**Journal of Phycology**
June 2021, Volume 57, Issue 3, Pages 831-848
https://doi.org/10.1111/jpy.13119
https://archimer.ifremer.fr/doc/00663/77554/



# Physiology of maerl algae: Comparison of inter- and intraspecies variations

Qui-Minet Zujaila Nohemy [1, *], Davoult Dominique [1], Grall Jacques [2], Delaunay Coralie [3], Six Christophe [3], Cariou Thierry [4], Martin Sophie [5]

[1] Sorbonne Université, CNRS, UMR 7144 Adaptation et Diversité en Milieu Marin, Station Biologique de Roscoff, Place Georges Teissier, 29688 Roscoff Cedex, France
[2] Université de Bretagne Occidentale, IUEM, Place Nicolas Copernic, 29280 Plouzané, France.
[3] Sorbonne Université, CNRS, UMR 7144 Adaptation et Diversité en Milieu Marin, Station Biologique de Roscoff, Place Georges Teissier, 29688 Roscoff Cedex, France.
[4] Sorbonne Université, CNRS, Fédération de Recherche FR2424, Station Biologique de Roscoff, Place Georges Teissier, 29680, Roscoff, France
[5] Sorbonne Université, CNRS, UMR 7144 Adaptation et Diversité en Milieu Marin, Station Biologique de Roscoff, Place Georges Teissier, 29688 Roscoff Cedex, France.

* Corresponding author : Zujaila Nohemy Qui-Minet, email address : zquiminet@sb-roscoff.fr

**Abstract :**

Free-living red coralline algae play an important role in the carbon and carbonate cycles of coastal environments. In this study, we examined the physiology of free-living coralline algae forming maerl beds in the Bay of Brest (Brittany, France), where Lithothamnion corallioides is the dominant maerl (i.e., rhodolith) species. Phymatolithon calcareum and Lithophyllum incrustans are also present (in lower abundances) at a specific site in the bay. We aimed to assess how maerl physiology is affected by seasonality and/or local environmental variations at the inter- and intraspecific levels. Physiological measurements (respiration, photosynthetic and calcification rates) were performed using incubation chambers in winter and summer to compare (1) the dominant maerl species at three sites and (2) three co-existing maerl species at one site. Comparison of the three co-existing maerl species suggests that L. corallioides is the best adapted to the current environmental conditions in the Bay of Brest, because this species is the most robust to dissolution in the dark in winter and has the highest calcification efficiency in the light. Comparisons of L. corallioides metabolic rates between stations showed that morphological variations within this species are the main factor affecting its photosynthetic and calcification rates. Environmental factors such as freshwater inputs also affect its calcification rates in the dark. In addition to interspecies variation in maerl physiology, there were intraspecific variations associated with direct (water physico-chemistry) or indirect (morphology) local environmental conditions. This study demonstrates the plasticity of maerl physiology in response to environmental changes, which is fundamental for maerl persistence.

**Keywords** : calcification, coralline algae, environmental conditions, field experiment, photosynthesis, physiology, plasticity, rhodoliths

*Abbreviations:* **$\Omega_{Ar}$, aragonite saturation state; CTD probe, conductivity temperature and depth probe; DIC, dissolved inorganic carbon; *E*, irradiance; *Ec*, irradiance of compensation; *Ek*, irradiance of saturation; *G*, net calcification; $G_D$, calcification in the dark; $G_{MAX}$, maximal gross calcification; $G_L$, calcification in the light; *GPP*, gross primary production; $GPP_{MAX}$, maximal gross primary production; KW, kruskall-wallis test; MPT, montecarlo permutation test; *NPP*, net primary production; $pH_T$, Total scale pH; *R*, respiration; R/V, research vessel; $Si(OH)_4$, silicate; $T_A$, Total Alkalinity; v/v, volume (solute) per volume (solvent); $Y_{MAX}$, maximal gross yield.**

## INTRODUCTION

Free-living non-geniculate coralline algae, also referred as maerl (or rhodoliths), are distributed worldwide in coastal systems stretching from the tropics to polar regions (Foster 2001). They can accumulate and form large beds of live and/or dead maerl (Birkett et al. 1998). Individual thalli can range from 1 to 10 cm in length and take on highly branched to spherical-shaped forms, depending on the species and environmental conditions (Bosence 1976, Steneck and Adey 1976, Birkett et al. 1998, Foster et al. 2013). Their tridimensional structure bestows them with a very important role as foundation species – species that harbor a diverse assemblage of flora and fauna (Cabioch 1969, Keegan 1974, Peña et al. 2014) and serve as nursery habitats for many juvenile invertebrates and fish (Grall 2002, Kamenos et al. 2004).

Coralline algae have significantly lower growth rates than non-calcareous algae and, among coralline algal species, maerl growth rates are among the lowest (Heijden and Kamenos 2015). Previous studies on maerl growth rates have shown variation between species, thallus ages, seasons and environmental conditions. Growth rates in temperate ecosystems can range from 0.01 to 2.5 mm per year (Adey and McKibbin 1970, Bosence 1980, Edyvean and Ford 1987, Potin et al. 1990, Fazakerley and Guiry 1998, Blacke and Maggs 2003, Wilson et al. 2004, Kamenos and Law 2010).

Despite their slow growth rate, their high abundance and spatial distribution indicates high carbon (C) and calcium carbonate ($CaCO_3$) production rates (Foster 2001). Due to their photosynthetic and calcification capacities, maerl species are thus considered as major contributors to

the C and $CaCO_3$ cycles in coastal systems all over the world (Martin et al. 2005, 2007, Schwarz et al. 2005, Nelson 2009, Amado-Filho et al. 2012, Basso and Granier 2012).

Recent advancements in molecular biological species identification have provided important information on coralline algal diversity and distribution (Pardo et al. 2014, Hernández-Kantún 2015). The distribution of the various maerl species is thought to be greatly influenced by irradiance, temperature, salinity, and substratum (Adey and McKibbin 1970, Birkett et al. 1998, BIOMAERL Team 1999). Although species with different geographic boundaries can coexist in the same location, their abundance and survival depend greatly on their environmental thresholds and plasticity to withstand variability in abiotic factors (Hurd et al. 2014). For instance, algae are subject to large and non-predictable fluctuations of irradiance due to cloud cover and turbidity, in addition to the predictable seasonal variations in light intensity and photoperiod (Williamson et al. 2014). Species located in the shallow photic zone usually use efficient mechanisms regulating light utilization to withstand seasonal and daily variations in irradiance, allowing them to harvest dim light irradiances (Kirk 2011) or to limit photo-inhibition and avoid photo-oxidative damage (Gomez et al. 2004, Burdett et al. 2014, Vasquez-Elizondo and Enriquez 2017). Algae may thus respond to variation in irradiance by acclimating and/or adapting their morphology and physiology, for example by modifying their pigment content and composition (Kim et al. 2013, Burdett et al. 2014). Interestingly, red coralline algae can occur under very high irradiance levels in the tropics (> 1500 μmol photons · $m^{-2}$ · $s^{-1}$) and also at the lower limit of the photic zone (< 1 μmol photons · $m^{-2}$ · $s^{-1}$; Burdett et al. 2014), suggesting that they have developed different adaptation mechanisms to colonize various light niches.

Maerl species may also have to cope with different ranges and variations in temperature, with a decrease towards higher latitudes and large seasonal variations at mid-latitudes (Hurd et al. 2014). At a local scale, temperature variations also affect the vertical distribution of maerl species (Williamson et al. 2014). Maerl beds located in mid-latitudes and shallow environments, such as those found in the Bay of Brest (western France), face high seasonal and daily temperature variability (Martin et al. 2006, Qui Minet et al. 2018). Seawater temperature regulates chemical reaction rates and thus metabolic pathways. Variation in temperature has an impact on carbon fixation, skeletal magnesium incorporation, growth rates, and reproduction (Williamson et al. 2014). Comprehension of

how the physiology within a species is regulated by temperature variations is necessary to understand their current distribution and how they will be affected by global change.

Salinity also affects algal physiology (King and Schramm 1982). Maerl beds located in coastal systems can experience freshwater inputs and thus bursts of extremely low salinity. Some maerl species such as *Lithothamnion corallioides* and *Phymatolithon calcareum* are known to be adapted to typical marine saline environments (Birkett et al. 1998), whereas other species such as *L. incrustans* are known to be euryhaline (Grall 2002). Extreme low salinity values have been linked to negative impacts on metabolic processes, particularly calcification rates (King and Schramm 1982, Schoenrock et al. 2018).

When located in shallow productive zones, maerl beds may require water movement to avoid sedimentation and to limit the colonization of their thalli by non-calcareous epiphytic algae (Steneck 1986). Furthermore, currents have been correlated with species distribution and maerl morphology (Bosence 1976, Qui-Minet et al. 2018).

The objective of this study was to provide information on how the physiology of coralline algal species forming maerl beds is affected by seasonality and/or local environmental variations at the inter- and intraspecific levels. Knowledge on how temperate maerl species physiology responds to current environmental conditions is essential to understand their evolution under local and global change scenarios. In this study, we studied variations in the physiology of *Lithothamnion corallioides,* the most abundant maerl species in the Bay of Brest (Britany, France) by comparing its physiological rates (i) between stations located in different maerl beds of the Bay of Brest and (ii) with other co-existing maerl species: *Phymatolithon calcareum* and *L. incrustans,* also present at a specific location, albeit at lower abundances (Qui-Minet et al. 2018; Fig. 1). Although the physiology of the dominant species *L. corallioides* has been previously studied (Martin et al. 2006), this is the first study to report *P. calcareum* and *L. incrustans* metabolic rates under *in situ* conditions. We hypothesized that physiological performance of maerl species in the Bay of Brest i) varies with local environmental factors and ii) affects their abundance and distribution.

## MATERIALS AND METHODS

*Biological material*

Live maerl algae were collected in maerl beds located in the Bay of Brest (Brittany, France), where mean tidal range is 4 m and maximum tidal range is 8 m. Sampling stations were located in the northern basin for Station A (48º21'57" N, 04º26'47'' W), and in the southern basin for Stations B (48º19'58'' N, 04º19'57'' W) and C (48º17'304'' N, 04º24'029'' W; Fig. 1). Chart datum depths are 2.5, 0.7, and 1.7 m at Station A, B and C, respectively. Incident irradiance at the bottom, calculated from light extinction coefficients in Qui-Minet et al. (2018), ranges at solar noon and high tide: from 10 (Station A) to 20 (C) µmol photons · $m^{-2}$ · $s^{-1}$ in winter and from 50 (A) to 170 (C) µmol photons · $m^{-2}$ · $s^{-1}$in summer. It ranges at solar noon and low tide: from 75 (A) to 190 (B) µmol photons · $m^{-2}$ · $s^{-1}$ in winter and from 270 (A) to 570 (B) µmol photons · $m^{-2}$ · $s^{-1}$in summer. We collected *Lithothamnion corallioides* specimens at all three stations (A, B and C) and *Phymatolithon calcareum* and *L. incrustans* specimens at Station B, the only station of the study where the three maerl species co-exist (Fig. 2). Algae were collected with a Van Veen grab (5 replicates of 0.1 $m^2$ per station). Maerl thalli were carefully cleaned with seawater to remove sediments and were gently brushed when needed to remove epiphytic organisms. Thalli size ranged from 1 to 2 cm, from 1.5 to 2.5 cm, and from 1.5 to 3 cm at stations A, B and C in *L. corallioides*; from 1.5 to 4 cm in *P. calcareum,* and from 2 to 4 in *L. incrustans*. It is worth noting that as a result of hydrodynamism (higher bottom currents), *L. corallioides* at Station C, presents a compact shape relative to *L. corallioides* arbuscular shape at Stations A and B (Qui-Minet et al. 2018).

*Pigment analyses*

A sub-sample of the algal specimens was rinsed with filtered autoclaved seawater to remove salt, stored in 2 mL cryotubes and frozen in liquid nitrogen. Samples were preserved at -80ºC prior to lyophilization until pigment analyses. To obtain a fine powder, samples placed in plastic tubes were ground with 0.5 cm stainless steel beads (Brammer) using a Tissue Lyser II (QIAGEN). Main pigments on maerl species were analyzed from 200-300 mg dry weight (DW) using high-performance liquid chromatography (HPLC) according to Noisette et al. (2013).

*Incubation procedure*

Maerl physiology was assessed in winter (February and March 2015) and in summer (September 2015) during two light and dark incubation experiments. In the first experiment (Exp. 1), *Lithothamnion corallioides* specimens from Stations A, B and C were incubated under six different levels of irradiance, including maximum surface irradiance (100%) and reduced irradiance levels (65%, 47%, 27%, 13%, 6%). Irradiance levels were implemented by using opacifying neutral filters in the dark (dark chambers), in winter (February 26-27, 2015) and summer (September 16-17, 2015). In the second experiment (Exp. 2), the three species (*L. corallioides*, *Phymatolithon calcareum* and *L. incrustans*) from Station B were incubated in parallel under surface irradiance and in the dark, in winter (16 March 2015) and summer (September 18, 2015). Maerl thalli (corresponding to a mass of 15-30 g DW) were incubated in 220 mL in-house-designed chambers filled with seawater collected ~ 1 m above the bottom of each station with a Niskin bottle. Incubations lasted around 1 hour in order to avoid oxygen saturation greater than 120% during light incubations and to maintain oxygen saturation above 80% at the end of dark incubations.

Incubations were performed on board the R/V *Albert Lucas* immediately after collecting the algae. Chambers were filled with bottom seawater from each station and kept in a water bath with a continuous flow of water coming from the bottom to maintain algae at the in situ temperature. For each irradiance level and in the dark, chambers without algae were used as controls.

*Environmental parameters*

Surface irradiance (PAR, μmol photons · $m^{-2}$ · $s^{-1}$) was measured every minute using a LI-COR quantum sensor (LI 192 SA) during the incubation. In Exp. 1, incubations of *Lithothamnion corallioides* were done between 10:00 and 16:00. In Exp. 2, incubations of the three species at surface irradiance were done between 15:00 and 17:00.

Seawater physico-chemical parameters were measured before each incubation at each station from bottom seawater (approximately 1 m above bottom). Salinity and temperature at the bottom were measured using a CTD probe (QSP-2300, Sea-Bird-Electronics). Seawater samples for nutrient assays ($NO_3^-$, $NH_4^+$, $PO_4^{3-}$ and $Si(OH)_4$) and total alkalinity ($T_A$) were filtered using 0.22 μm Sterivex cartridges (Millipore). $NH_4^+$ and $T_A$ samples were stored in 100 mL borosilicate glass bottles. Reagents (R1: phenol nitroprusside solution and R2: complexing alkaline solution with chlorine) were

added to samples intended for $NH_4^+$ analysis and then stored in the dark until further analysis. Samples for $T_A$ measurements were poisoned with mercuric chloride (0.02% v/v; Dickson et al. 2007) and then stored in a dark cool place. Samples for $NO_3^-$, $PO_4^{3-}$ and $Si(OH)_4$ analyses were stored at 4ºC on board and then frozen at -20ºC in the lab (4-8 h later) until further analysis. $NH_4^+$ concentration was determined using the Solorzano et al. (1969) method. $NO_3^-$, $NH_4^+$ $PO_4^{3-}$ and $Si(OH)_4$ concentrations in seawater were measured according to Aminot and Kérouel (2007). $T_A$ was measured using HCl 0.01 N potentiometric titration with an automatic titrator (Titroline alpha, Schott Si Analytics) calibrated on the National Bureau of Standards scale and by using the Gran method of non-linear least-squares fit applied to pH variations from 3.5 to 3.0 mEq · $L^{-1}$ (Dickson et al. 2007). Total scale pH ($pH_T$) and temperature were measured using a pH meter (HQ40D, Hatch Lange Ltd portable LDO™) standardized with Tris-HCl and AMP buffer solutions. Dissolved inorganic carbon (DIC) and aragonite saturation state ($\Omega_{Ar}$) were calculated from $pH_T$, $T_A$, salinity, temperature and $PO_4^{3-}$ concentrations, using $CO_2$sys software, version 2.1 (Lewis and Wallace 1998). Calculations were based on a set of constants K1 and K2 from Mehrbach et al. (1973) refitted by Dickson and Millero (1987). Dissolved oxygen ($O_2$) was measured using an oxygen probe (Oxymeter HQ40D, Hatch Lange, Ltd portable LDO™).

*Physiological measurements*

Net production (light incubation) and respiration (dark incubation) rates were determined by measuring $O_2$ concentration at the beginning and at the end of the incubations. Dark respiration referred to non-photorespiratory mitochondrial respiration. Calcification rates were calculated using the alkalinity anomaly technique (Smith and Key 1975); water samples were taken at the beginning and at the end of the incubations. Rates were corrected with those from control chambers containing only seawater. At the end of incubations, samples of *Lithothamnion corallioides*, *Phymatolithon calcareum* and *L. incrustans* were collected and dried (60ºC, 48 h) for biomass determination.

Rates of algal primary production (net primary production, *NPP* and gross primary production, *GPP*), respiration (*R*) and net calcification (G) in the light ($G_L$) and in the dark ($G_D$) were normalized to the algal biomass (g DW). To obtain the net and the gross yield (efficiency of Chl *a* in *NPP* and *GPP*), the *NPP* and the *GPP* were normalized to the algal Chl *a* content (mg Chl *a* $g^{-1}$ DW maerl). *G*

was calculated using the following relationship: $Ca^{2+} + 2\ HCO_3^- \rightarrow CaCO_3 + CO_2 + H_2O$, for which $T_A$ decreases by 2 equiv for each mol of $CaCO_3$ precipitated. *NPP* (or R), *GPP, Yield*$_{net\ (or\ gross)}$ and *G* were calculated according to Equations (1), (2), (3) and (4):

$$NPP\ or\ R\ (O_2) = \frac{\Delta O_2 \times V}{DW \times \Delta t} \quad (1)$$

$$GPP = NPP + R \quad (2)$$

$$Yield_{\ net\ (or\ gross)} = \frac{NPP\ (or\ GPP)}{Chl\ a} \quad (3)$$

$$G\ (CaCO_3) = \frac{\Delta T_A \times v}{2 \times DW \times \Delta t} \quad (4)$$

where *NPP* (µmol $O_2$ · g $DW^{-1}$ · $h^{-1}$) is the net primary production rate, *R* (µmol $O_2$ · g $DW^{-1}$ · $h^{-1}$) is the respiration rate, *GPP* (µmol $O_2$ · g $DW^{-1}$ · $h^{-1}$) corresponds to the sum of absolute values of *NPP* and *R, Yield* $_{net}$ (or $_{gross}$; µmol $O_2$ · mg Chl $a^{-1}$ · $h^{-1}$) is the *NPP* or *GPP* normalized to the chlorophyll *a* content (mg Chl · g maerl $DW^{-1}$), *G* (µmol $CaCO_3$ · g $DW^{-1}$ · $h^{-1}$) is the net calcification rate in the light ($G_L$) or dark ($G_D$), $\Delta O_2$ (µmol · $L^{-1}$) is the variation of dissolved oxygen concentration between the beginning and the end of the incubation, *v* (L) is the volume occupied by the seawater in the chamber, DW (g) the dry weight of algae in the chamber, Δt (h) is the incubation time, $\Delta T_A$ (µmol · $L^{-1}$) is the change in total alkalinity between the beginning and the end of the incubation.

The energy available from photosynthesis for growth was measured in the three species using the photosynthesis-to-respiration ratio (*GPP:R*). The level of interaction between photosynthesis and calcification was calculated for each species through the ratio of calcification (in the light) to photosynthesis ($G_l$: *GPP*). Both ratios were calculated in winter and summer under ambient irradiance for *Lithothamnion corallioides*, *Phymatolithon calcareum*, and *L. incrustans* (Station B).

The relationship between irradiance (*E;* µmol photons · $m^{-2}$ · $s^{-1}$) and *NPP, Yield*$_{Net}$ or *G* was

obtained using the Chalker (1981) equation:

$$NPP = GPPmax \times \left(1 - e^{\frac{-E}{Ek}}\right) - R \quad (5)$$

$$Yield_{net} = Ymax \times \left(1 - e^{\frac{-E}{Ek}}\right) - R \quad (6)$$

$$G = G_{max} \times \left(1 - e^{\frac{-E}{Ek}}\right) - G_D \quad (7)$$

where *NPP* (µmol $O_2$ · g $DW^{-1}$ · $h^{-1}$), $Yield_{Net}$ (µmol $O_2$ · mg Chl $a^{-1}$ · $h^{-1}$) and $G_L$ (µmol $CaCO_3$ · g $DW^{-1}$ · $h^{-1}$) are the net primary production, net yield, and net calcification rates at a given irradiance (*E*, µmol photons · $m^{-2}$ · $s^{-1}$), $GPP_{MAX}$ (µmol $CaCO_3$ · g $DW^{-1}$ · $h^{-1}$) and $Y_{MAX}$ (µmol $O_2$ · mg Chl $a^{-1}$ · $h^{-1}$) are the maximal gross primary production expressed in terms of total dry weight and chlorophyll *a* content (mg Chl *a* · g $DW^{-1}$), respectively. $G_{MAX}$ (µmol $CaCO_3$ · g $DW^{-1}$ · $h^{-1}$) is the rate of maximal gross calcification, *Ek* (µmol photons · $m^{-2}$ · $s^{-1}$) is the half saturation constant, *R* is the respiration term and $G_D$ is the dark calcification term. The compensation irradiance (*Ec*, µmol photons · $m^{-2}$ · $s^{-1}$) is the irradiance at which *NPP* = 0 (or *GPP* = *R*).

*Statistical treatment*

Statistical analyses were done using *R*. Because not all the data showed a normal distribution (Shapiro-Wilks test) and/or homogeneity of variances (Bartlett test), a one-way non-parametric ANOVA (Kruskal-Wallis test, KW) was used to compare the physiological rates (photosynthetic, respiration, and calcification in the light and in the dark) and pigment content of the algae between species/stations and seasons, tests were followed by a post-hoc Wilcoxon test (pairwise Wilcoxon test, Wp). Fitting of the curves was done using the Fisher test of significance. A Monte Carlo

permutation test (MPT) was used to compare *NPP-E* and *G-E* parameters ($GPP_{MAX}$, *R*, $G_{MAX}$, $G_D$, *Ek*, and *Ec*) between stations and seasons.

## RESULTS

The physico-chemical parameters of seawater at the beginning of the incubations at the three stations in both seasons are shown in Tables 1 and 2.

*Comparison of pigment content in* Lithothamnion corallioides *at the three stations (Exp. 1).*

Chl *a* content of *Lithothamnion corallioides* did not vary between winter and summer at any station. In winter, there was no difference in *L. corallioides* Chl *a* content among stations (Fig. 3a and Table 3), but in summer, Chl *a* content was significantly lower at Station C than at Stations A and B (Fig. 3b and Table 3). The xanthophyll content did not vary significantly between winter and summer. In winter, it was heterogeneous among stations and in summer, it was significantly lower at Station C relative to the other stations (Fig. 3, c and d and Table 3). The xanthophyll:Chl *a* ratio did not vary significantly, neither among seasons, nor among stations (Fig. 3, e and f and Table 3).

*Comparison of pigment content among the three maerl species at Station B (Exp. 2).*

Chl *a* content did not vary significantly between winter and summer, regardless of the maerl species. In both seasons, the species with the highest Chl *a* content ($\mu g \cdot g\,DW\ maerl^{-1}$) was *Lithothamnion incrustans* (Fig. 4, a and b and Table 3). In *L. corallioides* and *Phymatolithon calcareum* the major xanthophyll was identified as zeaxanthin (Z), whereas *L. incrustans* contained lutein (L). The xanthophyll content did not vary significantly between the two seasons in *L. corallioides* and *P. calcareum*, but in *L. incrustans* it was higher in winter than in summer (Fig. 4, c and d). Comparison among species showed that *L. incrustans* had the highest xanthophyll content in both seasons (Fig. 4, c and d and Table 3), although in summer its content was not significantly different from *P. calcareum* (Fig. 4d and Table 3). The xanthophyll : Chl *a* ratio did not vary in *L. corallioides* and *P. calcareum* between seasons, whereas it increased in winter for *L. incrustans* (Fig. 4, e and f). This ratio differed among species in both seasons: in winter, the ratio was higher in *L. incrustans* than in *L.*

*corallioides* (Fig. 4e and Table 3), but in summer it was higher in *P. calcareum* than in *L. incrustans* (Fig. 4f and Table 3).

*Comparison of* Lithothamnion corallioides *net primary production and irradiance relationships (Exp. 1).*

The relationships between net primary production (*NPP*) and irradiance (*E*) were significant at all three stations (Fig. 5, a and b; $R^2 > 0.95$ in winter and in summer). No photo-inhibition occurred in the range of irradiance levels tested, regardless of the season (Fig. 5). Respiration (*R)* was significantly higher in summer than in winter at the three stations (Tables 4 and 5). In both seasons, *R* showed a heterogeneous pattern among stations (Tables 4 and 6). Maximal gross primary production ($GPP_{MAX}$) was significantly higher in summer than in winter (Fig. 5 and Table 5). $GPP_{MAX}$ was lower at Station C in both seasons (Fig. 5 and Table 6). *Ek*, the irradiance at which photosynthesis saturates, did not vary significantly among seasons (Tables 4 and 5) or among stations (Tables 4 and 6). Irradiance of compensation (*Ec*) varied among seasons only at Station C, with higher values in summer than in winter (Tables 4 and 5). This parameter was lower at Station B than at Stations A and C in winter (Table 4 and 6). $Y_{MAX}$ was significantly higher in summer than in winter at Stations A and C, but no differences between seasons were observed at Station B (Table 4 and 5). No significant differences were observed between stations in winter, but a heterogeneous pattern among stations was observed in winter (Tables 4 and 6).

*Comparison of* Lithothamnion corallioides *calcification and irradiance relationships (Exp. 1).*

The relationship between net calcification (*G*) and irradiance (*E*) was significant at the three stations (Fig. 6; $R^2 > 0.95$). No light-induced inhibition of calcification was demonstrated, whatever the station or the season. Maximal gross calcification ($G_{MAX}$) was significantly higher in summer than in winter at Stations A and C, but no differences among seasons were recorded at Station B (Fig. 6 and Table 5). In both seasons, $G_{MAX}$ was significantly lower at Station C than at Stations A and B (Fig. 6 and Tables 4 and 6). In the dark, net dissolution was observed at the three stations at both seasons. It was significantly higher in winter than in summer at Station B, whereas no difference between seasons was observed at the other stations (Tables 4 and 5). In winter, net dissolution in the dark was

higher at Station B than at Stations A and C (Table 4). In summer, no difference was observed among stations (Tables 4 and 6). Irradiance of saturation (*Ek*) was significantly higher in winter than in summer at Station A (219 vs. 51 μmol photons · $m^{-2}$ · $s^{-1}$). No significant differences between seasons were observed at Stations B and C (Fig. 6 and Tables 5and 6). No difference in *Ek* was observed between stations regardless of the season (Fig. 6 and Table 7). Comparison between stations in each season showed heterogeneity, but there were no significant differences among them (Table 7).

*Comparison of gross primary production under surface irradiance and respiration rates among the three maerl species (Exp. 2).*

The mean gross primary production rate (*GPP)* was significantly higher in summer (at a mean irradiance of 279 ± 186 μmol photons · $m^{-2}$ · $s^{-1}$) than in winter (214 ± 65 μmol photons · $m^{-2}$ · $s^{-1}$) in *Lithothamnion corallioides* and in *L. incrustans,* whereas no difference between seasons was recorded in *Phymatolithon calcareum*. *GPP* was significantly higher in *L. incrustans* in both seasons relative to the other species and, in winter, it was significantly higher in *P. calcareum* than in *L. corallioides* (Fig. 7, a and b and Table 7). The gross yield (*GPP* normalized to the Chl *a* content, $Y_{MAX}$) in *L. corallioides* and in *L. incrustans* was significantly higher in summer than in winter but it did not vary between seasons in *P. calcareum*. It ranged from 6.0 to 8.6 μmol · mg Chl $a^{-1}$ · $h^{-1}$ in *L. corallioides*, from 5.4 to 7.7 μmol · mg Chl $a^{-1}$ · $h^{-1}$ in *L. incrustans* and from 7.9 to 8.1 μmol · mg Chl $a^{-1}$ · $h^{-1}$ in *P. calcareum*. Comparison among species showed that *P. calcareum* had a significantly higher gross yield relative to *L. incrustans* in winter, but there was no difference among species in summer (Table 7). Mean respiration rates (*R*) were higher in summer than in winter in the three species (Table 7). In winter, *R* was significantly lower in *L. corallioides* than in the other species (Fig. 7c and Table 7). In both seasons, it was significantly higher in *L. incrustans* (Fig. 7, c and d and Table 7). The ratio of gross primary production to respiration (*GPP:R* ratio) at surface irradiance was significantly higher in winter than in summer in the three species (KW, H = 6.82, p = 0.009; Fig. 8, a and b). In winter, significant differences were observed with the highest value measured in *L. corallioides* and the lowest in *L. incrustans* (KW, H = 12.5, p = 0.002), whereas in summer no difference was observed among species (KW, H = 4.88, p = 0.090; Fig. 8, a and b).

*Comparison of calcification rates under surface irradiance among the three maerl species (Exp. 2).* The mean net calcification rates ($G$) were higher in the light than in the dark and in summer than in winter for the three species (KW, $p < 0.01$). In winter, under a mean irradiance of 214 µmol photons · $m^{-2}$ · $s^{-1}$, $G_L$ was significantly lower in *Phymatolithon calcareum* than in *Lithothamnion corallioides* and *L. incrustans* (Table 7). In summer, under 279 µmol photons · $m^{-2}$ · $s^{-1}$, it was lower in *P. calcareum* than in *L. incrustans* (Fig. 9, a and b and Table 7). In winter in the dark, net dissolution was observed ($G_D < 0$), the highest net dissolution rate was measured in *L. incrustans* and the lowest in *L. corallioides* (Fig. 9c and Table 7). In summer, $G_D$ was positive (calcification > dissolution). *L. incrustans* $G_D$ was lower than that for *L. corallioides* and *P. calcareum* (Fig. 9d and Table 7).

*Comparison of the calcification-to-photosynthesis ratio under surface irradiance among the three maerl species (Exp. 1)*

The $G_L$:*GPP* ratio was calculated under ambient light intensity in winter (214 ± 65 µmol photons · $m^{-2}$ · $s^{-1}$) and in summer (279 ± 186 µmol photons · $m^{-2}$ · $s^{-1}$). It was significantly higher in summer than in winter in the three species (KW, $H = 6.82$, $p = 0.009$). It ranged from 0.40 to 0.65 in *Lithothamnion corallioides*, from 0.18 to 0.47 in *Phymatolithon calcareum*, and from 0.23 to 0.50 in *L. incrustans*. In winter, the $G_l$:*GPP* ratio was significantly different between the three species (KW, $H = 11.58$, $p = 0.003$), the highest ratio being measured in *L. corallioides* and the lowest in *L. incrustans* (Fig. 8c). In summer, differences were also observed (KW, $H = 9.62$, $p = 0.008$), the ratio was higher in *L. corallioides* than in *P. calcareum* (Fig. 8d).

## DISCUSSION

By comparing maerl respiration, photosynthetic and calcification rates between species and between contrasting stations in terms of physico-chemistry (salinity and carbonate chemistry) and hydrodynamism (Qui-Minet et al. 2018), we shed light on the physiological plasticity in maerl across species and locations.

*Respiration*

The seasonality of coralline algal respiration rates (*R*) depends on temperature (Hurd et al. 2014, Martin and Hall-Spencer 2017). In the present study, *R* was enhanced in summer relative to winter in the three maerl species, regardless of their location in the Bay of Brest. These results are in agreement with a previous report for *Lithothamnion corallioides* (Martin et al. 2006), although we found a weaker seasonal effect on *R* in this species: *L. corallioides R* was three-fold higher, across all stations, in summer than in winter in our study compared with almost five-fold higher in the previous study for similar seasons and temperature. At Station A, winter *R* in *L. corallioides* was two times higher than the rates reported in Martin et al. (2006) in the same season, whereas *R* was 10% (Station B) and 20% (Station C) higher in winter and 10% (Stations B and C) lower in summer. Given that the Martin et al. (2006) study was carried out at a different station in the Bay of Brest, such differences are most likely related to local environmental parameters varying with time and/or between stations. In our study, significant differences in *R* rates among stations were non-conclusive. Although mean values of R were higher at Station A, they varied strongly between replicates, possibly indicating a patchy/irregular distribution of maerl biofilm. In this context, the microflora and the bacteria forming maerl biofilm have an impact on the respiration rates of the holobiont system (host-microbiome; Longphuirt et al. 2007, Cavalcanti et al. 2014, 2018, Schoenrock et al. 2018). A previous study observed that Chl *a* belonging to maerl epiphytic microalgae varied among stations, being twice as high at Station A than at Stations B and C (Qui-Minet et al. 2018). With this in mind, we hypothesize that differences in pigment content may indicate differences in terms of maerl biofilm respiration rates among stations. Higher biofilm and related maerl respiration rates at Station A may be due to the lower abundance of grazers at this location, previously recorded by other authors (e.g., Guillou et al. 2002).

In addition, *R* depends on the energy produced by photosynthesis (Hurd et al. 2014) and may thus be related to algal morphology, because this parameter affects photosynthetic rates (see below). Comparison among species showed that the highest *R* values were found in *Lithothamnion incrustans* in both seasons. This may be related to higher growth rates and thus a higher metabolism for this species (Frantz and Bugbee 2005). Although algal respiration has not been as studied as photosynthesis, respiration is essential for providing the ATP, NADPH and C skeletons required for algal growth (Raven and Beardall 2003, Atkin et al. 2005). Moreover, this higher energy demand

corroborates the higher primary production and calcification rates found in *L. incrustans* relative to the other maerl species.

*Photosynthesis and pigments*

The increase in maerl photosynthetic rates in summer is attributed to the increase in temperature and irradiance levels (Martin et al. 2013, Egisildotir et al. 2016), and occurred despite seawater nutrient depletion that occurs during this period in the Bay of Brest (Le Pape et al. 1996, Qui-Minet et al. 2018). These results are in agreement with what Martin et al. (2006) previously observed: summer photosynthetic rates were about two-fold higher than in winter.

Differences in *Lithothamnion corallioides* photosynthetic rates also depended on local variability. During winter, physico-chemical parameters at Station B were significantly different from the other stations (Qui-Minet et al. 2018): an increase in nutrients (particularly nitrates and silicates) and a drop in salinity with a concomitant decrease in DIC concentration occurred. Although lower salinities have been correlated with deleterious effects for *L. corallioides* (Adey and McKibbin 1970), the lowest rates of maximum photosynthesis per unit mass ($GPP_{MAX}$) were observed at Station C. This appears to be linked to the compact morphology of *L. corallioides* at this location, because this morphotype has a lower surface-to-volume ratio than the branched morphology observed at Stations A and B. Given that photosynthetically active vegetative cells are only located in the surface layers of the thalli in coralline algae (McCoy and Kamenos 2015), compact morphotypes have lower Chl *a* content per unit mass and, consequently, lower photosynthetic rates. Conversely, when *L. corallioides* photosynthetic rates were normalized to the Chl *a* content, no differences were observed between the three stations. Therefore, differences in photosynthetic rates per unit mass between stations appear to be primarily related to differences in surface-to-volume ratio between morphotypes.

As shown for Chl *a* content, the main xanthophyll pigment in *Lithothamnion corallioides* was significantly lower at Station C. The carotenoid profile of coralline algae usually includes α-carotene and/or β-carotene, and one main xanthophyll: lutein, zeaxanthin, or antheraxanthin (Schubert et al. 2006, Esteban et al. 2009). There is no apparent relationship between phylogeny and main xanthophyll content (Schubert and García-Mendoza 2008), but differences in the type of the main xanthophyll among Rhodophyte species are related to their response to photo-inhibitory stress (Ursi et

al. 2003). Zeaxanthin is the main xanthophyll in *L. corallioides* (Noisette et al. 2013) and the main xanthophyll pigments in *L. incrustans* and *Phymatolithon calcareum* were described here for the first time, with *L. incrustans* being different from the other species, because it contains lutein instead of zeaxanthin. Interestingly, *L incrustans* rarely occurs below 15 m (Ford et al. 1983), whereas *L. corallioides* and *P. calcareum* can be found down to 20 m and 30 m, respectively (Birkett et al. 1998), where light is often limiting. Given that the three species here studied are located at the same depth, higher Chl *a* and xanthophyll content in *L. incrustans* may be related to its flat-branched morphology. Contrary to what has been reported in *L. corallioides* and *P. calcareum*, *L. incrustans* shows resistance to photo-inhibition under high light intensities and stressful conditions of pH and temperature (Qui-Minet et al. 2019). Nonetheless, zeaxanthin appears to have a major role against photo-inhibition, compared with lutein (Schubert and García Mendoza 2008); higher photoprotective pigment content and thallus reflectance have also been correlated with higher resistance to photo-inhibition (Schubert and García Mendoza 2008, Burdett et al. 2014).

Variations in photosynthetic rates are commonly observed among species and genotypes (Pallardy 2008). They can be linked to many parameters such as differences in metabolism and anatomy (Pallardy 2008, Heijden and Kamenos 2015, Hofmann and Heesch 2018). Accordingly, the higher photosynthetic capacities of *Lithothamnion incrustans* (it showed the highest *GPP* at both seasons) seem to be enhanced by a higher surface-to-volume ratio than the other species. Photosynthetic rates are also affected by environmental factors (Lee and Bazin 1991, Heijden and Kamenos 2015). In the Bay of Brest, the three maerl species studied here are located at a shallow depth (chart datum depth of 0.7 m and average tide of 4 m). Therefore, they are subject to high light intensities, particularly in summer at low tide, when bottom light intensity can reach extreme values of more than 500 μmol photons $\cdot$ $m^{-2}$ $\cdot$ $s^{-1}$ (Martin et al. 2006, Qui-Minet et al. 2018). In this context, mean ambient light intensities recorded during our incubations (~200 μmol photons $\cdot$ $m^{-2}$ $\cdot$ $s^{-1}$) are consistent with mean daily bottom irradiance for the summer season, but can be considered high for the winter season. Contrary to what was observed for the other study species (*L. corallioides* and *L. incrustans)* and previously reported for temperate coralline algae (Martin et al. 2013, Egilsdottir et al. 2015), *Phymatolithon calcareum GPP* did not vary significantly between winter and summer. Moreover, under the environmental conditions reported in this study, it showed the highest winter

$Y_{MAX}$ relative to the other species. According to its northern distribution and its presence in deeper environments, of the three species, *P. calcareum* seems to be the best adapted to lower depths and thus lower temperatures and irradiances (Adey and McKibbin 1970, Mendoza and Cabioch 1998). Nevertheless, this species did not display any photo-inhibition at an ambient light intensity of ~200-300 µmol photons · $m^{-2}$ · $s^{-1}$ regardless of the season.

Interestingly, the season did not induce any variation in the individual pigment content within a species at a given station. This suggests that they are already adapted to strong variations in light availability (tides, turbidity, cloudiness variability, etc.) throughout the year in the Bay of Brest. For instance, bottom irradiance at solar noon in the Bay of Brest has been estimated to reach almost 200 µmol photons · $m^{-2}$ · $s^{-1}$ at low tide in winter, which is within the order of magnitude of values found in summer at high tide (Qui-Minet et al. 2018). Similarly, the absence of significant differences between stations in terms of irradiance of saturation (*Ek*) for *Lithothamnion corallioides* is probably due to the high variability in bottom irradiance in the Bay of Brest.

*Calcification*

The main environmental parameters affecting $CaCO_3$ precipitation rates in coralline algae are irradiance, temperature and $CaCO_3$ saturation in seawater (Martin et al. 2006, Teichert and Freiwald 2014, Williamson et al. 2017). The amount of calcite produced by coralline algae is also known to vary with species, their morphology, growth rates and environmental conditions (Heijden and Kamenos 2015).

Although no dissolution was previously reported in *Lithothamnion corallioides* from the Bay of Brest (Martin et al. 2006), we observed net dissolution in *L. corallioides* in the dark. This is most likely linked to the different environmental conditions between stations and studies. The Bay of Brest is affected by freshwater inputs, which are higher at Station B. In winter, this station can reach extremely low values of salinity, alkalinity and carbonate availability (Qui-Minet et al. 2018), the latter being detrimental to the calcification process (King and Schramm 1982). Although winter pH at this station remained above 8.0, a decrease in $CO_3^{2-}$ availability at Station B during winter incubations may have been deleterious for *L. corallioides* calcification. Comparison between stations consistently showed that the net dissolution rate of *L. corallioides* in the dark was four- and five-fold higher at

Station B than at Stations A and C, respectively. However, no negative impact was observed on the calcification process in the light. A similar $G_{MAX}$ was observed at Stations A and B, which is consistent with the enhancement of calcification by photosynthesis previously reported for other coralline algal species (Chisholm 2000, Williamson et al. 2017, Hofmann et al. 2018). Effectively, photosynthesis increases the internal pH and therefore the $CO_3^{2-}$ at the site of calcification (Borowitzka and Larkum 1987, Williamson et al. 2017). This relationship indicates that the environment at the cellular level was thermodynamically favorable for inorganic $CaCO_3$ precipitation, regardless of the conditions in the surrounding water (Cyronak et al. 2016). On the other hand, differences in *L. corallioides* net calcification in the light observed among stations seem mostly explained by *L. corallioides* morphotypes and, as observed for $GPP_{MAX,}$ the lowest $G_{MAX}$ was observed at Station C in both seasons.

We also observed a seasonal effect on calcification rates. In summer, $G_{MAX}$ was enhanced at Stations A and C, but not at Station B. This may be related to a possible underestimation of summer $G_{MAX}$ at Station B due to the low maximal irradiance reached *in situ* during summer incubation experiments. The maximum surface irradiance reached at Station B was 370 μmol photons $m^{-2}$ $s^{-2}$ compared with 450 and 600 μmol photons · $m^{-2}$ · $s^{-2}$ at Stations A and C, respectively.

At Station B, *Phymatolithon calcareum* had the lowest net calcification rates among species in the light in both seasons. Growth rates being higher at the tips than at the basal parts (King and Schramm 1982), the more branched morphology of *Lithothamnion corallioides* with many apical tips compared with *P. calcareum,* may explain its higher calcification rates. More optimal environmental conditions in the bay for this species may also explain its dominance at this location. Various studies carried out in Northern Europe have reported higher growth rates in *L. incrustans* (Edyvean and Ford 1987) compared with *L. corallioides* and *P. calcareum* (Adey and McKibbin 1970, Blacke and Maggs 2003), which is in agreement with the higher calcification rates observed in our study in comparison to the other species. The highest $G_L$ measured in summer in *L. incrustans* may be related to its more southern geographical distribution (Hernández-Kantún et al. 2015) and preference for shallow depths (Ford et al. 1983). Higher summer temperatures and irradiances observed at Station B are likely favorable for its development at this location. Nevertheless, in the dark in both seasons, comparison between species showed that *L. incrustans* was the species with the highest net dissolution, suggesting

it is the most disadvantaged species in winter, when maerl beds remain in the dark most of the time. In the same manner, the lower maerl biomass at Station B relative to Stations A and C may be partly related to the significantly higher dissolution maerl species undergo in the dark following freshwater inputs (Qui-Minet et al. 2018).

In agreement with previous studies on the physiology of these maerl species (Martin et al. 2006, Qui-Minet et al. 2019), the three species presented higher calcification rates in summer, in the light they were more than two-fold higher than in winter. These results are consistent with the higher growth rates previously reported for *Lithothamnion corallioides* in summer (Potin et al. 1990).

In *Phymatolithon calcareum*, optimal temperature for growth is between 12-13ºC (Adey and McKibbin 1970). In this study, we observed higher calcification rates at 16ºC than at 10ºC (light intensity being comparable in winter and summer seasons). However, *NPP* was favored in winter (10ºC) relative to summer (16ºC). Therefore, our results suggest a seasonal decoupling between photosynthesis and calcification for this species. Despite differences in metabolic rates among species and seasons, the *GPP*:*R* ratio was higher in the three species in winter and the $G_L$ *:GPP* ratio was higher in summer. The latter relationship suggests greater tissue synthesis in winter than in summer and is in agreement with their higher energy demand for growth (respiration rates) in summer.

## CONCLUSIONS

According to our results, *Lithothamnion corallioides,* which is the dominant maerl species in the Bay of Brest, seems to be able to cope with the variable environmental parameters prevailing throughout the bay under winter and summer conditions. This species did not have the highest metabolic rates, but had the highest $G_L$*:GPP* ratio under ambient irradiance and the lowest dissolution rates relative to the other maerl species co-existing in the Bay of Brest, across all seasons.

Considering the light intensities of our study, a light:dark photoperiod of 8:16 h in winter, and 14:10 h in summer, estimations of net calcification ranged from 3 to 12 μmol $CaCO_3 \cdot g\ DW^{-1} \cdot d^{-1}$ in *Lithothamnion corallioides*, from 3 to 9 μmol $CaCO_3 \cdot g\ DW^{-1} \cdot d^{-1}$ in *Phymatolithon calcareum* and from 0.6 to 14 μmol $CaCO_3 \cdot g\ DW^{-1} \cdot d^{-1}$ in *L. incrustans*, in winter and in summer, respectively. Nevertheless, light calcification values were calculated at irradiances that are greater than average

incident bottom winter values, thus, under more realistic lower winter irradiances, *L. incrustans* may have had no growth or undergone dissolution.

In terms of local changes at small scales, freshwater inputs can negatively affect maerl calcification and thus the $CaCO_3$ budget through the enhancement of dissolution of maerl algae, notably in winter when maerl beds remain in the dark during most of the day. This is especially evident at Station B, where maerl biomass per unit surface remains the lowest (Qui-Minet et al. 2018). Interestingly, differences in metabolic rates at the three stations do not explain patterns in living maerl biomass among stations, Station C possessing the highest biomass (Qui-Minet et al. 2018). This lack of a clear pattern at the scale of the Bay of Brest highlights the importance of biotic and abiotic interactions, as well as any natural and anthropogenic disturbances previously undergone.

On the one hand, this work emphasizes the particular ability of *Lithothamnion corallioides* to maintain its photosynthetic and light calcification rates in unfavorable environmental conditions due to freshwater inputs. Photosynthesis seems to have a fundamental role in maintaining a favorable internal environment for calcification. With this in mind, the uncoupling of these processes under any future global change scenario may be detrimental for the calcification process under stressful conditions, because *Phymatolithon calcareum* is more vulnerable than the other species. On the other hand, *L. corallioides* was not able to prevent a significant increase in $CaCO_3$ dissolution in the dark following a drop in seawater carbonate parameters. Noteworthily, maintaining photosynthetic and calcification rates in the light under stressful conditions may have a metabolic cost.

Significant differences in production rates between different morphotypes have an impact on C and $CaCO_3$ budgets. Contrasting morphologies within the same species may affect maerl plasticity with regard to environmental changes. Morphology determines maerl photosynthetic capacity, which is closely interlinked with respiration and calcification rates. We verified that irradiance promotes calcification rates in the light and we speculate that low GPP:R ratios favor $CaCO_3$ dissolution in the dark. Morphology and photo-protective pigments also affect maerl ability to withstand different light intensities, which is fundamental under global change scenarios. Further work regarding other biological aspects at the inter- and intraspecies level, such as their photo-physiology, biofilm composition and reproduction mechanisms would allow a deep understanding of their fate in rapidly changing coastal systems.

**Acknowledgements**

We thank the crew of the R/V *Albert Lucas*, and Franck and Daniel in particular for their invaluable help with sampling. This work benefited from a grant of the Consejo Nacional de Ciencia y Tecnología (CONACyT) and from support from the French National Research Agency through the investment expenditure program IDEALG ANR-10-BTBR and the project EC2CO MAERLCHANGE.

## REFERENCES

Adey, W. H. & McKibbin, D. L. 1970. Studies on the maerl species *Phymatolithon calcareum* (Pallas) nov. comb. and *Lithothamnium corallioides* (Crouan) in the Ria de Vigo. *Bot. Mar.* 13:100-6.

Amado-Filho, G. M., Moura, R. L., Bastos, A. C., Salgado, L. T., Sumida, P. Y., Guth, A. Z., Francini-Filho, R. B., Pereira-Filho, G. H., Abrantes, D. P., Brasileiro, P. S., Bahia, R. G., Leal, R. N., Kaufman, L., Kleypas, J. A., Farina, M. & Thompson, F. L. 2012. Rhodolith Beds Are Major $CaCO_3$ Bio-Factories in the Tropical South West Atlantic. *PLoS ONE* 7 :e35171.

Aminot, A. & Kérouel, R. 2007. Dosage automatique des nutriments dans les eaux marines: méthodes en flux continu. Méthodes d'analyse en milieu marin. Ed. IFREMER -QUAE GIE, France. pp. 188.

Atkin, O. K., Bruhn, D., Hurry, V. M. & Tjoelker, M. G. 2005. The hot and the cold: unravelling the variable response of plant respiration to temperature. *Funct. Plant Biol*. 32:87-105.

Basso, D. & Granier, B. 2012. Calcareous algae in changing environments. *Geodiversitas* 34:5-11.

627 BIOMAERL Team.1998. Maerl grounds: habitats of high biodiversity in European waters. *In*
628 Barthel, K. G, Barth, H., Bohle-Carbonell, M., Fragakis, C., Lipiatou, E., Martin, P., Ollier, G. &
629 Weydert, M. [Eds.] *Third European marine science and technology conference, (Lisbon, Portugal,*
630 *23–27 May 1998),* vol 1, Marine systems. European Commission, Brussels, pp. 170–178
631
632 Birkett, D., Maggs, C., Dring, M., Boaden, P. & Seed, R. 1998. An overview of dynamic and
633 sensitivity characteristics for conservation management of marine SACs. Scott. Assoc. Mar. Sci. (UK
634 Marine SACs Project), 174 pp.
635
636 Blacke, C. & Maggs, C. A. 2003. Comparative growth rates and internal banding peridiocity of
637 maerl species (Corallinales, Rhodophyta) from northern Europe. *Phycologia* 42:606-12.
638
639 Borowitza, M. A. & Larkum, A. W. D. 1987. Calcification in algae: Mechanisms and the role of
640 metabolism. *Crit. Rev. Plant Sci*. 6:1-45.
641
642 Bosence, D. W. J. 1976. Ecological studies on two unattached coralline algae from Western Ireland.
643 *Palaeontology*. 19: 365–95.
644
645 Bosence, D. W. J. 1980. Sedimentary facies, production rates and facies models for recent coralline
646 algal gravels, Co. Galway, Ireland. *Geol. J.* 15:91-111
647
648 Burdett, H. L., Keddie, V., MacArthur, N., McDowall, L., McLeish, J., Spielvogel, E., Hatton, A. D.
649 & Kamenos, N. A. 2014. Dynamic photo-inhibition exhibited by red coralline algae in the red sea.
650 *BMC Plant Biol*. 14:139.
651
652 Cabioch, J. 1969. Les fonds de maerl de la Baie de Morlaix et leur peuplement vegetal. *Cah. Biol.*
653 *Mar*. 9:131–69
654

655 Cavalcanti, G.S., Gregoracci, G.B., dos Santos, E.O., Silveira, C. B., Meirelles, P. M., Longo, L.,
656 Gotoh, K., Nakamura, S., Lida, T., Sawabe, T., Rezende, C. E., Francini-Filho, R. B., Moura, R. L.,
657 Amado-Filho, G. M. & Thompson F. L. 2014. Physiologic and metagenomic attributes of the
658 rhodoliths forming the largest $CaCO_3$ bed in the South Atlantic Ocean. *ISME J.* 8:52–62.
659
660 Cavalcanti, G. S., Shukla, P., Morris, M., Ribeiro, B., Foley, M., Doane, M. P., Thompson, C. C.,
661 Edwards, M.S., Dinsdale, E.A. & Thompson, F.L. 2018. Rhodoliths holobionts in a changing ocean:
662 host-microbes interactions mediate coralline algae resilience under ocean acidification. *BMC*
663 *Genomics* 19:701.
664
665 Chisholm, J. R. M. 2000. Calcification by crustose coralline algae on the northern Great Barrier Reef,
666 Australia. *Limnol. Oceanogr*. 45:1476-84.
667
668 Cyronak, T., Schulz, K. G. & Jokiel, P. L. 2016. The Omega myth : what really drives lower
669 calcification rates in an acidifying ocean. *ICES J. Mar. Sci*. 73:558-62.
670
671 Dickson, A. G. & Millero, F. J. 1987. A comparison of the equilibrium constants for the dissociation
672 of carbonic acid in seawater media. *Deep-Sea Res.* 34:1733-43.
673
674 Dickson, A. G., Sabine, C. L. & Christian, J. R. 2007. *Guide to best practices for ocean $CO_2$*
675 *measurements PICES special publication 3*. North Pacific Marine Science Organization, Sidney,
676 British Columbia 191 pp.
677 Edyvean, R. G. J. & Ford, H. 1987. Growth rates of *Lithophyllum incrustans* (Corallinales,
678 Rhodophyta) from South West Wales. *Br. Phycol. J*. 22:139-46
679
680 Egilsdottir, H., Olafsson, J. & Martin, S. 2015. Photosynthesis and calcification in the articulated
681 alga Ellisolandia elongate (Corallinales, Rhodophyta) from intertidal rock pools. *J. Phycol*. 51:59-70
682

683 Esteban, R., Martinez, B., Fernández-Marín, B., Becerril, J. M. & Garcia-Plazaola, J. I. 2009.
684 Carotenoid composition in Rhodophyta: insights into xanthophyll regulation in Corallina elongata.
685 *Eur. J. Phycol.* 44:221-30.
686
687
688 Fazakerley, H. & Guiry, M. D. 1998. The distribution of maerl beds around Ireland and their
689 potential for sustainable extraction: phycology section. Report to the Marine Institute Dublin.
690 National University of Ireland, Galway, 34 pp.
691
692 Frantz, J. M. & Bugbee, B. 2005. Acclimation of plant population to shade: Photosynthesis,
693 respiration, and carbon use efficiency. *J. Am. Soc. Hortic. Sci*. 130:918-27
694
695 Ford, H., Hardy, F.G. & Edyvean, R. G. J. 1983. Population biology of the crustose red alga
696 *Lithophyllum incrustans* Phil. Three populations on the east coast of Britain. *J. Linn. Soc.* 23:353-63.
697
698 Foster, M. S. 2001. Rhodoliths: Between rocks and soft places. *J. Phycol.* 37: 659–67.
699
700 Foster, M. S., Amado-Filho G., Kamenos N., Riosmena-Rodriguez R. & Steller D. L. 2013.
701 Rhodoliths and rhodolith beds. *Smithson. Contrib. Mar. Sci*. 39:143-55.
702
703 Frantz, J.M. & Bugbee, B. 2005. Acclimation of plant population to shade: Photosynthesis,
704 respiration, and carbon use efficiency. *J. Am. Soc. Hortic. Sci*. 130:918-27.
705
706 Gomez, L. D., Noctor, G., Knight, M. R. & Foyer, C. H. 2004. Regulation of calcium signalling and
707 gene expression by glutathione. *J. Exp. Bot.* 55:1851-9.
708
709 Grall, J. 2002. Biodiversité spécifique et fonctionnelle du maerl: réponses à la variabilité de
710 l'environnement côtier. PhD dissertation, Université de Bretagne Occidentale, 340 pp.
711

712 Guillou, M., Grall, J. & Connan, S. 2002. Can low sea urchin densities control macro-epiphytic
713 biomass in a north-east maerl bed ecosystem (Bay of Brest, Brittany, France)? *J. Mar. Biol. Assoc.*
714 *UK* 82:867-76.
715
716 Heijden, L. H. & Kamenos, N. A. 2015. Reviews and syntheses: Calculating the global contribution
717 of coralline algae to total carbon burial. *Biogeosciences.* 12: 6429-41.
718
719 Hernández-Kantún, J., Rindi, F., Adey, W. H., Heesch, S., Peña, V., Le Gall, L. & Gabrielson, P. W.
720 2015. Sequencing type material resolves the identity and distribution of the generitype *Lithophyllum*
721 *incrustans*, and related European species *L. hibernicum* and *L. bathyporum* (Corallinales,
722 Rhodophyta) *J. Phycol.* 51:791-807.
723
724 Hofmann, L. C. & Heesch, S. 2018. Latitudinal trends in stable isotope signatures and carbon
725 concentrating mechanisms of northeast Atlantic rhodoliths. *Biogeosciences* 15:6139-49.
726
727 Hofmann, L. C., Schoenrock, K. & de Beer, D. 2018. Arctic coralline algae elevate surface pH and
728 carbonate in the dark. *Front. Plant Sci*. 9:1416.
729
730 Hurd, C. L., Harrison, P. J., Bischof, K. & Lobban, C. S. 2014. *Seaweed ecology and physiology*
731 (Second edition). Cambridge University Press, Cambridge, U. K. 551 pp.
732
733 Kamenos, N. A., Moore, P. G. & Hall-Spencer, J. M. 2004. Nursery-area function of maerl grounds
734 for juvenile queen scallops *Aequipecten opercularis* and other invertebrates. *Mar. Ecol. Prog. Ser.*
735 274:183-9.
736
737 Kamenos, N. A. & Law, A. 2010. Temperature controls on coralline algal skeletal growth. *J.*
738 *Phycol*. 46: 331-5.
739

Keegan, B. F. 1974. The macrofauna of maerl substrates of the West coast of Ireland. *Cah. Biol. Mar*. 15:513-30.

Kim, J. H., Lam, S. M. N. & Kim, K. Y. 2013. Photo-acclimation strategies of the temperate coralline alga *Corallina officinalis*: a perspective on photosynthesis, calcification, photosynthetic pigment contents and growth. *Algae* 28:355-63.

King, R. J. & Schramm, W. 1982. Calcification in the maerl coralline alga *Phymatolithon calcareum*: effects of salinity and temperature. *Mar. Biol*. 70: 197–204.

Kirk, J. T. O. 2011. *Light and Photosynthesis in Aquatic Ecosystems*, 3rd edn. Cambridge University Press, New York, 662 pp.

Lee, E. T. Y. & Bazin, M. J. 1991. Environmental factors influencing photosynthetic efficiency of the micro red alga *Porphyridium cruentum* (Agardh) Nageli in light-limited cultures. *New Phytol*. 118: 513-9

Le Pape, O., Del Amo, Y., Menesguen, A., Aminot, A., Quéquiner, B. & Tréguer, P. 1996. Resistance of a coastal ecosystem to increasing eutrophic conditions: the Bay of Brest (France), a semi- enclosed zone of Western Europe. *Cont. Shelf Res.* 16: 1885-907

Lewis, E. & Wallace, D. W. R. 1998. Program developed for $CO_2$ system calculations. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, Tennessee. doi: 10.3334/CDIAC/otg.CO2SYS_DOS_CDIAC 105

Longphuirt, S., Clavier, J., Grall, J., Chauvaud, L., Loc'h, F., Le Berre, I., Flye-Sainte-Marie, J. & Leynaert, A. 2007. Primary production and spatial distribution of subtidal microphytobenthos in a temperate coastal system, the Bay of Brest, France. *Estuar. Coast. Shelf Sci.* 74:367–80.

Martin, S., Clavier, J., Guarini, J. M., Chavaud, L., Hily, C., Grall, J., Thouzeau, G. 2005. Comparison

of *Zostera marina* and maerl community metabolism. *Aquat. Bot.* 83:161-74.

Martin, S., Castets, M. D. & Clavier, J. 2006. Primary production, respiration and calcification of the temperate free-living coralline alga *Lithothamnion corallioides*. *Aquat. Bot*. 85:121–8

Martin, S., Clavier J., Chauvaud L. & Thouzeau G. 2007. Community metabolism in temperate maerl beds I. Carbon and carbonate fluxes. *Mar. Ecol. Prog. Ser*. 335: 19-29.

Martin, S., Charnoz, A. & Gattuso, J. P. 2013. Photosynthesis, respiration and calcification in the Mediterranean crustose coralline alga *Lithophyllum cabiochae* (Corallinales, Rhodophyta). *Eur. Jour. Phycol*. 48:163-72.

Martin, S. & Hall-Spencer, J. M. 2017. Effects of ocean warming and acidification on rhodolith/maerl beds. *In* Riosmena-Rodriguez, R., Nelson, W. & Aguirre J. [Eds.] *Rhodolith/maerl beds: A global perspective*. Springfield International Publishing. Cham, Switzerland, pp. 87-102.

McCoy, S. J. & Kamenos, N. A. 2015. Coralline algae (Rhodophyta) in a changing world: integrating ecological, physiological, and geochemical responses to global change. *J. Phycol.* 51: 6-24.

Mehrbach, C., Culberson, C.H., Hawley, J.E. & Pytkowicz, R.N. 1973. Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. *Limnol. Oceanogr.* 18: 897–907.

Mendoza, M.L. & Cabioch, J. 1998. Étude comparée de la réproduction de *Phymatolithon calcareum* (Pallas) Adey & McKibbin et *Lithothamnion corallioides* (P & H Crouan) P & H Crouan (Corallinales, Rhodophyta), et reconsidérations sur la définition des genres. *Can. J. Bot.* 76:1433-45.

Nelson, W. A. 2009. Calcified macroalgae - critical to coastal ecosystems and vulnerable to change:

a review. *Mar. Freshwater Res.* 60:787-801.

Noisette, F., Egilsdottir, H., Davoult, D. & Martin, S. 2013. Physiological responses of three temperate coralline algae from contrasting habitats to near-future ocean acidification. *J. Exp. Mar. Biol. Ecol*. 448:179-87.

Pallardy, S.G. 2008. *Physiology of wood plants*. 3th Ed. Elsevier, Burlington, VT, 412 pp.

Pardo, C., Lopez, L., Peña, V., Hernández-Kantún, J., Le Gall, L., Bárbara, I. & Barreiro, R. 2014a. A multilocus species delimitation reveals a striking number of species of coralline algae forming maerl in the OSPAR maritime area. *PLoS ONE* 9:1-12.

Peña, V., Bárbara, I., Grall, J., Maggs, C.A. & Hall-Spencer, J.M. 2014. The diversity of seaweeds on maerl in the NE Atlantic. *Mar. Biodivers*. 44:533-51.

Qui-Minet, Z. N., Delaunay, C., Grall, J., Six, C., Cariou, T., Bohner, O., Legrand, E., Davoult, D. & Martin, S. 2018. The role of local environmental changes on maerl and its associated non-calcareous epiphytic flora in the Bay of Brest. *Estuar. Coast. Shelf Sci.* 208:140–52.

Qui-Minet, Z. N., Coudret, J., Davoult, D., Grall, J., Mendez-Sandin, M., Cariou, T. & Martin, S. 2019. Combined effects of global climate change and nutrient enrichment on the physiology of three temperate maerl species. *Ecol. Evol*. 9:13787–807.

Raven, J. A. & Beardall, J. 2003. Carbohydrate metabolism and respiration in algae. *In* Larkum, A.W.D., Douglas, S.E. & Raven, J.A. [Eds.] *Photosynthesis in Algae*, Advances in Photosynthesis and Respiration, Springer Netherlands, Dordrecht, pp. 205–224.

Schoenrock, K. M., Bacquet, M., Pearce, D., Rea, B. R., Schofield, J. E., Lea, J., Mair, D. & Kamenos, N. 2018. Influences of salinity on the physiology and distribution of the Arctic coralline algae, *Lithothamnion glaciale* (Corallinales, Rhodophyta). *J. Phycol*. 54:690–702.

Schubert, N. & Garcia-Mendoza, E. 2008. Photo-inhibition in red alga especies with different carotenoid profiles *J. Phycol*. 44:1437-46.

Schubert, N., Garcia-Mendoza, E. & Pacheco-Ruiz, I. 2006. Carotenoid composition of marine red algae *J. Phycol.* 42:1208-16.

Schwartz, Y. B., Kahn, T. G. & Pirrotta, V. 2005. Characteristic low density and shear sensitivity of cross-linked chromatin containing polycomb complexes. *Mol. Cell. Biol.* 25:432-9.

Smith, A. D., Key G. S. 1975. Carbon-dioxide and metabolism in marine environments. *Limnol. Oceanogr.* 20:493-5.

Solorzano, L. 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. *Limnol. Oceanog*. 14:799-801.

Steneck, R. S. & Adey, W. H. 1976. The role of environment in control of morphology in *Lithophyllum congestum*, a Caribbean algal ridge Builder. *Bot. Mar*. 19:197–216.

Steneck, R. S. 1986. The ecology of coralline algal crusts - convergent patterns and adaptative strategies. *Annu. Rev. Ecol. Syst.* 17:273–303.

Teichert, S. & Freiwald, A. 2014. Polar coralline algal $CaCO_3$-production rates correspond to intensity and duration of the solar radiation. *Biogeosciences* 11:833–42.

Vazquez-Elizondo, R. M. & Enriquez, S. 2017. Light absorption in coralline algae (Rhodophyta): A

854 morphological and functional approach to understanding species distribution in a coral reef lagoon.
855 *Front. Mar. Sci.* 4:297.
856
857 Williamson, C. J., Brodie, J., Goss, B., Yallop, M., Lee, S. & Perkins, R. 2014. *Corallina* and
858 *Ellisolandia* (Corallinales, Rhodophyta) photophysiology over daylight tidal emersion: interactions
859 with irradiance, temperature and carbonate chemistry. *Mar. Biol.* 161:2051-68.
860
861 Williamson, C.J., Perkins, R., Voller, M., Yallop, M. L. & Brodie, J. 2017. The regulation of coralline
862 algal physiology, an *in situ* study of *Corallina officinalis* (Corallinales, Rhodophyta).
863 *Biogeosciences*. 14: 4485-98
864
865 Ursi, S., Pedersén, M., Plastino, E. & Snoeijs, P. 2003. Intraspecific variation of photosynthesis,
866 respiration and photoprotective carotenoids in *Gracilaria birdiae* (Gracilariales: Rhodophyta). *Mar.*
867 *Biol.* 142:997–1007.
868
869 Wilson, S., Blake, C., Berges, J. A. & Maggs, C. A. 2004. Environmental tolerances of free-living
870 coralline algae (maerl): implications for European marine conservation. *Biol. Conserv*. 120:283-93.
871
872
873

**Figure 1.** Map of the Bay of Brest showing the surface covered by maerl beds and the sampled stations: Station A (*Lithothamnion corallioides*), Station B (*L. corallioides*, *Phymatolithon calcareum,* and *Lithophyllum incrustans*), and Station C (*L. corallioides*).

**Figure 2.** Photos of *Lithothamnion corallioides* from Station A, *L. corallioides*, *Phymatolithon calcareum* and *Lithophyllum incrustans* from Station B, and *L. corallioides* from Station C.

**Figure 3.** Experiment 1: Variations in Chlorophyll *a* (Chl *a*) content, main xanthophyll content (MX) and the MX:Chl *a* ratio in *Lithothamnion corallioides* at Stations A, B and C in winter (a, c, e) and in summer (b, d, f). Box plots extend from the 25% to the 75% percentiles of all the data, the central horizontal line represents the median, and bars extend to the 95% confidence limits.

**Figure 4.** Experiment 2: Variations in Chlorophyll *a* (Chl *a*) content, main xanthophyll content (MX) and the MX:Chl *a* ratio in *Lithothamnion corallioides*, *Phymatolithon calcareum* and *Lithophyllum incrustans* at Station B, in winter (a, c, e) and in summer (b, d, f). Box plots extend from the 25% to the 75% percentiles of all the data for each species, the central horizontal line represents the median, and bars extend to the 95% confidence limits.

**Figure 5.** Experiment 1: Relationship between net primary production (in μmol $O_2 \cdot g\ DW^{-1}$) and irradiance (μmol photons $\cdot\ m^{-2} \cdot s^{-1}$) in winter (a) and in summer (b) in *Lithothamnion corallioides* at Stations A (gray circles), B (black circles) and C (white circles).

**Figure 6.** Experiment 1: Relationship between net calcification (in μmol $CaCO_3 \cdot g\ DW^{-1}$) and irradiance (μmol photons $\cdot\ m^{-2} \cdot s^{-1}$) in winter (a) and in summer (b) in *Lithothamnion corallioides* at Stations A (gray circles), B (black circles) and C (white circles).

**Figure 7.** Experiment 2: Variations in *Lithothamnion corallioides*, *Phymatolithon calcareum* and *Lithophyllum incrustans* gross primary production (GPP) and respiration (R) rates at Station B in winter (a and c, respectively) and in summer (b and d, respectively). Box plots extend from the 25% to the 75% percentiles of all the data for each species, the central horizontal line represents the median, and bars extend to the 95% confidence limits.

**Figure 8.** Experiment 2: Variation in *Lithothamnion corallioides*, *Phymatolithon calcareum* and *Lithophyllum incrustans* (at Station B) gross primary production:respiration (GPP:R) and calcification in light:gross primary production ($G_l$:GPP) ratios in winter (a and c, respectively) and in summer (b and d, respectively). Box plots extend from the 25% to the 75% percentiles of all the data for each species, the central horizontal line represents the median, and bars extend to the 95% confidence limits.

**Figure 9.** Experiment 2: Variations in *Lithothamnion corallioides*, *Phymatolithon calcareum* and *Lithophyllum incrustans* calcification rates at Station B in the light and in the dark in winter (a and c, respectively) and in summer (b and d, respectively). Box plots extend from the 25% to the 75% percentiles of all the data for each species, the central horizontal line represents the median, and bars extend to the 95% confidence limits.

933 **Table 1.** Mean values (± SD) of seawater physico-chemical parameters measured in winter and summer on the days of incubations during
934 Experiment 1 at Stations A, B and C and Experiment 2 at Station B (n = 3). SW: seawater, $\Omega_{Ar}$: Aragonite Saturation State, $T_A$: Alkalinity
935 (mmol · kg $SW^{-1}$), DIC: Dissolved Inorganic Carbon (mmol · kg $SW^{-1}$).
936

| Season | Experiment | Station | Temperature (°C) | Oxygen (mg · $L^{-1}$) | Salinity | $pH_T$ | $\Omega_{Ar}$ | $T_A$ (mmol · kg $SW^{-1}$) | DIC (mmol · kg $SW^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|
| **Winter** | | | | | | | | | |
| February 27, 2015 | Exp. 1 | A | 9.3 ± 0.1 | 8.7 ± 0.0 | 34.0 ± 0.0 | 8.05 ± 0.00 | 1.7 ± 0.0 | 2.28 ± 0.00 | 2.10 ± 0.00 |
| February 27, 2015 | Exp. 1 | B | 8.9 ± 0.1 | 8.8 ± 0.0 | 23.0 ± 0.1 | 8.04 ± 0.01 | 1.2 ± 0.1 | 1.82 ± 0.22 | 1.73 ± 0.15 |
| February 27, 2015 | Exp. 1 | C | 9.7 ± 0.5 | 8.5 ± 0.0 | 32.0 ± 0.8 | 8.04 ± 0.06 | 1.6 ± 0.0 | 2.23 ± 0.01 | 2.07 ± 0.05 |
| March 27, 2015 | Exp. 2 | B | 10.5 ± 0.1 | 9.0 ± 0.0 | 33.3 ± 0.6 | 8.14 ± 0.00 | 2.3 ± 0.0 | 2.26 ± 0.00 | 2.04 ± 0.00 |
| **Summer** | | | | | | | | | |
| September 16, 2015 | Exp. 1 | A | 17.8 ± 0.2 | 9.8 ± 0.0 | 35.1 ± 0.0 | 7.92 ± 0.03 | 2.3 ± 0.1 | 2.37 ± 0.00 | 2.18 ± 0.01 |
| September 16, 2015 | Exp. 1 | B | 16.3 ± 0.0 | 7.4 ± 0.0 | 35.0 ± 0.0 | 8.03 ± 0.02 | 2.4 ± 0.1 | 2.30 ± 0.00 | 2.42 ± 0.12 |
| September 17, 2015 | Exp. 1 | C | 16.5 ± 0.1 | 7.5 ± 0.0 | 35.0 ± 0.0 | 8.01 ± 0.00 | 2.4 ± 0.0 | 2.34 ± 0.01 | 2.12 ± 0.01 |
| September 18, 2015 | Exp. 2 | B | 16.4± 0.1 | 7.4 ± 0.1 | 35.0 ± 0.0 | 8.00 ± 0.01 | 2.4 ± 0.0 | 2.29 ± 0.01 | 2.10 ± 0.01 |

937

939 **Table 2.** Mean values (± SD) of nutrient concentrations and irradiance measured in winter and summer on the days of incubations during
940 Experiment 1 at Stations A, B and C and Experiment 2 at Station B (n = 3).

| Season | Experiment | Station | $[NO_3^{-2}]$ ($\mu mol \cdot L^{-1}$) | $[NH_4^-]$ ($\mu mol \cdot L^{-1}$) | $[PO_4^-]$ ($\mu mol \cdot L^{-1}$) | $[SiO_4^{-4}]$ ($\mu mol \cdot L^{-1}$) | Irradiance ($\mu mol\ photons \cdot m^{-2} \cdot s^{-1}$) |
|---|---|---|---|---|---|---|---|
| **Winter** | | | | | | | |
| February 27, 2015 | Exp. 1 | A | 22.5 ± 2.7 | 0.6 ± 0.0 | 0.6 ± 0.1 | 7.2 ± 2.0 | 480 ± 65 |
| February 27, 2015 | Exp. 1 | B | 111.2 ± 4.8 | 1.2 ± 0.2 | 0.6 ± 0.1 | 34.1 ± 9.0 | 291 ± 25 |
| February 27, 2015 | Exp. 1 | C | 32.1 ± 0.8 | 0.7 ± 0.0 | 0.6 ± 0.0 | 13.2 ± 0.5 | 273 ± 102 |
| March 27, 2015 | Exp. 2 | B | 26.9 ± 0.4 | 0.2 ± 0.0 | 0.3 ± 0.0 | 5.5 ± 0.2 | 214 ± 65 |
| **Summer** | | | | | | | |
| September 16, 2015 | Exp. 1 | A | 2.0 ± 0.0 | 0.7 ± 0.0 | 0.2 ± 0.0 | 4.3 ± 0.0 | 600 ± 186 |
| September 16, 2015 | Exp. 1 | B | 1.5 ± 0.3 | 1.0 ± 0.2 | 0.4 ± 0.0 | 4.8 ± 0.0 | 370 ± 86 |
| September 17, 2015 | Exp. 1 | C | 1.2 ± 0.0 | 0.8 ± 0.1 | 0.4 ± 0.0 | 5.0 ± 0.0 | 446 ± 102 |
| September 18, 2015 | Exp. 2 | B | 2.6 ± 0.3 | 1.5 ± 0.4 | 0.5 ± 0.1 | 4.6 ± 0.0 | 279 ± 186 |

941
942

**Table 3**. Summary of the results of one-way non-parametric (Kruskal-Wallis) tests followed by post-hoc Wilcoxon tests (shown in italics), testing differences in Chlorophyll *a* content, main xanthophyll (zeaxanthin or lutein) content and Chlorophyll *a* : main xanthophyll ratio between Stations A, B and C in *Lithothamnion corallioides* (Exp. 1) and between the three maerl species: *L. corallioides* (*L.c.*), *Phymatolithon calcareum* (*P.c.*) and *Lithophyllum incrustans* (*L.i.*), at Station B (Exp. 2) in winter and summer. Comparison of the season effect at each station and for each species was done using a one-way non-parametric (Kruskal-Wallis) test (n=3).

| | **Chlorophyll *a*** | | **Main Xanthophyll** | | **Xanthophyll:Chlorophyll *a*** | |
|---|---|---|---|---|---|---|
| | F | p | F | p | F | P |
| **Exp. 1** | | | | | | |
| **Station comparisons** | | | | | | |
| Winter | 5.46 | 0.065 | 6.26 | **0.044*** | 0.06 | 0.970 |
| Post-hoc Wilcoxon | A = B = C | | A = B = C | | A = B = C | |
| | 9.42 | **0.009**** | 9.42 | **0.009**** | 3.02 | 0.221 |
| Summer | | | | | | |
| Post-hoc Wilcoxon | A & B > C | | A & B > C | | A = B = C | |
| | | | | | | |
| Season | | | | | | |
| Station A | 2.45 | 0.117 | 0.53 | 0.465 | 0.27 | 0.601 |
| Station B | 0.53 | 0.465 | 0.53 | 0.465 | 0.53 | 0.465 |
| Station C | 0.27 | 0.601 | 0.01 | 0.917 | 0.53 | 0.465 |
| **Exp. 2** | | | | | | |
| **Species comparisons** | | | | | | |
| Winter | 8.66 | **0.013*** | 8.96 | **0.011*** | 9.38 | **0.009**** |

| | | | | | | |
|---|---|---|---|---|---|---|
| Post-hoc Wilcoxon | *L.i.* > *L.c.* = *P.c.* | | *L.i.* > *L.c.* = *P.c.* | | *L.c.* = *P.c.* = *L.i.* | |
| Summer | 10.22 | **0.006**** | 7.46 | **0.024*** | 6.50 | **0.039*** |
| Post-hoc Wilcoxon | *L.i.* > *L.c.* = *P.c.* | | *L.i.* > *L.c.*<br>*P.c.*=*L.i.*, *P.c.*=*L.c.* | | *P.c.* > *L.i.*<br>*L.c.*=*P.c.*, *L.c.*=*L.i.* | |
| Season | | | | | | |
| *L. corallioides* | 0.01 | 0.917 | 0.53 | 0.465 | 0.53 | 0.465 |
| *P. calcareum* | 0.27 | 0.601 | 0.88 | 0.347 | 0.53 | 0.465 |
| *L. incrustans* | 0.88 | 0.347 | 4.81 | **0.028*** | 6.82 | **0.009**** |

**Table 4.** Parameters of primary production-irradiance (*NPP-E*) and calcification-irradiance (G-E) curves for *Lithothamnion corallioides* at Stations A, B, and C in winter and summer. Results are expressed as means ± SE (n = 5). $GPP_{MAX}$ (or $G_{MAX}$): maximum rates of gross photosynthesis (or calcification) (µmol $O_2$ or $CaCO_3$, g DW · $h^{-1}$); $Y_{MAX}$, $R$ (or $G_D$): dark respiration (or calcification) rates (µmol $O_2$ or $CaCO_3$, g DW · $h^{-1}$); *Ek*: saturating irradiance (µmol photons $m^{-2}$ $s^{-1}$); *Ec*: compensation irradiance (µmol photons · $m^{-2}$ · $s^{-1}$).

| | | **----------Primary production ----------** | | | | | **----------Calcification----------** | | |
|---|---|---|---|---|---|---|---|---|---|
| Season | Station | $GPP_{MAX}$ | $R$ | *Ek* | *Ec* | $Y_{MAX}$ | $G_{MAX}$ | $G_D$ | *Ek* |
| Winter | A | 1.59 ± 0.61 | 0.13 ± 0.02 | 290 ± 157 | 21 ± 2 | 8 ± 3 | 0.64 ± 0.13 | -0.02 ± 0.02 | 219 ± 127 |
| | B | 1.28 ± 0.37 | 0.09 ± 0.01 | 160 ± 92 | 11 ± 2 | 12 ± 6 | 0.64 ± 0.11 | -0.08 ± 0.02 | 162 ± 43 |
| | C | 0.66 ± 0.14 | 0.09 ± 0.02 | 136 ± 35 | 19 ± 3 | 10 ± 2 | 0.35 ± 0.03 | -0.04 ± 0.02 | 120 ± 19 |
| Summer | A | 3.10 ± 0.15 | 0.29 ± 0.14 | 171 ± 35 | 22 ± 5 | 22 ± 3 | 0.85 ± 0.09 | -0.03 ± 0.01 | 70 ± 10 |
| | B | 2.46 ± 0.18 | 0.29 ± 0.01 | 106 ± 19 | 15 ± 1 | 17 ± 2 | 0.63 ± 0.08 | -0.02 ± 0.02 | 93 ± 39 |
| | C | 1.10 ± 0.26 | 0.23 ± 0.03 | 190 ± 70 | 38 ± 10 | 15 ± 4 | 0.35 ± 0.06 | -0.03 ± 0.03 | 98 ± 38 |

980
981

**Table 5.** Summary of the results of one-way Monte Carlo permutation test (MPT) followed by a post-hoc permutation test to evaluate the effect of season on the primary production-irradiance (*NPP-E*) and calcification-irradiance (*G-E*) parameters in *Lithothamnion corallioides* at Stations A, B, and C (n = 5). $GPP_{MAX}$ is the maximal gross primary production (µmol $O_2$ g DW · $h^{-1}$), $G_{MAX}$ is the maximal gross calcification (µmol $CaCO_3$ g DW · $h^{-1}$), *Ek* is the irradiance of saturation (µmol photons · $m^{-2}$ · $s^{-1}$), *Ec* is the irradiance of compensation (µmol photons · $m^{-2}$ · $s^{-1}$), *R* is the respiration rate (µmol $O_2$ · g $DW^{-1}$ · $h^{-1}$), and $G_D$ is the dark calcification rate (µmol $CaCO_3$ · g $DW^{-1}$ · $h^{-1}$).

| | Parameter | Primary production | | Calcification | |
|---|---|---|---|---|---|
| | | F | P | F | P |
| **Season** | $GPP_{MAX}/G_{MAX}$ | | | | |
| | Station A | 2.39 | **0.030*** | 2.23 | **0.030*** |
| | Station B | 2.61 | **0.010**** | 0.87 | 0.499 |
| | Station C | 2.30 | **0.024*** | 1.87 | **0.048*** |
| | ***Ek*** | | | | |
| | Station A | -1.36 | 0.188 | -1.88 | **0.028*** |
| | Station B | -1.27 | 0.141 | -1.84 | 0.055* |
| | Station C | 1.43 | 0.136 | -0.34 | 0.819 |
| | ***Ec*** | | | | |
| | Station A | 0.38 | 0.736 | -0.36 | |
| | Station B | 1.14 | 0.278 | -2.88 | |
| | Station C | 2.55 | **0.008**** | 1.03 | |

| | | | | |
|---|---|---|---|---|
| $R/G_D$ | | | | |
| Station A | -2.13 | **0.029*** | -1-95 | 0.116 |
| Station B | -2.82 | **0.008**** | 2.55 | **0.016*** |
| Station C | -2.92 | **0.008**** | -1.47 | 0.058 |
| | | | | |
| $Y_{MAX}$ | | | | |
| Station A | 2.51 | **0.032*** | | |
| Station B | 1.57 | 0.142 | | |
| Station C | 2.18 | **0.007**** | | |

1010
1011
1012
1013

**Table 6.** Summary of one-way Mont Carlo Permutation test followed by a post-hoc permutation test to compare the primary production-irradiance (*NPP-E*) and calcification-irradiance (*G-E*) parameters in *Lithothamnion corallioides* between Stations A, B, and C (n = 5). $GPP_{MAX}$ is the maximal gross primary production (μmol $O_2$ · g $DW^{-1}$ · $h^{-1}$), $G_{MAX}$ is the maximal gross calcification (μmol $CaCO_3$ · g $DW^{-1}$ · $h^{-1}$), *Ek* is the irradiance of saturation (μmol photons · $m^{-2}$ · $s^{-1}$), *Ec* is the irradiance of compensation (μmol photons · $m^{-2}$ · $s^{-1}$), *R* is the respiration rate (μmol $O_2$ · g $DW^{-1}$ · $h^{-1}$), and $G_d$ is the dark calcification rate (μmol $CaCO_3$ · g $DW^{-1}$ · $h^{-1}$).

| | Parameter | Primary production | | Calcification | |
|---|---|---|---|---|---|
| | | F | P | F | P |
| **Winter** | | | | | |
| | $GPP_{MAX}/G_{MAX}$ | 6.75 | **0.011*** | 9.12 | **0.002**** |
| | | **A = B > C** | | A = B > C | |
| | ***Ek*** | 4.29 | 0.068 | 3.59 | 0.117 |
| | | A = B = C | | A = B = C | |
| | ***Ec*** | 9.14 | **0.002**** | 6.20 | **0.032*** |
| | | **A = C > B** | | A = B = C | |
| | $R/G_d$ | 5.75 | **0.035*** | 8.97 | **< 0.001***** |
| | | A = B = C | | B > A = C | |
| | ***Ymax*** | 1.81 | 0.446 | | |
| | | A = B = C | | | |
| **Summer** | | | | | |

| | | | | |
|---|---|---|---|---|
| $GPP_{MAX}/G_{MAX}$ | 12.41 | **< 0.001*****| 11.12 | **< 0.001***** |
| | **A = B > C** | | A = B > C | |
| ***Ek*** | 4.80 | **0.046*** | 2.18 | 0.369 |
| | A = B = C | | A = B = C | |
| ***Ec*** | 10.39 | **< 0.001***** | 6.19 | **0.009**** |
| | C = A > B | | A = B = C | |
| $R/G_d$ | 5.35 | **0.001***** | 6.70 | **0.017*** |
| | A = B = C | | A = B = C | |
| $Y_{MAX}$ | 7.11 | **0.012*** | | |
| | A = B = C | | | |

**Table 7**. Summary of the post-hoc Kruskal-Wallis and Wilcoxon tests to compare gross primary production (*GPP*), net primary production (*NPP*), respiration (*R*), calcification at light ($G_l$) and at dark ($G_d$) rates in *Lithothamnion corallioides (L.c.)*, *Phymatolithon calcareum (P.c.)* and *Lithophyllum incrustans (L.i.)* (n = 5).

| **Comparison** | ***GPP*** | | ***NPP*** | | ***$Y_{MAX}$*** | | ***R*** | | ***$G_L$*** | | ***$G_D$*** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Species** | H | p-value | H | p-value | H | p-value | H | p-value | H | p-value | H | p-value |
| Winter | 12.0 | **0.002**** | 9.3 | **0.010**** | 8.8 | **0.012*** | 12.5 | **0.002**** | 9.0 | **0.011*** | 12.5 | **0.002**** |
| *Post-hoc Wilcoxon* | | | | | | *P.c. > L.i.* | | | | | | |
| | *L.i. > P.c. > L.c.* | | *P.c. = L.i.* | | *L.c. = P.c.* | | *L.i. > P.c. > L.c.* | | *L.c. = L.i. > P.c.* | | *L.c. > P.c. > L.i.* | |
| | | | *L.i. > L.c.* | | *L.c. = L.i.* | | | | | | | |
| *Summer* | 9.8 | **0.007**** | 9.98 | **0.007**** | 3.4 | 0.179 | 9.4 | **0.009**** | 9.7 | **0.008**** | 10.8 | **0.007**** |
| *Post-hoc Wilcoxon* | | | | | | | | | *L.i. > P.c.* | | | |
| | *L.i. > P.c. = L.c.* | | *L.i. > P.c. = L.c.* | | *L.c. = P.c. = L.i.* | | *L.i. > P.c. = L.c.* | | *L.c.=L.i.,* | | *L.c.> L.i.* | |
| | | | | | | | | | *L.c.=P.c.* | | *P.c. > L.i.* | |
| **Season at Station B** | | | | | | | | | | | | |
| *L. corallioides* | 4.8 | **0.028*** | 0.5 | 0.465 | 5.8 | **0.016*** | 6.8 | **0.009**** | 6.8 | **0.009**** | 6.8 | **0.009**** |
| *P. calcareum* | 0.0 | 0.916 | 4.8 | **0.028*** | 0.0 | 0.917 | 6.8 | **0.009**** | 6.8 | **0.009**** | 6.8 | **0.016*** |
| *L. incrustans* | 5.8 | **0.016*** | 0.1 | 0.754 | 6.9 | **0.009**** | 6.8 | **0.009**** | 6.8 | **0.009**** | 6.8 | **0.009**** |