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Contribution of biofilm to ecosystem functioning in rock pools with different macroalgal assemblages

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ABSTRACT: Research into the role of biodiversity in the functioning of rocky shore ecosystems has ignored the microscopic component of the biota, in particular the microbial films coating most marine surfaces. Yet in other habitats, biofilms are known to be major contributors to ecosystem functioning. The objective of the present study was thus to understand the contribution of epilithic biofilms to rock pool metabolism (oxygen fluxes) and to identify whether this varies under different macroalgal assemblages. A 2-way factorial experiment was run in artificial rock pools on a moderately exposed rocky shore, combining different macroalgal treatments with the presence/absence of biofilm. Biofilm made significant contributions to rock pool community primary productivity and rock pool residual respiration (rock pool metabolism measured without the macroalgal treatment), independently of the composition of the overlying macroalgal treatment. In rock pools with no macroalgae, biofilm appeared to dominate community metabolism, while the planktonic community was a minor contributor. There was no evidence of any significant effect of the different macroalgal assemblages tested on the metabolism of the biofilm. The presence of both *Mastocarpus stellatus* and *Fucus serratus* in pools, however, resulted in a lower photosynthetic activity of the biofilm than any of the other macroalgal treatments. Our results suggest that biofilm is a compartment that contributes significantly to the functioning of rock pool ecosystems, whether in terms of its heterotrophic or autotrophic activity, and should thus be taken into account in future research on these ecosystems.

KEY WORDS: Microbial film · Metabolism · Rock pool · Macroalgae · Oxygen fluxes · Photosynthetic efficiency

INTRODUCTION

Recently, there has been a considerable effort to understand how ecosystem processes are influenced by different components of biodiversity (e.g. Stachowicz et al. 2007, Naeem et al. 2009). Research to date has tended to focus on particular groups, often chosen for practical reasons, such as ease of manipulation. Many authors have focused on the productivity of macroscopic plants (e.g. Duffy et al. 2001, Isbell et al. 2008) and algae (e.g. Arenas et al. 2009). With some notable exceptions (Cardinale et al. 2005, Leary & Petchey 2009, Vanelslander et al. 2009), there has been a general tendency to ignore the microscopic component of ecosystems. Given the widely acknowledged importance of microorganisms in ecosystem processes, this is an important deficiency.

Experimental studies of the role of biodiversity in rocky shore ecosystems have only ever involved manipulation of macrobiota and have either measured productivity using proxies, such as percentage cover or accumulated biomass of macrobiota (e.g. O'Connor & Crowe 2005, Long et al. 2007), or have measured ecosystem metabolism (productivity and respiration) as fluxes of carbon dioxide (Golléty et al. 2008) or oxygen (Martins et al. 2007, Masterson et al.

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2008). The use of proxies disregards the contribution of microbial films entirely, and measurements of fluxes cannot distinguish fluxes of this compartment from that of others in the community. Yet, almost all marine surfaces are coated with biofilms. These biofilms comprise variable amounts of organic molecules, fungi, microalgae, cyanobacteria, heterotrophic bacteria, protists and macroalgal early stages (Raffaelli & Hawkins 1999). They are known to be at the base of major trophic pathways in many coastal habitats (Galván et al. 2008, Jaschinski et al. 2008, Evrard et al. 2010) and also play a role in stabilising substrata (Le Hir et al. 2007, Gerbersdorf et al. 2008, Lubarsky et al. 2010). Microbes have also been suggested to be a significant actor in the nitrogen metabolism of rock pools (Pfister 2007). While the contribution of biofilm to carbon fluxes is relatively well understood in sedimentary habitats (Cahoon & Cooke 1992, Spilmont et al. 2005), the contribution of epilithic biofilm to the metabolism of rocky shores remains unknown.

The distribution and composition of biofilms on rocky shores is known to be affected by abiotic and biotic factors. For example, the elemental chemistry of granite has been shown to exert a selective pressure on *in situ* bacterial communities (Gleeson et al. 2006), substrate roughness can affect small-scale patchiness (Hutchinson et al. 2006), and season, tidal elevation and exposure to wave action can all affect photosynthetic biomass (Thompson et al. 2005). The presence of grazers can also affect the relative contribution of the major taxonomic groups of the biofilm (Skov et al. 2010), changes in grazer densities can modify its photosynthetic biomass (Stafford & Davies 2005), and differences in grazer densities and/or macroalgal canopy cover can affect the abundances of diatoms and cyanobacteria and their photosynthetic biomass (Thompson et al. 2005). Macroalgae themselves are surfaces that host different microbial communities that vary among macroalgal species (e.g. Chan & McManus 1969, Cundell et al. 1977). Given that different kinds of macroalgae modify the environment in different ways (e.g. in terms of shading, nutrient use or habitat provision for animals), the composition of biofilms could also be affected by the composition of macroalgal assemblages, as would the biofilms' contribution to ecosystem processes. The extent of this variation may be a key consideration in predicting consequences of changes in biodiversity for rocky shore ecosystems but remains to be characterised.

Rock pools offer the great advantage for research into intertidal ecosystem processes of acting like mesocosms or incubating chambers in which oxygen fluxes can be measured (Noël et al. 2010). Manipulations of the different organisms present in rock pools allow the metabolism of different compartments to be characterised separately. The biofilm, however, is permanently associated with the rock and thus cannot be isolated from some of the other compartments naturally present in rock pools (e.g. plankton, scraps of macroalgae and small animals). In the present study, we thus distinguished 3 compartments: (1) community metabolism, which is the metabolism of everything present in the rock pool, which in addition to the macroalgae and biofilm can include the contribution of bacteria, phytoplankton and/or meiofauna and macrofauna hidden in rock crevices; (2) residual metabolism, which is the metabolism of everything present in the rock pool but with the macroalgal treatments removed; and (3) biofilm metabolism, which can only be inferred indirectly by comparing the metabolism of pools where the biofilm is left intact to the metabolism of pools where the biofilm has been removed (Table 1).

The main objective of the present study was to better understand the contribution of each compartment to ecosystem processes in rock pools and in particular the role of the epilithic biofilm in the ecosystem functioning of macroalgae-dominated rock pools. For this purpose, we designed a field experiment to address the following questions: (1) Do biofilms contribute significantly to rock pool ecosystem processes, and if so, what is their percentage contribution? (2) Do the ecosystem processes of biofilms vary under different macroalgal assemblages?

MATERIALS AND METHODS

Study area

The experimental site was a moderately exposed rocky shore at Carnsore Point, County Wexford, at the south-eastern tip of Ireland (52° 10′ 23″ N, 6° 21′ 48″ W). The shore is composed of a stacked boulder field and fractured bedrock. Rock pools of a wide range of sizes are naturally present. The most abundant macroalgae dominating these rock pools are the encrusting red alga *Phymatolithon lenormandii*, the turf-forming green alga *Cladophora rupestris*, the understorey alga *Mastocarpus stellatus* and the canopy *Fucus serratus*. Other turf and understorey species, such as *Ulva* spp., *Corallina elongata* and many other red macroalgal species, and a few other canopy species, such as *Halidrys siliquosa*, can

Table 1. For each combination of macroalgae and biofilm treatment, the compartments that are present in the rock pools when measuring either the community or the residual metabolism. The treatments are NM: no macroalgae, M: *Mastocarpus stellatus*, FM: *Fucus serratus* and *M. stellatus*, NB: no biofilm, B: biofilm. 'Potential macrofauna' refers to the fact that there was no control over the access of the rockpool to small macrofauna such as crustacean mesograzers, gastropods (but no limpets), juve-nile crabs and fish. –: not present

	Macroalgae × Biofilm treatment					
	NM.NB	NM.B	M.NB	M.B	FM.NB	FM.NB
Compartments contributing to community metabolism						
Macroalgae	–	–	√	√	√	√
Biofilm	–	√	–	√	–	√
Plankton	√	√	√	√	√	√
Potential macrofauna	√	√	√	√	√	√
Compartments contributing to residual metabolism						
Biofilm	–	√	–	√	–	√
Plankton	√	√	√	√	√	√
Potential macrofauna	√	√	√	√	√	√

also be found. Animals present in the pools included grazing gastropods, such as *Patella ulyssiponensis, Littorina littorea* and *Gibbula umbilicalis*, amphipods, barnacles and occasional fish and crabs.

Interspersed on the shore where natural rock pools are found, the rock has been drilled to create an array of 24 artificial and cylindrical rock pools of 30 cm diameter, which are separated from each other and the natural pools. The pools were diamond drilled by Holemasters in 2009 for research of this kind. The experiment prior to this one finished 2 mo before the start of the current project. These pools ranged between 9.5 and 14.5 cm deep resulting in volumes ranging from 6.7 to 10.2 l. Prior to the experiment, the only macroalgae that had time to colonise these artificial pools were *Ulva* spp., which were all scraped off 1 wk before the start of the experiment. Although mesh was not placed to limit the access of the pools to macrofauna, very few species and individuals of crustacean mesograzers, gastropods (but no limpets), juvenile crabs and fish could be found in these pools before and throughout the experiment.

Experimental design

The experiment ran for 21 d, from 26 July to 16 August 2010. A factorial design was established with the following factors (Table 1): (1) Macroalgal treatment, with 3 levels to represent a range of naturally occurring assemblages: no macroalgae (NM), *Mastocarpus stellatus* (M), and *Fucus serratus* with *M. stellatus* (FM); (2) Biofilm, with 2 levels: presence (B) and absence (NB). Each combination of macroalgal treatment and biofilm was replicated 4 times, so the design required $4 \times 2 \times 3 = 24$ experimental units. These experimental units were randomly assigned to the artificial rock pools. Because of tidal constraints, ecosystem functioning could only be sampled in a maximum of 9 rock pools per day, so the experiment was run in 3 batches, 1 batch of 9 pools and 2 of 8 pools, sampled 2 d apart (Fig. 1). Pools were assigned to batches such that each batch included at least 1 representative of each treatment to ensure that temporal variability would not confound comparisons among treatments.

The macroalgal treatments were established on 26 and 27 July by collecting algae and attaching them with gardening wire to 21 × 21 cm squares of stainless steel mesh (40 mm openings) that covered 63% of the surface area of the rock pool bottom; this allowed for the best compromise between the largest mesh that could fit in the pools and a large enough area available to ensure that the percentage cover of macroalgae would be representative of that found in adjacent natural pools. The bottom of each pool was drilled in 4 places so that the mesh on which the macroalgal treatment was attached could be fixed with stainless steel screws. For *Mastocarpus stellatus*, the understorey species, strands were collected and fixed on different points of the mesh to establish 90% cover of the mesh, corresponding to 397 cm^2 or 57% of the bottom of a rock pool. For *Fucus serratus*, the canopy species, a single thallus was attached to the centre of the mesh so that it also covered 90% of the mesh. Since the canopy was combined with understorey, this resulted in 90% of primary cover (*F. serratus*) plus 90% of secondary cover (*M. stellatus*), making 180% cover in total. For the NM treatment, an empty square of mesh was screwed into the pools.

Biofilm removal and measurements of ecosystem functioning were done from 9 to 16 August 2010. The biofilm treatments were applied the day before measurements of ecosystem functioning (Fig. 1). Pools assigned to treatments with biofilm absent were first emptied into a bucket using a compact bilge and waste water pump (Gusher Urchin model BP9013, Whale® water system specialist). The mesh of the

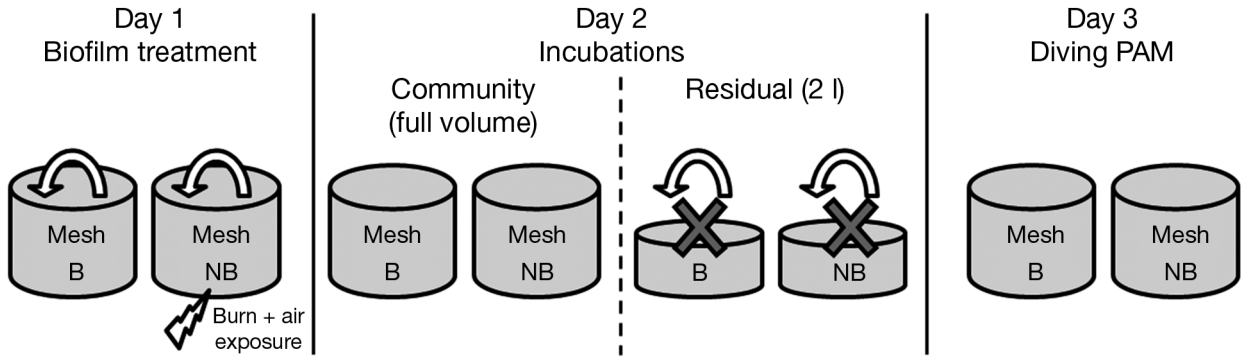

Fig. 1. Diagram of the experimental procedure followed for each batch of pools. Grey cylinders represent the artificial rock pools that were used. Mesh: mesh used for the macroalgal treatments, B: biofilm, NB: no biofilm. On Day 1, the arrows indicate that the pools were refilled with the same water that was present when the pools were uncovered by the tide. Burn: removing the biofilm of the empty pools using a blow torch and leaving the pools to dry for 55 min. On Day 2, the community metabolism was first measured using the full volume, then the pools were emptied to remove the mesh holding the macroalgae (cross) and refilled (arrows) with only 2 l of the water initially present to measure the residual metabolism. On Day 3, the diving PAM measurements were performed on full pools. Note that Day 3 of the first batch became Day 1 of the next one and so on

macroalgal treatment was removed and placed in the bucket containing the seawater from that pool, and the biofilm in the pool was eliminated by burning the entire surface area of the pool using a blowtorch (Campingaz® TH 2000 PZ Torch). The pool was then left empty for 55 min, after which the macroalgal assemblage was screwed back into the pool, and the pool was refilled with its seawater (Fig. 1). Pools assigned to treatments with biofilm present were also emptied into buckets, and the mesh was removed and set aside in that same bucket. Immediately after that, however, the pools were refilled with that same seawater so that after 50 min they had to be emptied again in order to screw back the mesh and refill them in the same way as the pools from which biofilm had been removed.

Ecosystem functioning measurements

Community gross primary productivity (GPP) and community respiration (CR) were estimated by measuring oxygen fluxes in light and dark conditions (Nielsen 2001, Noël et al. 2010). As a proxy for biofilm activity, we also measured the photosynthetic efficiency of the biofilm using pulse amplitude modulated (PAM) fluorometry (Consalvey et al. 2005). The O_2 fluxes at the substratum–water interface were measured using a luminescent dissolved oxygen meter (Hach, HQ30D). Based on linearity tests performed prior to the experiment, the change in O_2 (mg l^{-1}) was measured as follows: after a 20 min dark adaptation, obtained by covering the pools with opaque polyethene sheets, an initial measurement was performed followed by a 30 min dark incubation after which a final measurement was performed in the dark; after 17 min, the pools were uncovered, left under ambient light for an 8 min light adaptation, and the initial light measurement was then performed; the final light measurement was performed after a 25 min light incubation. This sequence of incubations avoided supersaturation of the pools, which would have inhibited photosynthesis (Noël et al. 2010). On the same day, the same sequence of incubations was performed first with the full rock pool volume and the macroalgal treatments present, in order to assess the community metabolism, and then repeated without the macroalgal treatment in 2 l of seawater, to assess the residual metabolism of the rock pools (Fig. 1). This was done to ensure that the smaller fluxes of oxygen due to the microphytobenthos, compared to that of macroalgae, could be measured in the time frame available during a single low tide period. Dark incubations allowed measurement of CR and residual respiration (RR), i.e. respiration with and without the macroalgal treatments respectively, while those under ambient light allowed measurement of community net primary productivity (NPP) and residual net primary productivity (RNPP), i.e. net primary productivity with and without the macroalgal treatments respectively. Community GPP and residual gross primary productivity (RGPP) were then calculated as the sum of NPP and CR and of RNPP and RR respectively. The data, measured in mg O_2 l^{-1} min^{-1}, were then converted in mg O_2 m^{-2} h^{-1} to standardise the data as a function of the amount of substratum covered with biofilm; for the residual metabolism, which was measured using only 2 l of water, this takes into ac-

count the fact that a smaller pool height, i.e. with a smaller surface area for the biofilm to grow, was covered in water. In the absence of any information regarding the saturating irradiance of the communities, care was taken to perform the measurements with photosynthetically active radiation (PAR) (400 to 700 nm) above 1500 µmol photons m^{-2} s^{-1} since saturating irradiance values for intertidal algae have often been measured in this range (e.g. Johnston & Raven 1986, Kirk 2000). This ensured that measurements could be compared among treatments despite variations in irradiance levels.

The day following the metabolism measurements, the photosynthetic efficiency of the biofilm was assessed by measuring the maximum quantum yield of photosystem II (F_v/F_m) by PAM fluorometry (Consalvey et al. 2005), using a Diving-PAM (Heinz Walz). A 15 min dark adaptation, obtained using the same opaque polyethene sheets over the pools as for the metabolism, allowed measurement of the minimum fluorescence yield (F_0), which represents the fluorescence yield in the absence of any photochemical and non-photochemical quenching (Consalvey et al. 2005). The maximum fluorescence yield (F_m) was then measured after application of a short pulse of saturating irradiance (>3000 µmol photons m^{-2} s^{-1}). The difference between F_m and F_0 is the variable fluorescence F_v, such that the maximum quantum yield of photosystem II is calculated as follows:

$$F_v / F_m = \frac{F_m - F_0}{F_m} \quad (1)$$

The photosynthetic efficiency of the biofilm of each rock pool was sampled the day after the metabolism measurements, with the macroalgal assemblages still present in the pools (Fig. 1). A single measurement per pool was performed on the side of the rock pools to ensure that any debris covering the bottom of the pool would not interfere with the measurement.

Statistical analysis

Both 1- and 2-way analyses of variance (ANOVA) were used to test the effect of the different macroalgal treatments and the presence of the biofilm on ecosystem metabolism, residual metabolism and the photosynthetic efficiency of the biofilm.

To test whether the biofilm made a significant contribution to rock pool community ecosystem metabolism under different macroalgal treatments, we performed a 2-way ANOVA on the ecosystem metabolism data (GPP and CR) with the macroalgal treatments (NM, M and FM) as a first random factor and the biofilm presence/absence (B and NB) as a fixed orthogonal second factor. To test whether, under different macroalgal treatments, the biofilm had a significant contribution to (1) the residual metabolism and (2) the biofilm photosynthetic efficiency, the same analysis was performed on the residual metabolism data (RGPP and RR) and the F_v/F_m data respectively. To test whether the different compositions of macroalgal treatments had an effect on the metabolism of the biofilm, we performed a 1-way ANOVA on the residual metabolism data (RGPP and RR) and on the F_v/F_m data measured when biofilm was present on each of the different macroalgal treatments (NM + B, M + B and FM + B).

Computations were done using WinGMAV5 (Underwood et al. 1998). Normality of the residuals was verified visually using Q-Q plots, and homogeneity of variance was tested using Cochran's test. When ANOVA showed a significant difference, the Student-Newman-Keuls (SNK) procedure was used to identify which means differed from one another (Underwood 1997). Results of these tests are discussed in the text but not given in a table. Where appropriate, terms that were not significantly different ($p > 0.25$) were pooled to enable tests to be made on previously untestable terms and to provide more powerful tests of additional terms ('post-hoc pooling'; Underwood 1997). In general, however, the use of pooling was kept to a minimum. Only terms of relevance to the hypotheses being tested were re-evaluated after pooling.

Finally, the contribution of biofilm to the community and residual metabolism was estimated. In each case, only the equations relating to GPP are given, but each time, the same applies for respiration, which can be calculated by replacing GPP with CR and RGPP with RR.

First, the contribution of the biofilm to the community metabolism ($B\%GPP_j$) was estimated for each treatment as the percentage that the difference between the community metabolism of pools with biofilm and of pools without biofilm represents for the community metabolism of pools with biofilm, which for the community GPP was estimated as follows:

$$B\%GPP_j = \frac{\left[\left(\sum_{i=1}^{n} GPP_{Bij}/n\right) - \left(\sum_{i=1}^{n} GPP_{NBij}/n\right)\right] \times 100}{\sum_{i=1}^{n} GPP_{Bij}/n} \quad (2)$$

where GPP_B and GPP_{NB} are the community gross primary productivity measured in pools with and with-

out biofilm respectively, j is each macroalgal treatment (NM, M and FM), and n is the number of replicates per macroalgal treatment. Secondly, the contribution of the biofilm to the residual metabolism ($B\%RGPP_j$) was estimated in the same manner as for the community metabolism, which for the RGPP was estimated as follows:

$$B\%RGPP_j = \frac{\left[\left(\sum_{i=1}^{n} RGPP_{Bij}/n\right) - \left(\sum_{i=1}^{n} RGPP_{NBij}/n\right)\right] \times 100}{\sum_{i=1}^{n} RGPP_{Bij}/n} \quad (3)$$

where $RGPP_B$ and $RGPP_{NB}$ are the residual gross primary productivity measured in pools with and without the biofilm respectively, while j and n are defined as previously.

RESULTS

The community metabolism ranged from 24 to 497 mg O_2 m^{-2} h^{-1} in terms of GPP and from 26 to 288 mg O_2 m^{-2} h^{-1} in terms of CR. RGPP ranged from 21 to 334 mg O_2 m^{-2} h^{-1}, and RR ranged from 14 to 127 mg O_2 m^{-2} h^{-1} (Fig. 2).

Variation in effects of biofilm on community metabolism

In the pools assigned to the NM treatment, pools with biofilm tended to have greater GPP (231.0 ± 98.0 mg O_2 m^{-2} h^{-1} vs. 64.2 ± 8.7 mg O_2 m^{-2} h^{-1}) and CR (73.2 ± 28.0 mg O_2 m^{-2} h^{-1} vs. 34.2 ± 1.7 mg O_2 m^{-2} h^{-1}) than pools without biofilm (Fig. 2). These differences, however, were only significant for GPP (Table 2a; SNK procedure). In fact, this pattern was consistent for all macroalgal treatments.

As expected, the presence or absence of macroalgae was a strong driver of metabolism in the pools; both GPP and CR were significantly greater with macroalgae present (M or FM) than without (NM). This was true independently of the presence of biofilm, as indicated by the lack of statistical interaction between the 2 factors (Table 2a).

Variation in effects of biofilm on residual metabolism

The 2-way ANOVA showed that different macroalgal treatments did not result in any significant differences in RGPP, RR or F_v/F_m. This was true inde-

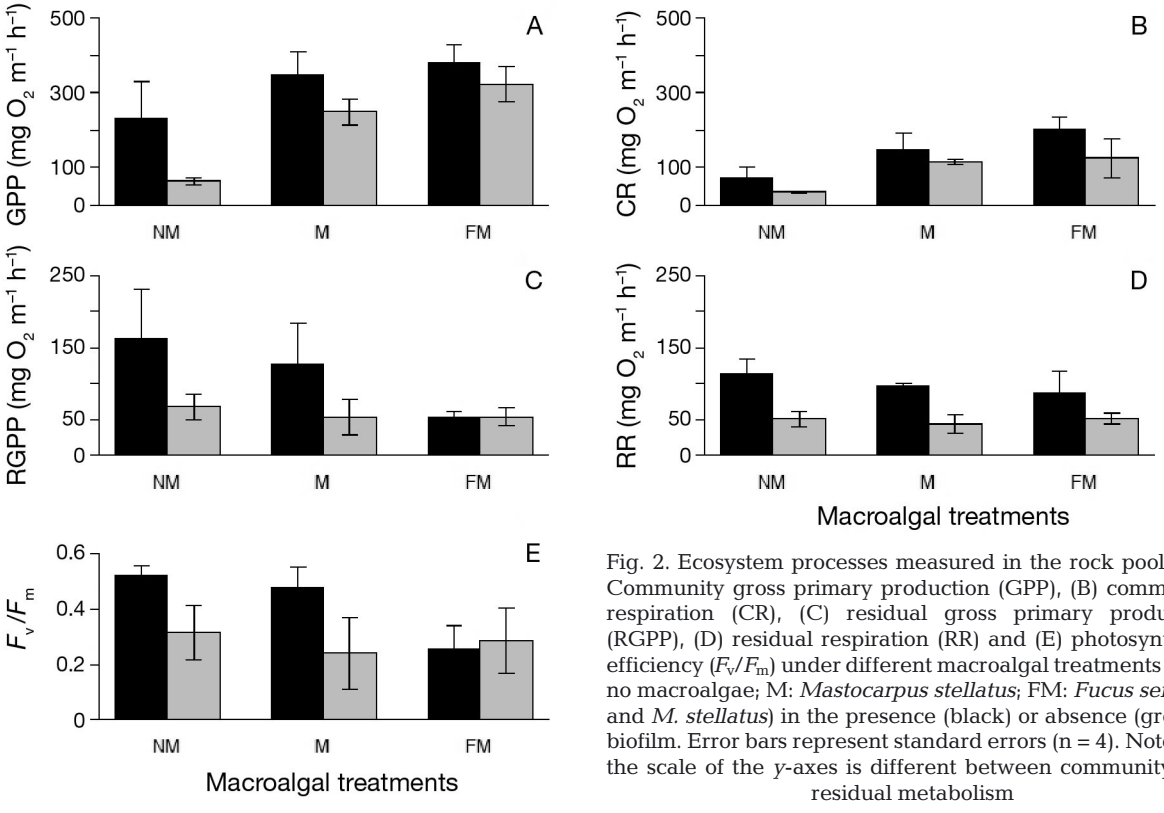

Fig. 2. Ecosystem processes measured in the rock pools. (A) Community gross primary production (GPP), (B) community respiration (CR), (C) residual gross primary production (RGPP), (D) residual respiration (RR) and (E) photosynthetic efficiency (F_v/F_m) under different macroalgal treatments (NM: no macroalgae; M: *Mastocarpus stellatus*; FM: *Fucus serratus* and *M. stellatus*) in the presence (black) or absence (grey) of biofilm. Error bars represent standard errors (n = 4). Note that the scale of the *y*-axes is different between community and residual metabolism

Table 2. Results of the 2-way analysis of the effect of macroalgal treatments and the presence of the biofilm on the (a) community gross primary productivity (GPP) or community respiration (CR) and (b) the residual gross primary productivity (RGPP) or the residual respiration (RR) and the photosynthetic efficiency (F_v/F_m); (c) the 1-way analysis of variance of the effect of the different macroalgal assemblages on the biofilm metabolism measured as RGPP, RR or F_v/F_m. For all analyses, n = 4 pools; data are untransformed; Cochran's test: ns. Significant values at $p < 0.05$ given in **bold**

Source	df	MS	*F*	p-value
(a) Community metabolism				
GPP				
Macroalgae	2	90399	7.07	**0.0054**
Biofilm	1	70417	5.82[a]	**0.0256**
Macroalgae × Biofilm	2	5909	0.46	0.6374
Residual	18	12793	–	–
CR				
Macroalgae	2	25751	5.78	**0.0115**
Biofilm	1	14553	3.53[a]	0.0749
Macroalgae × Biofilm	2	1115	0.27	0.7657
Residual	18	4455	–	–
(b) Residual metabolism				
RGPP				
Macroalgae	2	6891	1.54	0.2412
Biofilm	1	39204	7.17	0.1157
Macroalgae × Biofilm	2	5466	1.22	0.318
Residual	18	4473	–	–
RR				
Macroalgae	2	565	0.94	0.4074
Biofilm	1	3851	6.76[a]	**0.0171**
Macroalgae × Biofilm	2	315	0.53	0.5992
Residual	18	598	–	–
F_v/F_m				
Macroalgae	2	0.0434	1.2	0.3255
Biofilm	1	0.1139	2.73	0.2401
Macroalgae × Biofilm	2	0.0417	1.15	0.3392
Residual	18	0.0363	–	–
(c) Biofilm metabolism				
RGPP				
Macroalgae	2	11799.25	1.52	0.2703
Residual	9	7772.42	–	–
RR				
Macroalgae	2	845.08	0.99	0.4083
Residual	9	852.61	–	–
F_v/F_m				
Macroalgae	2	0.0796	4.35	**0.0476**
Residual	9	0.0183	–	–

[a]*F* calculated using pooled MS, (Macroalgae × Biofilm) + Residual

pendently of the presence or not of the biofilm, as indicated by the lack of interaction effect between the 2 factors (Table 2b). The presence of the biofilm only significantly affected RR (Table 2b), with pools with biofilm displaying a greater RR than pools without biofilm (65.8 ± 14.6 mg O_2 m^{-2} h^{-1} vs. 40.5 ± 8.5 mg O_2 m^{-2} h^{-1}). Although the same trend was true for RGPP (148.2 ± 46.1 mg O_2 m^{-2} h^{-1} vs. 67.4 ± 16.3 mg O_2 m^{-2} h^{-1}) and F_v/F_m (0.418 ± 0.086 vs. 0.280 ± 0.107), it was not significant for these variables.

Effects of macroalgal assemblages on biofilm metabolism

When comparing ecosystem processes among the 3 different macroalgal treatments of pools where biofilm was left intact, there was no significant difference among macroalgal treatments for RGPP and RR (Table 2c). The F_v/F_m of biofilms, however, was significantly influenced by macroalgal treatments (Table 2c). Although SNK procedure could not fully resolve the differences, F_v/F_m was markedly lower when *Mastocarpus stellatus* and *Fucus serratus* were present together (mean F_v/F_m ± SE: 0.257 ± 0.081) than with *M. stellatus* alone (0.477 ± 0.077) or no macroalgal assemblage (0.520 ± 0.035).

Estimated contribution of biofilm to metabolism

The percentage contribution of the biofilm to community metabolism decreased in the presence of macroalgal species for both productivity and respiration. The contribution of the biofilm to residual metabolism decreased markedly in the presence of macroalgal species in the case of respiration only (Fig. 3).

DISCUSSION

The present study is the first to estimate the contribution of biofilm to the community metabolism of rock pools. It appears that the biofilm has a significant contribution to rock pool community primary productivity and to residual respiration, independently of the composition of overlying macroalgal assemblages. In fact, there is no evidence of any significant effect of the different macroalgal treatments tested on the metabolism of the biofilm. There seems to be, however, an effect of different macroalgal treatments on the photosynthetic activity of the biofilm.

The wide range of methods and conditions under which metabolism measurements are performed

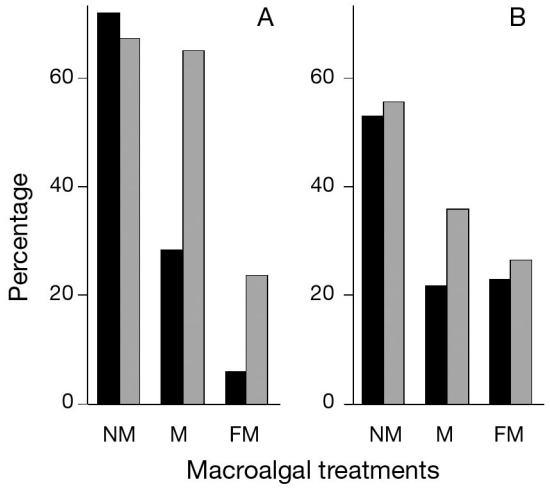

Fig. 3. Contribution of the biofilm metabolism to (A) gross primary productivity and (B) respiration in the presence of macroalgae (NM), *Mastocarpus stellatus* (M), or *Fucus serratus* and *M. stellatus* (FM): barplots show contribution of biofilm to community metabolism (black) and of biofilm to residual metabolism (grey). The contribution of biofilm metabolism is not based on the calculation of a single mean but on the difference of 2 means (metabolism of pools with biofilm minus metabolism of pools without biofilm); thus, errors could not be estimated for these calculations

when dealing with rocky shore photosynthetic organisms requires caution when comparing our measurements to the few available data in the literature. In particular, comparisons of CO_2 to O_2 fluxes are not straightforward since photosynthetic and respiratory quotients are usually unknown. Keeping this in mind, rock pool GPP and CR measured in the absence of macroalgae in the present study were comparable to the metabolism of mid-intertidal immersed biofilm-covered rocks (Magalhães et al. 2003). When macroalgae were present, both GPP and CR values were comparable to the lower range of the metabolism of canopy-covered mid-intertidal emergent rocky reefs (Golléty et al. 2008) and to measurements performed on individual intertidal algae, including *Fucus* spp. and *Chondrus crispus* (Roman et al. 1990, Dudgeon et al. 1995, Kinney & Roman 1998, Kawamitsu & Boyer 1999).

Contribution of biofilm to rock pool ecosystem processes

The rates of metabolism of the biofilm are comparable to those of sediment microphytobenthos or phytoplankton and much lower than those of macroalgae, seagrasses or marsh plants (Charpy-Roubaud & Sournia 1990, Duarte & Cebrián 1996, Ouisse et al. 2010). Yet, the contribution of the biofilm was significant for GPP and for RR. This suggests that biofilm is an important compartment in the functioning of rock pools, as could be suspected from the contribution of biofilms in sedimentary systems (e.g. Cahoon & Cooke 1992). Biomass was not estimated in the present study, but microscopic autotrophic organisms are known to display high turnover rates or assimilation numbers (Falkowski 1981, Migné et al. 2004). The biofilm compartment is thus especially responsible for substantial productivities despite the small biomasses involved compared to those of macroalgae. On canopy-covered rocky shores, it appears that the biofilm formed on the surface of canopy macroalgae can also contribute significantly to the CR measured at low tide (Golléty et al. 2008). Clearly, this compartment cannot be ignored in analysing the functioning of these systems in terms of sources and sinks of carbon. In rock pools, the biofilm seems to be a significant source of carbon for the food web, thus likely contributing to the complexity that characterises rocky shores in terms of sources of organic matter and numbers of trophic pathways (Golléty et al. 2010).

For each of the 3 macroalgal treatments, percentages of the contribution of the biofilm to either the community or the residual metabolism were inferred. These calculations are only indicative, however, since they compared measurements made in different pools. In addition, the residual metabolism was measured using a smaller volume than the total rock pool volume. This meant that a smaller surface area of the biofilm community present in each rock pool was taken into account. It also meant that, when the water was poured back into each pool before measuring the residual metabolism, the debris that had accumulated under the mesh during the 2 wk period of 'exposure' to the macroalgal treatment was suspended, potentially affecting the biofilm that had developed. We do not believe this created an artificial condition since sand and macroalgal debris always accumulate amongst the macroalgae covering rockpools. Keeping that in mind, it is worth noting that in the treatment with no macroalgae, the biofilm contributed 50 to 70% of the community metabolism (Fig. 3). This suggests that, in rock pools with no macroalgae, microbenthic organisms dominate community metabolism, while the planktonic community is a minor contributor. The results of this study emphasise the predominant role of benthic organisms to community metabolism, as previously noted in other intertidal systems (Mann 1973, Branch & Griffiths 1988, Barranguet et al. 1996).

Effects of different macroalgal assemblages on ecosystem processes of biofilm

The presence of different macroalgal assemblages did not have an effect on the residual metabolism of the rock pools, either in terms of primary productivity or respiration. In fact, the presence of macroalgal assemblages did not significantly affect the contributions of the biofilm to either the overall or the residual metabolism. To our knowledge, no other study has looked at the effect of the presence of different macroalgal assemblages on the contribution of the biofilm to the metabolism of rock pools or indeed rocky shores in general. Analyses of chlorophyll *a* content have shown that the presence of *Fucus vesiculosus* had no effect on the biomass of biofilm on emergent rocky shores (Hill & Hawkins 1991). Our study takes a further step in the study of the effect of macroalgae on the metabolism of biofilms by measuring actual fluxes, both for gross primary productivity and respiration. Considering the small magnitude of the oxygen fluxes due to the biofilm, a valuable next step would be to use finer techniques, such as microelectrodes, to improve the resolution of the contribution of biofilm to community metabolism.

There was variation in the photosynthetic activity of the biofilm (measured as F_v/F_m) depending on the presence and identity of macroalgal treatments. The presence of both *Mastocarpus stellatus* and *Fucus serratus* in pools resulted in a markedly lower photosynthetic efficiency of the biofilm than with *M. stellatus* alone or no macroalgae. Variation in photosynthetic efficiency of the biofilm could be the result of changes in either the photosynthetic activity of that biofilm or a decrease in photosynthetic biomass, either of which could be associated with a change in composition of the biofilm. F_0, which is also a proxy for the amount of chlorophyll *a* (Consalvey et al. 2005), showed no significant differences among macroalgal treatments (df = 3, 12; F = 2.81; p = 0.0843), indicating that the mechanism underpinning variation in this case was variation in the photosynthetic activity of the biofilm, not a reduction in its biomass.

On emergent rocky shores, the photosynthetic activity of the biofilm has been shown to be affected by abiotic and/or biotic factors (Stafford & Davies 2005, Thompson et al. 2005, Hutchinson et al. 2006, Skov et al. 2010). Several patellid limpets, littorinids and trochids were present in and outside of the natural rock pools of the study sites. The presence of these grazers was not recorded as part of the experiment, but limpets were never observed in the experimental rockpools at low tide. If any did enter the rockpools at high tide, their effect was much smaller than the one they can have in pools where they stay throughout the tidal cycle. Yet, we cannot discount the possibility that the combined presence of *Fucus serratus* and *Mastocarpus stellatus* may have increased the foraging activity of grazers in the rock pools, which in turn could have changed the biofilm composition resulting in a reduced photosynthetic activity of the biofilm (Kaehler & Froneman 2002). While the present study cannot address this hypothesis, further studies should explore the interactive role of grazers and macroalgae in controlling the photosynthetic activity of rock pool biofilm.

Conclusion

Clearly, the metabolism of intertidal ecosystems still needs to be explored in detail before generalisations can be made on the contribution of different compartments to the functioning of these habitats. The contribution of biofilms to rock pool community metabolism is likely to be influenced by many factors that affect the biofilm's composition and/or photosynthetic efficiency. Nevertheless, the present study has shown that the contribution of biofilms to rock pool metabolism is significant and underlines concerns about their general omission from current debates about the role of biodiversity in ecosystem functioning (but see Vanelslander et al. 2009, Peter et al. 2011).

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