



HAL
open science

Physiological differences among cryptic species of the Mediterranean crustose coralline alga *Lithophyllum stictiforme* (Corallinales, Rhodophyta)

Sophie Martin, Virgile Calvert, Anne Chenuil

► To cite this version:

Sophie Martin, Virgile Calvert, Anne Chenuil. Physiological differences among cryptic species of the Mediterranean crustose coralline alga *Lithophyllum stictiforme* (Corallinales, Rhodophyta). *European Journal of Phycology*, 2024, Volume 59 (2024 - Issue 2), pp.Pages 184-195. 10.1080/09670262.2023.2281484 . hal-04329191v2

HAL Id: hal-04329191

<https://hal.science/hal-04329191v2>

Submitted on 20 Feb 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

**PHYSIOLOGICAL DIFFERENCES AMONG CRYPTIC SPECIES OF THE
MEDITERRANEAN CRUSTOSE CORALLINE ALGA *LITHOPHYLLUM*
STICTIFORME (CORALLINALES, RHODOPHYTA)**

Sophie MARTIN*

CNRS, Sorbonne Université,

AD2M, Adaptation et Diversité en Milieu Marin, UMR 7144, Station Biologique de Roscoff,
Place Georges Teissier, 29680 Roscoff, France

E-mail address: sophie.martin@sb-roscoff.fr, Phone / Fax: +33 (0)2 98 29 56 59 / 23 24

Virgile CALVERT

Aix Marseille Univ, Avignon Univ, CNRS, IRD,

IMBE, Institut Méditerranéen de Biodiversité et d'Ecologie, Station Marine d'Endoume,
Chemin de la Batterie des Lions, 13007 Marseille, France

Anne CHENUIL

Aix Marseille Univ, Avignon Univ, CNRS, IRD,

IMBE, Institut Méditerranéen de Biodiversité et d'Ecologie, Station Marine d'Endoume,
Chemin de la Batterie des Lions, 13007 Marseille, France

Short running title: CCA cryptic species physiology

* Corresponding author: smartin@sb-roscoff.fr

ORCID: <https://orcid.org/0000-0002-1256-3674>

ABSTRACT

The crustose coralline alga *Lithophyllum stictiforme* is an important reef builder, forming coralligenous concretions in the Mediterranean Sea. This algal species complex is composed of eight cryptic species (or clades). The objective of our study was to compare light-dependent physiological processes within and between the two most abundant cryptic species (C1 and C4) of *L. stictiforme* from the Bay of Marseille (France). Physiological rates of respiration, photosynthesis and calcification were measured using incubation chambers under various irradiance levels and in the dark during the summer period. We compared the physiology of the C1 and C4 cryptic species of *L. stictiforme* among three algal groups: C1 (the most common in the Marseille area) from 28 m depth, C4 from the same site and depth, and C1 from a deeper site at 45 m depth. We found both interspecific (between C1 and C4) and intraspecific (within C1) physiological differences. Photosynthetic parameters showed acclimation to light (or depth) with higher values of initial slope of photosynthetic light response curve (α) and lower values of saturating irradiance (E_k) and compensating irradiance (E_c) for C1 from 45 m than for C1 and C4 from 28 m depth. At the shallower depth, significant physiological differences were observed between C1 and C4 with a higher value of maximum rate of gross photosynthesis ($P_{g\max}$) in C4, suggesting better ability to grow under high irradiance. Light and dark calcification rates differed only between C4 from 28 m and C1 from 45 m depth, being intermediate in C1 from 28 m depth. On a 24 h basis, diel net calcification rates were significantly higher in specimens from the shallower waters than from the deeper waters. Physiological differences suggest physiological plasticity within the regionally dominant cryptic species C1 and species-specific physiological traits between the cryptic species C1 and C4.

HIGHLIGHTS

- Photosynthesis and calcification rates differ among *Lithophyllum stictiforme* cryptic species
- The regionally dominant cryptic species exhibits light-dependent physiological plasticity
- Physiological differences occur between cryptic species found in similar environments

KEYWORDS: Calcification, coralline algae, cryptic species, depth, light, *Lithophyllum*, photo-acclimation, photosynthesis, physiology

INTRODUCTION

Crustose (nongeniculate) coralline algae (CCA) are marine red algae (Rhodophyta) of the family Corallinaceae. They are characterized by a calcareous thallus formed by the extensive crystalline calcium carbonate deposits found in the walls of their vegetative cells. CCA occur worldwide from polar regions to the tropics (Johansen, 1981; Steneck, 1986) and occupy the entire depth range inhabited by photosynthetic organisms, from the surface to depths below 200 m (Littler *et al.*, 1991); some grow under intense illumination, others grow in very dim light conditions. They are key ecosystem engineers that produce or consolidate biogenic benthic habitats (Nelson, 2009). In the Mediterranean Sea, CCA are the main builders of a typical habitat: coralligenous reefs, unique calcareous formations of biogenic origin (Ballesteros, 2006). The complex structure of the calcareous coralligenous concretions fosters the development of high species diversity comprised of taxonomic groups as diverse as algae, sponges, gorgonian corals, molluscs, echinoderms, bryozoans, polychaetes, tunicates, crustaceans or fishes, which live on these concretions as epiflora and epifauna, in the small holes and crevices as cryptofauna, inside the concretions as borers, and even in the sediment deposited in cavities and holes as endofauna (Ballesteros, 2006). Coralligenous reefs are considered the second most important Mediterranean benthic ecosystem in terms of biodiversity, after *Posidonia oceanica* meadows (Boudouresque, 2004) and provide a wide range of ecosystem services including original resources for local fisheries and beautiful seascapes for recreational scuba diving (Thierry de Ville d'Avray *et al.*, 2019).

Mediterranean coralligenous concretions develop under low-light conditions, at depths ranging from 20 to 120 m, where irradiance is reduced to 0.05-3% of the surface irradiance (Ballesteros, 2006). In these environments of limited light, the main coralligenous reef-forming CCA are species of the genus *Lithophyllum*, previously reported as *L. stictiforme* (previously known as “*stictaeforme*”) (Areschoug) Hauck (1877) and *L. cabiochiae* (previously known as “*cabiochae*”) (Boudouresque & Verlaque) Athanasiadis (1999) (Sartoretto *et al.*, 1996). The recent molecular phylogenetic assessment of this group of algae suggests that *L. cabiochiae* is not a taxonomically distinct species and is part of the *L. stictiforme* species complex (De Jode *et al.* 2019, Pezolesi *et al.* 2019). These common CCA species are known to play a major role in the development and biodiversity of Mediterranean coralligenous assemblages. They are the main *Lithophyllum* species that contribute to coralligenous concretions (Pezolesi *et al.*, 2019) and they favour establishment of other coralligenous reef species, with the recruitment of algae and invertebrates on their thalli (Piazzi *et al.*, 2022).

The *L. stictiforme* species complex is the single most important reef builder that is widespread across the whole Mediterranean Basin. The complex is made up of numerous genetically isolated, but morphologically similar species, named cryptic species (De Jode *et al.*, 2019; Pezolesi *et al.*, 2019). Not accounting for these cryptic species — a frequent phenomenon among marine species — may lead to an overestimation of the distribution range size (or niche width) when distinct cryptic species have distinct distributions (or niches, respectively) (Chenuil *et al.*, 2019). Such errors, particularly when they involve keystone species, may have serious consequences for ecosystem monitoring/management. In *L. stictiforme*, eight cryptic species (C1 to C8) are found in sympatry in the Bay of Marseille (north-western Mediterranean Sea; De Jode *et al.*, 2019). Spatial distributions of these cryptic species overlap, but their relative abundances are significantly differentiated according to depth and irradiance, suggesting that their niches are differentiated (De Jode *et al.*, 2019). Moreover, irradiance is the most important environmental factor shaping coralligenous communities (Ballesteros, 2006). The distribution of the cryptic CCA engineering species according to irradiance can thus potentially have a great influence on the composition of these communities (De Jode *et al.*, 2019). Although the cryptic species of the *L. stictiforme* complex are adapted to low irradiances, their light-dependent ecological and physiological traits are likely to vary among species.

Before the presence of cryptic species was revealed, Martin *et al.* (2013a,b) had studied physiological responses to irradiance of *L. stictiforme* (reported as *L. cabiochae* in that study) collected in the Bay of Villefranche (NW Mediterranean) at 25 m depth. They showed that both photosynthesis and calcification depend strongly on irradiance in this species. Although poorly documented, physiological differentiation has already been observed among cryptic marine red macroalgal species of the families Delesseriaceae and Rhodomelaceae (Kamiya *et al.*, 2014; Muanmai *et al.*, 2015). If this physiological differentiation is a response promoting adaptation to particular environments, then cryptic species may perform in different ways in response to specific environmental factors. As previously reported for other temperate coralline algae (Martin *et al.*, 2006; Qui-Minet *et al.* 2021), respiration, photosynthesis and calcification processes in *L. stictiforme* are strongly influenced by seasonal variations in irradiance and temperature and are the highest in summer when temperature and irradiance levels are maximal (Martin *et al.*, 2013b). In addition to their metabolism, both survival and reproduction have also been reported to depend on temperature and irradiance in *L. stictiforme* (as *L. stictaeforme* in that study) collected at 30-35 m depth in the NW Mediterranean (Rodríguez-Prieto, 2016). As

those investigations were carried out without reference to cryptic species, they thus overlooked potential differences in the responses among cryptic species to these environmental factors.

In the present study, we measured the physiological rates of *L. stictiforme* in the summer period, probably under optimal conditions of temperature and irradiance. In their natural environment along Marseille's coastline, the various *L. stictiforme* cryptic species are exposed to comparable temperature values because they generally thrive below the summer thermocline, located at a depth of between 16 and 20 m (Harmelin, 2004; Haguenaer *et al.*, 2013). Therefore, De Jode *et al.* (2019) considered irradiance as the main environmental factor differentiating between cryptic species. Here, we compared light-dependent physiological processes (i.e. photosynthesis and calcification) of two of the cryptic species of the *L. stictiforme* complex, one, clade C1, which is the most abundant along the Marseille coastline at depths ranging from 28 to 45 m and, the other, clade C4, which is less abundant and mainly found in the northern sector of the Bay of Marseille at relatively shallow depths (De Jode *et al.*, 2019). Because photosynthetic and calcification processes depend on the availability of light (Martin *et al.*, 2013a,b), we assessed the physiological performances of *L. stictiforme* cryptic species by measuring their specific photosynthetic parameters derived from photosynthesis-irradiance curves and their specific calcification rates under representative *in situ* irradiances. Photosynthetic and calcification responses under various irradiance levels allowed us (i) to identify potential physiological differences between the cryptic species and/or depths and (ii) to assess whether these reflect differences in the relative abundance of cryptic species across our study area. Two sites at two distinct depths were chosen to allow interspecific comparisons at similar depths and intraspecific comparisons at different depths.

MATERIALS AND METHODS

Studied area and biological material

Specimens of the crustose coralline algae *Lithophyllum stictiforme* species complex were collected in coralligenous habitats of the Bay of Marseille (France), a semi-opened embayment (about 40 km by 25 km, from the Gulf of Fos to the Riou Archipelago) located in the north-western Mediterranean Sea. Two cryptic species (or clades, C1 and C4) were chosen among the eight cryptic species found along the Marseille coastline, based on (i) their relative abundance, to obtain sufficient individuals of each species for the experiment and (ii) their field distribution, to have cryptic species coexisting in the same environment in at least one site (in syntopy) and being present in contrasting environments (different sites and depths). C1 and C4 are the most abundant cryptic species in the Bay of Marseille (De Jode *et al.*, 2019). On the one hand, they can be found at the same site. On the other hand, they have different environmental preferences: C1 is rare (~1% of sampled C1) in the shallowest waters (15-20 m depth) along the French Mediterranean coastline, but well represented in the deepest waters at depths ranging from 30 to 45 m (~80% at 28-31 m and ~20% at 37-46 m, Table S1). Conversely, C4 displays preference for the shallowest waters (~30% at 15-20 m and ~65% at 28-31 m), but is rare (~5%) below 30 m depth (Table S1). Individuals of the C1 and C4 cryptic species were sampled by scuba divers in two sites of the Bay of Marseille at distinct depths: (i) “La Couronne” (LC) in the northern sector of the Bay of Marseille at 28 m (43°19.668’N, 5°1.775’E) on 9 July 2018 and (ii) “Riou-Moyade” (RM) in the Riou archipelago in the southern sector of the Bay of Marseille at 45 m (43°10.574’N, 5°22.208’E) on 11 July 2018 (Fig. 1). According to De Jode *et al.* (2019), two cryptic species (C1 and C4) have been found at LC, while only one cryptic species (C1) was found in deep waters at RM. Light and depth were homogeneous for all collected specimens at each sampling site, even at LC, where C1 and C4 co-occur in similar light conditions. The slope of the substrate was similarly inclined at LC and RM, while light exposure of the substrate was to the south-west at LC and east at RM (De Jode, 2018).

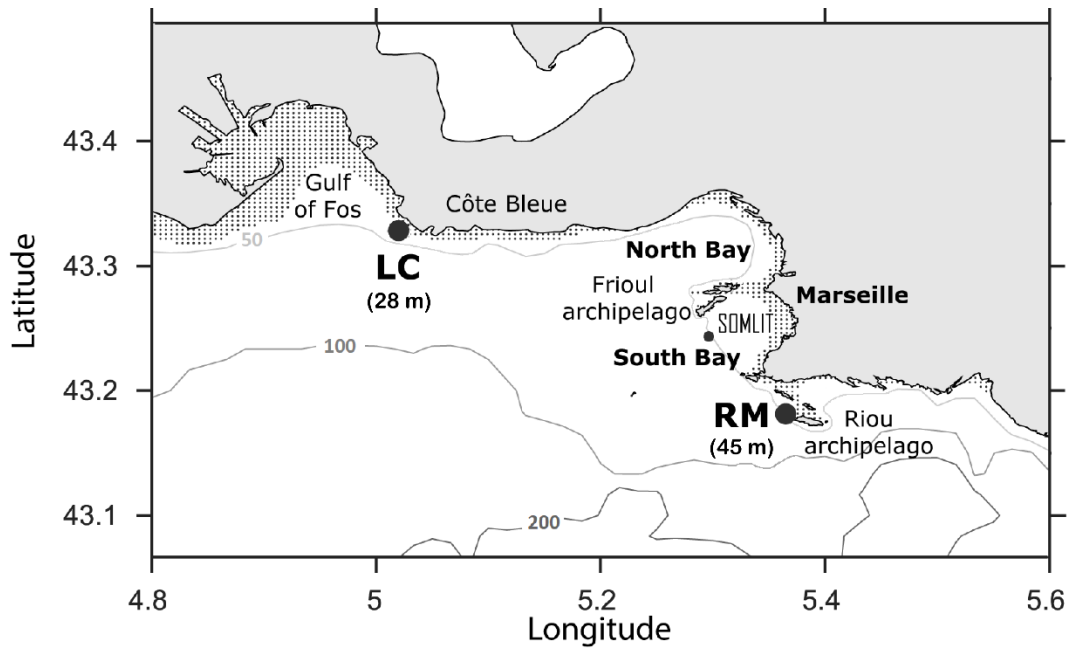


Fig. 1. Map of the study area, showing the locations of the two sampling sites in the Bay of Marseille: La Couronne (LC) at 28 m depth and Riou-Moyade (RM) at 45 m depth. SOMLIT: Frioul station (c. 65 m depth) of the French national network of coastal observation CNRS-INSU SOMLIT (*Service d'Observation en Milieu Littoral*).

Photosynthetically available radiation (PAR; $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) at 28 and 45 m in the Bay of Marseille was calculated from surface PAR and vertical PAR attenuation coefficient (K_{PAR}) for a typical month of July. K_{PAR} was calculated according to Kirk (1994) from vertical irradiance profiles obtained in the Bay of Marseille at the SOMLIT Frioul Station on four separate dates (11 and 25 July 2018, and 11 and 23 July 2019). The mean K_{PAR} were 0.094 and 0.085 m^{-1} at 28 m and 45 m depth, respectively. They were used in combination with the incident PAR at the surface to determine the daily cycle of irradiance at 28 and 45 m depth (Fig. 2). The incident PAR at the surface was measured on July 2007 using a flat quantum sensor (LI-COR, LI-192SA) at Saint-Jean-Cap-Ferrat located at similar latitude ($43,7^\circ$), 150 km east of Marseille on the French Mediterranean coast (Fig. 2). In the Bay of Marseille in July, the mean maximal PAR at noon was 135 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ at 28 m depth and 40 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ at 45 m depth and the mean daily values were 70 and 20 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, respectively.

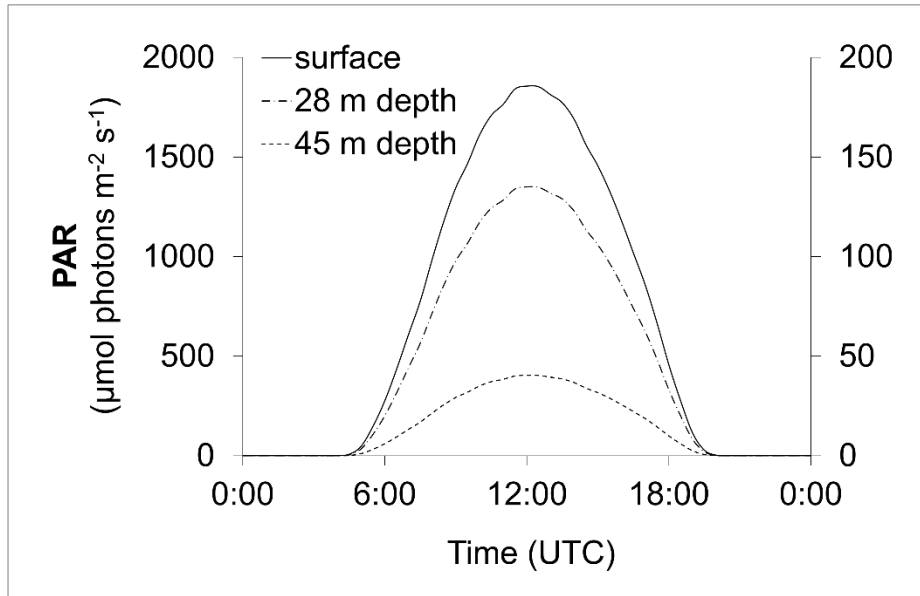


Fig. 2. Daily mean changes in incident photosynthetically available radiation (PAR) at the surface (left axis) and at 28 and 45 m depth (right axis) in July in the Bay of Marseille.

Algae were transported to the *Mediterranean Institute of Biodiversity and Ecology* (IMBE) in seawater in a thermostated tank to maintain samples at their *in situ* temperature (*c.* 16°C). Algae were thoroughly cleaned of epiphytic organisms without causing any damage to the thalli. LC samples were divided in two groups of algae according to their morphology. The first group of algae from LC was characterized by a thinner and slightly more purple and rougher thallus. It was identified as clade C4 by genetic analysis (see below). The second group of algae from LC was characterized by a thicker and slightly pinker and smoother thallus; it was then identified as clade C1 (Fig. 3). RM samples were all morphologically similar with a pink, smooth thin thallus. Their identification as clade C1 at this location by De Jode *et al.* (2019) was confirmed by genetic analysis. Differences in thickness, measured as surface-to-volume (S/V) and surface-to-weight (S/W) ratios were significant among algal groups ($p \leq 0.002$, Table 1, Fig. S1). Both ratios were the lowest in the clade C1 from LC (28 m depth) and significantly (50%) higher in the clade C1 from RM (45 m depth). The S/V and S/W ratios of clade C4 (LC) were intermediate and did not differ significantly from C1 from LC and RM.

From each of the three groups of algae (LC-C4, LC-C1 and RM-C1), 12 specimens with a flat thallus of *c.* 20 cm² were selected for the experiments. At the end of the experiments, the algal volume was measured by displacement of water, considering that the algal volume submerged equals the volume of seawater displaced. It was determined on a balance to the nearest 0.01 g and converted to ml using the density of seawater. The algal fresh weight was

determined to the nearest 0.01 g after drying with an absorbent paper to remove seawater. The algal surface area was determined from photographs using the software Image J Version 1.8.0.

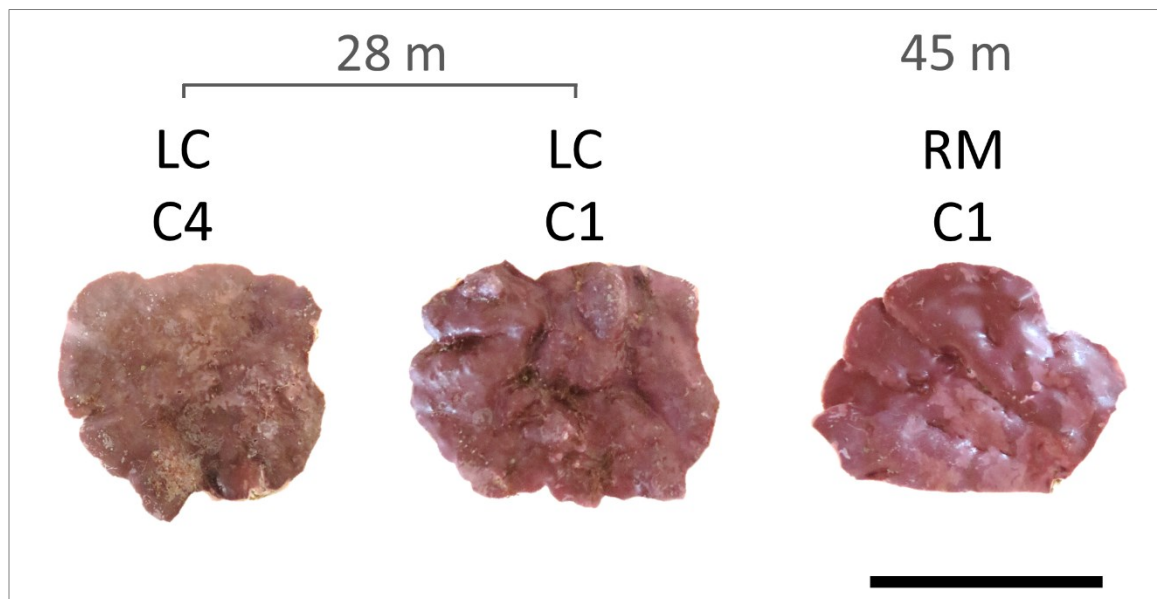


Fig. 3. Morphology of the *Lithophyllum stictiforme* cryptic species C1 and C4 from “La Couronne” (LC) at 28 m depth and “Riou-Moyade” (RM) at 45 m depth. Scale = 5 cm. (Photo © S. Martin).

Genetic analyses

To identify the cryptic species for each sample, a piece of thallus was cut from each individual and frozen at -20°C until DNA extraction after physiological experiments were achieved. DNA extraction, PCR (of partial 28S ribosomal DNA) and Sanger sequencing were performed according to De Jode *et al.* (2019).

Experimental setup

Algae were maintained in aquaria continuously supplied with natural Mediterranean seawater pumped at 5 m depth in the Bay of Marseille to prevent any change in seawater composition. Temperature in the aquaria was adjusted to 16.0°C and controlled in the aquaria to within $\pm 0.1^\circ\text{C}$ using a temperature controller (Model Teco TK 150). Salinity was measured with a conductivity probe (Horiba U-5000) and remained constant (37.9) during the experiment. The

pH on the total scale (pH_T) and total alkalinity (A_T) were measured according to the methods described in Qui-Minet *et al.* (2021). Temperature, salinity and parameters of the seawater carbonate system (pH_{T-16°C} = 8.11 ± 0.02, corresponding to pH_{T-25°C} = 7.98 ± 0.02, and A_T = 2560 ± 2 μEq kg⁻¹, *n* = 8) in the aquaria were in the range of those measured at the SOMLIT (*Service d'Observation en Milieu Littoral*) Frioul Station in the Bay of Marseille (Fig. 1) on 11 July 2018, at 32 m depth (T = 16.6°C, S = 38.1, pH_{T-25°C} = 7.97, and A_T = 2559 μEq kg⁻¹) and 55 m depth (T = 14.7°C, S = 38.2, pH_{T-25°C} = 7.94, and A_T = 2562 μEq kg⁻¹) (Wimart-Rousseau *et al.* 2020). They were representative of those measured in July at these depths in the Bay of Marseille (T = 15.6 ± 0.4°C, S = 38.2 ± 0.01, pH_{T-25°C} = 7.95 ± 0.01, and A_T = 2584 ± 8 μEq kg⁻¹, *n* = 12; SOMLIT dataset from 2016 to 2018).

The irradiance in the aquaria was set to mimic average daylight irradiance values in deep waters (30-45 m depth) of the Bay of Marseille in July. It was adjusted to *c.* 20 μmol photons m⁻² s⁻¹ with an Apogee SQ-120 quantum sensor under a controlled photoperiod of 12 h. The light was provided by a combination of Blue (450 nm) and Cool White (6500 K) LEDs (Alpheus Radiometrix EVO) above the aquaria.

Physiological measurements

Physiological measurements were performed after a one-day acclimation period under laboratory conditions. Algal physiology (photosynthesis, respiration and calcification) was assessed through two independent sets of light and dark incubation measurements. Physiological rates were measured in 100 or 185 ml transparent acrylic respirometry chambers (Engineering & Design Plastics Ltd, Cambridge, UK) filled with filtered seawater and continuously stirred with a magnetic stirring bar. The chambers were placed in a thermostated bath at 16.0°C, above which was installed similar light sources (Blue and Cool White LEDs) as above the aquaria. The intensity of photosynthetically active radiation (PAR) was adjusted using an Apogee SQ-120 quantum sensor and a computer-controlled system (Alpheus Radiometrix EVO).

Control incubations without algae were performed to check for biological activity in seawater. No significant activity (no difference from 0; t-test, *p* > 0,05) was detected in the seawater, whatever the irradiance levels.

Two sets of complementary measurements were performed in each group of algae: (i) a first set of incubations to determine photosynthetic parameters derived from the response

curves of photosynthesis to irradiance (P-E) under a range of irradiance levels from 0 to 200 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ and (ii) a second set of incubations to determine calcification rates in the dark and under representative *in situ* irradiances at *c.* 30 m and 45 m depth (i.e. 100 and 50 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, respectively).

(i) Photosynthetic parameters

The 12 selected specimens of each group of algae (LC-C4, LC-C1 and RM-C1) were incubated individually through independent dark and light incubations in 185 and 100 ml chambers, respectively. Respiration rates (R_d) were measured in the dark for 30 minutes after 1-hour exposure to the dark. Net primary production rates (P_n) were measured for 10 minutes at each irradiance level provides by the controlled LED-based light source in increasing orders (5, 10, 25, 50, 100, 150, and 200 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$). Oxygen concentrations were measured at the beginning and at the end of each irradiance level using a non-invasive optical fibre system (FIBOX 3, PreSens, Regensburg, Germany). Reactive oxygen spots in the chambers were calibrated with 0% and 100% oxygen buffers.

As recommended by Martin & Gattuso (2009) and Martin *et al.* (2013a,b) for species of the *L. stictiforme* complex (as *L. cabiochae*), physiological rates were normalized to thallus surface area since photosynthetic tissues are present in the living upper thallus layer of the crusts. Indeed, CCA metabolic rates are usually expressed per unit of crust surface to avoid any artefact due to the thickness of skeletal carbonate below the living coralline layer (Chisholm, 2003). This enables comparison of the physiological rates between algal groups of different surface-to-volume (or weight) ratios.

The rates of net photosynthesis (P_n) and dark respiration (consumption of O_2 , R_d) (in $\mu\text{mol O}_2 \text{ cm}^{-2} \text{ thallus h}^{-1}$) were calculated following Eq. (1):

$$P_n \text{ (or } -R_d) = (\Delta\text{O}_2 \times V) / (\Delta t \times S) \quad (1)$$

where ΔO_2 is the difference between the initial and final oxygen concentrations ($\mu\text{mol O}_2 \text{ l}^{-1}$), V is the volume of seawater in the chamber (the volume of the chamber minus the volume of the alga, l), Δt is the incubation time (h) and S is the thallus planar surface area (cm^2).

The response of the photosynthetic oxygen-evolving rate to irradiance (E , $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) was fitted with the model of Chalker *et al.* (1981). Photosynthesis versus irradiance (P_n - E) curve parameters were obtained for each specimen according to Eq. (2):

$$P_n = P_{g \max} \times (1 - e^{-\frac{E}{E_k}}) - R_d \quad (2)$$

where $P_{g \max}$ is the maximum rate of gross photosynthesis ($\mu\text{mol O}_2 \text{ cm}^{-2} \text{ thallus h}^{-1}$), E_k ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) is the saturating irradiance (irradiance at which the initial slope intercepts the horizontal asymptote, $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$). The initial slope of the P_n - E curve (α) was calculated as $\alpha = P_{g \max} / E_k$.

The compensation irradiance (E_c , $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) is the irradiance at which $P_n = 0$ (or $P_g = R_d$).

(ii) Net calcification rates

The rates of net calcification (gross calcification – dissolution) were measured through longer incubation times (from 1 to 2 h, depending on the chamber volume) necessary to obtain a significant signal to quantify the amount of CaCO_3 precipitated, based on measurements previously performed in this species at 16.0°C by Martin *et al.* (2013a). The first 6 of the 12 specimens of each group of algae (LC-C4, LC-C1 and RM-C1) were incubated individually in independent incubations in the dark and in the light at two irradiance levels of 50 and 100 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. Net calcification rates in the dark (G_d) were measured for 2 h in 185 mL chambers after 1-h exposure to the dark. Net calcification rates in the light (G) were measured at 50 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ for 1 h in 100 mL chambers and at 100 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ for 1.5-2 h in 185 mL chambers. G and G_d were estimated using the alkalinity anomaly technique (Smith & Key, 1975), which is a good estimator for short-term incubations. It is based on a decrease of 2 moles total alkalinity (A_T) by two equivalents per molecule of CaCO_3 precipitated (Wolf-Gladrow *et al.*, 2007). Seawater was sampled at the beginning and at the end of incubation, and A_T analyses were performed as previously described.

Net calcification rates (G or G_d) were calculated as Eq. (3):

$$G \text{ (or } G_d) = (\Delta A_T \times V) / (2 \times \Delta t \times S) \quad (3)$$

where ΔA_T is the difference between initial and final A_T ($\mu\text{Eq l}^{-1}$), V is the volume of the chamber (l), Δt is the incubation time (h) and S is the thallus surface of the algae (cm^2).

Diel carbon and carbonate production

Diel (24 h) P_n (or G) was calculated by summing daily P_n (or G) during the daylight period and R_d (or G_d) during the night-time period. Daily P_n was calculated by integrating the P_n versus E curves against the incident irradiance at 28 m (LC) or 45 m (RM) depth in July in the Bay of Marseille during the daylight period (Fig. 1). The rates of P_n and R_d in $\mu\text{mol O}_2 \text{ cm}^{-2} \text{ thallus h}^{-1}$ were converted to $\mu\text{mol C cm}^{-2} \text{ thallus h}^{-1}$ using the photosynthetic ($\text{PQ} = P_n \text{ O}_2 / P_n \text{ CO}_2 = 1$) and respiratory ($\text{RQ} = R_d \text{ CO}_2 / R_d \text{ O}_2 = 1$) quotients previously reported for species of the *L. stictiforme* complex (as *L. cabiochae*) (Martin *et al.*, 2013a). Daily G was estimated from linear relationship between 0 and mean maximal *in situ* incident irradiance (i.e. $100 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ at LC and $50 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ at RM) since the rate of calcification increases linearly with light until saturating around maximal *in situ* light intensities (Martin *et al.*, 2013a,b).

Statistical analyses

Results are expressed as mean \pm standard error of the mean (SE).

Differences in morphological and physiological parameters between algal groups were tested by using one-way ANOVA when normal distribution (Shapiro-Wilk test) and equality of variance (Levene's test) were verified. When necessary, data were log transformed to meet ANOVA assumptions. Otherwise, Kruskal-Wallis tests were performed. ANOVAs were followed by Tukey's honestly significant difference (HSD) post hoc tests and Kruskal-Wallis tests were followed by Mann-Whitney U-tests to separate sets of homogeneous data.

RESULTS

Genetic analyses

Nucleotide sequences confirmed that all specimens belonged to the clades C1 or C4 and that the three algal groups morphologically identified a priori were homogeneous (containing only C1 or only C4 specimens) so that our sample sizes for physiological analyses were balanced accordingly.

Photosynthetic parameters

Dark respiration (R_d) did not differ significantly among the three algal groups and averaged $0.11 \pm 0.01 \mu\text{mol O}_2 \text{ cm}^{-2} \text{ thallus h}^{-1}$ in terms of O_2 consumption (Table 1). The response of net photosynthesis in terms of O_2 production to irradiance (P_n - E curves) is presented in Fig. 4 for each algal group. Maximal gross photosynthesis ($P_{g \text{ max}}$) differed significantly between algal groups (Table 1). It was minimal in clade C1 from LC and RM, averaging $0.64 \pm 0.03 \mu\text{mol O}_2 \text{ cm}^{-2} \text{ thallus h}^{-1}$ and significantly higher in clade C4 from LC, reaching $0.93 \pm 0.08 \mu\text{mol O}_2 \text{ cm}^{-2} \text{ thallus h}^{-1}$. The mean ratio of $P_{g \text{ max}} / R_d$ was 6 ± 1 both in clade C1 from LC and RM. It was 2-fold higher (12 ± 3) in clade C4 from LC (Table 1). The saturating irradiance (E_k) and the compensation irradiance (E_c) were significantly lower in RM (45 m depth) samples than in LC (28 m depth) algal groups. Mean E_k was $25 \pm 2 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ in specimens from RM and was 3- to 4-fold higher in specimens from LC. It did not differ between clades C1 and C4 from LC, in which E_k averaged $88 \pm 11 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$. Mean E_c was 2-fold lower in specimens from RM ($5 \pm 1 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) than those from LC ($12 \pm 2 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$). The initial slope of the P_n - E curve (α) was 2-fold higher in specimens from RM than those from LC (Table 1).

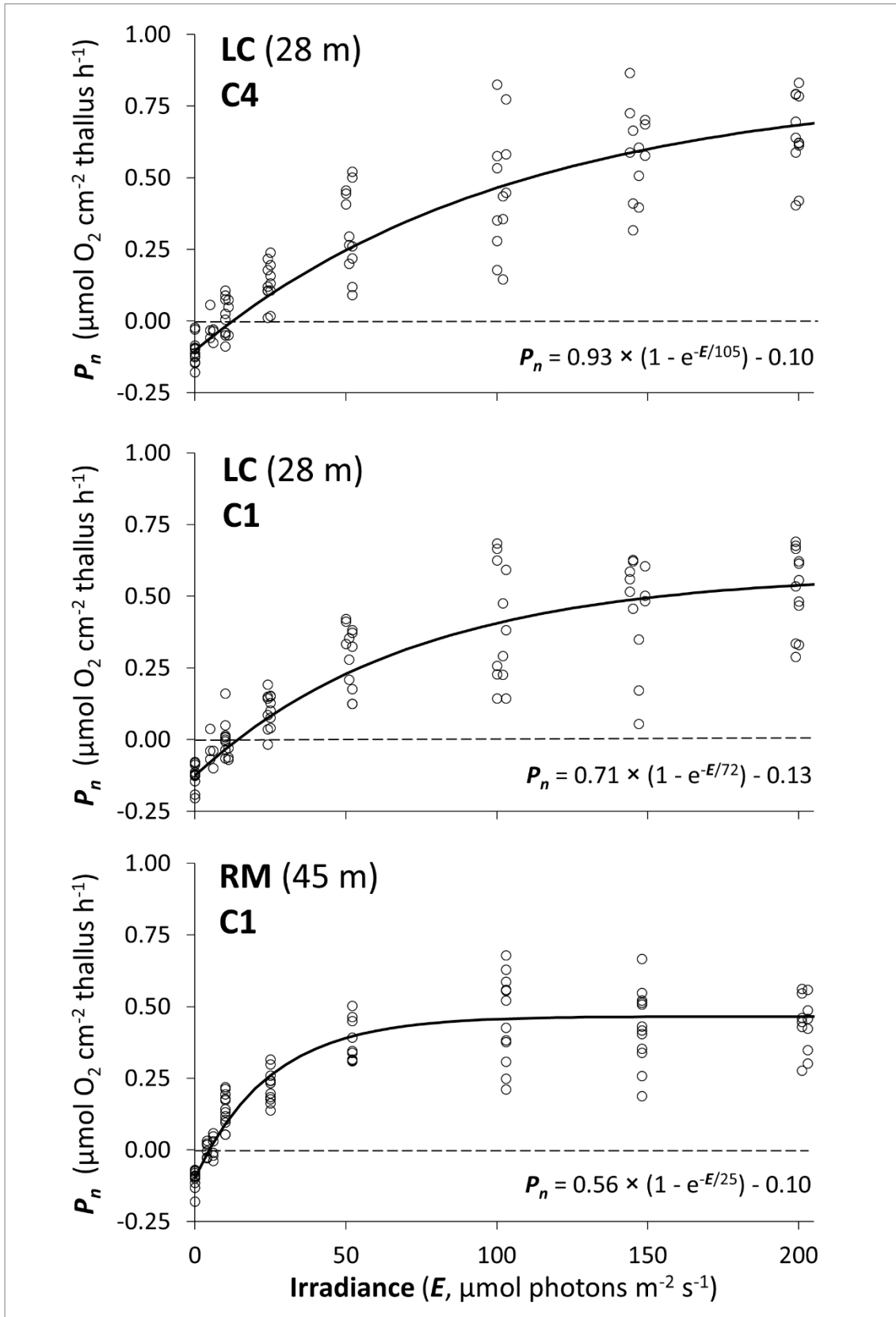


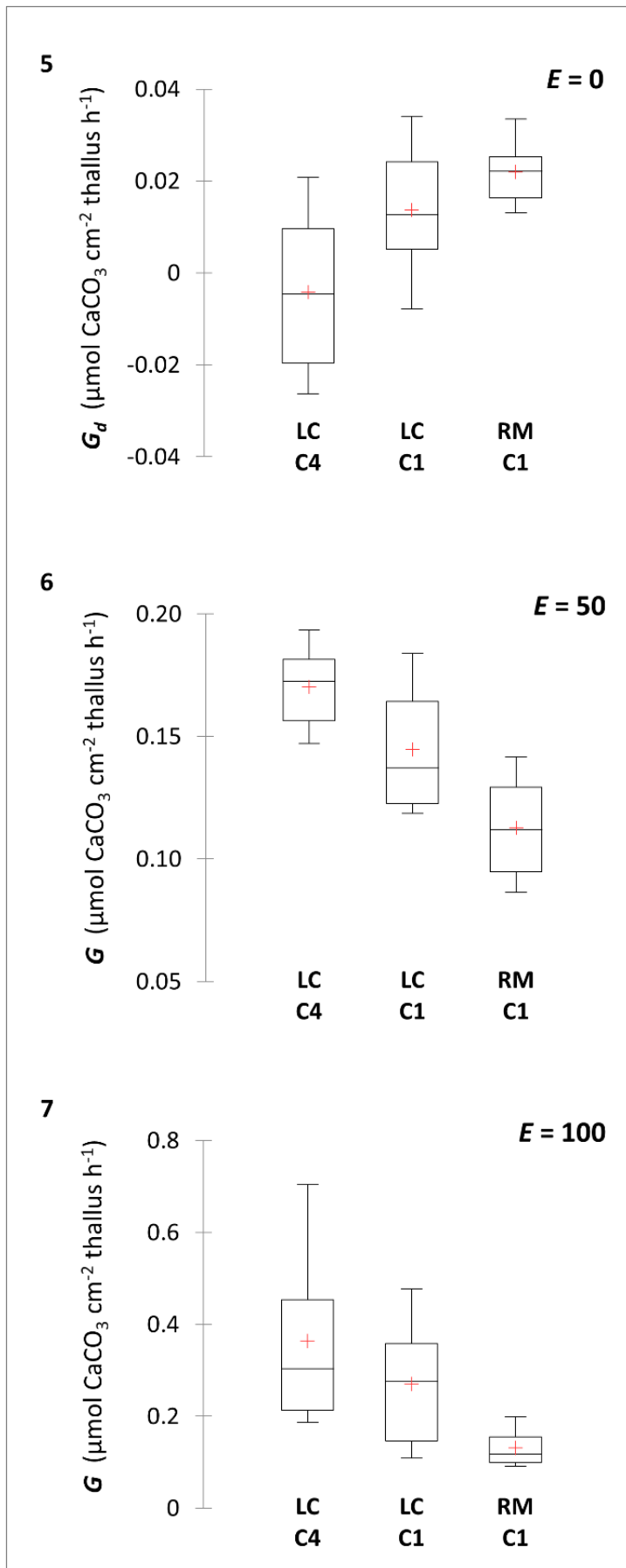
Fig. 4. Net photosynthesis versus irradiance curves in the three algal groups of the *Lithophyllum stictiforme* species complex: clade C4 from LC (LC-C4), clade C1 from LC (LC-C1) and clade C1 from RM (RM-C1). LC = 28 m depth and RM = 45 m depth.

Net calcification rates

Net calcification rates in the dark (G_d) significantly differed among algal groups. G_d were lowest in clade C4 from LC, intermediate in clade C1 from LC and highest in clade C1 from RM, averaging -0.004 ± 0.008 , 0.014 ± 0.006 and 0.022 ± 0.003 $\mu\text{mol CaCO}_3 \text{ cm}^{-2}$ thallus h^{-1} , respectively (Fig. 5, Table 1). Net calcification rates in the light (G) followed the opposite trends as G_d with the lowest rates in clade C1 from RM, intermediate in clade C1 from LC and highest in clade C4 from LC, averaging 0.11 ± 0.01 , 0.14 ± 0.01 and 0.17 ± 0.01 $\mu\text{mol CaCO}_3 \text{ cm}^{-2}$ thallus h^{-1} , respectively, under $50 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ and 0.13 ± 0.02 , 0.27 ± 0.06 and 0.36 ± 0.08 $\mu\text{mol CaCO}_3 \text{ cm}^{-2}$ thallus h^{-1} , respectively, under $100 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (Figs 6-7, Table 1).

Diel carbon and carbonate production

Diel (24 h) net organic C production (Diel P_n) was estimated to be highest in clade C4 from LC, intermediate in clade C1 from LC and lowest in clade C1 from RM (Table 1). Diel P_n was 2-fold higher in clade C4 from LC (4.6 ± 0.6 $\mu\text{mol C cm}^{-2}$ thallus d^{-1}) than in clade C1 from RM (2.3 ± 0.3 $\mu\text{mol C cm}^{-2}$ thallus d^{-1}). Diel net inorganic C production (Diel G) did not differ significantly between clades C1 and C4 from LC and averaged 3.7 ± 0.5 $\mu\text{mol CaCO}_3 \text{ cm}^{-2}$ thallus d^{-1} . Diel G was estimated to be 3-fold lower in specimens from RM than those from LC (Table 1).



Figs 5-7. Net calcification in the dark (G_d) and light (G) under irradiances (E) of 50 and 100 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ in the three algal groups (LC-C4, LC-C1 and RM-C1) of the *Lithophyllum stictiforme* species complex.

Fig. 5. G_d .

Fig. 6. G under 50 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$.

Fig. 7. G under 100 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$.

Each box plot has mean ("+" red cross in the box-plot), median (solid bar in the box plot), 25th-75th percentile (rectangular box) and 1.5*interquartile range (non-outlier range of the box whiskers).

DISCUSSION

In the present study, we identified differences in physiological performance between the two cryptic species C1 and C4 from the same depth and light environment, and within the dominant and ubiquitous cryptic species C1 from different depths and light environments. Light is indeed one of the most crucial factors that drive the distribution and physiological activities of macroalgae (Gattuso *et al.*, 2006). Red coralline algae are the deepest known marine benthic macroalgae. They are found down to depths of more than 200 m, where irradiance levels are less than 0.001% of the surface irradiance (*ca.* 0.01 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) (Littler *et al.* 1985). Their ability to acclimate/adapt to low light conditions is attributed to diverse physiological mechanisms. For example, algae living in deep habitats have low respiratory activity, a strategy to avoid excessive carbon losses (Littler *et al.*, 1986; Gomez *et al.*, 1997). Here, dark respiration rates (R_d) measured in *L. stictiforme* (0.10-0.13 $\mu\text{mol O}_2 \text{cm}^{-2} \text{thallus h}^{-1}$) collected at 28-45 m depths in the Bay of Marseille were in the low range of those reported for temperate CCA (see Martin *et al.*, 2013a for a review) and consistent with R_d values reported for species of this complex (as *L. cabiochae*) collected at 25 m depth in the Bay of Villefranche (NW Mediterranean Sea) and measured in similar temperature conditions (0.1-0.2 $\mu\text{mol O}_2 \text{cm}^{-2} \text{thallus h}^{-1}$; Martin *et al.*, 2013a,b).

Photosynthetic parameters derived from photosynthesis-irradiance curves indicate how well the three algal groups are adapted to their light environment. As a sublittoral species growing in deep water (28-45 m depth), *L. stictiforme* is typically adapted to chronic low light conditions, as indicated by low values of compensation irradiance (E_c). In the three groups of algae, the energy balance between gross photosynthesis (supply of energy) and respiration (energy used for maintenance and growth) was positive at very low irradiance levels ($E_c < 15 \mu\text{mol photons m}^{-2} \text{s}^{-1}$). The photosynthetic parameters E_c , E_k and α of *L. stictiforme* were strongly related to water depth and concomitant changes in light availability. The compensation irradiance E_c averaged 12 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ in C1 and C4 specimens from 28 m depth, whereas an irradiance of 5 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ was sufficient for photosynthesis to exceed respiration in C1 specimens growing at 45 m depth. Similarly, the saturating irradiance E_k was only 25 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ in deeper specimens, being 3.5-fold lower than in shallower specimens. The initial slope α was 2-fold higher in specimens from 45 m depth than in those from 28 m depth. Here, we demonstrated changes in E_k , E_c and α as an acclimation to ambient light within one cryptic species (C1). Photo-acclimation within species has already been observed in soft red macroalgae (Gomez *et al.*, 1997; Marquardt *et al.*, 2010) and CCA (Payri,

2001) from different light environments. This ability for photo-acclimation allows the *L. stictiforme* cryptic species C1 to grow in a wide range of light environments, as confirmed by its broad vertical distribution, at least from 20 to 46 m depth, and its ubiquitous distribution in the region of Marseille, where it is dominant in all sites sampled (De Jode *et al.*, 2019). Its ability to acclimate to various light niches may thus contribute to its large spatial distribution in this region.

Plasticity could not be studied in the cryptic species C4, which is very rare in the deepest sites along the French Mediterranean coasts (De Jode *et al.*, 2019). The C4 species appeared less adapted to low light environments than C1, as indicated by its high light requirements to saturate photosynthesis (E_k : 105 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$). In shallower waters of the Bay of Marseille (LC), where irradiance can reach up to 135 $\text{photons m}^{-2} \text{ s}^{-1}$ at noon in July, the cryptic species C4 appeared particularly efficient, as shown by the 30% higher rate of maximum gross photosynthesis ($P_{g \text{ max}}$) and the two-fold higher ratio of $P_{g \text{ max}}/R_d$ relative to C1. Such increased photosynthetic performance under higher irradiance levels could give the C4 cryptic species a competitive advantage in situations with higher incident irradiance, such as in summer, when algae are exposed to irradiance levels of more than 100 $\text{photons m}^{-2} \text{ s}^{-1}$ for several hours during daytime at 28 m depth in the Bay of Marseille. Accordingly, on a 24 h basis, we estimated diel net primary production to be approximately 30% higher in C4 than in C1 in shallow waters (LC). The C4 cryptic species is the second most abundant species after C1 in shallower waters in the northern area of the Bay of Marseille (De Jode *et al.*, 2019). Unsurprisingly, this species is relatively abundant at depths of less than 30 m (> 90% of all C4 individuals sampled along the French Mediterranean coastline), but rare at depths below 37 m, where ~20% of all C1 individuals were sampled (Table S1). In contrast, C1 is nearly absent (~1%) in the relatively shallow waters, from 15 to 20 m depth, where 30% of C4 were sampled along the French Mediterranean coastline.

We also observed differences among the three algal groups in their ability to calcify in response to light. The C4 cryptic species appeared well adapted to calcifying under *in situ* irradiance levels of 50-100 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, with the highest rates of calcification found in the *L. stictiforme* species complex, 20-30% higher than C1 collected in a similar light environment, and 2- to 3-fold higher than C1 sampled at 45 m depth. Conversely, C4 was the least efficient species for dark calcification, with negative rates of net calcification (dissolution) in the dark, where the C1 cryptic species from both depths exhibited positive rates of net dark calcification. Differences in calcification rates can be explained by species-specific differences in the ability of coralline algae to physiologically control the calcification process (Cornwall *et*

al., 2017). In particular, recent observations support a species-specific control of calcification traits in Mediterranean *Lithophyllum* cryptic species. Differences in cell-wall skeletal ultrastructure have recently been demonstrated in two morphologically similar, but genetically distinct sympatric species, *L. racemus* and *L. pseudoracemus*, even though they grow in similar conditions and depths (Basso *et al.*, 2022). In the light, strong correlations between calcification and photosynthetic processes have been reported in coralline algae (Pentecost, 1978; Borowitzka & Larkum, 1987; Chisholm, 2000; Martin *et al.* 2006, 2007, 2013a,b; Williamson *et al.*, 2017), and in particular in species of the *L. stictiforme* complex (Martin *et al.*, 2013a,b). Photosynthesis can effectively influence calcification by providing energy and substrates (Chisholm, 2003) or favourable conditions for CaCO₃ precipitation by locally raising the pH at the calcification sites and in the boundary layer (Koch *et al.*, 2013; Hofmann *et al.*, 2016; Cornwall *et al.*, 2017). Indeed, light calcification responses among our three *L. stictiforme* algal groups exhibited patterns similar to those of photosynthesis ($P_g \text{ max}$), with maximal rates observed in C4, minimal rates in C1 from 45 m depth and intermediate rates in C1 from 28 m depth. The highest calcification rates in C4 appear to confirm the competitive advantage of this cryptic species in high light conditions. Accordingly, C4 is dominant over C1 in the relatively shallow waters, from 15 to 20 m depth, along the French Mediterranean coastline (Table S1). At 28 m depth, C4 is less abundant than C1 in spite of notable light availability and could thus be restricted by other factors.

We demonstrated here that *L. stictiforme* calcification rates increase with increasing light intensity and with a concomitant increase in photosynthetic or light-triggered processes. However, the calcification process is not totally light-dependent because calcification can also occur in darkness. The continuation of calcification in the dark apparently relies on accumulated energy stored during periods of irradiance (McCoy & Kamenos, 2015). We have previously shown that calcification continues to occur in the dark in species of the *L. stictiforme* complex (as *L. cabiochae*) both during day and night (Martin *et al.*, 2013b). Here, net calcification rates in the dark were reduced, but remained positive in C1, while they became net negative (net CaCO₃ dissolution) in C4. The deeper specimens of C1 carried out calcification the most efficiently in the dark, with the highest rates of dark calcification. They were able to maintain 20% of their calcification rates in the dark, whereas specimens from 28 m depth had calcification rates that were more than 10-fold lower in the dark, compared with in the light. The better performance of the deeper specimens (45 m depth) for dark calcification confirms that they have very low light requirements for growth and are better acclimated to grow in deeper waters. Conversely, the shallower-depth C4 cryptic species was not able to maintain

positive net calcification in the dark in comparison to C1. However, on a 24 h basis, diel net carbonate production did not significantly differ between C1 and C4 from shallow waters, being 3- to 4-fold higher than in specimens from 45 m depth. The high rate of diel net calcification estimated for C4 is explained by its ability to compensate for night-time dissolution by high calcification rates during daylight. This can occur in summer under favourable conditions of light intensity and photoperiod length. However, C4 may be disadvantaged at the other seasons, when exposed to lower light levels and shorter photoperiod.

Despite physiological acclimation to low light conditions, specimens from deeper waters showed reduced metabolic activities and significantly lower diel (24 h) net carbonate production than those found in shallower waters. *L. stictiforme* thus appears to grow faster at 28 m depth (LC) than at 45 m depth (RM). Associated differences in morphological traits observed between C1 from different depths likely result from acclimation to depth, with reduced thallus thickness (higher surface-to-volume or -weight ratio) in the deeper specimens. Thallus thickness in coralline algae can reflect their growth strategies, i.e. vertical versus horizontal growth. The capacity of fast horizontal (marginal) growth of thin thalli, such as those found at 45 m depth, may thus be an advantage in deep water where light is reduced (Hanelt & Figueroa, 2012).

In conclusion, our results revealed physiological plasticity within the *L. stictiforme* cryptic species C1, which may contribute to its large spatial distribution from shallow to deep habitats. Physiological differences between cryptic species are compatible with the observed depth preferences of each species. C4 indeed displays a preference for relatively shallow waters, where it may hold an advantage over C1 through its better photosynthetic performance under high irradiance conditions (higher rate of maximum gross photosynthesis). Conversely, C4 is rare in deeper environments where it may be disadvantaged due to lower calcification rates where light is less available (lower ability to compensate for night-time dissolution).

To increase our knowledge on acclimation and differential adaptations in cryptic *L. stictiforme* species, future research should focus on physiological performances on an annual basis, considering seasonal changes in daylength, temperature and irradiance levels. The physiological activities under optimal summer conditions of temperature and irradiance need to be high enough to promote growth, reproduction and energy storage to cope with the reduced temperature and light availability during less favourable seasons (Hanelt & Figueroa, 2012). Cryptic *L. stictiforme* species may also differ in terms of their competitive ability or resistance to environmental stresses, which can also affect their distribution. Identifying cryptic species-specific physiological and ecological traits can help determine species' susceptibility to local

and global environmental stresses (Kato *et al.*, 2014). Studies on the specific cryptic species responses to environmental changes of *L. stictiforme* are thus needed for a better prediction of future changes in coralligenous habitats and Mediterranean coastal ecosystems.

ACKNOWLEDGEMENTS

We thank Dorian Guillemain from the diving service of the Observatory of Sciences of the Universe (OSU) Pytheas Institute and Bruno Belloni, Pascal Mirleau, and Sandrine Ruitton for their help in sample collection by scuba diving. We also thank Sacha Molinari for technical help during lyophilization and T rence Legrand for providing a map image file he has previously built. Data used in this work were partly produced through the technical facilities of the Molecular laboratory facility (SCBM) and the Experimental Aquaria facility (SCDE) of the IMBE (Marseille).

This work was funded by the CNRS-INEE initiative “Projets Exploratoires Premier Soutien (PEPS Adaptation 2017)”.

No potential conflict of interest was reported by the authors.

AUTHOR CONTRIBUTIONS

S. Martin: original concept, designed and performed the experiment, physiological measurements, data analyses, drafting and editing manuscript; V. Calvert: set up and performed the experiment; A. Chenuil: original concept, molecular analyses, editing manuscript.

REFERENCES

- Athanasiadis, A. (1999). The taxonomic status of *Lithophyllum stictaeforme* (Rhodophyta, Corallinales) and its generic position in light of phylogenetic considerations. *Nordic Journal of Botany*, 19: 735-745.
- Ballesteros, E. (2006). Mediterranean coralligenous assemblages: a synthesis of present knowledge. *Oceanography and Marine Biology: An Annual Review*, 44: 123-195.
- Basso, D., Piazza, G. & Bracchi, V.A. (2022). Calcification traits for cryptic species identification: Insights into coralline biomineralization. *PLOS One*, 17(10): e0273505.
- Borowitzka, M.A. & Larkum, A.W.D. (1987). Calcification in algae: Mechanisms and the role of metabolism. *Critical Reviews in Plant Sciences*, 6(1): 1-45.
- Boudouresque, C.F. (2004). Marine biodiversity in the Mediterranean: status of species, populations and communities. *Scientific Reports of Port-Cros National Park*, 20: 97-146.
- Chalker, B.E. (1981). Simulating light-saturation curves for photosynthesis and calcification by reef-building corals. *Marine Biology*. 63: 135-141.
- Chenuil, A., Cahill, A.E., Délémontey, N., Du Salliant Du Luc, E. & Fanton, H. (2019) Problems and Questions Posed by Cryptic Species. A Framework to Guide Future Studies. In *From Assessing to Conserving Biodiversity: Conceptual and Practical Challenges* (Casetta, E., Marques da Silva, J. & Vecchi, D., editors), pp. 77–106. Vol. 24. Springer, Cham.
- Chisholm, J.R.M. (2000). Calcification by crustose coralline algae on the northern Great Barrier. *Limnology and Oceanography*, 45(7): 1476-1484.
- Chisholm, J.R.M. (2003). Primary productivity of reef-building crustose coralline algae. *Limnology and Oceanography*, 48(4): 1376-1387.
- Cornwall, C. E., Comeau, S. & McCulloch, M. T. (2017). Coralline algae elevate pH at the site of calcification under ocean acidification. *Global Change Biology*, 23(10): 4245-4256.

De Jode, A. (2018). A study of coralligenous habitats biodiversity and of the influence of environmental factors using genetic tools: from engineer species populations to communities. / Etude de la biodiversité des habitats coralligènes et de l'influence des facteurs environnementaux par des approches génétiques : des populations d'espèces ingénieuses aux communautés. PhD thesis, Aix-Marseille Université, Marseille, 352 p.

De Jode, A., David, R., Haguenaer, A., Cahill, A. E., Erga, Z., Guillemain, D., Sartoretto, S., Rocher, C., Selva, M., Le Gall, L., Féral, J.-P. & Chenuil, A. (2019). From seascape ecology to population genomics and back. Spatial and ecological differentiation among cryptic species of the red algae *Lithophyllum stictiforme*/*L. cabiochiae*, main bioconstructors of coralligenous habitats. *Molecular Phylogenetics and Evolution*, **137**: 104-113.

Gattuso, J.-P., Gentili, B., Duarte, C. M. et al, Kleypas, J. A., Middelburg, J. J. & Antoine, D. (2006). Light availability in the coastal ocean: impact on the distribution of benthic photosynthetic organisms and their contribution to primary production. *Biogeosciences*, **3**, 489–513.

Gomez, I., Weykam, G., Kloser, H. & Wiencke, C. (1997). Photosynthetic light requirements, metabolic carbon balance and zonation of sublittoral macroalgae from King George Island (Antarctica). *Marine Ecology Progress Series*, **148**: 281-293.

Haguenaer, A., Zuberer, F., Ledoux, J.B. & Aurelle, D. (2013). Adaptive abilities of the Mediterranean red coral *Corallium rubrum* in a heterogeneous and changing environment: from population to functional genetics. *Journal of Experimental Marine Biology and Ecology*, **449**: 349-357.

Hanelt, D. & Figueroa, F.L. (2012). Physiological and photomorphogenic effects of light on marine macrophytes. *Seaweed Biology. Novel Insights into Ecophysiology, Ecology and Utilization*. B. K. Wiencke C. Berlin, Heidelberg, Springer 219.

Harmelin, J.G. (2004). Environnement thermique du benthos côtier de l'île de Port-Cros (Parc national, France, Méditerranée nord-occidentale) et implications biogéographiques. *Scientific Reports of Port-Cros National Park*, **20**: 173-194.

Hauck, F. (1877). Beiträge zur Kenntnis der adriatischen Algen. V. *Österreichische Botanische Zeitschrift*, 27: 292-293.

Hofmann, L.C., Koch, M. & de Beer, D. (2016). Biotic control of surface pH and evidence of light-induced H⁺ pumping and Ca²⁺-H⁺ exchange in a tropical crustose coralline alga. *PLOS One*, 11(7): e0159057.

Johansen, H. W. (1981). *Coralline Algae, a First Synthesis*, CRC Press, Boca Raton, FL.

Kamiya, M. & West, J.A. (2014). Cryptic diversity in the euryhaline red alga *Caloglossa ogasawaraensis* (Delesseriaceae, Ceramiales). *Phycologia*, 53(4): 374-382.

Kato, A., Hikami, M., Kumagai, N.H., Suzuki, A., Nojiri, Y. & Sakai, K. (2014). Negative effects of ocean acidification on two crustose coralline species using genetically homogeneous samples. *Marine Environmental Research*, 94: 1-6.

Kirk, J. (1994). *Light and Photosynthesis in Aquatic Ecosystems* (2nd ed.). Cambridge University Press, Cambridge. 400 pp.

Koch, M., Bowes, G., Ross, C. & Zhang, X.-H. 2013. Climate change and ocean acidification effects on seagrasses and marine macroalgae. *Global Change Biology*, 19(1): 103-132.

Littler, M.M., Littler, D.S., Blair S.M., & Norris, J.N. (1985) Deepest known plant life discovered on an uncharted seamount. *Science*, 227: 57-59.

Littler, M.M., Littler, D.S., Blair, S.M. & Norris, J.N. (1986). Deep-water plant communities from an uncharted seamount off San Salvador Island, Bahamas: distribution, abundance, and primary productivity. *Deep Sea Research*, 33: 881-889.

Littler, M.M., Littler, D.S. & Dennis Hanisak, M. (1991). Deep-water rhodolith distribution, productivity, and growth history at sites of formation and subsequent degradation. *Journal of Experimental Marine Biology and Ecology*. 150(2): 163-182.

- Marquardt, R., Schubert, H., Varela, D.A., Huovinen, P., Henríquez, L. & Buschmann, A.H. (2010). Light acclimation strategies of three commercially important red algal species. *Aquaculture*, **299**(1): 140-148.
- Martin, S., Castets, M.D. & Clavier, J. (2006). Primary production, respiration and calcification of the temperate free-living coralline alga *Lithothamnion corallioides*. *Aquatic Botany*, **85**: 121-128.
- Martin, S., Clavier, J., Chauvaud, L. & Thouzeau, G. (2007). Community metabolism in temperate maerl beds. I. Carbon and carbonate fluxes. *Marine Ecology Progress Series*, **335**: 19-29.
- Martin, S. & Gattuso, J.-P. (2009). Response of Mediterranean coralline algae to ocean acidification and elevated temperature. *Global Change Biology*, **15**(8): 2089-2100.
- Martin, S., Charnoz, A. & Gattuso, J.-P. (2013a). Photosynthesis, respiration and calcification in the Mediterranean crustose coralline alga *Lithophyllum cabiochae* (Corallinales, Rhodophyta). *European Journal of Phycology*, **48**(2): 163-172.
- Martin, S., Cohu, S., Vignot, C., Zimmerman, G. & Gattuso, J.-P. (2013b). One-year experiment on the physiological response of the Mediterranean crustose coralline alga, *Lithophyllum cabiochae*, to elevated $p\text{CO}_2$ and temperature. *Ecology and Evolution*, **3**(3): 676-693.
- McCoy, S.J. & Kamenos, N.A. (2015). Coralline algae (Rhodophyta) in a changing world: integrating ecological, physiological, and geochemical responses to global change. *Journal of Phycology*, **51**(1): 6-24.
- Muangmai, N., Preuss, M. & Zuccarello, G.C. (2015). Comparative physiological studies on the growth of cryptic species of *Bostrychia intricata* (Rhodomelaceae, Rhodophyta) in various salinity and temperature conditions. *Phycological Research*, **63**(4): 300-306.

Nelson, W.A. (2009). Calcified macroalgae – critical to coastal ecosystems and vulnerable to change: a review. *Marine and Freshwater Research*, **60**: 787–801.

Payri, C.E., Maritorena, S., Bizeau, M. & Rodière, M. (2001). Photoacclimation in the tropical coralline alga *Hydrolithon onkodes* (Rhodophyta, Corallinacea) from a French Polynesian reef. *Journal of Phycology*, **37**: 223-234.

Pentecost, A. (1978). Calcification and photosynthesis in *Corallina officinalis* L. using $^{14}\text{CO}_2$ method. *British Physiological Journal*, **13**: 383-390.

Pezzolesi, L., Peña, V., Le Gall, L., Gabrielson, P.W., Kaleb, S., Hughey, J.R., Rodondi, G., Hernandez-Kantun, J.J., Falace, A., Basso, D., Cerrano, C. & Rindi, F. (2019). Mediterranean *Lithophyllum stictiforme* (Corallinales, Rhodophyta) is a genetically diverse species complex: implications for species circumscription, biogeography and conservation of coralligenous habitats. *Journal of Phycology*, **55**(2): 473-492.

Piazzzi, L., Pinna, F. & Ceccherelli, G. (2022). Crustose coralline algae and biodiversity enhancement: The role of *Lithophyllum stictiforme* in structuring Mediterranean coralligenous reefs. *Estuarine, Coastal and Shelf Science*, **278**: 108121

Qui Minet, Z.N., Davoult, D., Grall, J., Delaunay, C., Six, C., Cariou, T. & Martin, S. (2021). Physiology of maerl algae: Comparison of inter- and intraspecies variations. *Journal of Phycology*, **57**(3): 831-848.

Rodríguez-Prieto, C. (2016). Light and temperature requirements for survival, growth and reproduction of the crustose coralline *Lithophyllum stictaeforme* from the Mediterranean Sea. *Botanica Marina*, **59**(2–3): 95–104.

Sartoretto, S., Verlaque, M. & Laborel, J. (1996). Age of settlement and accumulation rate of submarine 'coralligene' (-10 to -60 m) of the northwestern Mediterranean Sea; Relation to Holocene rise in sea level. *Marine Geology*, **130**(3-4): 317-331.

Smith, S. & Key, G. (1975) Carbon dioxide and metabolism in marine environments. *Limnology and Oceanography*, 20:493–495.

Steneck, R.S. (1986). The ecology of coralline algal crusts: Convergent Patterns and Adaptive Strategies. *Annual Review of Ecology, Evolution, and Systematics*, 17: 273-303.

Thierry de Ville d'Avray, L., Ami, D., Chenuil, A., David, R. & Féral, J.P. (2019). Application of the ecosystem service concept at a small-scale: The cases of coralligenous habitats in the North-western Mediterranean Sea. *Marine Pollution Bulletin*, 138: 160-170.

Williamson, C.J., Perkins, R., Voller, M., Yallop, M.L. & Brodie, J. (2017). The regulation of coralline algal physiology, an *in situ* study of *Corallina officinalis* (Corallinales, Rhodophyta). *Biogeosciences*, 14(19): 4485-4498.

Wimart-Rousseau, C., Lajaunie-Salla, K., Marrec, P., Wagener, T., Raimbault, P., Lagadec, V., Lafont, M., Garcia, N., Diaz, F., Pinazo, C., Yohia, C., Garcia, F., Xueref-Remy, I., Blanc, P.-E., Armengaud, A. & Lefèvre, D. (2020). Temporal variability of the carbonate system and air-sea CO₂ exchanges in a Mediterranean human-impacted coastal site. *Estuarine, Coastal and Shelf Science*, 236: 106641.

Wolf-Gladrow, D.A., Zeebe, R.E., Klaas, C., Körtzinger, A. & Dickson, A.G. (2007). Total alkalinity: The explicit conservative expression and its application to biogeochemical processes. *Marine Chemistry*, 106(1): 287-300.

Table 1

Comparison of morphological and physiological characteristics between the three algal groups (LC-C4, LC-C1 and RM-C1) of the species complex *Lithophyllum stictiforme*.

		Morphological characteristics			p-values
		LC-C4	LC-C1	RM-C1	
<i>Surface / Volume</i>		5.7 ± 0.4 ^{ab}	4.6 ± 0.4 ^a	7.0 ± 0.3 ^b	< 0.001
<i>Surface / Weight</i>		3.4 ± 0.3 ^{ab}	2.6 ± 0.2 ^a	3.9 ± 0.2 ^b	0.002
<i>P_n-E</i> curves parameters					
		LC-C4	LC-C1	RM-C1	
<i>R_d</i>		0.10 ± 0.01 ^{ns}	0.13 ± 0.01 ^{ns}	0.10 ± 0.01 ^{ns}	0.189
<i>P_{g max}</i>		0.93 ± 0.08 ^a	0.71 ± 0.06 ^b	0.56 ± 0.04 ^b	0.001
<i>P_{g max} / R_d</i>		12 ± 3 ^a	6 ± 1 ^b	6 ± 1 ^b	0.013
<i>E_k</i>		105 ± 22 ^a	72 ± 16 ^a	25 ± 2 ^b	< 0.001
<i>E_c</i>		12 ± 3 ^a	13 ± 2 ^a	5 ± 1 ^b	0.002
<i>α</i>		0.013 ± 0.002 ^a	0.013 ± 0.001 ^a	0.023 ± 0.001 ^b	< 0.001
Net calcification rates					
<i>E</i>		LC-C4	LC-C1	RM-C1	
<i>G_d</i>	0	-0.004 ± 0.008 ^a	0.014 ± 0.006 ^{ab}	0.022 ± 0.003 ^b	0.023
<i>G</i>	50	0.170 ± 0.007 ^a	0.145 ± 0.011 ^{ab}	0.113 ± 0.009 ^b	0.002
<i>G</i>	100	0.364 ± 0.082 ^a	0.271 ± 0.059 ^{ab}	0.131 ± 0.017 ^b	0.016[‡]
Diel (24-h) net carbon and carbonate production					
		LC-C4	LC-C1	RM-C1	
Diel <i>P_n</i>		4.6 ± 0.6 ^a	3.5 ± 0.4 ^{ab}	2.3 ± 0.3 ^b	0.008
Diel <i>G</i>		4.2 ± 0.8 ^a	3.3 ± 0.6 ^a	1.2 ± 0.1 ^b	<0.001[‡]

Results are expressed as mean ± SE ($n = 12$ for morphological characteristics, P_n - E curves parameters and diel P_n , and $n = 6$ for net calcification rates and diel G).

G_d are rates of net calcification (in $\mu\text{mol CaCO}_3 \text{ cm}^{-2} \text{ thallus h}^{-1}$) measured in the dark ($E = 0 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) and G are rates of net calcification measured in the light under 50 or 100 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. $P_{g \text{ max}}$ ($\mu\text{mol O}_2 \text{ cm}^{-2} \text{ thallus h}^{-1}$) is the maximum rate of gross photosynthesis, E_k ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) is the saturating irradiance, E_c ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) is the compensation irradiance and α is the initial slope of the P_n - E curve. Diel P_n and diel G are 24-h net photosynthesis and calcification rates (in $\mu\text{mol C or CaCO}_3 \text{ cm}^{-2} \text{ thallus d}^{-1}$).

The p-values from one-way analyses of variance (ANOVAs) ($df = 2,33$ for morphological characteristics, P_n - E curves parameters and diel P_n and $df = 2,15$ for net calcification rates and diel G) are shown at right. Bold type indicates significance ($p < 0.05$). Different subscripts (a and b) indicate significant difference between algal groups ($p < 0.05$, Tukey HSD post hoc tests); ns, non-significant difference ($p > 0.05$). Transformed data are indicated: [‡] $\log(x)$. Since E_k data did not meet the assumptions of equality of variance (Levene test), differences between groups were assessed by Kruskal-Wallis ANOVA H-tests followed by Mann-Whitney U-tests to separate sets of homogeneous data.

Supplementary Material

Table S1

Abundances of the eight cryptic species (or clades) of the *Lithophyllum stictiforme* complex found (a) along the French Mediterranean coastline and (b) along the Marseille coastline according to depth (data from Fig. 2 and Fig. 3 of De Jode *et al.*, 2019). The cryptic species C1 and C4 are presented in bold. We emphasize that sampling effort was much higher for sites between 28 and 31 m depth so the relevant information in this table is the relative abundances among depth categories. This does not represent absolute depth distributions.

Clades	C1	C2	C3	C4	C5	C6	C7	C8
(a) French Mediterranean coastline								
Depth 0 (15-20 m)								
Number of individuals (%)	4 (1%)	2 (4%)	11 (100%)	15 (31%)	0	7 (78%)	5 (36%)	0
Depth 1 (28-31 m)								
Number of individuals (%)	302 (81 %)	50 (93%)	0	31 (65%)	9 (100%)	2 (22%)	3 (21%)	0
Depth 2 (37-46 m)								
Number of individuals (%)	65 (18%)	2 (4%)	0	2 (4%)	0	0	6 (43%)	3 (100%)
Total number of individual samples	371	54	11	48	9	9	14	3
(b) Marseille coastline								
Depth 0 (15-20 m)								
Number of individuals (%)	0	0	0	0	0	0	0	0
Depth 1 (28-31 m)								
Number of individuals (%)	214 (77%)	41 (98%)	0	28 (93%)	9 (100%)	1 (100%)	3 (38%)	0
Depth 2 (37-46 m)								
Number of individuals (%)	65 (23%)	1 (2%)	0	2 (7%)	0	0	5 (63%)	3 (100%)
Total number of individual samples	279	42	0	30	9	1	8	3

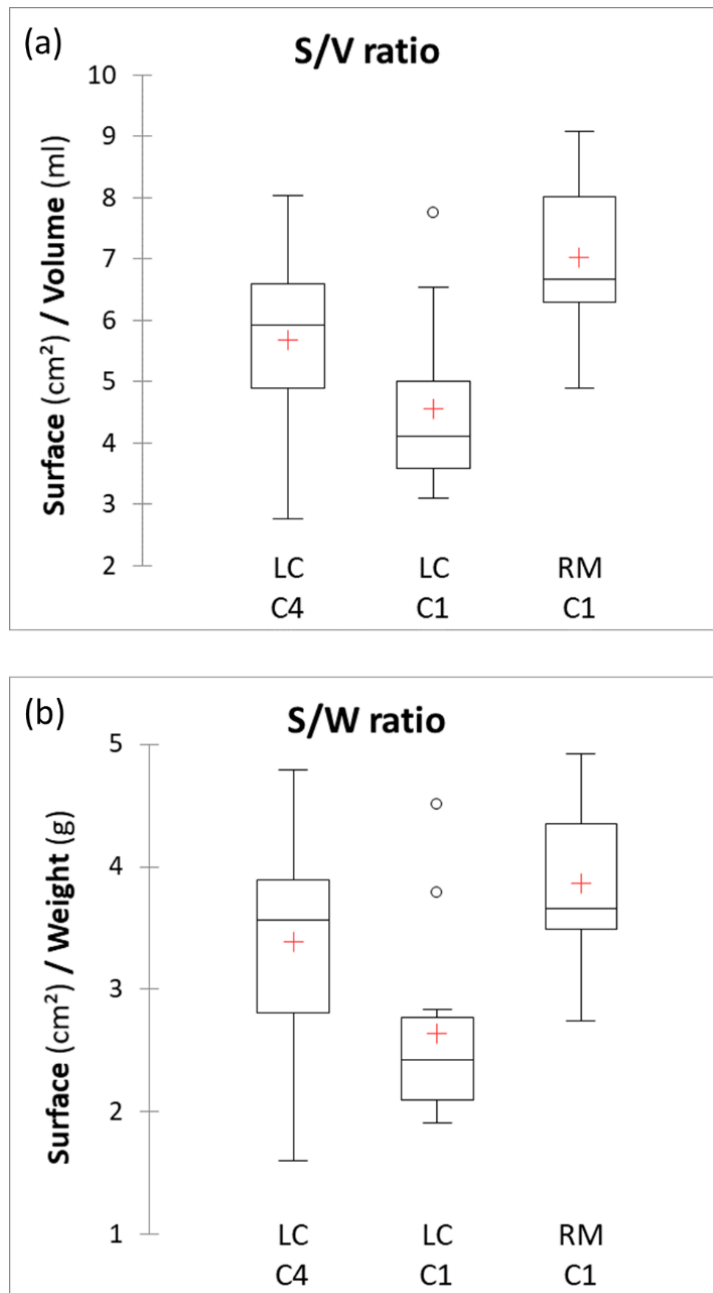


Fig. S1. Morphological characteristics of the three algal groups (LC-C4, LC-C1, and RM-C1) of the species complex *Lithophyllum stictiforme*: (a) surface area to volume ratio and (b) surface area to weight ratio. Each box-plot has mean ("+" red cross in the box-plot), median (solid bar in the box-plot), 25th to 75th percentile (rectangular box), 1.5*interquartile range (non-outlier range of the box whiskers), and outlier values (outside box whiskers). LC = 28-m depth and RM = 45-m depth.

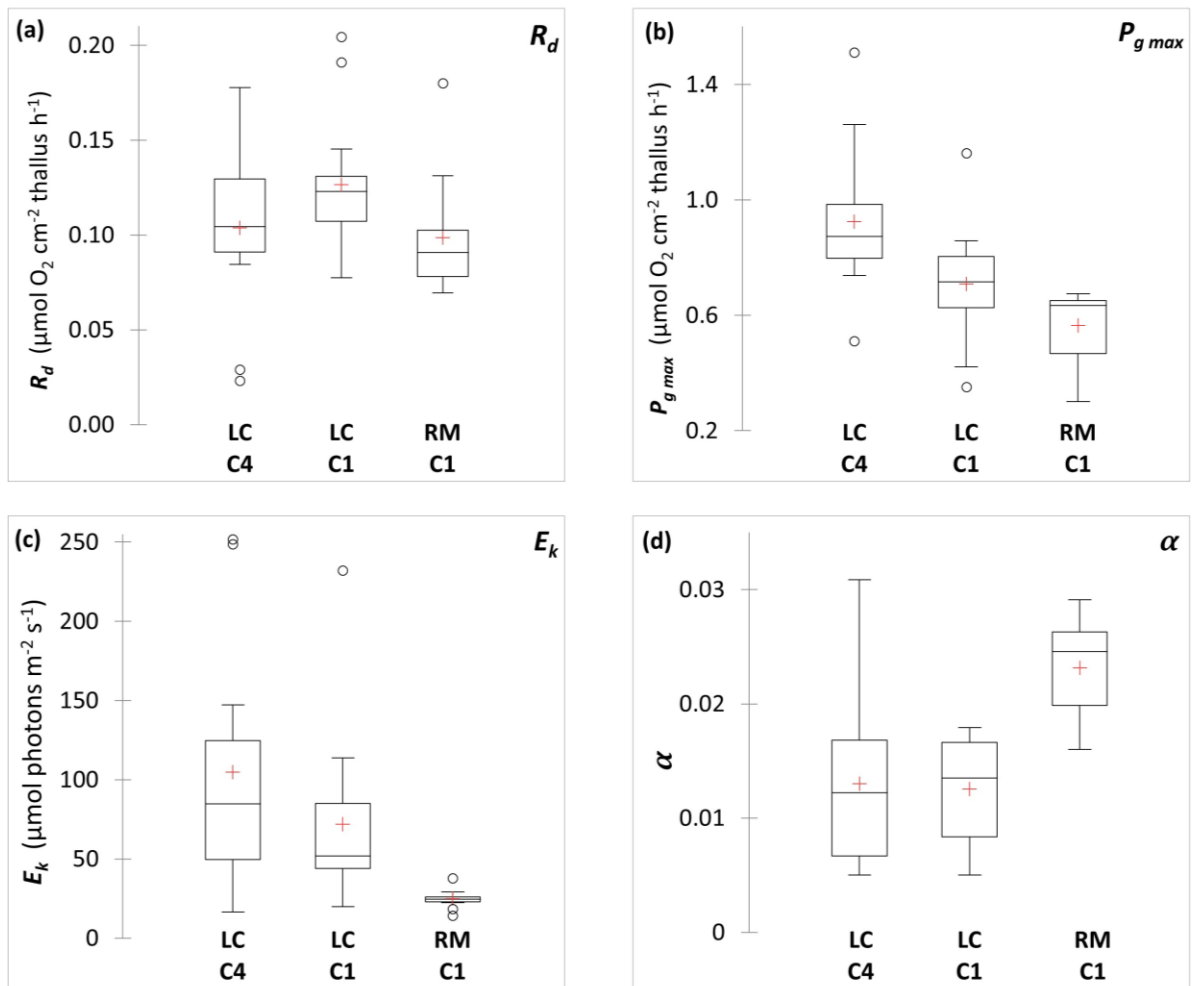


Fig. S2. Photosynthetic parameters of the P_n - E curves in the three algal groups (LC-C4, LC-C1, and RM-C1) of the species complex *Lithophyllum stictiforme*: (a) respiration rate (R_d), (b) maximal rate of gross photosynthesis ($P_{g\ max}$), (c) saturating irradiance (E_k) and (d) initial slope of the photosynthesis-irradiance curve (α). LC = 28-m depth and RM = 45-m depth.