Vol. 416: 115–125, 2010
doi: 10.3354/meps08781

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published October 14

# Primary productivity of intertidal macroalgal assemblages: comparison of laboratory and *in situ* photorespirometry

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ABSTRACT: Photorespirometry has long been used to examine primary production of aquatic micro- and macroalgae. Despite a growing number of studies examining *in situ* primary production in soft sediment ecosystems, little has been done to test *in situ* primary production on rocky reefs. This disparity may be due, in part, to a lack of a suitable photorespirometry apparatus able to be sealed to rocky reef surfaces. To compare laboratory and field-based primary production we designed and tested a photorespirometer. Because the device fits securely to rocky reefs, we were able to test natural assemblages and monospecific stands during immersion *in situ*. These were then compared to similar algal assemblages in laboratory conditions over irradiance levels from 100 to 2000 µmol $m^{-2}$ $s^{-1}$. Primary productivity of both canopy-forming fucoid algae and understory corallines was greater *in situ* than under lab conditions, particularly at irradiance levels above 1500 µmol $m^{-2}$ $s^{-1}$. Comparisons between laboratory and *in situ* conditions suggest fundamental differences in dynamics of productivity and point to a vital role of light delivery. Furthermore, the increasing productivity down a shore-height gradient shows that a combination of greater biomass and greater numbers of macroalgal species significantly increases primary productivity of these macroalgal assemblages.

KEY WORDS: Primary productivity · Macroalgae · *In situ* · Irradiance · Rocky intertidal · Fucoids



## INTRODUCTION

Oxygenic photosynthesis is responsible for virtually all biochemical production of organic matter in marine and terrestrial ecosystems. Transfer of energy through most food webs can be directly linked to the fixation of carbon at the primary producer level (Field et al. 1998). It is vital, therefore, to understand the quantity and quality of primary production in various natural assemblages and the factors affecting it. At the global scale, terrestrial net primary production is one of the most modelled ecological parameters and is often the primary metric for ecosystem function (Field et al. 1998, Loreau et al. 2001). However, estimations of primary production of the nearshore benthic marine environment are poorly represented in the ecological literature (Stachowicz et al. 2007). Although marine macrophytes make up a small proportion of ocean primary production, they undoubtedly supply a majority of biomass to nearshore ecosystems. Macroalgal subsidies are documented in analyses of stable isotopes, which show that the signature of marine algae extends far outside of areas where they occur, including to non-vegetated nearshore areas and terrestrial landscapes (Anderson & Polis 1998, Hyndes & Lavery 2005), intertidal mud flats (Riera & Hubas 2003), offshore communities (Hill et al. 2006) and deep offshore basins (Fischer & Wiencke 1992). Estimating the potential primary production of macroalgal assemblages is therefore essential to understanding the role of macroalgae in reef ecology and the transfer of energy through food webs.

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Primary production of marine macrophytes has typically been examined in laboratory incubations (Littler & Littler 1980, Littler & Arnold 1982) or by *in situ* measurements of change in biomass (Mann 1973, Reed et al. 2008). Although both techniques are useful under certain circumstances, they have numerous shortcomings. Laboratory incubations often fail to scale up primary production to that which occurs in nature (Binzer & Middleboe 2005). Furthermore, laboratory incubations are performed under artificial conditions and often use excised tissue samples (Flores-Moya et al. 1995, Gómez et al. 1997), which do not necessarily give an accurate estimate of production by whole plants. Measurement of change in biomass (or growth increments) is one way to estimate primary production *in situ* and has the potential to predict large-scale variation in production over time. Reed et al. (2008), for example, showed using growth increment techniques that primary production of the giant kelp *Macrocystis pyrifera* depended largely on the foliar standing crop of algae. However, *in situ* measurements of changing biomass can either fail to factor processes of natural loss into estimations of primary production, or else require elaborate and time-consuming field-based measurements to be realistic (as in Reed et al. 2008). If possible, it is far better to estimate benthic primary production of macroalgae assemblages by measuring physiological primary production directly in natural assemblages, as is common practice for the estimation of production by microalgae in soft sediments (Migné et al. 2004).

Estimations of primary production by assemblages are increasingly relevant, given the growing interest in research on primary productivity in assemblages containing natural compositions of species (e.g. Bracken et al. 2008). Levels of primary production seen in diverse communities indicate that photosynthesis does not show a typical saturation curve, as is usually seen in productivity-irradiance curves for single species (Lobban et al. 1985), but increases in a more linear fashion (Binzer & Sand-Jensen 2002a, Binzer & Sand-Jensen 2002b, Middleboe & Binzer 2004; Binzer & Middleboe 2005). In fact, primary production within diverse assemblages has been shown to increase in a relatively linear fashion with increasing irradiance up to maximum levels of natural irradiance (Middleboe & Binzer 2004). Understanding the dynamics of primary production across a range of irradiances is particularly relevant to macroalgae in the intertidal zone, where changes in light regimes can be large over short time spans through changing tidal height and repeated immersion and emersion. The relationships between these environmental changes and primary production levels have important implications for how primary productivity should be measured in natural assemblages, since they relate directly to how a diverse canopy structure (i.e. layering of algae in natural assemblages), and therefore species diversity, may affect this essential function.

To estimate benthic primary productivity of a macroalgal assemblage effectively, it is necessary to have a means of assaying a whole assemblage and not just a species in isolation. *In situ* photorespirometry incubations have been used for several decades (e.g. Carpenter 1985, Chisholm et al. 1990, Cheshire et al. 1996, Golléty et al. 2008), for example in seagrass beds (Moncreiff et al. 1992, Eyre & Ferguson 2002) and other soft sediment systems (Dalsgaard 2003, Migné et al. 2004), where the low profile (i.e. very low standing biomass and short height of autotrophs) of communities and quiescent conditions enable incubation chambers to be readily sealed. Photorespirometry of benthic communities on rocky reefs has received some attention, but incubations have usually been on displaced algae (Littler & Littler 1980, Littler & Arnold 1982, Cheshire et al. 1996), or on algae cultured on settlement plates (Carpenter 1985). Only a few studies have analyzed photosynthesis within intact, *in situ* macroalgal assemblages (Chisholm et al. 1990, Golléty et al. 2008, Miller et al. 2009, Noël et al. 2010). The system used by Chisholm et al. (1990) was designed primarily to examine tropical encrusting turf assemblages, but it was small, at only 50 $cm^2$. More recently, several studies have used larger chambers, integrating productivity in whole macroalgal assemblages. For example, Golléty et al. (2008) tested primary productivity during emersion on *in situ Ascophyllum nodosum* dominated assemblages. Miller et al. (2009) developed and used a larger chamber for benthic subtidal assemblages. This had a weighted skirt to create a seal with the reef. Noël et al. (2010) used an open incubation method to determine productivity of rockpool communities. With the exceptions of Miller et al. (2009) and Noël et al. (2010), few studies describe physiological *in situ* primary production of macroalgal assemblages during immersion on rocky reefs. Despite some progress, a major impediment has been the unavailability of an adequate chamber that can be readily attached with an effective seal, be used in multiple conditions from calm to more wave-swept and be reused on the same assemblages over time.

We wished to account for primary production in intertidal macroalgal assemblages dominated by fucoid algae and with a diverse understory of red and green algae. We needed a device capable of estimating total net and gross primary production using dark respiration, which measurements of growth increments can miss. With this in mind, a novel incubation chamber was designed and tested on natural benthic communities. This chamber, unlike other photorespirometers,

was fitted around attached assemblages and was firmly attached to a reef surface, providing a quick and effective means of analyzing net and gross primary production. This provided a platform to examine assemblages at various stages of succession and could also be used to test effects of disturbance on primary productivity and the subsequent recovery of natural assemblages. We used this device to test the hypothesis that similar assemblages tested *in situ* and using laboratory methods would have similar productivity dynamics. *In situ* and laboratory methods were tested in analogous incubation chambers to compare any differences in productivity dynamics.

## MATERIALS AND METHODS

**Photorespirometer design, materials, and deployment.** Primary production of natural macroalgal assemblages was examined *in situ* using custom-built incubation chambers fixed to the substratum of rocky reefs. Chambers were designed to be secured around established assemblages of benthic intertidal macroalgae, without displacing them. The chambers were made of a clear Perspex tube of 2 heights/volumes (25 cm/12.3 l or 30 cm/14.7 l) with a clear Perspex attachment plate and lid (Fig. 1). They were attached to a reef using a separate base plate to which the main chambers were bolted. The Perspex tubing had a diameter of 25 cm and covered a reef surface area of approximately 491 $cm^2$.

Before chambers could be effectively fixed to a reef, the area around the target assemblage needed to be cleared of algae and invertebrates. This was done by scraping the substratum with a chisel and a wire brush. Cracks and concavities in the rocky substratum were filled with a 2-compound epoxy resin (Expocrete) to ensure that the surface was flat enough to allow a watertight seal. The use of this resin allowed chambers to be refitted during subsequent visits, for example to measure successional events. However, as a short-term fix for leakage problems, a silicon sealing compound (window sealant that binds to moist surfaces) can be used to fill in gaps. In either case, a flat base plate (with a central hole the diameter of the Perspex chamber) was placed around the assemblage and sealed to the reef using inset rawl plugs and four 10 cm long bolts. Base plates were made from 1 cm thick PVC to which a 5 cm thick piece of closed-cell polystyrene foam was glued. When tightened to the reef, the foam compresses and fills slight irregularities in the reef, forming a good seal between the rock and the base plate. Four long, threaded bolts were used to attach the main chamber to the base plate. The seal between a chamber and base plate was maintained using 2 rubber O rings that compress when the long bolts are tightened (Fig. 1A). The use of the base plates was essential to stop flexing from occurring in the main chamber when tightened to an irregular reef surface and the O rings on the main chamber compensate for any slight flexing in the base plate. The lid of the chamber was attached by 4 long, threaded bolts which extended from the base plate to the lid; the use of wing nuts allowed quick fixing and removal of the lid. The lid itself was a flat piece of Perspex (10 mm thick) with a circular groove lathed into it, to which an O ring or a 2 mm thick piece of closed-cell foam was fitted. Once chambers were filled with seawater for an incubation, the lid could be quickly secured; any slight leaks from the base plate usually stop because of the vacuum created within the filled chamber. Water samples were taken using 2 taps in the lid, one to take the sample, the other to replace water (if necessary). The taps were fitted into 2 hollow, plastic, threaded plugs that were screwed into threaded holes in the lid (Fig. 1A).

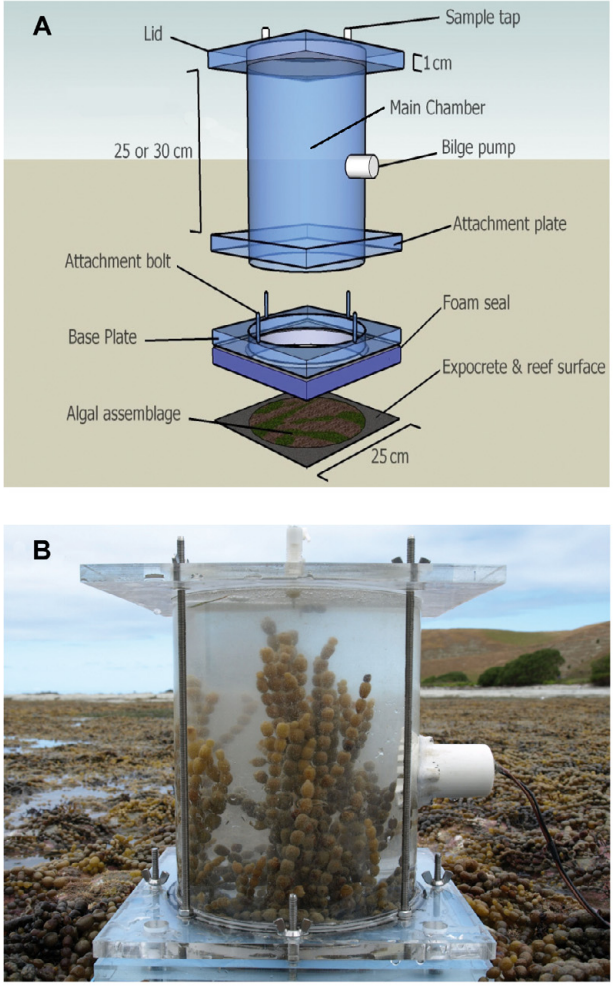


Fig. 1. *In situ* incubation chamber and its components. (A) Exploded diagram. (B) Chamber in place on a reef platform

To stop boundary layers from establishing during the sampling period, the water within chambers was mixed using a battery-powered bilge pump (made for use in small boats) that pumped ~800 l $h^{-1}$ and was fitted so that the chamber was stirred in a circular vortex motion. It was powered by a 12V sealed lead-acid battery (a small motorcycle battery) housed in a watertight case.

Because most production-irradiance curves are derived in controlled laboratory conditions, we tested the same species in the laboratory to enable a direct comparison with field incubations. Laboratory incubations were done using the same experimental procedure as for *in situ* incubations, and in identical chambers, except with a Perspex sheet fixed to the underside of each chamber. Temperature was controlled by immersing chambers in a temperature-regulated water bath (set to 15°C). Chambers were mixed internally using the same bilge pumps as in the field chambers. Productivity-irradiance curves were generated using 5 levels of irradiance (150, 300, 800, 1500 and 2000 µmol $m^{-2}$ $s^{-1}$), with the light generated by metal halide lamps calibrated to PAR (photosynthetically active radiation). Dark respiration was measured in the laboratory by covering chambers to exclude light. For these laboratory incubations, macroalgae were taken from the field using a hammer and chisel to remove the substratum, to which the macroalgae remained attached. The attached macroalgae were, therefore, at similar densities, biomass and percentage cover as the field assemblages used in incubations. Data from both laboratory and *in situ* conditions were standardized to a benthic surface area of reef (i.e. the section of substratum removed) to allow direct comparisons, as well as in g dry wt of algal material to compare photosynthetic characteristics of the algae. For the purposes of these experiments, algae were harvested following *in situ* and laboratory incubations in order to standardize data by dry biomass of algae.

**Incubation protocol.** Once a chamber was secured to a reef, the effectiveness of the seal was tested by filling the chamber with seawater (during low tide) and looking for any significant leaks (Fig. 1B). If the chamber held water when the lid was secured and no bubbles were observed, the seal was deemed adequate to prevent water flux between the chamber and surrounding seawater. Incubations were done while the tide covered the chambers to ensure that the internal temperature remained stable and so prevented the formation of oxygen bubbles, and also to ensure that the light regime was as natural as possible. Light intensity and temperature within the chambers were measured throughout experiments using HOBO (Onset Corporation™) data loggers. The loggers were placed on the inside of the chamber lid to measure irradiance reaching the algal canopy. Light intensity was cross calibrated and regularly checked for accuracy with a LiCor light meter (LiCor LI 192 quantum sensor). Dissolved oxygen concentration within a chamber was obtained by extracting water samples from the tap on the chamber lid using a syringe, and then immediately measuring with a Hach LDO meter (Model HQ40d). To measure productivity, samples were taken from the chamber at 20 min intervals. Oxygen production was converted to carbon fixation using a photosynthetic quotient of 1.1 (Kirk 1994). Syringes were kept cool using the surrounding water and were kept in the dark after extraction to limit post-extraction effects on oxygen concentration (i.e. to prevent warming and the formation of oxygen bubbles). Each replicate reef plot was tested across a wide range of natural irradiance, from very dull to full sunlight (~100–300 to < 2000 µmol $m^{-2}$ $s^{-1}$) to gain a productivity-irradiance curve for the target species or assemblage. For this reason, experiments on a single replicate plot were often done over several days.

Macroalgal assemblages can contain a variety of sessile and mobile invertebrates. To limit their respiration, visible invertebrates were removed from the target area. However, removing all invertebrates from *in situ* assemblages was often impossible because of their small size (some are much smaller than 1 mm) and the presence of overlying algae. Dark respiration of the entire assemblage was measured, therefore, to obtain an estimate of gross primary productivity. Dark respiration was measured by covering the chambers with a layer of black cloth with an overlay of tin foil to stop chambers from becoming heated. Following periods of photosynthesis, the relative respiration rate of macroalgae can be substantially elevated, a phenomenon known as photorespiration (Reiskind et al. 1989). Therefore, once chambers were covered they were allowed to settle for 30 min before respiration measurements were started to limit photorespiration. Respiration rates recorded during these shaded experiments were also compared to night-time samples of respiration and revealed no significant difference. Oxygen concentration was measured every 20 min for up to 2 h.

**Study sites and *in situ* photorespirometry experiments.** Incubations were done during austral spring to summer 2007, and summer 2008 at Wairepo Reef, Kaikoura and North Reef, Moeraki. The 2 study sites are located ~400 km apart on the east coast of South Island, New Zealand. The sites had a similar suite of macroalgal species, but differed in rock type and wave exposure. Wairepo Reef is composed predominantly of sandstone and mudstone, whereas reefs at Moeraki are limestone and various conglomerates. Moeraki is exposed to greater and more frequent wave force. Therefore, chambers were used at sites under quite different wave conditions and rock types.

First, it was necessary to calibrate the chambers to *in situ* conditions. This was done through a series of experiments that tested the limitations and effectiveness of the chambers. To understand the limitations of the chambers for measuring oxygen evolution, an experiment was done to test the potential for decline in productivity over time. The discrete volume of water contained within the chambers has the potential to become depleted of essential nutrients, supersaturated with oxygen during photosynthesis or depleted of oxygen during respiration.It would therefore be necessary to replace seawater periodically in order to maintain natural conditions during incubations for sufficient time to allow an appreciable decline in primary production to occur. To determine the effective time for incubations, we tested the decline in production over time in sealed chambers. This was done on relatively dense stands of *Hormosira banksii* (a fucoid alga reaching ~30 cm height and with a biomass of <8 kg fresh biomass $m^{-2}$ or ~2 kg dry biomass), because an assemblage with large biomass was most likely to saturate. Change in oxygen concentration in chambers was sampled every 20 min for 80 min at 4 levels of irradiance: 800, 1000, 1200, and 2000 µmol $m^{-2}$ $s^{-1}$, with 4 replicates for each. Following this, the experiment was repeated on the same plots, but with the water inside the chambers being replaced every 20 min.

Respiration by invertebrates may potentially have a large effect on measurements of net productivity due to consumption of oxygen. To account for the potential influence of invertebrates that could not be removed *in situ*, a laboratory experiment was designed to test the effects of invertebrate density on overall community respiration. It was difficult to remove all invertebrates from *in situ* assemblages without causing a major disturbance. However, removing all invertebrates in a laboratory experiment was relatively easy. Therefore, the effects of invertebrate density on respiration rates was tested under laboratory conditions. Respiration was measured by covering chambers with a dark cloth to exclude light. For density treatments 0, 5 and 10 invertebrates per chamber were used (n = 6). The invertebrate species used were a combination of the trochid snail *Diloma aethiops* and the chiton *Sypharochiton pelliserpentis*. Macroalgae and invertebrates attached to sections of rock were removed from the field and manipulated in the lab. All invertebrates were removed from the substratum and counted. Once all invertebrates were removed, the macroalgal assemblage alone was incubated. After the zero-invertebrate respiration, 5 invertebrates (approximate natural density) removed from the assemblage were added back into the incubation chamber (excluding any microgastropods). These were the most common mobile invertebrates found within these assemblages. For the final treatment, the density was doubled to 10 invertebrates per incubation chamber. These incubations were done at 3 temperatures (10, 15 and 20°C) to examine potential differences in respiration across a range of natural temperatures.

Daily variation in ambient oxygen concentration may cause limitations in primary production of macroalgal assemblages under conditions of minimal mixing. Supersaturation of oxygen under quiescent conditions may cause inhibition of photosynthesis within the algae. Therefore, the variation in ambient oxygen concentration and primary productivity throughout the day were analyzed. Data were collected from several experiments done over different days and under various irradiance regimes. To ensure consistency, data were taken on incoming tides during mid tide when water height ranged between 20 and 60 cm above the reef. This was done on several days across 3 wk during October 2008. Data were collected only on relatively calm days, where supersaturation was most likely. Incubations were done on *Hormosira banksii*-dominated assemblages using the best experimental procedure identified above (i.e. 20 min incubation duration). Data on ambient oxygen concentrations were collected adjacent to chambers where productivity measurements were taken.

Productivity-irradiance curves were generated for 2 species. Monospecific stands were found in natural assemblages and were used to avoid any complications involved with having different compositions of species in replicate plots. The species were *Hormosira banksii* and *Corallina officinalis*; the latter is a turf-forming calcareous alga dominant in the understory of canopies. Incubations were done (n = 4 for each species at each site) between September 2007 and February 2008. However, the curves for the 2 sites were identical, so results were pooled for presentation. For laboratory incubations, *H. banksii* and *C. officinalis* attached to rocks that were chipped away from the reef surface were collected from the field. These monospecific stands were then bought to the lab where photorespiration incubations were done. To compare *in situ* and laboratory-based incubations, *in situ* data were pooled into several irradiance ranges (0, 150, 300, 800, 1500 and 2000 µmol $m^{-2}$ $s^{-1}$), the same as those at which the laboratory algae were incubated. We analyzed primary production across an intertidal height gradient using assemblages at 3 shore heights (high, mid and low). Different canopy species occurred at the various shore heights, with *H. banksii* dominating the high zone, equal cover of the fucoids *H.banksii* and *Cystophora torulosa* in the mid zone, and *C. torulosa* dominating the low zone. There were also several understory species. Diversity of macroalgae, biomass and relative covers of assemblages were also determined at

each site. Data were analyzed at irradiance levels above 1000 µmol $m^{-2}$ $s^{-1}$ to test differences in maximal productivity.

## RESULTS

Tests to determine when primary productivity began to decline when internal water was not replaced showed that at 2000 and 1200 µmol $m^{-2}$ $s^{-1}$, this occurred after 40 min (Fig. 2A). Also at high irradiance, the saturation of oxygen reached over 200% after 60 min of incubation time, indicating excessive levels of dissolved oxygen. This effect was less pronounced at lower irradiance levels but still occurred after a similar time. A 2-way ANOVA indicated a significant effect of irradiance ($F_{3,48}$ = 221, p < 0.0001), time ($F_{3,48}$ = 97.3, p < 0.0001) and an interaction effect (irradiance × time, $F_{9,48}$ = 27.7, p < 0.0001). Bonferroni post-hoc tests of the data indicated that at both 2000 ($t$ = 10.9, p < 0.0001) and 1200 µmol $m^{-2}$ $s^{-1}$ ($t$ = 4.7, p < 0.016) primary productivity was significantly less at 60 min than at 20 min. Productivity at 80 min was not significantly less than at 20 min in the 2 lower irradiance treatments (800 and 1000 µmol $m^{-2}$ $s^{-1}$). However, when seawater was replaced at 20 min intervals (just after each water sample was taken for analysis), primary production at all irradiance levels remained constant throughout an experiment (Fig. 2B). There was no significant effect of time on production, but there was a significant effect of irradiance ($F_{3,48}$ = 24.19, p < 0.0001). The reliability of results depends, therefore, on the length of the incubation and the level of irradiance. In particular, at higher irradiance levels, incubations should not exceed 40 min, but for efficient sampling, 20 min intervals would provide greater resolution.

Effects of density of the invertebrates *Diloma aethiops* and *Sypharochiton pelliserpentis* on respiration showed a general trend of increasing respiration rate with increasing density (Fig. 3). Respiration rates also increased at elevated temperatures. However, the results indicate considerable variability, with a large amount of overlap between respiration rates at different densities at the same temperature. Two-way ANOVA indicated no significant effect of temperature and no interaction effect, but a significant effect of invertebrate density ($F_{2,45}$ = 4.3, p < 0.05). Invertebrate density has the potential to influence productivity, but large variations in density are required to to produce this effect.

Daily production data were derived from incubations performed over different days on *Hormosira banksii*-dominated assemblages and compared to daily ambient oxygen concentration. As expected, there was a strong relationship between oxygen evolution and primary production that corresponded to the hours of highest irradiance between 11:00 and 13:00 h at 2000 µmol $m^{-2}$ $s^{-1}$ (Fig. 4). Ambient oxygen concentration generally increased throughout the day; it peaked at 13:00 to 14:00 h, then declined in the evening. The greatest observed oxygen saturation was 150%, which

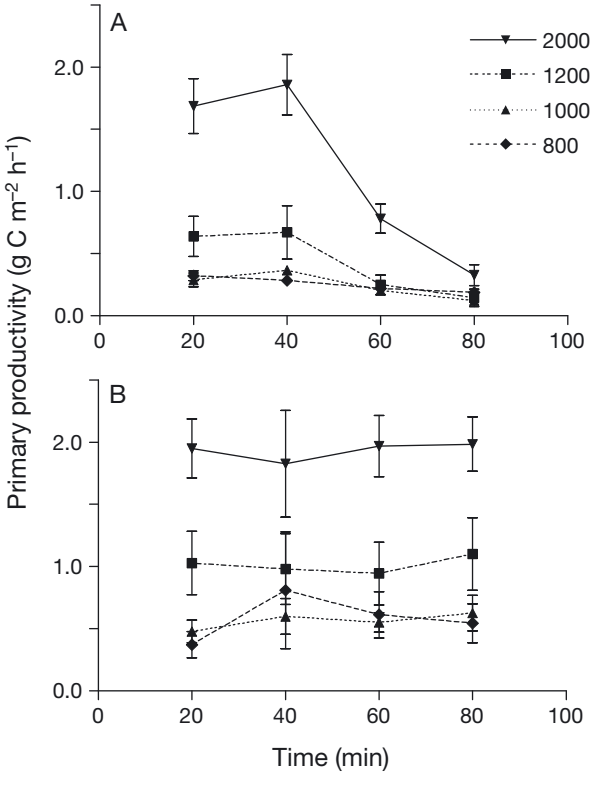


Fig. 2. Net primary production (mean ±SE, n = 4) over time when water is (A) not replaced and (B) replaced at ca. 20 min intervals (just after each reading) within chambers at various levels of irradiance 2000, 1200, 1000 and 800 µmol $m^{-2}$ $s^{-1}$

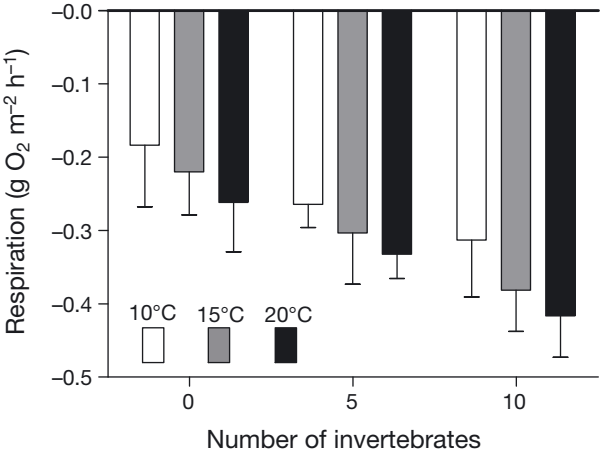


Fig. 3. Overall respiration by macroalgal assemblages (mean ±SE, n = 6). Effects of temperature (10, 15 and 20°C) and density of invertebrates (0, 5 and 10 per chamber)

occurred at ~13:00 h. Primary productivity showed a similar relationship throughout the day but with a peak between 11:00 and 13:00 h. Because ambient oxygen concentration increased throughout the day, production late in the day could potentially have been affected by supersaturation of oxygen. However, these data show that the peak in production was related to sunlight rather than to water chemistry.

For both *Hormosira banksii* and *Corallina officinalis*, laboratory data showed saturation at lower levels of irradiance compared to *in situ* data. On a per-area basis, the *in situ* curves reached peak values around 20% greater than those derived in the lab, and inflection points were at a lower irradiance in the lab (Fig. 5A,C). For *H. banksii*, *in situ* maximum primary productivity $P_{max}$ was significantly greater when standardized by area (2-tailed *t*-test, $t = 2.6$, df = 7, $p = 0.024$), but not by biomass. *In situ* $P_{max}$ for *C. officinalis* was significantly greater than laboratory measurements when standardized by area (2-tailed *t*-test, $t = 3.7$, df = 7, $p = 0.0036$) and by biomass ($t = 4.3$, df = 7, $p = 0.0011$). Refraction of light through surface waters *in situ* may have delivered light more consistently through these assemblages than to those in the lab. To control for potential differences in angles of incidence, the laboratory light source was moved to various locations, but this caused no significant difference in primary productivity. $P_{max}$ of *H. banksii* was almost double that of *C. officinalis* on a per area basis (2-tailed *t*-test, *in situ* $t = 6.4$, df = 7, $p < 0.0001$; laboratory $t = 5.7$, df = 7, $p = 0.0002$) and on a per dry biomass basis (*in situ* $t = 2.5$, df = 7, $p = 0.03$; laboratory $t = 3.7$, df = 7, $p = 0.004$; Fig. 5). Since saturation of photosynthesis did not always occur, $P_{max}$ in these monospecific stands was considered as primary productivity at the highest irradiance analyzed (i.e. 2000 µmol $m^{-2}$ $s^{-1}$). *H. banksii* reached an average *in situ* production of 1.0 g C $m^2$ $h^{-1}$, whereas *C. officinalis* reached levels of 0.5 g C $m^2$ $h^{-1}$. Per biomass, both species were in the range of 0.2 to 0.3 mg C g dry $wt^{-1}$ $h^{-1}$. Comparisons of the *in situ* and laboratory-derived irradiance curves showed that they were not identical for either species (all curves were fitted using 1-phase associations).

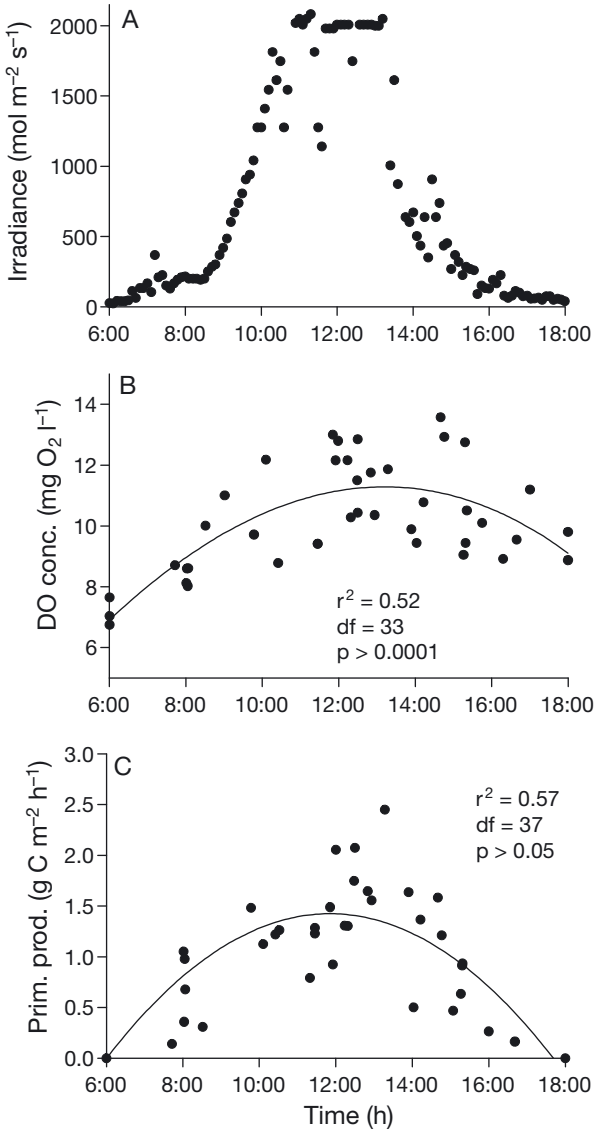


Fig. 4. Daily cycles of (A) *in situ* irradiance, (B) ambient dissolved oxygen (DO) concentration and (C) primary productivity (prim. prod.). 06:00 h/18:00 h = approximate time of sunrise/sunset. Lines are fitted using a non-linear regression (third order polynomial)

Composition of assemblages down the shore showed a change in the dominance of fucoid species, with the canopy changing from *Hormosira banksii* on the high-shore zone to *Cystophora torulosa* on the low shore (Fig. 6). Generally, the assemblages across shore zones were dominated by *H. banksii*, *C. torulosa*, or both, in association with a large cover of the turf-forming coralline alga *Corallina officinalis*. Furthermore, low-shore assemblages had greater numbers of macroalgal species and more species with greater covers. Along with the total number of species, the average number of species and biomass were greater in low-shore assemblages (Table 1). There was a clear trend in production down a reef gradient (Fig. 7). Primary production at high irradiances (>1000 µmol $m^{-2}$ $s^{-1}$) was greatest in the low shore assemblage and least on the upper shore (1-way ANOVA, $F_{2,6} = 17.8$, $p < 0.003$). Tukey's multiple comparison post-hoc tests showed significant differences between high shore and mid shore ($q = 6.1$, $p < 0.05$), as well as high shore and low shore ($q = 8.1$, $p < 0.01$). This gradient in primary production represents a physiological capacity for production only and does not take into account the immersion times at each shore height.

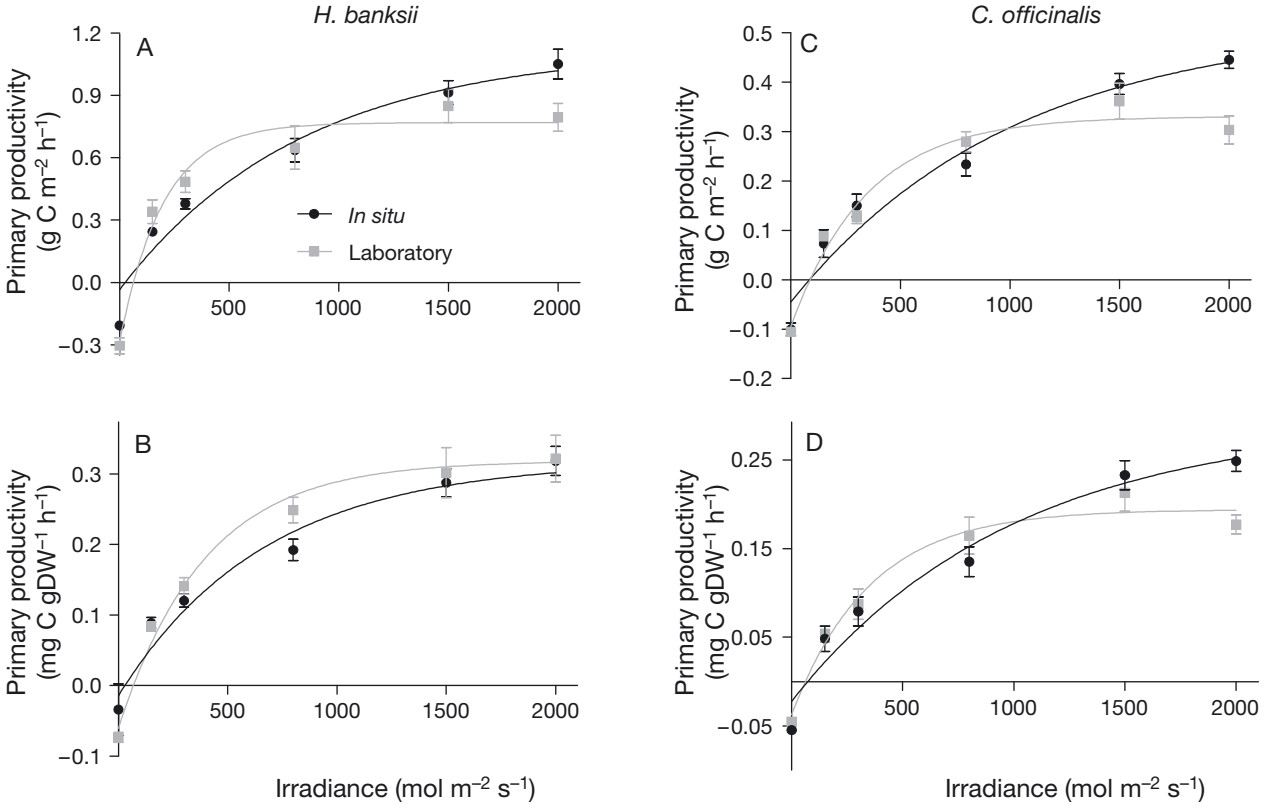


Fig. 5. Net primary productivity (mean ±SE, n = 8) vs. irradiance in stands of 2 dominant species, the fucoid *Hormosira banksii* (A,B) and the calcareous turf *Corallina officinalis* (C,D) under *in situ* or laboratory conditions. Data were standardized by surface of reef area (m$^{-2}$) (A,C) or by dry weight (g DW) of alga (B,D)

## DISCUSSION

These incubation chambers are some of the first documented devices of their kind capable of sealing around large, attached macroalgae on intertidal rocky reefs during immersion. Although many experiments using similar principles have tested primary productivity on macrophytes *in situ*, they have mostly focused on sediment-based ecosystems (Eyre & Ferguson 2002, Dalsgaard 2003), on macroalgae during emersion (Golléty et al. 2008) or on much smaller algae (Chisholm et al. 1990). One of the major studies on macroalgae attached to hard substrata (Chisholm et al. 1990) fixed the incubation chamber to the reef using masonry tools, which tore away sections of the substratum, and was difficult and time consuming. Golléty et al. (2008) used a sophisticated incubation chamber that was able to be attached to intertidal rocky reefs, but was only able to measure primary productivity during emersion. The apparatus used by Miller et al. (2009), although similar to our device, was used in relatively deep water with the aid of divers. Their chamber was made of flexible Teflon sheeting and held down by a weighted skirt around the bottom edge. Although suited to the deep reef conditions it was designed for, it is unlikely to be suitable in the intertidal zone, where frequent wave disturbances would likely dislodge it. The high velocity of water currents in the intertidal zone may also make a weighted seal ineffective at keeping the internal water isolated. Our chambers overcome these problems, are a quick and effective tool for assaying existing algal assemblages, and involve no major modifications to the reef surface (with the exception of using a small amount of epoxy resin). The ability to seal these chambers around existing assemblages makes them very useful for studying patterns and processes across a diverse range of conditions and habitats and because they can be repeatedly deployed on the same assemblages, for including longer-term processes.

Our study showed that monospecfic stands of *Corallina officinalis* can fix ~0.1 to 0.3 g C m$^2$ h$^{-1}$. Chisholm et al. (1990) found that tropical crustose coralline algae had an average *in situ* production of 0.18 g C m$^{-2}$ h$^{-1}$. Data from our study match this closely, indicating that these techniques produce results comparable across different habitats and reef systems. Primary production by *Hormosira banksii* had a range between 0.3 and 1.0 g C m$^{-2}$ h$^{-1}$ (or ~3.3 g C m$^{-2}$ d$^{-1}$), much greater than that of *C. officinalis*. Productivity of *H. banksii*

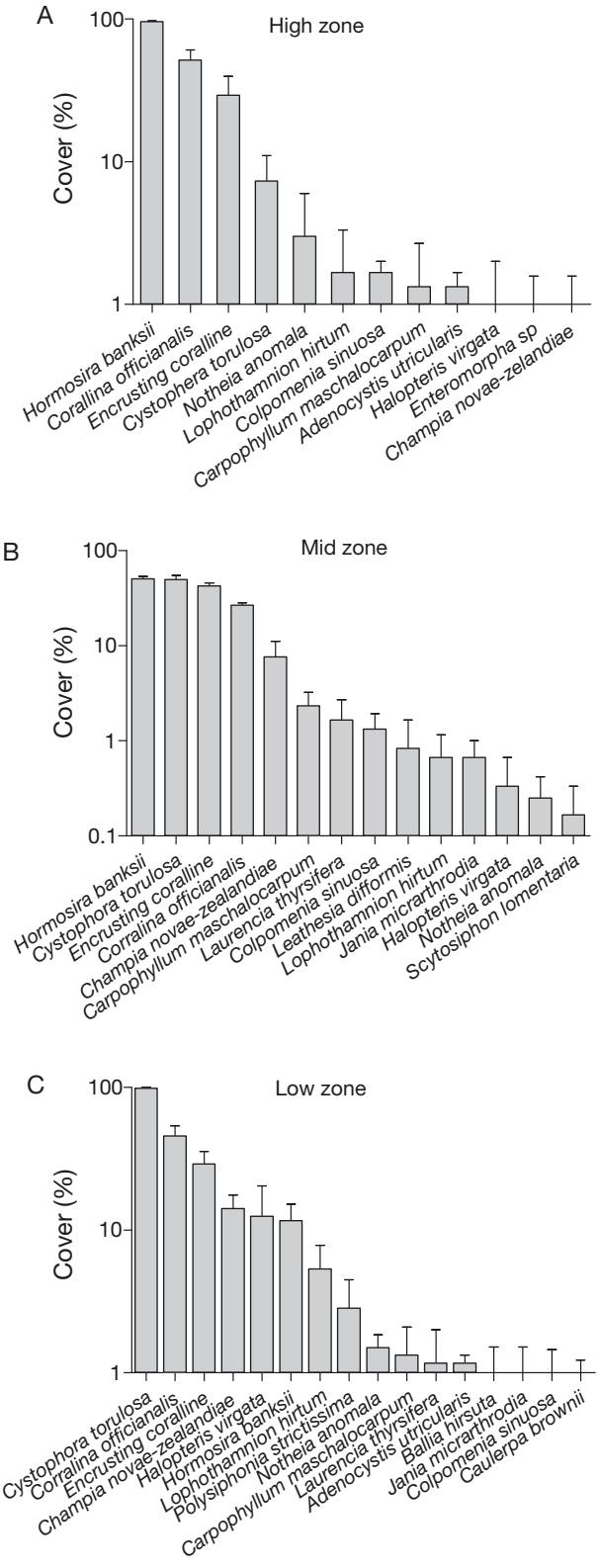


Fig. 6. Cover (%; mean + SE, n = 3) and number of macroalgal species at 3 shore heights. (A) High zone: *Hormosira banksii*-dominated assemblages. (B) Mid zone: equal covers of *H. banksii* and *Cystophora torulosa*. (C) Low zone: *C. torulosa*-dominated assemblages. The *y*-axis is shown as a log scale

Table 1. Dry biomass and average number (mean ± SE) of macroalgal species in 3 assemblages dominated by *Hormosira banksii* (high shore), equal amounts of *H. banksii* and *Cystophora torulosa* (mid shore), and *C. torulosa* (low shore)

| Assemblage dominant | Dry biomass (kg $m^{-2}$) | Number of species |
|---|---|---|
| *H. banksii* | 2.2 ± 0.15 | 6.3 ± 0.41 |
| *H. banksii* and *C. torulosa* | 2.5 ± 0.29 | 7.8 ± 0.31 |
| *C. torulosa* | 3.1 ± 0.32 | 8.3 ± 0.42 |

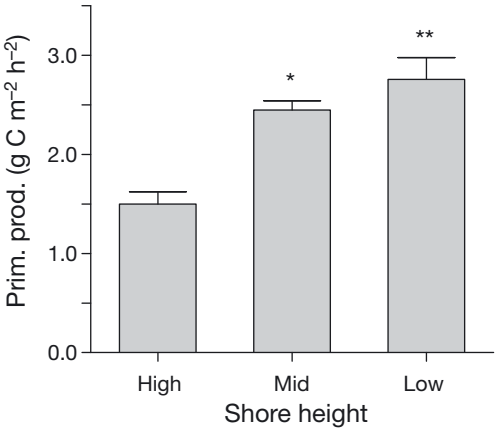


Fig. 7. Primary production (mean +SE, n = 3) of *in situ* assemblages at Wairepo Reef, Kaikoura, across a shore-height gradient at irradiances > 1000 µmol $m^{-2}$ $s^{-1}$. Significant difference from high shore indicated by *p < 0.05, **p < 0.01, using 1-way ANOVA and Tukey's post-hoc test

monocultures is similar to the gross carbon fluxes of *Ascophyllum nodosum* shown by Golléty et al. (2008), at between 0.2 and 0.8 mg C $m^{-2}$ $h^{-1}$. Average daily production of *Macrocystis pyrifera* was estimated by Jackson (1977) at 9.5 g C $m^{-2}$ $d^{-1}$ (~1.2–1.6 g C $m^{-2}$ $h^{-1}$ on average, depending on h of sunlight) which, as its large size and extensive foliage would lead one to expect, is much greater than that of *H. banksii*. Nevertheless, primary production potential of *H. banksii* is within a similar range as that of some large, very productive macroalgae (e.g. Jackson 1977; Golléty et al. 2008).

Differences between laboratory and *in situ* results suggest there are fundamental differences in how light in these 2 environments may be affecting algae. Probably the most obvious difference between laboratory and *in situ* incubations is the delivery of light, with laboratory light produced by a beam source and irradiance varied by using filters. In contrast, light from the sun can vary significantly from seasonal to second-by-second scales. One clear inconsistency is the change from beam to diffuse irradiance *in situ*, with the presence of cloud cover significantly altering density of

shadows through a canopy. Studies in terrestrial ecosystems suggest that productivity may in fact be greater during days of diffuse radiation due to the decreased volume of shade within a forest canopy (Roderick et al. 2001). Although this cannot be unequivocally concluded from our study, our results indicate the intricacies of light delivery *in situ* and its importance to production. In the marine environment, small-scale fluctuations in irradiance by diffraction through waves add a further dimension to light delivery. Increasing frequency of light fluctuations is associated with an enhancement of photosynthesis, possibly due to post-illumination bursts of $CO_2$, and variation in irradiance over periods shorter than 1 s can be responsible for the greatest levels of productivity (Dromgoole 1988). Thus, how light is delivered to macroalgal assemblages appears to play an important role in determining productivity levels.

Variation in primary productivity with shore height may represent underlying differences among species or possibly differences in assemblage composition and diversity. The pattern observed in this study is different from those found in some other studies. For example, 2 studies found greater photosynthetic capacity in high-shore algae compared to low-shore algae (Gómez et al. 1997, Skene 2004). This may be an issue of standardization, as many studies use dry biomass to standardize primary productivity, whereas we used surface area of reef (as did Miller et al. 2009), which more directly relates to other ecological processes that are assessed on a per-area basis. Furthermore, these studies considered single species as opposed to whole assemblages. Lower-shore assemblages from our study tend to have a greater biomass than those higher up, which is the most obvious explanation for the elevated productivity at lower shore levels. However, the number of species is also greater in low-shore assemblages, suggesting a potential role of biodiversity in the enhancement of productivity (Schiel 2006). Structure and composition of assemblages, including diversity and layering, may play vital roles in the dynamics of light use in complex assemblages, and evidence suggests that increasing complexity supports a more linear increase in productivity with increasing irradiance, as opposed to saturation of productivity (Middleboe & Binzer 2004; Binzer & Middleboe 2005). Although this argument is disputable without further research, it does suggest a potential relationship between biomass and species diversity, giving impetus to the argument that diversity may enhance primary productivity in macroalgal assemblages (Bruno et al. 2005).

The application of *in situ* techniques can, therefore, extend beyond dynamics of primary production alone. For example, they have potential uses in biodiversity-ecosystem function research (BEF), a developing field in ecology where research on marine assemblages is still relatively sparse compared to terrestrial communities (Stachowicz et al. 2007). *In situ* photorespirometry can be used in experimental conditions where assemblage structure is altered and resultant effects, both spatial and temporal, can be followed over time. Currently, much research has been done in artificial mesocosms (Bruno et al. 2005), but real-world examples will provide new insights to research on function in diverse assemblages (O'Conner & Crowe 2005, Naeem 2006, Stachowicz et al. 2007), including successional processes and recovery of function after various forms of disturbance. These *in situ* techniques can therefore be used to test a wide variety of ecological theories pertinent to marine ecosystems.

*Acknowledgements.* We thank Nick Etheridge of the University of Canterbury for help in designing and constructing the chambers, the Marine Ecology Research Group for help in the field, the University of Canterbury for scholarship support, Clive Howard-Williams and Malcolm Forster for helpful comments on the draft manuscript, 3 anonymous reviewers for their helpful and constructive comments, and the Andrew W Mellon Foundation of New York and the New Zealand Foundation for Research, Science and Technology (Coasts and Oceans OBI programme) for supportive funding.

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*Editorial responsibility: Antony Underwood, Sydney, New South Wales, Australia*

*Submitted: June 22, 2010; Accepted: August 23, 2010*
*Proofs received from author(s): October 10, 2010*