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Estimation of photosynthesis and calcification rates of *Corallina elongata* Ellis and Solander, 1786, by measurements of dissolved oxygen, pH and total alkalinity*

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SUMMARY: Experiments were conducted on the calcareous red alga, *Corallina elongata*, a species representative of shallow water vegetal cover in Mediterranean areas with biomass ranging from 820 to 2544 gDW.m^{-2}, in order to estimate its productivity and calcification rates. Carbonate and oxygen budgets were estimated on samples incubated *in situ* under natural light cycles, by measuring initial and final dissolved oxygen, pH and alkalinity levels. In light conditions, oxygen concentrations and pH values increased as a consequence of oxygen production and carbon dioxide consumption due to the productivity process, and were a direct function of sample biomass. Strictly-reverse dynamics were recorded in dark conditions. A comparison of photosynthetic performances was conducted on a non-calcareous green alga, *Ulva rigida*, which showed higher rates of oxygen production and pH modification than *C. elongata,* but no significant change in total alkalinity. For *C. elongata*, a significant decrease in total alkalinity with incubation time was observed under light conditions, which was directly related to the algal sample biomass ($R^2 = 0.95$; n=16). Light to dark calcification ratio (L/D) was about 3.6. In these experiments, the photosynthetic quotient of *C. elongata* was 0.89, its net carbon productivity was 2.5 g C.m^{-2}.d^{-1}, gross production to daily respiration (Pg/R) was about 4.9 and its calcification rate was estimated at 13.8 g $CaCO_3$.m^{-2}.d^{-1}.

Key words: photosynthesis, calcification, dissolved oxygen, pH, total alkalinity, inorganic carbon, *Corallina elongata*, *Ulva rigida*.

RESUMEN: ESTIMACIÓN DE TASAS DE FOTOSÍNTESIS Y DE CALCIFICACIÓN EN *CORALLINA ELONGATA* ELLIS Y SOLANDER, 1786, A TRAVÉS DE MEDIDAS DE OXÍGENO DISUELTO, PH Y ALCALINIDAD TOTAL. – Los experimentos se efectuaron sobre el alga calcárea roja, *Corallina elongata*, un representante de aguas someras en áreas mediterráneas con biomasa comprendida entre los límites de 820 GDW.m^{-2} y 2544 GDW.m^{-2} para estimar su productividad y las tasas de calcificación. Las concentraciones de carbonato y oxígeno fueron estimados sobre muestras incubadas *in situ* bajo ciclos naturales de luz, midiendo el oxígeno disuelto al inicio y al final, así como los niveles de alcalinidad y pH. En condiciones de luz, las concentraciones de oxígeno y el pH aumentaron como consecuencia de la producción de oxígeno y el consumo de dióxido de carbono debido al proceso de productividad, y eran una función directa de la biomasa de la muestra. La dinámica estrictamente inversa fue registrada en condiciones de oscuridad. Una comparación del funcionamiento fotosintético se llevó a cabo sobre un alga no-calcárea verde, *Ulva rigida*, que presentó tasas más altas de producción de oxígeno y variación de pH que *C. elongata*, pero ningún cambio significativo en alcalinidad total. Para *C. elongata*, una disminución significativa en alcalinidad total con el tiempo de incubación fue observada en condiciones de luz, que directamente fueron relacionadas con la biomasa de muestra algal ($R^2 = 0.95$; n=16). La relación entre la calcificación a la luz y a la oscuridad (L/D) fue aproximadamente 3.6. En estos experimentos, el cociente fotosintético de *C. elongata* fue 0.89, su productividad neta de carbono, 2.5 g C.m^{-2}.d^{-1}, la relación producción total respecto a la respiración diaria (Pg/R), 4.9 y su tasa de calcificación, 13.8 g CaCO3.m^{-2}.d^{-1}.

Palabras clave: fotosíntesis, calcificación, oxígeno disuelto, pH, alcalinidad total, carbón inorgánico, *Corallina elongata*, *Ulva rigida*.

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INTRODUCTION

Calcification process studies in calcareous macroalgae are recent and mainly focus on coral-reef ecosystems which are the most striking example of calcifying ecosystems (Gattuso *et al.,* 1999). In the northwestern Mediterranean Sea, recent research (Canals and Ballesteros 1997; Massé 1999; Cebrián *et al.,* 2000) has studied coastal benthic ecosystems with a relatively high carbonate content and high carbonate production. These authors highlighted the need for a better knowledge of the macrophytobenthic and macrofaunal role in the carbon budget.

Photosynthetic and respiratory activities of marine organisms have an impact on the sea water content of various compounds such as oxygen, CO_2 and carbonate ionic forms (Smith and Key, 1975; Sournia 1982). Among the latter, bicarbonate ions (HCO_3^-) may be used as a potential carbon source for photosynthetic processes (Larkum *et al.*, 1989; Johnston *et al*., 1992; Bjôrk *et al*., 1992). However, in most cases, it is considered that in the natural environment photosynthetic and respiratory budgets do not change TA values due to the $CaCO_3$ oversaturation situation in surface waters (Sournia, 1982; Copin-Montégut, 1996). Moreover, during organic-matter biosynthesis, changes in the concentration of bicarbonates, one of the several ionic forms involved in TA values, are counterbalanced by equivalent changes in H^+ ionic forms. Thus, it is generally considered that only calcification or decalcification processes (due to the activities of calcareous living organisms and eroders) may significantly change the seawater TA values (Smith and Key, 1975; Chisholm and Gattuso, 1991). These organisms either assimilate HCO_3^- to precipitate $CaCO_3$ in their tissues in order to build their hard biological structures (e.g. coral, calcareous algae), or enhance carbonate dissolution by eroding and perforating the substrate for feeding or protection as performed by cyanobacteria, sponges, urchins, and fishes either in coral reefs (Le Campion-Alsumard *et al*., 1993; Harmelin-Vivien *et al*., 1992; Chazottes *et al*., 1995; Peyrot-Clausade *et al*., 1995a and b; Chazottes, 1996; Peyrot-Clausade *et al*., 2000; Tribolet and Payri, 2001) or in concretioning biota (Sartoretto, 1998).

The rhythm and nature of calcification/dissolution processes may be estimated in different ways but the measurement of TA changes in seawater is considered as the most convenient for short duration experiments (Smith and Key, 1975; Barnes, 1983; Chisholm and Gattuso, 1991). The respective influence of both bioconstruction and bioerosion activities of living organisms on dissolved carbonate budget and their natural rhythms have been extensively studied in coral reef environments (Smith and Kinsey, 1978; Sournia, 1982; Le Campion-Alsumard *et al*., 1993; Payri, 1997). On the other hand, in Mediterranean coastal marine environments, where calcareous algae thrive and dominate in a large range of habitats from the mediolittoral to the deepest part of the photic zone (Canals and Ballesteros, 1997; Cebrián *et al.*, 2000; Garrabou and Ballesteros, 2000), little is known about their potential influence on the TA budget and their carbonate utilisation. *C. elongata* is one of the most abundant calcareous macroalgae in the shallow waters of the northwestern Mediterranean, and forms the "infralittoral *Corallina elongata* community". It is considered as a major contributor to the calcium carbonate deposition in these media (Ballesteros, 1988). Unlike the other species studied by Garrabou and Ballesteros (2000), *C. elongata* is not a deep-water species but lives between 0 and 5 m depth. Many authors have indicated the presence of communities dominated by *C. elongata* on the coasts of the western Mediterranean (see Boudouresque, 1973; Gili and Ros, 1985; Ballesteros, 1988; Soltan *et al*., 2001 for reviews). But among recent works only those of Verlaque (1977), Ballesteros and Catalán (1983), Ballesteros (1988) and Soltan *et al*. (2001)—for the polluted zones—offer complete inventories of this type of community. A few works (Levavasseur, 1980 and 1987; Häder *et al*., 1996 and 1997) have previously reported photosynthetic rates of *C. elongata.* However, despite their ecological importance, to our knowledge *in situ* calcification rates have not yet been studied.

In this paper, we present the evolution of dissolved oxygen, pH and total alkalinity during dark or light *in-situ* incubation experiments of a calcareous *C. elongata* collected in the shallow waters of the Marseilles area, in relation to the biomass employed for the experiment. For comparison of the specificity of metabolic activity budget, its photosynthetic and respiratory rates were compared to those of a non-calcareous macroalgae, *Ulva rigida* C. Agardh, which is considered here as a blank for carbonate assimilation activity.

TABLE. 1. – Conditions of *Corallina elongata* and *Ulva rigida* incubations. n_i: number of samples per experiment; Lab: laboratory experiment; N: total number of samples. Irradiance values showed for each group of data are the averages. N_{DO}, N_{pH} and N_{TA} are total numbers of measurement averages for the parameters DO, pH and TA respectively.

Species	Light/dark	Date	T°C	Duration	Irradiance (W m^{-2})	n_i	Medium
Corallina elongata							
N = 57	L	January-98	13.5	5 h 30 min	378	4	*in situ*
N_{DO} = 41	L	February-98	13.0	7 h 30 min	382-443	10	*in situ*
N_{pH} = 23	L	March-98	13.0	2 h	193-756	10	*in situ*
N_{TA} = 16	L	March-98	14.0	6 h 30 min	134-578	10	*in situ*
	L	April-98	14.5	3 h 30 min	697	8	*in situ*
	L	January-99	16.0	6 h	367	7	*in situ*
	L	June-99	17.0	3 h 5 min	256	8	*in situ*
N = 13	D	March-98	13.5	14 h	0	5	*in situ*
N_{DO} = 13	D	January-99	17.0	14 h	0	5	lab.
N_{pH} = 16	D	May-99	17.5	12 h	0	6	lab.
N_{TA} = 14							
Ulva rigida							
N = 60	L	February-98	13.0	2 h	180-581	13	*in situ*
N_{DO} = 42	L	February-98	14.0	2 h 30 min	179-491	12	*in situ*
N_{pH} = 26	L	March-98	13.0	2 h	88-756	10	*in situ*
N_{TA} = 18	L	March-98	14.0	5 h 30 min	380	6	*in situ*
	L	April-98	14.5	2 h	347	8	*in situ*
	L	April-98	14.5	7 h 30 min	116	5	*in situ*
	L	January-99	16.0	6 h	367	6	*in situ*
N = 22	D	February-98	13.0	7 h 30 min	0	8	*in situ*
N_{DO} = 19	D	April-98	14.0	16 h	0	4	*in situ*
N_{pH} = 13	D	April-98	15.5	18 h	0	3	*in situ*
N_{TA} = 10	D	January-99	12.0	14 h	0	7	lab.

MATERIALS AND METHODS

Algae collection and seawater sampling

All the biological samples of *Corallina elongata* and *Ulva rigida* were collected at the same site (Anse des Cuivres-Gulf of Marseilles French Mediterranean coasts, 43.280°N, 5.347°E), a little creek of 5 m depth, always in the morning (between 07.00 and 09.00 h) and they were immediately examined under binocular in order to discard epiphytic organisms and damaged samples as well as possible. Bulk water samples used for incubation experiments were collected at the same moment and at the same place as biological material, filtered on a GF-C (Whatman) filter and twice assayed for their dissolved oxygen, pH and TA contents, which represent incubation initial values (To). Glass flasks (250 or 1000 ml) containing pieces of algal material were filled with the analysed seawater. For each experiment, a blank was realised by using the same seawater but without any algae samples, and was incubated and assayed at the end of the experiments in the same manner (twice assayed for DO, pH and TA).

Experimental conditions

The light experiments were conducted by immersion of the flasks at the sampling place (0.5-1 m depth). In order to provide a gentle flask agitation by the waves, they were left free at the end of a rope. The dark experiments were conducted under regulated temperature conditions corresponding to *in situ* temperature. Since agitation is suspected to play an important role in incubation results, a set of experiments was conducted for which samples were hand shaken every 30 min. Comparison between results (n=8) showed no significant difference.

Table 1 shows the experimental conditions. *In situ* temperatures were recorded by a sensor located near the flask and the light values by a LICOR pyranometer located on the roof of the laboratory and connected to a LICOR-185 fitted with a dedicated CALCOMETER to translate sensor output into light units ($W.m^{-2}$). Temperature and light measurements were sampled every 1.5 min and averaged every 30 min. The temperature of the experiments ranged from 13 to 17°C (temperature values of seawater between February and May) and in most cases incident light was higher than 300 $W.m^{-2}$. For light

experiments, duration ranged from 2 to 7 hours to cover different irradiance values. For long incubation times, irradiance changed considerably and the values shown in Table 1 for each group of data are the averages. When a group contains two experiments—or more—with different conditions, we give the minimal average of the experiment for which the irradiance was weakest followed by the maximal average. In order to obtain a significant response for changes in oxygen, pH and alkalinity in dark experiments, their time durations were sometimes continued up to 14 to 18 h. At the end of the incubations the incubated seawater was twice assayed for DO, pH and TA final determinations.

Biomass determination

The conversion of a metabolic activity rate in terms of production requires the determination of the biomass. Many temperature procedures have been used in previous studies (most of them for a 24 h drying duration): 60°C (Dudgeon *et al*., 1995 and Rosenberg *et al.,* 1995), 70–75°C (Buesa 1977), 80°C (Payri, 1987), 100°C (Ballesteros, 1991) and 105°C (Levavasseur, 1980 and 1987).

We used a temperature of 60°C (for 24 h), as recommended by Dudgeon *et al*. (1995) and Rosenberg *et al.* (1995). Results were expressed per gram of dry weight at 60°C (gDW). The alga samples were rinsed with distilled water and treated in a furnace at 60°C for 24 h for a precise dry weight (DW) determination and at 105°C for comparison between the 60°C and 105°C drying methods. The ratio of algal DW (g) to volume of incubated seawater (l) was lower than unity, except for 13 samples of *C. elongata* (the ratio was from 1 to 4).

The mineral fraction was estimated from the determination of the ash weight (AW) by treating samples (n=55) at 450°C for 24 h. We also conducted comparison with hydrochloric acid (HCl, 0.1 N) for 24 h. The weight of dry organic matter (DOMW) was deduced by the equation: DOMW=DW - AW.

In order to try to extrapolate our results of organic and inorganic daily productions of natural populations of *C. elongata* in the experimental area (Anse des Cuivres-Gulf of Marseilles), we estimated month-to-month the coverage of *C. elongata* living biomass using a monthly dual subsampling (10/10 cm average of the two subsamples) inside the same sampling place (70/70 cm). *C. elongata* biomass coverage varied from 820 to 2544 g DW.m^{-2}, with 1564±626 g DW.m^{-2} (n=10) as the mean value.

Chemical analysis and calculations

The pH measurements (two for each water samples) were performed on a Beckman phmeter (mod. PHI-34 with automatic temperature compensation) fitted with a combined pH sensor (internal Ag/AgCl reference). Prior to all the pH measurements, a calibration procedure was achieved by using a commercial buffer (pH 4.01 ref. 9437, pH 7.01 ref. 9439 and pH 10.01 ref. 9461, MERCK). The manufacturer's technical specifications of the pH meter are lower than 5.10^{-4} for resolution and 2.10^{-3} for precision (standard error). A test for the stabilisation of the readings is achieved electronically by the hardware (the value is retained when no change in the reading occurs within 30 sec). For all the pH measurements presented here, the recorded precision (standard error) is 0.001 (n = 93).

The pH values were corrected (Culberson, 1980; Aminot and Chaussepied, 1983) for *in-situ* and laboratory temperature differences, which never exceeded 2.0 to 3.0°C.

The total alkalinity (TA) is defined as the excess of dissolved basic radicals in seawater which can be balanced by an excess of acid and expressed in milliequivalent per litre (mEq.l^{-1} e.g. 2 equivalent per mole of $CaCO_{3)}$. The method used to measure total alkalinity (TA) is that currently known as the "pH-method" or "alkalinity anomaly" of Strickland and Parsons (1972). The mixture [seawater sample + acid (HCl, 0.01N, titrisol-Merck)] is bulled by air, which had previously passed first through an NaOH (10 N) solution and then an HCl (1 N) solution, in order to remove free CO_2 as recommended by Smith and Kinsey (1978). For each seawater sample to be examined (one at the start, the other at the end of incubations), TA values were measured on two subsamples and for each of them two pH measurements were made. Out of ten (10) replicates, we obtained a standard deviation of 0.003 mEq.l^{-1} (n=10).

After correction for temperature and salinity (tables in Strickland and Parsons, 1972), mean values were expressed in milliequivalent per litre (mEq.l^{-1}), or in mg of $CaCO_3$, by the multiplication of TA values by _$CaCO_3$ molecular weight (50.05).

Dissolved oxygen concentrations in seawater were measured by Winkler method's as modified by Carpenter (1965) and Carritt and Carpenter (1966) on two subsamples for each seawater sample. In the figures and tables presented here, all the experimental values are the average of the two aliquots. For all

the DO measurements presented here, the recorded precision is 0.004 (n= 93).

Assuming that the hourly production rates may be considered as stable for calcareous algae, the respective estimations of daily net productivity (on DO) and daily calcification were conducted on a 12/12 photoperiod basis using an extrapolation of the hourly production rates measured during a few hours in daylight to a calculation of daily production rates.

With the hourly net-photosynthetic activity rates (p, in ml $DO.g^{-1}DW.h^{-1}$) and the mean value of the hourly respiration rates measured in dark conditions (r, ml $DO.g^{-1}DW.h^{-1}$), the gross production (Pg) was calculated by the following equation:

$$Pg = (p + r) * 12 \text{ (in ml } DO.g^{-1}DW.d^{-1})$$

Then R (the 24 h respiratory activity) was subtracted from Pg in order to obtain the 24 h net production (Pn) based on DO measurements:

$$Pn = Pg - R \text{ (in ml } DO.g^{-1}DW.d^{-1})$$

(with: $R = r * 24$ (in ml $DO.g^{-1}DW.d^{-1}$), assuming r constant over 24 h).

The carbon mass budget from Pn values was calculated by the following equation:

$$mg\ C = mg\ DO*(12/32).Qp^{-1}$$

(Where 12 and 32 are the respective carbon and oxygen mass and Qp is the above-mentioned photosynthetic ratio).

Photosynthetic quotients calculation

In order to express photosynthetic activities in milligrams of carbon (mg C), we calculated photosynthetic quotients according to the following method:

The total CO_2 change or $dTCO_2$ (TCO_2: the sum of CO_2, H_2CO_3, HCO_3^- and CO_3^{2-}) in the incubation flask is the sum of total inorganic carbon changes due to calcification (dC_i_calc) and to photosynthesis/respiration (dC_i_p-r). The carbon dioxide budget may be expressed as

$$dTCO_2 = dC_i\_calc + dC_i\_p\text{-}r \qquad (1)$$

The proper contribution of the calcification process (dC_i_calc) to this budget can be deduced from the general calcification formula:

$$2HCO_3^- + Ca^{2+} \leftrightarrow CaCO_3 + CO_2 + H_2O \qquad (2)$$

where the TA value is decreasing by 2 equivalent per litre when one mole of $CaCO_3$ is biologically mineralised, with the formation of one mole of CO_2. This is stoichiometrically true in freshwater, but the CO_2 formation in marine waters is slightly lower than the unit (≈ 0.6 -Frankignoulle *et al.* 1994; Copin-Montégut, 1996) as a consequence of the salt buffer effect. Equation 1 can be now written as

$$dTCO_2 = 1/2\ dTA + dC_i\_p\text{-}r \qquad (3)$$

By measuring initial and final pH and TA values in our experiments, and knowing temperature, salinity, by using Strickland and Parsons tables (1972) we can estimate the total inorganic carbon levels (TCO_2). The changes in carbon dioxide related only to photosynthesis and respiration (dC_i_p-r) can now be calculated from Equation (3). Knowing the changes in CO_2 levels in this way and the dDO production by direct measurements, we now calculate the photosynthetic quotient $Qp=dO_2/-dC_i$_p-r for all our experiments.

RESULTS

Mineral and organic Biomass determination

A comparison between the 60°C and 105°C drying methods over 56 samples of *C. elongata* shows the mean ratio DW105/DW60 to be 0.989 ± 0.017 (m±sd), a value which is not significantly different to the unit. By using successively a temperature of 60°C for the DW determination and 450°C for the ash weight (AW), it was found for *C. elongata* that the carbonate content of dry algal samples is 82.8±3.10% (n=56) and dry organic matter (DOMW) is 17.2±3.10% (n=56). The ratio of dry matter (DW) to organic matter (DW/DOMW) for *C. elongata* is 5.97±0.94 (n=55), a ratio which is close to the value of 6 mentioned by Levavasseur (1980).

Comparative decalcification with hydrochloric acid (HCl, 0.1 N) for 24 h was conducted on samples of *C. elongata*. It showed equivalent values of the carbonate content (85±2.45%; n=10) and organic matter (15±2.45%; n=10) of dry algal samples.

Productivity and respiratory estimation

Figure 1 shows the variations of DO (dDO) during light and dark experiments with *C. elongata* and

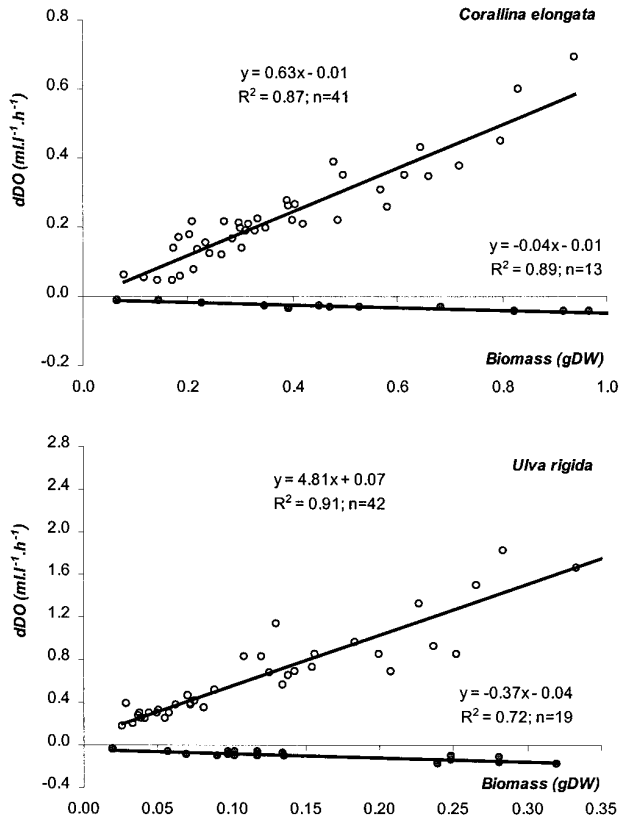

FIG. 1. – Hourly rate of dissolved oxygen variations in millilitres per litre of seawater (DO in ml·l^{-1}) as a function of the biomass (in grams of dry weight, DW) of *C. elongata* and *U. rigida* (respectively) placed in experiment during light (open circle) and dark (dots).

U. rigida respectively. For *C. elongata*, with biomass dry weight ranging from 0.1 to 9.6 g, the dDO per hour *vs* biomass under light exhibited a highly-significant positive relationship (R^2=0.87; n=41; p<0.0001), with a value of the intercept to the origin (-0.008) that was not significantly different from zero. The mean productivity calculated was 0.608±0.165 ml DO.g^{-1}DW.h^{-1} (n=41). During the dark experiments, the difference between final and initial oxygen values was always negative (oxygen consumption), with a highly significant negative relationship (R^2=0.89; n=13). The mean hourly respiration calculated was –0.069±0.031 ml DO.g^{-1}DW.h^{-1} (n=13).

For *U. rigida*, the hourly rate of oxygen production per biomass unit at light was almost 9 times higher than for *C. elongata*: 5.610±1.120 ml DO.g^{-1} DW.h^{-1} (n=42). This regression line between dDO and biomass dry weight (Fig. 1) exhibited a positive intercept (0.07) with a highly significant relationship (R^2=0.91; n=42; p<0.0001). In the dark experiments, DO concentrations decreased with a highly significant negative relationship with biomass dry weight (R^2=0.72; n=19). The mean hourly calculated respiration for this non-calcareous alga was –0.800±0.383 ml DO.g^{-1}DW.h^{-1} (n=19), which was 11.6 times higher than for *C. elongata*.

The daily net productivity (calculated from mean values of hourly rate oxygen productivity and respiration as indicated in Materials and Methods) was 6.47 ml DO.g^{-1}DW.d^{-1} for *C. elongata* and 57.72 ml DO.g^{-1}DW.d^{-1} for *U. rigida*. The Pg/R ratios (i.e. gross photosynthesis to daily respiration) were 4.9 for *C. elongata* and 4.0 for *U. rigida*.

PH variations and DO-pH coupling

During the light experiments of *C. elongata,* the pH variations increased regularly over time and the dpH.dt^{-1} (differences between pH initial and final values) were highly-significantly linked (Fig. 2) to the biomass amount (R^2=0.89; n=23; p<0.001). The mean hourly rate of pH increasing per biomass unit of *C. elongata* was 0.042±0.014 UpH.g^{-1}DW.h^{-1} (n=23). In dark experiments, opposite trends were registered for the two species, with a significant

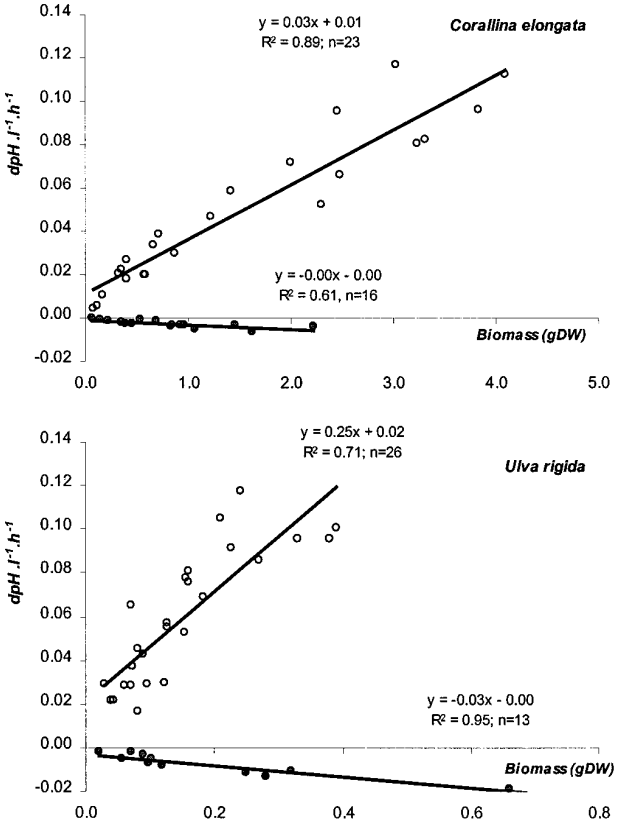

FIG. 2. – Hourly rate of pH variations per litre of seawater as a function of the biomass (in grams of dry weight, DW) of *C. elongata* and *U. rigida* (respectively) placed in experiment during light (open circle) and dark (dots).

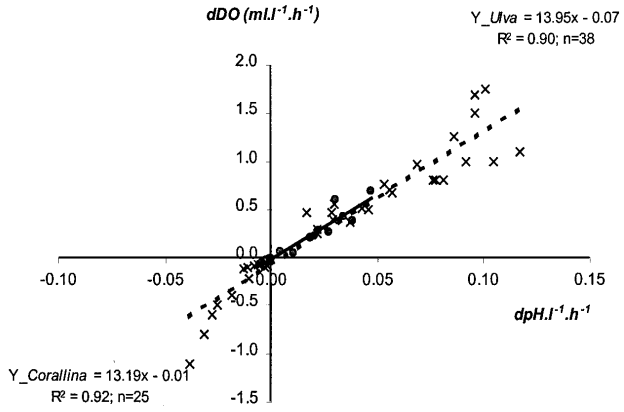

FIG. 3. – Regression plot between pH and DO variations of *C. elongata* (dots) and *U. rigida* (cross).

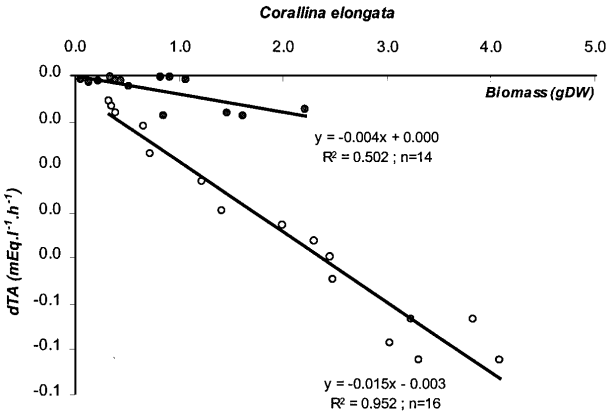

FIG. 4. – Hourly rate of total alkalinity (TA in mEq.l^{-1}) per litre of seawater as a function of the biomass (in grams of dry weight, DW) of *C. elongata* placed in experiment during light (open circle) and dark (dots).

decrease in pH values between the start and the end of incubations. The hourly rate of pH decrease as a function of the biomass (Fig. 2) was weaker than the increase occurring under light but significant at a $p<0.002$ level ($R^2=0.61$; n=16).

During the *U. rigida* light experiments, the same hourly pH variation was recorded (Fig. 2), with a highly-significant relationship with biomass ($R^2=0.71$; n=26; $p<0.001$). But for this alga, the mean hourly pH increasing rate (0.453±0.184 UpH.g^{-1}DW.h^{-1}; n=26) was 10.8 times higher than for *C. elongata*. For the *U. rigida* dark experiments the pH decrease was highly significant as a function of biomass amount ($R^2=0.95$; n=13; $p<0.0001$), and the range variation in dark was also smaller (Fig. 2) than in light experiments. The mean hourly rate of pH variations per biomass unit was 12 times higher for *U. rigida* than for *C. elongata*: -0.05±0.022 UpH.g^{-1}DW.h^{-1} (n=13) and -0.004±0.002 UpH.g^{-1}DW.h^{-1} (n=16) respectively.

In light experiments of *C. elongata* and *U. rigida* oxygen production and pH increase were highly linked to the reactive biomass. Figure 3 plots the dpH *vs* dDO between final and initial values for each experiment with *C. elongata* and *U. rigida*. In both cases the linear relationships were highly significant ($p < 0.0001$), with a slope value of 13.2 ($R^2=0.92$; n=25) and 14.0 ($R^2=0.90$; n=38) respectively.

Calcification estimated by total alkalinity variations (dTA)

During all the light experiments with the calcareous algae *C. elongata,* the TA difference between final and initial values decreased as a function of the incubated biomass. The hourly TA variations are presented in Figure 4. They were significantly linked ($R^2=0.95$; n=16; $p<0.0001$) to the tested range of biomass (0.02 to 4.1g), with a weak value of the intercept (-0.0018) which was not statistically different from zero. The mean hourly daylight TA variation calculated was –0.018±0.002 mEq.l^{-1}.g^{-1}DW.h^{-1} (n=16).

In *C. elongata* dark experiments, the TA values also decreased but at a lower rate and the relationship between dTA.h^{-1}and biomass amounts (Fig. 4) remained significant at $p<0.02$ ($R^2=0.50$; n=14). The weak value of the intercept to the origin (6.00E-05) was not statistically different from zero. The distribution of experimental points in dark conditions was cohesive with the conservation of a small but clear disappearance of calcium carbonate from seawater, but in three cases (Fig. 4), the differences between initial and final TA values were lower than -0.0003 mEq.l^{-1}.h^{-1}. The mean hourly dark TA variation was –0.005 mEq.l^{-1}.g^{-1}DW.h^{-1} (n=14), which was 3.6 times lower than in natural light conditions (L/D).

Thus, the mean carbonate assimilation rate can be estimated as 0.892 mg CaCO3.g^{-1}DW.h^{-1} in daylight and 0.225 mg CaCO3.g^{-1}DW.h^{-1} in dark, and extrapolated to 13.8 mg CaCO3.g^{-1}DW.d^{-1} (on the basis of a 12/12 photoperiod).

On the other hand, as was expected, with the non-calcareous alga *U. rigida*, under the same light conditions, no significant changes appeared between final and initial TA concentrations. More than 80% of the measuring points were randomly distributed around the x-axis and were included in an interval of ±0.002 mEq.l^{-1}.h^{-1}. In dark conditions and with incubated biomass ten times higher than under natural light, no more changes were recorded.

Photosynthetic quotient calculation

In *C. elongata* experiments the Qp ranged from 0.68 to 1.05, with an average value of 0.89 ± 0.10 (m±sd; n = 16). For *U. rigida* , the Qp exhibited a wider range of variations, from 0.65 to 1.60, with a mean value of 1.01 ± 0.25 (n = 19).

DISCUSSION

The main topic of this paper is to establish and estimate the carbonate assimilation by a calcareous algae in natural conditions using the "alkalinity anomaly" method, and to compare its rate to a more currently employed physiological index such as DO light production/dark consumption or pH modifications in relation to CO_2 utilisation. The assessment that these TA decreases are the consequence of the carbonate assimilation rate by the calcareous algae *C. elongata* is confirmed by the use of the non-calcareous and well-studied algae (Levavasseur 1980, 1987), *U. rigida* as a blank for this physiological activity. From a methodological point of view, we can note that the range of variations for all the measured parameters (DO, pH and TA) is largely higher than the resolution and accuracy of the respective assaying methods.

The experiments which have been conducted with these two species show that the decreasing TA is only recorded in calcareous algae *C. elongata* experiments and is the consequence of its specific metabolism. The two species, each of them with its specific rate, modify DO and pH values of the incubation seawater as a consequence of their photosynthetic activity and organic production: enhancement of DO levels in light, decrease in dark, increase of pH value in light (assimilation of CO_2) and decrease in dark conditions (CO_2 releasing). However, only *C. elongata* also exhibits a carbonate consumption that can be assimilated to an "inorganic production". This activity does not stop in dark conditions, even if the L/D (light/dark) ratio reaches 3.6. This L/D value is close to those reported in the literature for some algae species: i.e. 3-4 for *Corallina officinalis* (Pentecost 1978), 1-4 for *Halimeda* sp. (Jensen *et al.,* 1985; Borowitzka and Larkum 1976a,b,c,d), and 0-5 for zooxanthellate scleractinian corals, with a median value of 3.0 (Gattuso *et al.,* 1999).

One can suspect that this weak but clear "dark carbonate assimilation" may be the consequence of a residual "light" metabolism of the samples which would start their biological activity before the morning collection and continue after they are placed in dark. Such a metabolic activity continuation has been reported previously: Pearse (1972) and Pentecost (1978) noted low carbonate assimilation in apical segments of other calcareous macroalgae (*Bossiella orbigniana, Amphiroa tribulus* and *Corallina officinalis*) when they were passed from light to dark.

The significant decrease in TA values in the incubated seawater containing *C. elongata*, as compared to the lack of change measured in flasks containing *U. rigida* , corresponds to a significant carbonate assimilation by the calcareous algae. The sum of mean carbonate assimilation rate in daylight and dark is 13.8 mg $CaCO_3.g^{-1}DW.d^{-1}$.

This amount of assimilated mineral matter is important (almost 1% of the sample dry weight) and a part of this input contributes to the construction of the organism. The good recorded agreement between the experimental biomass of *C. elongata* and the rate of its carbonate assimilation may provide an original way to compare inorganic and organic carbon production in the calcareous algae group. But opinions are divergent about whether this metabolic activity must be considered as a biological production (*stricto sensu*, e.g. increase of carbon biomass) or as an energy consumption. The calcareous macroalgae are often considered as one of the less efficient primary producers (Lewis, 1977) in terms of carbon productivity. This opinion is based on the fact that in this group the energy requirements for inorganic "production" are added to those of organic carbon. A part of the energy provided by the photosynthetic activity is used for the carbonate assimilation and fixation process and not for organic carbon production (Digby, 1977). Nevertheless, in some calcareous species (such as *Halimeda* sp.), using classical methods for primary production measurements, Payri (1987) reported a potential production which can be considered to be as high as that of other macroalgae groups. If the respective rates of oxygen or pH variations measured in our experiment for *C. elongata* and *U. rigida* are compared, the metabolic-activity level of the non-calcareous algae appears to be significantly higher, within comparable biomass and light levels, when the whole dry weight is taken as reference. However, when the contribution of the mineral fraction (AW, which represents 83-85% of the DW of *C. elongata*) to the dry weight values is subtracted, the differences in metabolic rates are significantly

reduced between these two species, but for *C. elongata* they remain lower than for *U. rigida*. In fact, the mean hourly productivity was 1.9-2.16 ml $DO.g^{-1}DW.h^{-1}$ for *C. elongata* (15-17% of DOMW) and 3.22 ml $DO.g^{-1}DW.d^{-1}$ for *U. rigida* (≈ 90% of DOMW).

The comparison between data of the literature is often difficult due to the fact that in natural conditions several environmental factors influence these activities (Riccardi and Solidoro, 1996), particularly light levels (Häder *et al.*, 1997; Häder and Figueroa, 1997; Hanelt, 1996) and temperature (Orfanidis, 1993). Moreover, little is generally known about the sampling conditions and most of the time the experiments are conducted *in vitro* under variable light conditions, not only in intensity but also with a different spectral composition. In opposition to pH and TA measurements, which are scarce or absent in other works, the utilisation of the dissolved oxygen production or consumption rates is one the most widely-used methods for measuring algae metabolic activity. Thus, DO variations may provide an efficient tool for comparing our results with those found in the literature when the metabolic rates are expressed in the same units (per litre, per hour and per gram of dry weight). For example, for *U. rigida* Levavasseur (1980) reported an average DO production value of 8 $ml.g^{-1}DW.h^{-1}$ from samples collected in Channel, but Enriquez *et al*. (1995) mentioned 6 $ml.g^{-1}DW.h^{-1}$ from biological material of the same species collected in the gulf of Gascogne, a value which is close to our mean value (5.6 $ml.g^{-1}DW.h^{-1}$). For *C. elongata* the range of data is greater: 0.28-1.03 $ml.g^{-1}DW.h^{-1}$ (this study), 0.57 $ml.g^{-1}DW.h^{-1}$ for *C. elongata* and 1.03 $ml.g^{-1}DW.h^{-1}$ for *C. elongata var. compacta* in the Atlantic Ocean (Levavasseur, 1980) and 1.0 $ml.g^{-1}DW.h^{-1}$ (Häder *et al*., 1997). If we consider that the cited papers concerned *in vitro* studies conducted in laboratory conditions on samples collected in other seas and environmental conditions, we can consider that the DO reference method provides a solid estimation of activity rates. The calculated mean daily productivity was 3.89 mg $C.g^{-1}DW.d^{-1}$ for *C. elongata* and 34.8 mg $C.g^{-1}DW.d^{-1}$ for *U. rigida*.

The range of photosynthetic production values reported here for *C. elongata* is first the consequence of *in situ* differences in natural conditions during incubation experiments (irradiance, temperature). But we cannot discard also a seasonal effect on *C. elongata* activity. From incubations conducted over various periods in the year, indeed we have noticed that *C. elongata* exhibited seasonal changes in its photosynthetic activity under a comparable level of light, with a maximum in spring and a minimum in autumn, and a 40 % significant difference on average (results not reported here, El Haikali, *in prep*.). Such a seasonal variability was previously reported by Levavasseur (1980) for two other species of macroalgae (*Ulva* sp. and *Palmaria palmata*), but these laboratory experiments are too different from ours for a conclusive comparison at this stage.

The two other variables (pH and total alkalinity) measured in our incubation experiments have been previously studied, but at the cellular level, in tissue examinations for inorganic carbon source in relation to the enzymatic activity of carbonic anhydrase (Israel and Beer, 1992; Giordano and Maberly, 1989). The results of these authors cannot be directly extrapolated to the whole calcareous organism. However, pH and TA changes during incubations may also provide information on the CO_2 concentrations. As presented in Figure 3, DO and pH variations exhibited a close linear relationship in both *C. elongata* and *U. rigida* experiments. The pH variations are dependent on carbon dioxide concentrations in seawater, but it is not possible to directly deduce the photosynthesis/respiration contribution to CO_2 levels from the pH values alone because their equilibrium with the carbonate pool is dependent on two biological antagonistic processes (photosynthesis/respiration and calcification/decalcification, Smith and Kinsey 1978).

The results presented here demonstrate that under natural light and temperature conditions calcareous macroalgae may provide a specific signature of their activity in seawater as compared with non-calcareous algae by inducing a significant decrease in TA levels. Unlike in coral reefs, where the influence of calcareous organisms on seawater carbonate budget has been extensively studied, to our knowledge no such environmental approach has previously been conducted in the Mediterranean coastal waters. Nevertheless, in many places where shoreline and sea bottom are densely covered by calcareous algae, it is likely that they influence the carbonate budget of Mediterranean seawater. Given that these communities are considered as some of the most important producers of carbonate in the Mediterranean (Ballesteros, 1988; Garrabou and Ballesteros, 2000), and some of them may build impressive hard rock-cemented structures (i.e. concretions) in mediolittoral levels (Pérès and Picard,

1952; Laborel *et al.,* 1983; Laborel 1987; Verlaque, 1987; Laborel-Deguen *et al.,* 1992; Boudouresque, 1996), it is particularly important that further studies are conducted in this area. By coupling with pH and dissolved oxygen, measurement of total alkalinity is an indirect way of estimating the specific contribution of various compartments of marine environment to the whole carbon budget.

Natural populations of *C. elongata* in the experimental area (Anse des Cuivres-Gulf of Marseilles) showed high biomass values ranging between 820 and 2544 g DW.m^{-2}. These values are in the same order of magnitude as those reported by Ballesteros (1988) for infralittoral communities on the Costa Brava (north-west Spain), which ranged from 1110 to 4015 g DW.m^{-2}.

In order to extrapolate the organic and inorganic daily productions of natural populations of *C. elongata* of the northwestern Mediterranean coasts from our experimental results, we need to correct daily net photosynthesis calculated in this work from short term incubations (i.e. 2 to 7 h) to account for the daily mean value (but we did not correct daily calcification).

Indeed, the estimation of the net photosynthesis and calcification daily rates was calculated on a 12/12 photoperiod basis under the currently employed hypothesis which assumes that the hourly rates are nearly constant all day long. However, these rates may change with hour-to-hour irradiance variations. This can be demonstrated by using an *in situ* incubator which follows DO and pH increments each 30 min (results not reported here. El Haikali, *in prep*.). The mean 24 h production value calculated from these nearly-continuous records represents only 63% of the corresponding production during the 11:00-18:00 period, the part of the day in which most of the experiments reported here were conducted.

Also, the mean productivity rate reported here for *C. elongata* was obtained under light-saturated conditions if we consider that it corresponds to an average of 200-300 W/m^{-2} (the irradiance average of all the experiences was 360 W/m^{-2}).

For 1kg DW.m^{-2}, a daily *C. elongata* photosynthetic and calcification production may be about 2.5 g C.m^{-2}.d^{-1} and 13.8 g $CaCO_3$.m^{-2}.d^{-1} respectively.

If we consider the low and high biomass hypothesis of 1 and 4 kg DW.m^{-2} of *C. elongata*, a 5 m high belt of *C. elongata* occupying one kilometre of rocky shores may produce between 4.5 and 17.9 tons C.yr^{-1} by photosynthesis and 25.2-100.8 tons $CaCO_3$ yr^{-1} by calcification.

These estimations, based on calcification rates measurements, highlight the importance of *C. elongata* as a major contributor to calcium carbonate deposition in the shallow waters of the northwestern Mediterranean, as was already stated by Ballesteros (1988). With other deeper calcified-macroalgae communities such as the Coralligenous community, Maërl and *Peyssonnelia* beds (Ballesteros, 1994; Canals and Ballesteros, 1997), these littoral communities located in shallow waters therefore constitute a key element of carbonate particle production in Mediterranean coastal waters.

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