Variability in diel and seasonal *in situ* metabolism of the tropical gastropod *Tectus niloticus*

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ABSTRACT: Researchers often use metabolic measurements in the field over narrow time periods to estimate an organism's metabolism over large time scales. Here, we measured *in situ* respiration, calcification and excretion rates of the tropical gastropod *Tectus niloticus* L. through benthic chamber experiments. Our samples spanned a 21 h time frame and were taken during both the warm and cool seasons. We assessed diel and seasonal variability in metabolic rates, as well as the effect of individual size and the contribution of shell epi- and endobionts. Our results show that metabolic rates vary through time at both diel and seasonal scales, as measured fluxes for respiration and calcification were significantly higher at night during the warm season. This nocturnal pattern was not significant in the cool season. Size effects were significant with higher respiration and calcification rates for small individuals regardless of the season, although the difference tended to be more pronounced in the warm season. We also found that shell epi- and endobionts made an important contribution to respiration, as 40 and up to 100 % of total measured fluxes for night and day, respectively, could be attributed to the shell community. More importantly, the direction of the measured flux was occasionally opposite that of the individual trochus, highlighting that the contribution of shell epi- and endobionts must be accounted for in order to achieve an accurate understanding of individual metabolism. Lastly, depending on the time of day and season when measurements are taken, ignoring diel or seasonal variations in metabolic rates could result in important under- or overestimation of the contributions of gastropods to carbon and calcium carbonate fluxes in coastal ecosystems.

KEY WORDS: Gastropod · Seasonal variations · Diel variations · Size · Carbon fluxes · CO_2 · $CaCO_3$ · New Caledonia

INTRODUCTION

Respiration and biocalcification of marine animals such as molluscs can be a source of CO_2 into the ecosystem and their role in the carbon cycle has been demonstrated in temperate environments (Chauvaud et al. 2003, Clavier et al. 2009, Lejart et al. 2012, Mistri & Munari 2013). This contribution can be assessed empirically through oxygen respiratory fluxes converted to carbon using available respiratory quotients (Boucher & Clavier 1995) or by directly assessing carbon metabolism through respiration and calcification measurements (e.g. Martin et al. 2006). While most studies have focused on temperate molluscs, either to infer their role in the carbon cycle (Martin et al. 2006, Lejart & Hily 2011, Lejart et al. 2012, Mistri & Munari

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2013) or to gain better insight into their adaptive strategies (Tagliarolo et al. 2012, 2013a,b), few studies are available for tropical molluscs (but see Boucher-Rodoni & Boucher 1993).

Tectus niloticus L. is a large tropical marine snail commonly known as topshell or trochus. The species is widespread throughout Southeast Asia and the Western Pacific, and has been introduced in many South Pacific islands (Purcell & Cheng 2010). Trochus meat is a source of protein, while its shells have been traditionally used for ornaments by indigenous people (Castell & Sweatman 1997, Hoang et al. 2008). This species is also one of the most valuable and sought-after reef gastropods because its shell can be used in the manufacture of mother-of-pearl buttons (Castell & Sweatman 1997), which can be a valuable source of income for people in the Indo-Pacific region. However, overharvesting is causing a rapid decline in its numbers (Nash 1993, Castell 1997, Castell & Sweatman 1997). Because of commercial interest and the risk of overfishing, this species has been the subject of numerous biological studies (e.g. Bour 1989, Castell & Sweatman 1997, Pakoa et al. 2010, Purcell & Cheng 2010).

Trochus can be found in both intertidal and sub-tidal reef areas, generally in high energy sections of the reef (Bour 1990, Pakoa et al. 2010). While the optimum depth for *T. niloticus* is between 0 and 15 m, individuals can be found as deep as 25 m (Pakoa et al. 2010). Juveniles are quite cryptic but larger individuals are found easily on tropical coral reef flats (Castell 1997). *T. niloticus* has a large, thick shell and can grow to over 15 cm (basal diameter). Typical lifespan is 15 to 20 yr, and reproductive maturity is reached after 2 yr and at a basal diameter of ca. 6 cm (Castell 1997). Trochus are herbivores and tend to eat turf algae and biofilm by grazing on corals and rocks (Villanueva et al. 2013). They are mainly nocturnal (Bour 1989), with feeding rates increasing significantly at night (Heslinga & Hillmann 1981).

Together with the objective of increasing general knowledge of an exploited tropical gastropod, examining trochus diel activity patterns provide an excellent opportunity to consider variations of carbon metabolism over a 24 h cycle. Variations in respiration or calcification of marine organisms have already been measured over different seasons (Lejart et al. 2012), but diel variations are rarely considered since measurements tend to be taken only during the day, even for large-scale extrapolations of carbon metabolism. Furthermore, trochus shells, similar to carbonate substrates (Tribollet 2008), are colonized by epi- and endobionts such as bacterial biofilm, turf algae, encrusting calcareous algae and endolithic flora (Heisterkamp et al. 2013). Until now, the influence of these epi- and endobionts on respiration and calcification in molluscs has never been considered. The objectives of this study were therefore to (1) estimate *in situ* diel variations of trochus underwater respiration and calcification in a tropical ecosystem, (2) test the influence of individual size and seasons on the corresponding carbon, calcium carbonate and ammonium fluxes, and (3) determine if shell epi- and endobionts have a significant impact on these different fluxes.

MATERIALS AND METHODS

Study site

Field work was conducted from the RV 'Alis' in the southwest New Caledonian lagoon on the Tabou reef (22° 28.861′ S; 166° 26.793′ E; Fig. 1) on 4–5 February (warm season) and 23–24 August 2012 (cool season), to sample 2 seasons with contrasting temperatures. In this region, lower water temperatures occur during the cool season from May to November while high water temperatures are seen in January and February (22.3 vs. 28.1°C; Clavier & Garrigue 1999). Trochus individuals were harvested by SCUBA divers at between 2 and 7 m depth at the Amédée reef (22° 28.804′ S; 166° 27.877′ E; Fig. 1), where the highest average density occurs for this species in New Caledonia (0.69 ± 0.43 ind. m^{-2}; P. Dumas pers. comm.). Specimens were then transferred to the study site at a depth of 7 m in February, and 3 m in August. Sea temperatures for the Amédée reef station (Fig. 1) were obtained at 30 min intervals via a Seabird SBE56 from the ReefTEMPS network (http://webgops.ird. nc/geoserver/www/reeftemps_network.html).

Incubation protocol

Dissolved inorganic carbon (DIC) and calcium carbonate (CaCO$_3$) fluxes associated with *Tectus niloticus* were measured *in situ* using transparent Plexiglas benthic chambers 0.19 m in diameter (Fig. 2). The enclosed volume ranged from 6.3 to 6.9 l (depending on the exact length of hoses connecting the different parts of the experimental system and on trochus volume). Once during the warm and the cool season, 4 chambers were deployed simultaneously and a series of 8 incubations with the same specimens were performed over a 24 h period to encom-
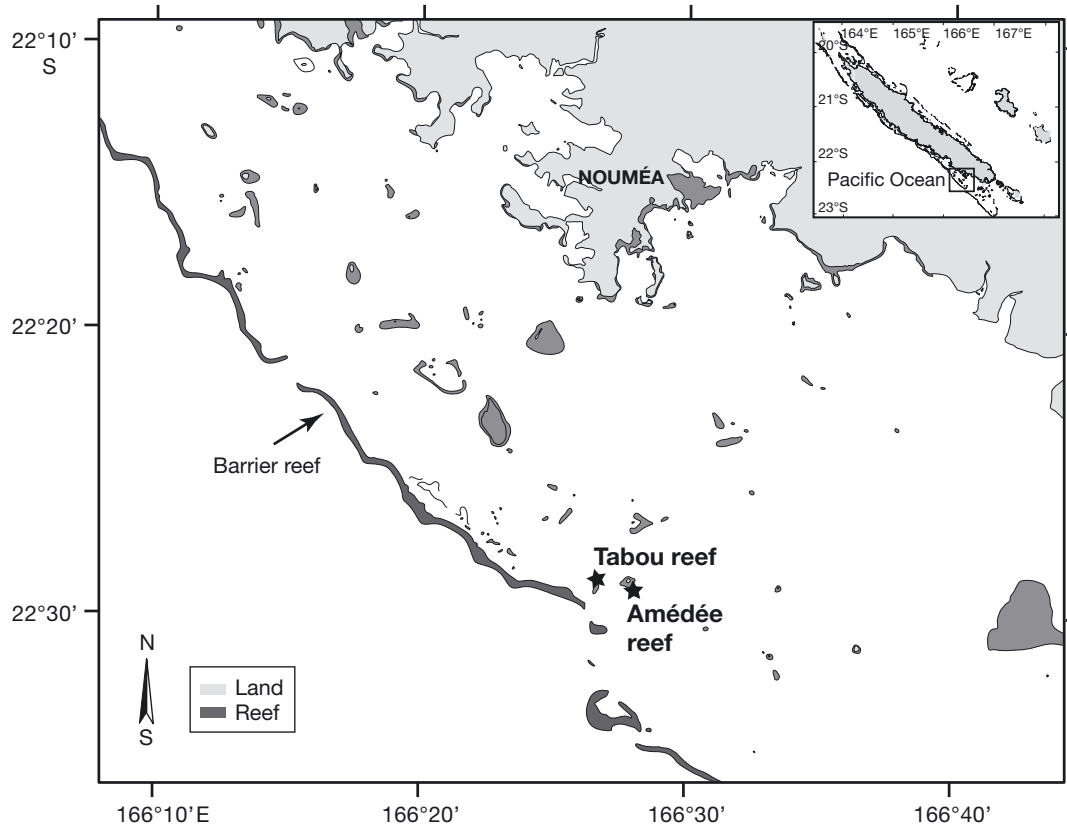
Fig. 1. Sampling site for *Tectus niloticus* metabolism measurements (Tabou reef) and environmental survey (Amédée reef) in the southwest lagoon of New Caledonia
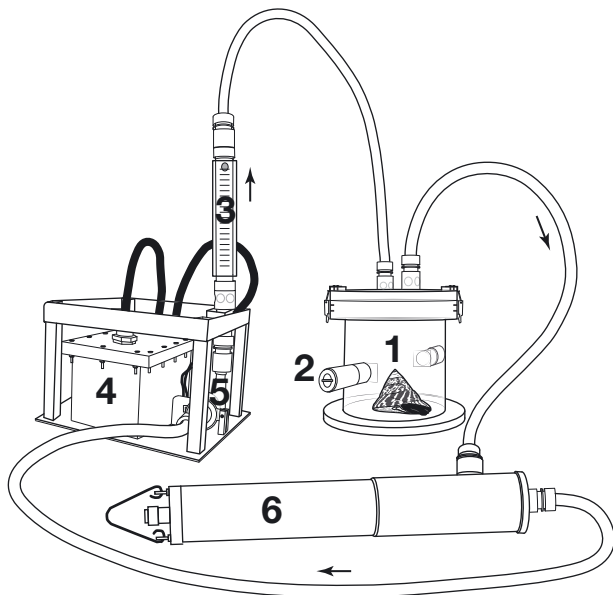

Fig. 2. Schematic representation of the experimental system for *Tectus niloticus* metabolic measurements, showing 1: benthic chamber containing a trochus; 2: seawater sampling port; 3: flow meter; 4: battery in a waterproof housing; 5: adjustable submersible pump; 6: multiparameter probe. Arrows indicate direction of water flux

pass the full range of daily irradiance levels. Each of these incubations was carried out for 1 h. The enclosures were then opened for at least 30 min between subsequent incubations to restore ambient conditions in the chamber. Adjustable submersible pumps connected to waterproof batteries mixed the water inside the enclosures at a water flow rate of $2 \, l \, min^{-1}$ (see Fig. 2). To assess the effect of animal size on fluxes, the chambers contained either small trochus (max. basal diameter 51 to 75 mm) or large trochus (112 to 121 mm) (2 chambers per size treatment). The small size treatment chambers contained 4 small trochus and the large size treatment chambers contained one large trochus to maintain the same amount of total biomass in each benthic chamber.

The contribution of epi- and endobionts colonizing trochus shell surfaces (hereafter referred to as the 'shell community') to the fluxes was assessed by hermetically closing the aperture of the same trochus shells with sealing wax and measuring the fluxes over the same range of irradiance covered by the previous experiment. All individuals had approximately the same epi- and endobiont coverage. At the end of the experiments, the biomass of the specimens

was determined by drying trochus flesh at 60°C for 24 h and combusting at 450°C for 4 h. The ash mass was then subtracted from the dry mass to obtain the ash-free dry weight (AFDW). All biomass results are expressed as g AFDW.

Respiration and calcification measurements

Photosynthetically active radiation (PAR, 400 to 700 nm) irradiance (I, μmol photons m^{-2} s^{-1}) was measured underwater at the same depth as benthic chambers using a quantum sensor (LI-192 SA coupled to Li1400, LI-COR). Dissolved oxygen concentration, temperature, salinity and depth were recorded every minute inside each chamber with a multiparameter probe (YSI 6920). We assessed the system's performance by checking that the temporal pattern of oxygen concentration remained linear during the incubations ($r^2 > 0.99$). Oxygen saturation was always above 90% at the end of the experiments, indicating the absence of hypoxic stress in the benthic chambers. Seawater samples were collected with 450 ml syringes inside chambers at the beginning and end of each incubation to obtain measurements of pH, total alkalinity (TA), and ammonium and nitrate concentrations. The pH was measured immediately on-board using a pH-meter (Radiometer pH240) standardised with TRIS/HCl (2-amino-2-hydroxymethyl-1, 3-propanediol) and 2-aminopyridine/HCl buffer solutions in synthetic seawater of salinity 35. TA samples were filtered through 0.7 μm Whatman glass-fiber filters (GF/F) and stored in 250 ml bottles in the dark. TA (mmol kg^{-1}) was further determined on 20 ml subsamples (6 replicates) by Gran automatic potentiometric titration (Radiometer, Titrilab TIM 865) using 0.01 M HCl. The mean standard deviation of replicate measurements was less than 0.003 mmol kg^{-1}.

The concentration of DIC was calculated from pH, TA, temperature, salinity, phosphate and silicate concentrations (Pierrot et al. 2006). Southwest lagoon phosphate (0.04 μmol l^{-1}) and silicate (2 μmol l^{-1}) concentrations from Jacquet et al. (2006) were used for calculations. The values of the CO_2 dissociation constants K1 and K2 were taken from Roy et al. (1993). Ammonium ions (NH_4^+), the major nitrogenous waste product of aquatic molluscs (Bishop et al. 1983), potentially caused an increase in TA by 1 equivalent per mole, resulting in an underestimation of calcification fluxes. The rates of ammonia excretion were therefore determined for large and small individuals to correct TA fluxes, and secondarily to estimate

nitrogen excretion of trochus individuals. Ammonium concentrations were analyzed on-board using the fluorometric method (Holmes et al. 1999; Turner Trilogy fluorometer). Nitrates were also measured, but no significant variations were observed during incubations, and therefore these fluxes were not considered further.

Calcification was estimated using the alkalinity anomaly technique (Smith & Key 1975) based on the fact that TA decreases by 2 equivalents for each mole of $CaCO_3$ precipitated. Calcification (G, μmol $CaCO_3$ h^{-1}) and respiration (R, μmol DIC h^{-1}) rates per chamber were estimated using the following equations:

$$G = -\frac{\Delta TA \times v}{\Delta t \times 2} \qquad (1)$$

$$R = -\frac{\Delta DIC \times v}{\Delta t} - G \qquad (2)$$

where ΔTA is the variation in TA during incubation (μmol l^{-1}) corrected from the ammonium fluxes, ΔDIC is the variation in DIC concentration (μmol l^{-1}), v is the volume of the benthic chamber (l) and Δt is the incubation time (h). Positive $CaCO_3$ fluxes refer to calcification while negative or null fluxes express dissolution or no calcification.

At the individual scale, estimations of the mollusc contribution to carbon and carbonate fluxes are complex as the shell offers habitat for other organisms. The total metabolic rate (total respiration rate and total calcification rate) then results from the gastropod activity itself (respiration, shell calcification, shell dissolution), and from shell community, whose activity also might vary between night and day. Epibiont primary production should decrease DIC concentration during the day, resulting in underestimates of trochus respiration for that time period. In the same way, epibiont respiration at night should inflate DIC levels and result in overestimates of gastropod respiration. The same is true with $CaCO_3$ dissolution from epibionts, which would lead to an underestimation of trochus calcification, while calcification of calcareous algae would lead to overestimation of $CaCO_3$ fluxes during the day. To correct trochus fluxes from epi- and endobiont influence, wax-sealed shell production vs. irradiance curves and calcification vs. irradiance curves were established (i.e. the Michaelis & Menten model) according to the following equation (Vermaat 2009):

$$P = \frac{V_m \times I}{K_m + I} - R \qquad (3)$$

where P is the shell community production or calcification rate (μmol C or $CaCO_3$ h^{-1}), V_m is the maximal

production or calcification rate (µmol C or $CaCO_3$ h^{-1}), I is the *in situ* PAR irradiance (µmol photons m^{-2} s^{-1}), K_m is the half saturation constant (µmol photons m^{-2} s^{-1}), and R is the respiration rate (µmol C h^{-1}) or calcification rate in the dark (i.e. dissolution, µmol $CaCO_3$ h^{-1}). Total fluxes were then corrected by subtracting the shell community fluxes to estimate trochus respiration and calcification rates at any irradiance.

The respiratory quotient was calculated as RQ = $|\Delta CO_2 / \Delta O_2|$ by functional regressions in which the slope of the RQ is the ratio of 2 standard deviations (SD): RQ = SD production CO_2 / SD consumption O_2.

Statistical treatment

To determine RQ, slopes of the functional regressions were compared and tested against the null hypothesis of isometry ($b = 1$) using a Z-test (Scherrer 1984). Statistical analyses were performed using the R statistical software (R Development Core Team 2013). An information–theoretic approach (Burnham et al. 2011) was taken to investigate the effects of our experimental treatments on the 3 variables measured in the field (respiration, calcification, ammonification). The experiment featured 3 fixed factors with 2 levels each: season (warm, cool), time (day, night), and trochus individual size (small, large). We did not include a random effect for the experimental chamber as only 4 chambers were used, which is less than the minimum number recommended for the inclusion of a random effect (Bolker et al. 2009). A set of candidate models was defined using all possible combinations of factors and 2-way interactions (Table 1). Generalised linear models (GLMs) were

fitted for each response variable on all candidate models. Normality in the model residuals was verified using Q–Q plots and histograms of the residuals. Model selection for fixed terms was performed by selecting the candidate model with the lowest corrected Akaike Information Criterion (AIC_c, a version of AIC corrected for small sample size; Symonds & Moussalli 2011) with the R package 'AICcmodavg' (Mazerolle 2013). AIC_c results are presented for the top 3 ranked models. Unless otherwise specified, parameter estimates and statistical significance of model terms are presented for the top-ranked model only.

RESULTS

Environmental parameters

As recorded by the *in situ* multiparameter probe at the Tabou reef station, average bottom temperatures were 27.1 ± 0.2 and 22.3 ± 0.3°C for the warm and cool season, respectively (i.e. a 4.8°C difference between February and August). Maximum temperature variation between night and day was 0.4°C in the warm season vs. 0.6°C in the cool season. Oxygen measurements from the *in situ* multiparameter probes showed no clear diel pattern, but in general lower values were observed at night (5.7 mg l^{-1}) while higher values (7.5 mg l^{-1}) were observed at the highest irradiances, between 10:00 and 13:00 h. Temperature data were available at the Amédée reef from April 2012 to April 2013, and showed good correspondence between temperatures at Tabou and Amédée reefs on 23 and 24 August 2012 (22.3 ± 0.3 vs. 21.6 ± 0.4°C for Tabou and Amédée reefs, respec-

Table 1. Model selection results for respiration, calcification and excretion in *Tectus niloticus*. The top 3 models are included for each response variable, as well as the model structure, number of estimated parameters, Akaike's information criterion corrected for small sample size (AIC_c), difference in AIC_c score between the current model and the top-ranking model (ΔAIC_c), the relative model likelihood given the data, the AIC_c model weight, the negative log likelihood (LL), the cumulative model weight (Cum.Wt) starting from top-ranking model and the evidence ratio (ER) of the top-ranking model compared to the current model

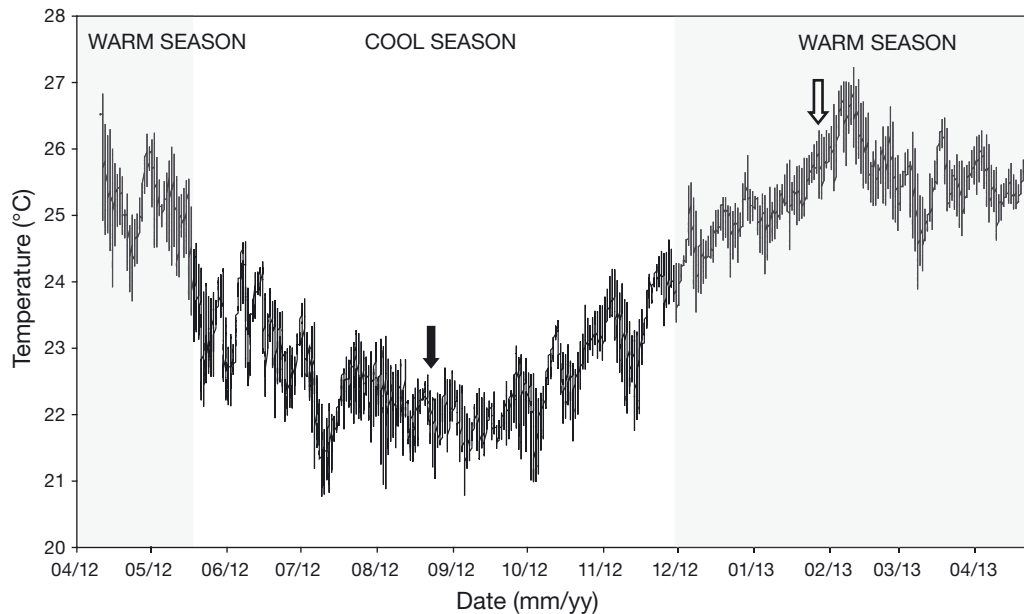| Variable	Model	K	AIC_c	ΔAIC_c	ModelLik	AIC_cWt	LL	Cum.Wt	ER
Respiration	Time × season + size	6	335.659	0	1	0.908	−161.079	0.908	1
	Time × size + season	6	340.434	4.775	0.092	0.083	−163.467	0.991	10.886
	Time + season + size	5	346.479	10.82	0.004	0.004	−167.713	0.995	223.68
Calcification	Time × season + size	6	279.729	0	1	0.991	−133.114	0.991	1
	Time × season	5	289.092	9.363	0.009	0.009	−139.02	1	107.92
	Time + season + size	5	328.472	48.743	0	0	−158.71	1	>1000
Excretion	Size × season	5	11.674	0	1	0.663	−0.32	0.663	1
	Size × season + time	6	13.162	1.488	0.475	0.315	0.156	0.978	2.105
	Size	3	20.774	9.1	0.011	0.007	−7.187	0.985	94.644

Fig. 3. Temperature at the Amédée reef station from April 2012 to April 2013. Black arrow: August cruise during the cool season. White arrow: period of the warm season cruise (although it was actually done in February of the previous year i.e. Feb 2012)

tively) (Fig. 3). Fig. 3 also shows that our sampling periods were representative of average temperatures found during the cool and warm seasons. Measured bottom seawater irradiances were higher in the cool season than in the warm season (1099 vs. 839 µmol photons m^{-2} s^{-1} at 12:00 h), mainly due to meteorological conditions (i.e. sunniest during the cool season experiment), but also to the shallower depth of the experiment in the cool season (3 vs. 7 m).

Shell community contribution

Shell community fluxes were assessed in both seasons for DIC and $CaCO_3$ but only in August 2012 for ammonium. A clear decrease in DIC fluxes and increase in $CaCO_3$ fluxes was observed with increasing irradiance (Figs. 4a & 5a). To obtain DIC and $CaCO_3$ fluxes from trochus alone (Figs. 4c & 5c), the shell community contribution was subtracted from the total flux at each irradiance (Figs. 4b & 5b). Shell community ammonium fluxes were very low, and displayed a non-consistent relationship with irradiance. The average value of −0.04 µmol NH_4^+ h^{-1} was used to correct all ammonium fluxes from the influence of the shell, both in February and in August.

Fig. 4a shows shell community positive DIC fluxes at night (up to 100 µmol C h^{-1}) due to epi- and endobiont respiration, and increasing CO_2 consumption (max. negative flux ca. −200 µmol C h^{-1}) with irradiance, reflecting photosynthesis by epibionts. Fig. 4b

shows total respiration rates (i.e. uncorrected from shell contribution), revealing contrasting night and day patterns with positive fluxes at night (up to 400 µmol C h^{-1}), and negative fluxes (−150 µmol C h^{-1}) during the day, reflecting epibiont photosynthesis. At night, respiration from epi- and endobionts contributed to ~30% of the whole community respiration flux in both seasons. Shell contribution was higher for large than for small individuals (44 and 39% vs. 23 and 26% in the cool and warm seasons, respectively). Conversely, during the day, primary production of calcareous algae and other primary producers on the shell fully counterbalanced CO_2 production from trochus respiration, strongly underestimating its respiration (Fig. 4b,c). As an example for small individuals in the warm season, Fig. 6 illustrates the diel pattern of the respective contribution of the shell community and the trochus itself to observed DIC fluxes. The shell community contribution is particularly strong during the day.

For calcification, shell carbonate dissolution (up to −15 µmol $CaCO_3$ h^{-1}) was observed at night, and $CaCO_3$ fluxes then increased with irradiance (up to 20 µmol $CaCO_3$ h^{-1}; Fig. 5a). Whole individual rates varied from −40 to 160 µmol $CaCO_3$ h^{-1} (Fig. 5b). Shell contribution to $CaCO_3$ fluxes was minor for high fluxes; i.e. at night in the warm season (6%) but more important in the cool season for lower fluxes (100%). Fig. 6 illustrates that the shell contribution is particularly minor at night when most of the calcification occurs, but more important during the day for
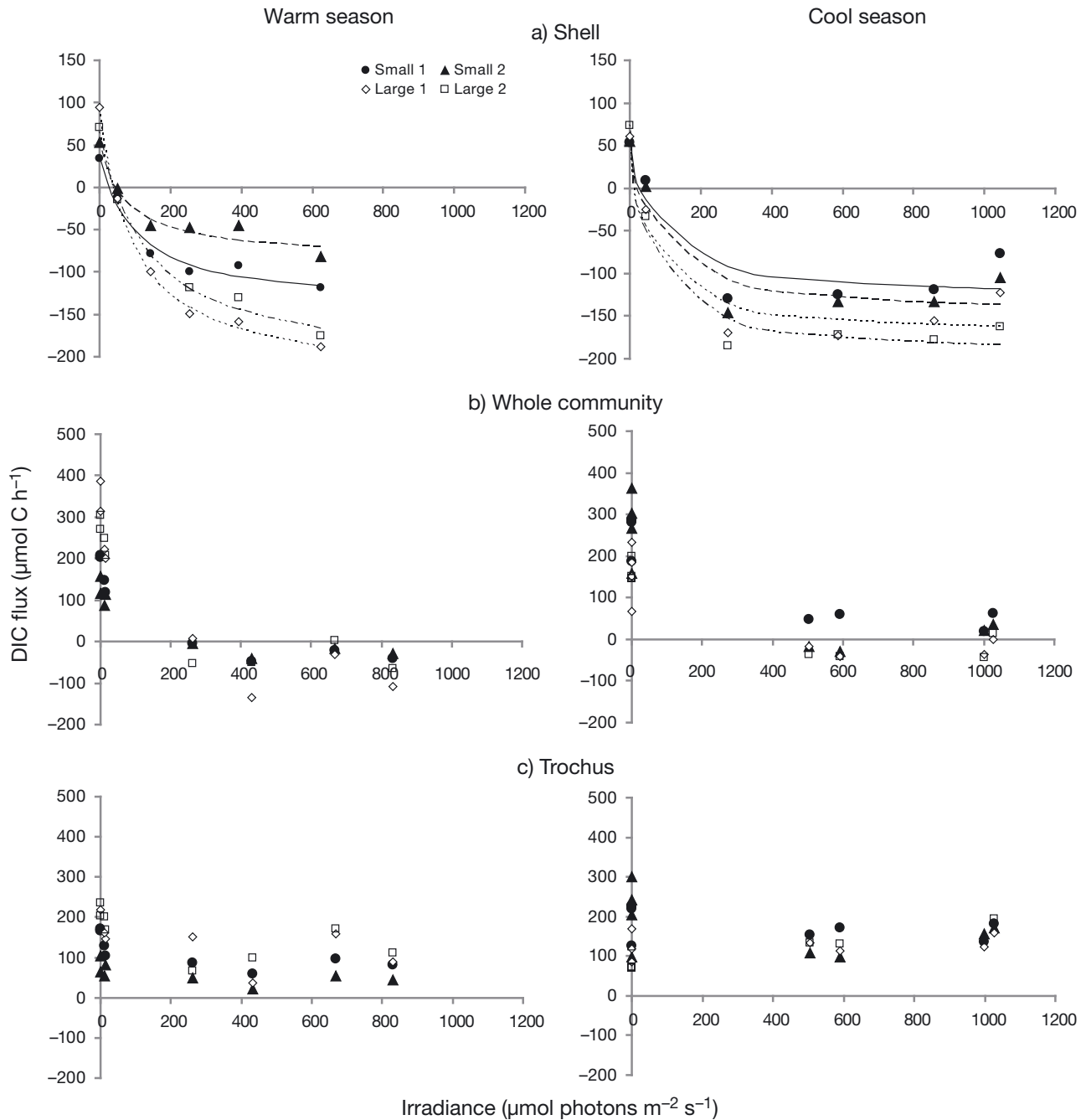
Fig. 4. Total dissolved inorganic carbon (DIC) fluxes vs. irradiance in the warm and cool season for (a) shell epi- and endo-bionts (shell contribution), (b) the whole community (shell and trochus), and (c) the trochus (shell contribution removed) for both small and large individuals. Each symbol represents a different chamber containing either small or large individuals

small $CaCO_3$ fluxes. During the day, epi- and endo-biont calcification, mainly due to calcification by calcareous algae or bryozoans, overestimated the $CaCO_3$ fluxes of the gastropod in the cool and warm season, respectively, by 52 to 75%. The corrections then lead to smaller fluxes during the day, and even negative values at some specific irradiances (Figs. 5c & 6).

Respiration

Treatment effects

Corrected DIC fluxes varied from 2.5 to 23.6 µmol C g^{-1} AFDW h^{-1} (Figs. 7 & 8). Patterns differed between night and day according to season but also

Warm season

Cool season

a) Shell

b) Whole community

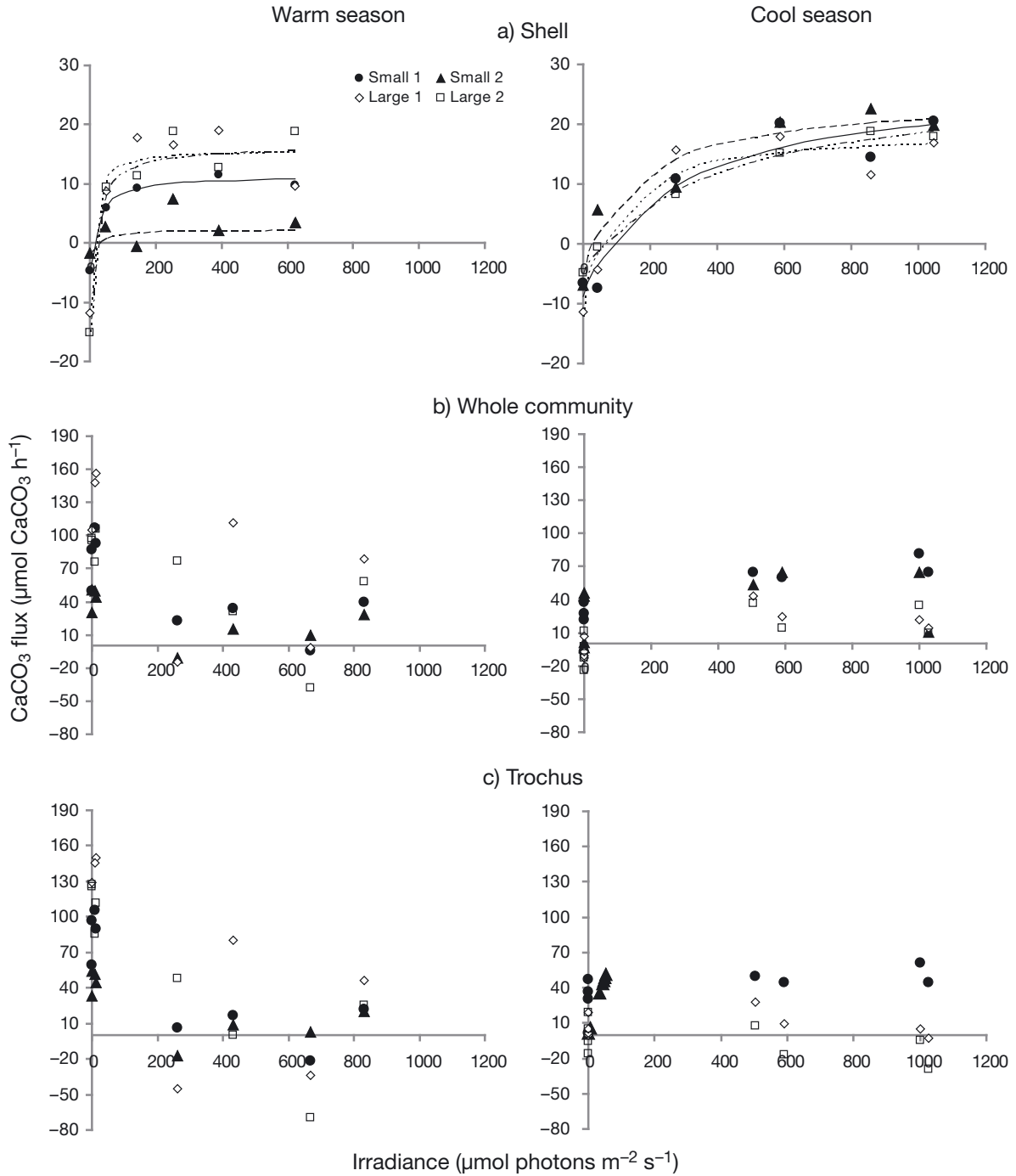c) Trochus

Irradiance (µmol photons m^{-2} s^{-1})

Fig. 5. Total CaCO$_3$ fluxes vs. irradiance in the warm and cool season for (a) shell epi- and endobionts (shell contribution), (b) the whole community (shell and trochus), and (c) the trochus (shell contribution removed) for both small and large individuals. Each symbol represents a different chamber containing either small or large individuals

varied with the size of the individuals. Night and day respiration rates tended to be more variable in the warm season, showing a more pronounced diel difference (14.8 ± 4.4 vs. 8.3 ± 2.1 µmol C g^{-1} AFDW h^{-1} for night and day, respectively) compared to the cool season (9.1 ± 3.1 vs. 8.8 ± 1.2 µmol

C g^{-1} AFDW h^{-1} for night and day, respectively). In addition, small individuals tended to have higher respiration rates than large ones especially during the night (17.6 vs. 9.9 and 12.7 vs. 8.6 µmol C g^{-1} AFDW h^{-1} for the warm and cool season, respectively; Fig. 8).
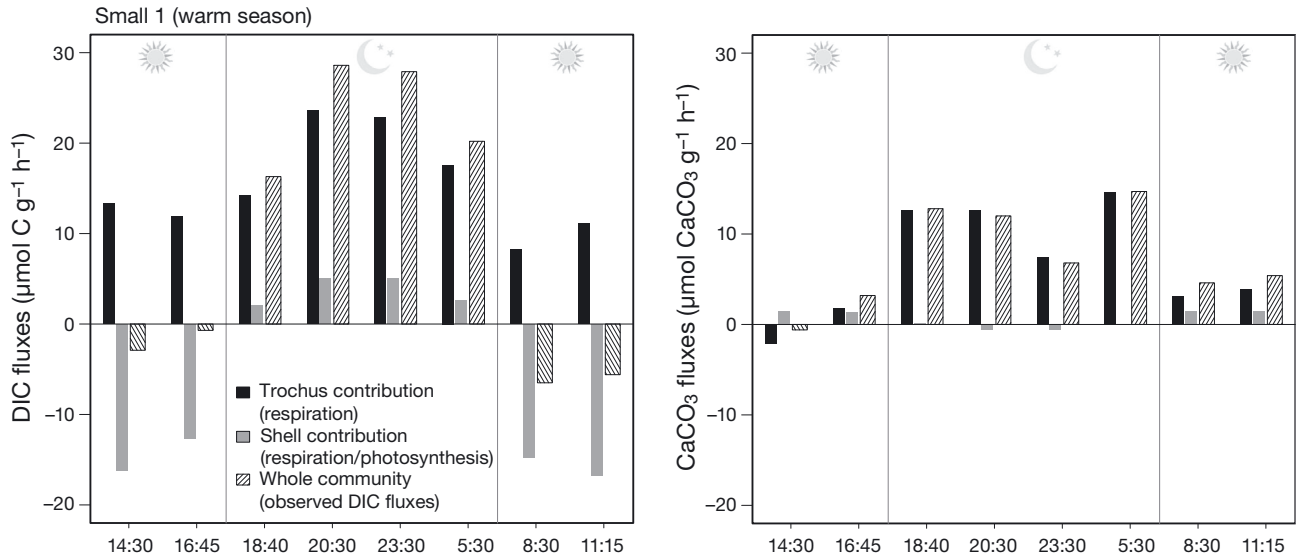
Fig. 6. Illustration of trochus and shell epi- and endobiont contribution to the observed whole community dissolved inorganic carbon (DIC) and $CaCO_3$ fluxes over the day/night cycle for small individuals during the warm season
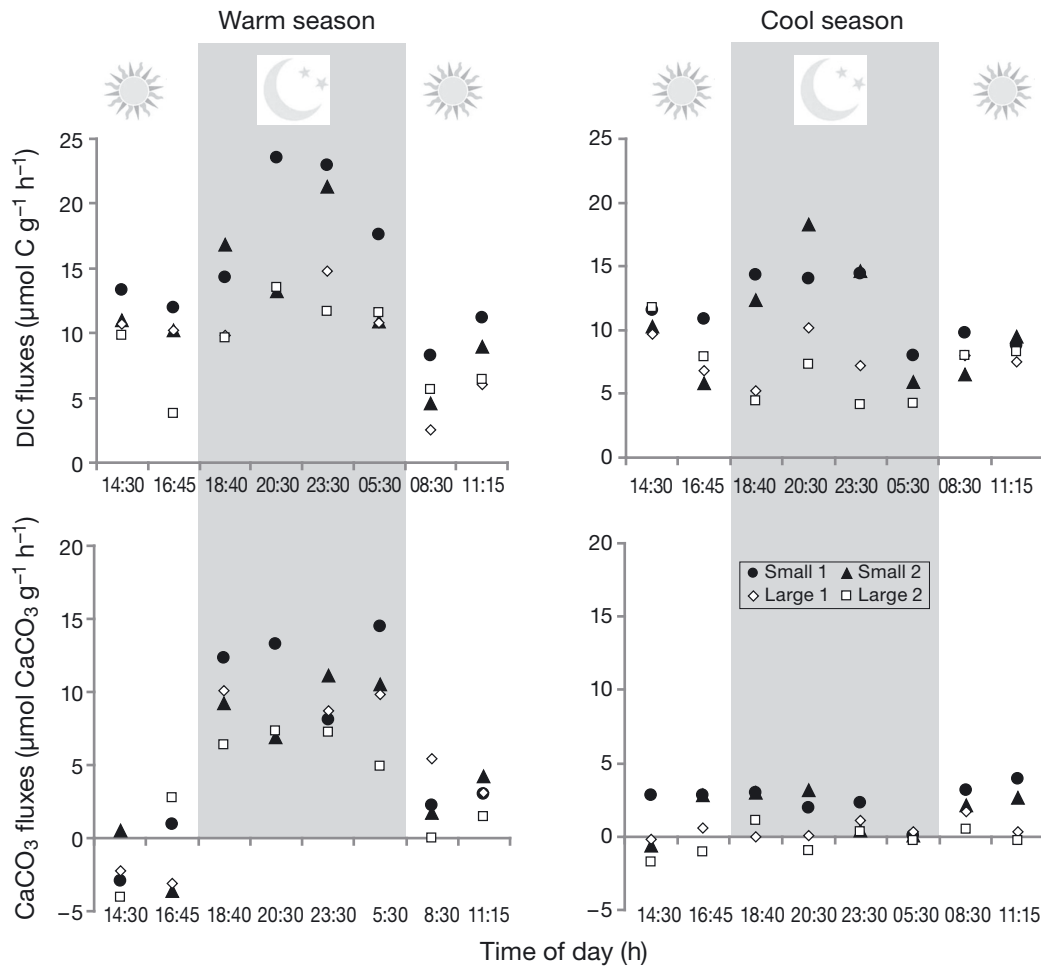

Fig. 7. Dissolved inorganic carbon (DIC) and $CaCO_3$ fluxes per gram during the day/night cycle for the small and large trochus. Each symbol represents a different chamber containing either small or large individuals
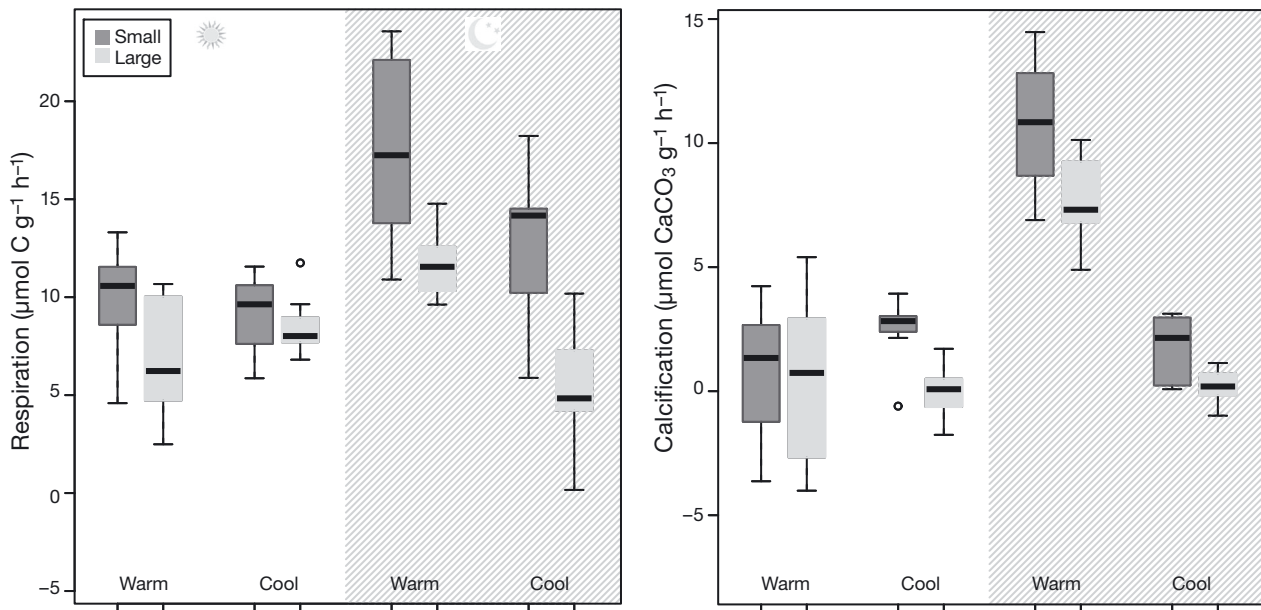
Fig. 8. Day and night respiration and calcification rates for small (dark grey) and large trochus (light grey) during both seasons (warm and cool). Boxplots show the median (middle line) and the interquartile range (IQR, box). The whiskers extend to the minimum and maximum observations, or to a distance equal to 1.5 x IQR from the lower or upper hinges of the box, whichever is closer. Observations are shown as outlier points if they occur beyond the whiskers

The top-ranked AIC_c candidate model predicted respiration as a function of time, season and size, with an interaction between season and time (Table 1). The second-ranked model had a relatively high Akaike weight and indicated an interaction between time and size. There is thus high support for an interaction with time, but the response data does not contain enough information to distinguish with high certainty whether this interaction is with size or season—though support is higher for the former, with an AIC evidence ratio of 10.89 (that is, the first model is ca. 10 times more likely than the second). Focusing on the top-ranked model, small size had a positive effect on respiratory rates (4.212, p < 0.0001), and there was a positive interaction between night and the warm season (6.046, p = 0.0005; Table 2).

Respiratory quotients

The RQ ranged between 0.6 and 0.7 for the cool and warm seasons, respectively. These values are significantly different from 1, but the 2 slopes are not significantly different (Z-test, p < 0.05) and the resulting RQ combining both seasons is 0.7. Interestingly, calculating a RQ without removing the shell community influence would have led to more elevated values much closer to 1 (0.9 vs. 0.7 for the warm and cool seasons, respectively).

Calcification

Shell $CaCO_3$ fluxes were generally small with dissolution/calcification rates ranging from −4.0 to 14.5 µmol $CaCO_3$ g^{-1} AFDW h^{-1} (Fig. 7). Most of the

Table 2. Results from generalised linear model (GLM) respiration rates; intercept ('baseline') levels were time: day, season: cool, size: large

	Estimate	SE	t	p-value
Intercept	6.7096	0.9105	7.3695	<0.0001
Time (night)	0.2413	1.1497	0.2098	0.8345
Season (warm)	−0.4125	1.1497	−0.3588	0.7211
Size (small)	4.2121	0.8198	5.1378	<0.0001
Time:season (night:warm)	6.0459	1.6396	3.6873	0.0005

Table 3. Results from generalised linear model (GLM) calcification rates; intercept ('baseline') levels were time: day, season: cool, size: large

	Estimate	SE	t	p-value
Intercept	0.3194	0.5841	0.5469	0.5865
Time (night)	−0.2582	0.7376	−0.3501	0.7275
Season (warm)	−0.6327	0.7376	−0.8578	0.3945
Size (small)	1.8188	0.5259	3.4581	0.001
Time:season (night:warm)	8.9695	1.0519	8.527	<0.0001

calcification occurred at night during the warm season for both small and large trochus (10.7 and 7.8 µmol CaCO$_3$ g^{-1} AFDW h^{-1} for small and large trochus, respectively; Figs. 7 & 8). Some dissolution was observed at 14:30 and 16:45 h during the warm season, and to a lesser extent during the cool season independent of the hour.

The top-ranked AIC$_c$ GLM model predicted calcification rates as a function of time, season, and size with an interaction between time and season (Table 1). This model had a much greater probability than the other candidate models (about 100 times more probable than the second-highest ranked model; AIC evidence ratio = 107.9). For this top-ranked model, time and season had little effect, but their interaction was highly significant with a positive effect of 8.9695 (p < 0.0001) for calcification rates at night during the warm season (Table 3). Size had a small effect, with small individuals having slightly higher calcification rates (1.8188, p = 0.0010). The value for the intercept was not statistically significant (p = 0.5865), meaning that large individuals in the day during the cool season had calcification rates not significantly different from 0.

Ammonium excretion

Ammonium fluxes (after subtracting the shell community influence) varied from 0 to 0.6 and from 0 to 1.2 µmol NH$_4^+$ g^{-1} AFDW h^{-1} for small and large trochus, respectively. The largest ranges were observed during the warm season for the 2 large individuals (0.0 to 1.2 µmol NH$_4^+$ g^{-1} AFDW h^{-1}). Both small and large individuals exhibited a much lower range in ammonium fluxes during the cool season (0.0 to 0.6 µmol NH$_4^+$ g^{-1} AFDW h^{-1}; Table 4). The AIC$_c$ top-ranking model for ammonium excretion used size, season and their interaction as model terms (Table 1). The second top-performing model also had a high probability but will not be discussed here. For

Table 4. Average night and day ammonium fluxes (µmol NH$_4^+$ g^{-1} AFDW h^{-1}, ± SD, n = 8 for each value) for small and large *Tectus niloticus* during the warm and cool season

| | | Ammonium excretion rates | |
		Small	Large
Warm season	Day	0.1 ± 0.1	0.4 ± 0.3
Warm season	Night	0.1 ± 0.1	0.5 ± 0.5
Cool season	Day	0.2 ± 0.2	0.2 ± 0.1
Cool season	Night	0.4 ± 0.2	0.2 ± 0.1

Table 5. Results from generalised linear model (GLM) excretion rates; intercept ('baseline') levels were time: day, season: cool, size: large

	Estimate	SE	t	p-value
Intercept	0.1913	0.0628	3.0459	0.0034
Size (small)	0.1019	0.0888	1.1473	0.2558
Season (warm)	0.2669	0.0888	3.0054	0.0039
Size:season (small:warm)	−0.4719	0.1256	−3.7576	0.0004

the top-ranking model, excretion rates in the warm season were higher (0.2669, p = 0.0039) but the interaction between size and season was such that small individuals had significantly lower excretion rates than large individuals in the warm season (estimate of the interaction = −0.4719, p = 0.0004; Table 5). Overall, estimated effects were small, with the lower bound of the 95% confidence interval close to zero for all terms.

DISCUSSION

Shell community contribution to carbon metabolism in the trochus

One of the most striking results of our study was the large influence of shell epi- and endobionts on observed CO$_2$ and (to a lesser extent) CaCO$_3$ fluxes. Because of epibiotic primary production, uncorrected DIC fluxes strongly overestimated respiration of the gastropod at night, by up to 44% for the large individuals in the cool season. Conversely, gastropod respiration was underestimated during the day. This had an impact on the observed diel variations, which were much more pronounced for total fluxes compared to corrected ones (e.g. Fig. 4b,c). The influence of the shell community on CaCO$_3$ fluxes was less important. When calcification was the highest for the trochus (at night during the warm season), there was only a 6% underestimation of the fluxes. The shell contribution to CaCO$_3$ is therefore only substantial when trochus fluxes are small; i.e. during the day in both seasons, and at night in the cool season. This uncertainty might be compounded by the added difficulty of determining small carbonate fluxes accurately via the alkalinity anomaly technique.

To our knowledge, our study is the first to discriminate these fluxes, and we have shown that their contribution is not negligible. Future studies on organisms bearing epi- and endobionts on their shells (i.e. gastropods, bivalves) should account for the influ-

ence of the shell community on measured carbon and $CaCO_3$ fluxes. However, these communities were not explored in detail during our study. The taxonomic composition and quantity of these organisms is probably highly variable between trochus individuals, seasons or localities (Wernberg et al. 2010), and assessing their diversity and structure would help to understand their role.

Respiration

Few studies have been conducted on tropical gastropod respiration rates, and most of them measured oxygen fluxes without removing the shell community influence (e.g. Baldwin et al. 2007) or by manually cleaning the shells from epibionts (e.g. Baums et al. 2003). On average, corrected oxygen fluxes in our study varied from 8 to 19 µmol O_2 g^{-1} AFDW h^{-1} (ranging from 3 to 29 µmol O_2 g^{-1} AFDW h^{-1}), which is lower than what was found for small gastropods such as the corallivorous gastropod *Coralliophila abbreviata* (27 to 37 µmol O_2 g^{-1} AFDW h^{-1}; Baums et al. 2003) or *Nerita articulata* (40 to 57 µmol O_2 g^{-1} AFDW h^{-1}; Houlihan 1979). This disparity is probably due to the large size differences between gastropods in these 2 studies (1 to 3 cm) compared to trochus (5 to 12 cm), as we expect higher respiration rates for small individuals (see below). More similar respiration rates were found for the similarly sized gastropod *Haliotis asinina* (a tropical abalone), at 2 to 10 µmol O_2 g^{-1} AFDW h^{-1} for resting and crawling individuals, respectively (Baldwin et al. 2007). Average carbon fluxes measured in our study (5 to 18 µmol C g^{-1} AFDW h^{-1}) are also similar to those previously published on temperate gastropods such as *Patella vulgata* (7 to 17 µmol C g^{-1} AFDW h^{-1}; Tagliarolo et al. 2013b) and *Crepidula fornicata* (2 to 44 µmol C g^{-1} AFDW h^{-1}; Martin et al. 2006).

Our study demonstrates significant diel and seasonal variations in CO_2 production. There was also a pronounced size effect: small trochus had higher respiration rates than larger individuals at night (Fig. 8), as previously reported for other mollusc species, and this size effect was independent of the time of the day or the season (e.g. Baums et al. 2003, Martin et al. 2006). This is not surprising since body size is one of the most important factors affecting metabolism. Young animals respire at higher rates per unit body mass since their higher growth rates increase metabolic demands (Marsden et al. 2012).

Respiration data also highlighted nocturnal behaviour in trochus, with enhanced respiration at night in the warm season (February). During the cool season, small trochus apparently released more CO_2 at night than during the day (12.7 vs. 8.6 µmol C g^{-1} AFDW h^{-1}; Fig. 8) although we could not distinguish with full certainty whether the interaction was between time and season, or time and size. This result is in agreement with previous studies that observed nocturnal behaviour in this species (Bour 1989). The enhanced activity at night would then increase oxygen requirements, and subsequently, CO_2 release. In abalone for example, crawling individuals have higher respiration rates than resting ones (Baldwin et al. 2007). No detailed study on trochus activity rhythms is available to interpret the difference we observed between seasons, which could be related to differences in foraging strategies or movement patterns. Temperature variations between night and day are small in the lagoon (between 0.4 and 0.6°C in this study) and would probably not generate the observed trend. Nevertheless, respiration of benthic molluscs is generally only studied during daytime for short periods of time. Our study shows the importance of also considering nighttime respiration rates, in particular when extrapolations of carbon fluxes at large scales are required.

Seasonal variation in respiration rates was observed as well, with increased respiration rates during the warm season, but only at night (11.7 vs. 5.4 µmol C g^{-1} AFDW h^{-1} for large individuals and 17.6 vs. 12.7 µmol C g^{-1} AFDW h^{-1} for small individuals). These variations are comparable to what has been found in temperate environments such as the Bay of Brest, where a 2-fold increase in respiration rates was observed between seasons for the slipper limpet *C. fornicata* (Martin et al. 2006). Such differences were expected to be lower as smaller temperature differences between seasons (4.8°C) occur in the southwest lagoon of New Caledonia compared to the ~8°C seasonal variations in the Bay of Brest (France). Temperature is arguably the most important environmental variable driving the physiological rates of ectotherms (Lewis 1971, Hochachka & Somero 2002), however other factors can also explain increases in respiration rates, such as enhanced food availability and timing of the reproduction period (Santini et al. 2000, Marsden et al. 2012). For instance, the reproductive period for trochus lasts from October to April in New Caledonia (Bour et al. 1982) and could generate higher respiration rates during the warm season. Alternatively, benthic production and micro- and macroflora biomasses (on which trochus feed) are higher in the warm season (Garrigue 1991, Clavier & Garrigue 1999), which could also explain the seasonal signal observed here.

Respiratory quotient

The RQ is often used to estimate CO_2 released by mollusc respiration from O_2 consumption (Hawkins & Bayne 1985). Indeed, measurements of CO_2 from the pH/alkalinity method and DIC variations are more difficult to conduct than continuous oxygen measurements. The average RQ of trochus was 0.7. Usually, RQs are related to the catabolic substrate used in respiration. Catabolism of proteins and lipids theoretically results in RQ values of 0.8 and 0.7, respectively (Richardson 1929), which would indicate that lipids are used as metabolic substrates. Values around 0.7 have previously been reported for other tropical molluscs such as the cephalopod *Nautilus macromphalus* in New Caledonia (Boucher-Rodoni & Boucher 1993). Such low values are mainly representative of starving animals that have to use their lipid reserves (Emerson & Duerr 1967). However, these theoretical values apply to tissues and not necessarily to whole organisms (Mayzaud et al. 2005).

Furthermore, it has been suggested that for calcifying organisms, CO_2 produced by respiration might be re-routed into the shell for calcification, which could artificially lower the numerator in the RQ formula (Tagliarolo et al. 2013b). This CO_2 can not be measured by our method, as it is directly incorporated within the shell during calcification via the extrapallial fluid. The actual RQ of small trochus could then be higher than the measured value of 0.7. Interestingly, uncorrected RQs (including fluxes from the shell) would have led to much higher—and typical—RQ values close to 0.9 (i.e. catabolism from proteins), showing again the need to consider epi- and endobiont contributions.

Calcification

Calcification rates of trochus varied on average from 0 to 14.5 µmol $CaCO_3$ g^{-1} AFDW h^{-1} from the cool to the warm season, respectively. In a few cases, we also observed dissolution (from −0.1 to −4 µmol $CaCO_3$ g^{-1} AFDW h^{-1}), with the highest values occurring during the day in the warm season, but we have no explanation for this pattern. Similar studies of temperate gastropods found calcification rates between 0 and 44 µmol $CaCO_3$ g^{-1} AFDW h^{-1} for *C. fornicata* (Martin et al. 2006), and between 1 and 8 µmol $CaCO_3$ g^{-1} AFDW h^{-1} for *P. vulgata* (Tagliarolo et al. 2013b). Our values are therefore in agreement with these studies, though we might have expected higher calcification rates since tropical species tend to have thicker shells (Vermeij 1978). Our study demonstrates season, size and diel variations in $CaCO_3$ production. Small individuals precipitated more $CaCO_3$ than large specimens, and $CaCO_3$ production rates were higher in the warm season and at night. The importance of age and temperature is well documented in mollusc growth (Rhoads & Lutz 1980). The decreasing growth pattern with age and during the cool season is in agreement with shell growth for this tropical species, as well as for many others species (Bour et al. 1982, Appeldoorn 1988).

The increased $CaCO_3$ fluxes at night during periods of intense calcification (i.e. the warm season) have been less documented in the literature and are particularly interesting. It is well known that pH decreases at night, favouring $CaCO_3$ dissolution (Shaw et al. 2012). Therefore environmental pH variations at night can not, on their own, explain the higher calcification rates observed here. Enhanced calcification at night is then most probably biologically induced, perhaps resulting from the nocturnal activity of trochus individuals (i.e. foraging activity). This result is of particular interest for sclerochronologists working on growth rhythms and proxy calibration (Schöne & Gillikin 2013). Sclerochronology is analogous to dendrochronology for trees, that is, it uses information provided by the skeleton of aquatic organisms to reconstruct records of environmental and climatic change through space and time, based on growth patterns observed at various temporal scales (see Gröcke & Gillikin 2008). As an example, Lazareth et al. (2007) explained night and day differences in the magnesium content (a seawater temperature proxy) in the shell of the gastropod *Concholepas concholepas* by the nocturnal feeding activity of the species. Schwartzmann et al. (2011) also provided an instructive example of the benefits of coupling growth rate and animal behaviour studies with the giant clam *Hippopus hippopus* in New Caledonia. Better proxy calibration between observed patterns in shells and environmental parameters such as temperature could be achieved by understanding the animal's daily growth patterns.

Ammonium excretion

In this study, we measured ammonium fluxes to correct alkalinity and properly assess trochus calcification fluxes. These ammonium fluxes were minor (0 to 1.2 µmol NH_4^+ g^{-1} AFDW h^{-1}), as already reported for limpets (0.5 to 2.3 µmol NH_4^+ g^{-1} AFDW h^{-1}; Martin et al. 2006). Furthermore, estimated size

or season effects for these fluxes were small. Still, from our analysis it appears that excretion is slightly higher in the warm compared to the cool season when both calcification and respiration rates are at the highest. Martin et al. (2006) found no correlation between the seasonal cycle of ammonium excretion and respiration. More experiments are needed to properly assess excretion patterns for trochus individuals.

CONCLUSIONS

Corrected respiration and calcification rates from our study revealed (1) a nocturnal activity reflected in higher respiration and calcification rates of *Tectus niloticus* during the warm season (February) for both small and large individuals, (2) higher respiration and calcification rates for small individuals, and (3) a significant contribution of shell epi- and endobionts to the fluxes. Ignoring these variations could severely bias estimates of the contribution of gastropods to carbon and $CaCO_3$ fluxes. These results are not only relevant to our fundamental knowledge of tropical gastropods (which is necessary to predict how, for instance, this group will be affected by climate change), but also provide important information for sclerochronologists working on shell proxy calibration. Indeed, knowledge of respiration and calcification circadian rhythms on a daily basis is important for a mechanistic understanding of proxy incorporation. Studies of activity rhythms over a 24 h cycle on small and large trochus in both seasons would be particularly helpful to substantiate our results. Finally, examining respiration and calcification rates for the whole mollusc community (animal + shell epi- and endobionts) would be beneficial, as long as the study aimed to quantify rates at the ecosystem scale. If the goal is to study metabolism at the individual scale, however, further experiments on epi- and endobiont contribution would be required, including precise quantification of the different species involved.

Many field studies have measured metabolic rates of individuals in the field over small time periods, ignored the contribution of the shell community, and assumed that these rates are representative of the population over the year. Our work has shown that metabolic rates extrapolated from such measurements are likely to be biased, and that variables known to affect metabolism, such as time of day, season, individual size and epi- and endobiont activity, should not be ignored.

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