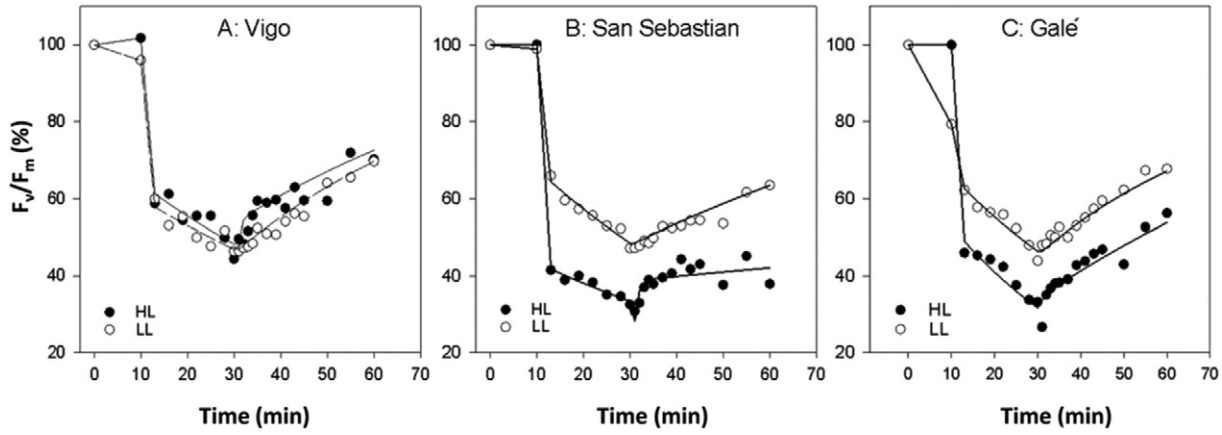

Fig. 5. Time course in PSII quantum efficiencies (F_v/F_m in the dark, $\Delta F/F_m'$ in the light) during the HL stress experiment with strains acclimated to HL and LL. A – Strain from Vigo. B – Strain from Galé. C – Strain from San Sebastian. At $t = 0$ a sample was collected from the culture and left in the dark for 10 min. From $t = 10$ to $t = 30$ the sample was exposed to HL for 20 min. At min 30, the sample was put back in the dark in order to measure the cells recovery. In order to make the patterns more clear, all values were normalized and expressed as percentage of F_v/F_m at $t = 0$. Some traces were corrected for a shift between $t = 30$ (end light stress) and $t = 33$ (3 min after dark acclimation).

between strains of the same genus and stated that cells could grow from 10 °C up to 35 °C, which explains their wide global distribution (David et al., 2014; Jeong et al., 2012; Leaw et al., 2016; Momigliano et al., 2013; Penna et al., 2005; Rhodes et al., 2014). This behavior contrasts with that of other epibenthic dinoflagellates such as species of the genera *Ostreopsis* and *Gambierdiscus*, which are only found in tropical or warm temperate areas (Aligizaki and Nikolaidis, 2008; Mangialajo et al., 2011; Parsons et al., 2012). Nutrients such as nitrogen and phosphorous are known to have an enormous influence on marine microalgae growth (Clark et al., 2002) and dinoflagellates, according to Hu et al. (2008), are known to prefer lower N/P ratios when comparing to diatoms. These changes may be used to predict algal succession, and forecast the occurrence of harmful algae blooms (Zhang and Hu, 2011).

Fluorescence techniques have been used to study the diurnal changes in the photochemical efficiency of dinoflagellates (Brown et al., 1999; Fraga et al., 2012; Jones and Hoegh-Guldberg, 2001) as well as other taxa (Hanelt, 1996, 1998; Ihnken et al., 2014a). Those indicated that the lowest F_v/F_m values reported in microalgae in response to the highest irradiances (Ihnken et al., 2014a; Jones and Hoegh-Guldberg, 2001) usually occurred between noon and early afternoon (Hanelt, 1996, 1998). This agrees well with the values observed in Fraga et al. (2012), where the rETR was seen to change throughout the course of a day in field samples during a *Gambierdiscus* bloom, with higher rETR values observed around midday. Conversely, no changes were seen in the photosynthetic fluorescence parameters of $rETR_{max}$, α or E_k, from our strains. No changes during the light cycle might be expected since cultured algae were under the same light intensity during the course of the day, in contrast with the environment where radiation changes throughout the day. Jones and Hoegh-Guldberg (2001) demonstrated that diurnal changes are light dependent and not due to a circadian periodicity. The only noticeable difference was between the strains acclimated to HL and LL where the latter presented higher $rETR_{max}$ and α values and lower E_k values. Hence the cells seemed to show a classic light-shade acclimation pattern. High-light adapted organisms generally have higher maximal photosynthetic rates and their apparatus saturates at higher irradiances compared with low-light photoacclimated cultures when normalized to chlorophyll (Cruz and Serodio, 2008; Fraga et al., 2012), a result partly driven by a decrease in the cellular chlorophyll content.

Algae adapted to higher irradiances are known to react much faster to changes in irradiances in the course of the day (Hanelt, 1998). Organisms should have efficient photoprotective mechanisms such as high dynamic photoinhibition and photoprotection strategies to tolerate light-induced stress (Betancor et al., 2015). The microalgae from this study showed high photoinhibition signals as expected in comparison with shaded-adapted species. At the start of the HL stress experiment, higher initial values of F_v/F_m of strains adapted to LL suggest that these cells were in better conditions than those acclimated to HL as discussed by Dimier et al. (2007) and Cruz and Serodio (2008). During the inhibition phase, all strains showed a very rapid decrease in the effective PSII quantum efficiency ($\Delta F/F_m'$) due to RCII closure and an augmentation in non-photochemical quenching (NPQ). The very slow and incomplete recovery of F_v/F_m demonstrated that part of this NPQ, was most likely due to the increase of the photoinhibitory quenching (qI), which suggested an increase in photodamage (Giovagnetti et al., 2014). This was also noted for the largest P_2 (slow) by Hanelt (1998). The biphasic behavior seen in the HL acclimated cells indicated that part of the recovery was due to thermal energy dissipation (qE). After the initial rapid decrease in $\Delta F/F_m'$, the slower decrease during the remainder of the inhibition phase is likely associated with the difference

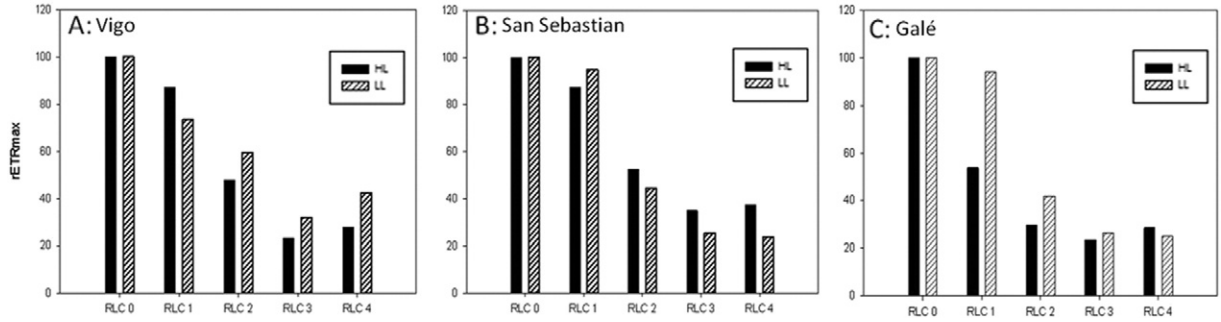

Fig. 6. The $rETR_{max}$ values of the rapid light curves of the HL stress experiment. All data were normalized to the initial values. RLC 0 = min 0, RLC 1 = min 10, RLC 2 = min 30, RLC 3 = min 45, RLC 4 = min 60.

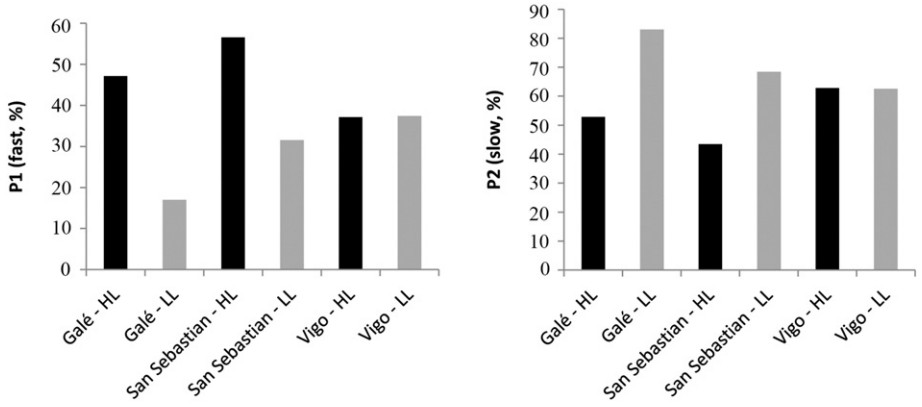

Fig. 7. Proportion of the fast (P_1) and slow (P_2) reacting PSII centres during the 20 min inhibition phase of the HL stress experiment.

between the degradation and replacement of the D1 protein reaction center, which is known to accumulate non-functional PSII (Yakovleva and Hidaka, 2004). This is supported by the RLCs parameters, although a minored-activation was observed especially in the HL acclimated San Sebastian strain after 10 min in the dark. This behavior has also been observed in the Chlorophyte *Dunaliella tertiolecta* (Ihnken et al., 2014b).

A recovery from exposure to high irradiances in macroalgae, was seen to be slower and less effectively, as deeper the occurrence of a species (Hanelt, 1998). In the inhibition phase of our experiment, cells downregulated their photosynthetic activity but in the following dark recovery phase, F_v/F_m never reached their initial conditions and no recovery at all was observed in the parameters describing the light curves, showing that all strains suffered from photodamage, and that dynamic downregulation (qE) was not sufficient to prevent this. Interesting differences were observed between the different strains: whereas the inhibition and recovery kinetics did not differ for HL and LL in Vigo, HL strains of San Sebastian and Galé showed a more pronounced decrease in $\Delta F/F_m'$ than LL cells. Cells of San Sebastian at HL showed a very poor recovery, whereas the rates of recovery in the HL and LL of Galé were similar. The kinetics of recovery can be divided into two phases according to Hanelt et al. (1997), where the fast phase appears to be independent of the slow phase, also known as the repair phase, via D1-turnover protein (Hanelt, 1998; Huner et al., 1993) and most likely related to the xanthophyll cycle. The fraction of the fast reaction centres was about 15% higher than the fraction of the slow reaction centres (P_2), with exception of the HL of Galé where P_1 was approximately 75% of the total (Fig. 8). Hence, our results do not resemble those of Hanelt (1998), where, in samples from shallow waters, the fraction of P_2 (slow) in the recovery was small and consequently P_1 (fast) was large, and the opposite was observed in deep-water algae. The largest differences were found in the rate constant of slow recovery k_2, which was about 2 min^{-1} in the HL strains and 9 in the LL strains. Nevertheless, the effect of this on the recovery kinetics is marginally, whereas a doubling of k_1 from 0.1 to 0.01 has a relative large effect on the final value of F_v/F_m. Although data were not significant, results seemed to indicate that the strain of Galé had a faster recovery than the other two strains. Even so, the harm to the PSII function and its process of recovery were

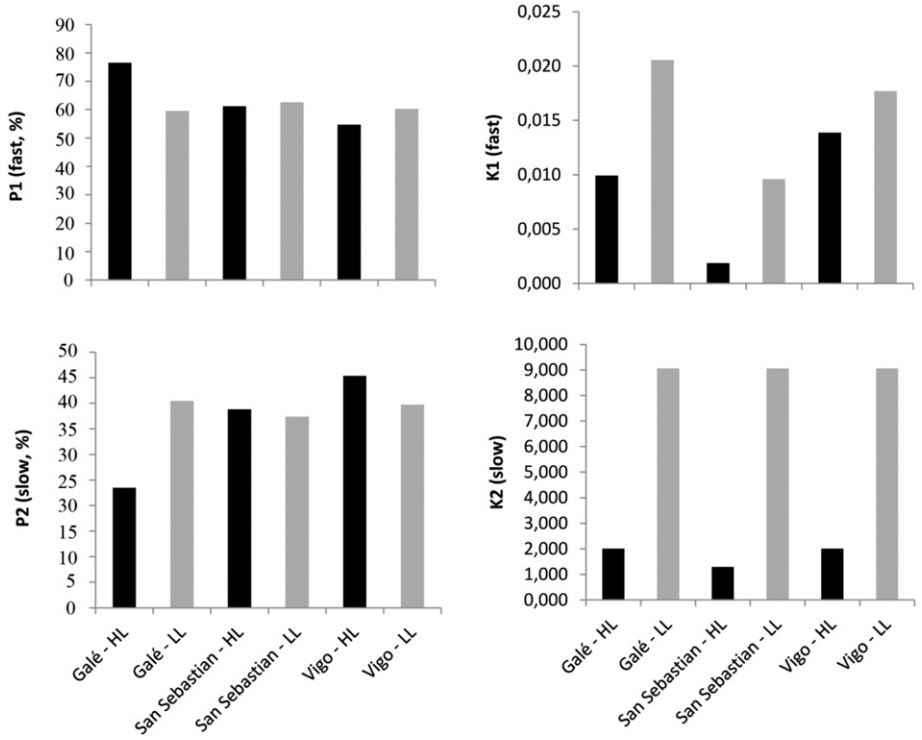

Fig. 8. Recovery parameters of the HL stress experiment. Both left panels show the proportion (%) of the fast (top, P_1) and slow (bottom, P_2) recovering PSII centres. The right panels show the rate constants (min^{-1}) for recovery of the fast (top, k_1) and slow (bottom, k_2).

apparently similar in all the strains with the exception that in the strain from Vigo the HL and LL acclimated cells reacted similar, whereas in the other two strains the HL cells showed, contrary to our expectation, a more pronounced inhibition, but with similar rates of recovery. As the starting F_v/F_m values were lower in the HL cells, this might point to light stress during the acclimation period.

Algae sampled near the sea surface are known to have a fast reaction to the photoinhibition and recovery (Hanelt, 1998) but, photosynthesis-irradiance (P-E) curves obtained from benthic dinoflagellates showed more features of shade-adapted organisms rather than high-lighted ones (Fraga et al., 2012). This was also confirmed by the FRRF data. A suggestion by Villareal and Morton (2002) was that *Gambierdiscus* might have a strategy to exploit the algae host three-dimensional structure for protection of light exposure and perhaps other similar dinoflagellates as *Coolia* or *Ostreopsis* might do the same for protection. The present results showed that high light exposure produced significant reduction in both F_v/F_m and $rETR_{max}$ in the *Coolia monotis* strains and, although cells were all collected attached to macroalgae, slightly differences such as the ones described above were seen in strains from different localities. Total recovery was not achieved within the 30 min time frame investigated, and some studies reported the recovery time to be within 24 h (Yakovleva and Hidaka, 2004). According to results, it seemed that cells would probably have a total recovery within 2 h in those specific conditions. The high photophysiological diversity among the strains did not match their phylogenetic relationships where no genetic divergence was seen (David et al., 2014). The different growth rates suggest an adaptation to different ecological properties responsible for photophysiological differences. Perhaps the use of other molecular markers or the study of epigenetics should be able to support the previous statement.

Contributions declaration

All authors have participated in all aspects of the article nearly equally.

Helena David – Experimental set up, data acquisition, analysis and data interpretation, drafting and revising the article, approved final version.

Jacco C. Kromkamp – Design of the study, Experimental set up, analysis and data interpretation, statistical expertise, drafting and revising the article, approved final version.

Emma Orive – The conception of the study, interpretation of data, drafting and revising the article, approved final version.

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