

Respiration, calcification, and excretion of the invasive slipper limpet, *Crepidula fornicata* L.: Implications for carbon, carbonate, and nitrogen fluxes in affected areas

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Abstract

We measured in situ respiration, calcification, and excretion of the slipper limpet, *Crepidula fornicata* L., and considered both seasonal variations and individual size, to estimate the effects of this exotic species on annual budgets of carbon, carbonate, and nitrogen in the Bay of Brest (France). Respiration, calcification, and excretion rates changed significantly with size and season. Oxygen consumption varied from 6 to 63 $\mu\text{mol O}_2 \text{ g}^{-1}$ ash-free dry weight (AF dry wt) h^{-1} , which corresponded to a carbon dioxide release that ranged from 2 to 44 $\mu\text{mol CO}_2 \text{ g}^{-1}$ AF dry wt h^{-1} . Maximum respiration rates were observed in summer, and minimum rates were observed in winter. CaCO_3 production ranged from -4 to 44 $\mu\text{mol CaCO}_3 \text{ g}^{-1}$ AF dry wt h^{-1} from winter to summer, respectively. Ammonium release varied from 0.7 to 3.1 $\mu\text{mol NH}_4^+ \text{ g}^{-1}$ AF dry wt h^{-1} , with the highest excretion rate in spring. Total carbon release by *C. fornicata* in highly colonized zones in the Bay of Brest averaged 290 $\text{g C m}^{-2} \text{ yr}^{-1}$, carbonate production was $\sim 515 \text{ g CaCO}_3 \text{ m}^{-2} \text{ yr}^{-1}$, and nitrogen production by ammonium excretion was $\sim 25 \text{ g N m}^{-2} \text{ yr}^{-1}$. *C. fornicata* respiration and excretion account for 55% and 85% of the benthic community respiration and excretion, respectively. These results illustrate the importance of this invasive species to carbon and nitrogen cycles, including biogenic carbonate production, in coastal ecosystems.

Since the end of the nineteenth century, the suspension-feeding gastropod *Crepidula fornicata* L. (slipper limpet) has invaded European coastal waters from the Mediterranean to the Baltic Sea (Blanchard 1997). This exotic gastropod was introduced into waters along the western French coast, including the Bay of Brest, in the 1950s and now colonizes more than half the subtidal surface area of the bay. Its extensive spread in European waters induces both economic and ecologic consequences. Dense *C. fornicata* populations disturb oyster cultures and *Pecten maximus* fisheries and can make expensive cleaning operations necessary (Blanchard 1997). Ecologic impacts include sediment silting by biodeposit accumulation, modifications of the composition and trophic structure of benthic communities, and likely reduced primary production (Chauvaud et al. 2000). Reaching several thousands of individuals m^{-2} in bays and estuaries, *C. fornicata* proliferation may greatly alter ecosystem structure and functioning. Invasive mollusks can affect carbon and nutrient cycles by enhanced filtration, excretion, and respiration activities and, thus, by recycling nutrients and

dissolved carbon back to the pelagic ecosystem (Arnott and Vanni 1996; Lavrentyev et al. 2000; Chauvaud et al. 2003).

High filtration rates by invasive suspension-feeding mollusks may strongly decrease suspended particulate-matter concentrations in bottom water, such as in the well-studied case of zebra mussels in North America. Benthic filter feeders can selectively remove large quantities of particles from the surrounding water, especially phytoplankton and small zooplankton, and cause subsequent changes in the composition and dynamics of organisms that normally recycle carbon and nutrients in the water column (Gardner et al. 1995). They have major effects on pelagic organic-matter production and degradation and divert primary production and energy flow from planktonic to benthic food webs. They affect the microbial food web by removal of the predominant grazers of bacteria from the water and by production of biodeposits that provide an organic substrate for heterotrophic bacteria in the sediments (Gardner et al. 1995; Lavrentyev et al. 1995, 2000). Invasive suspension-feeding mollusks also affect carbon and nutrient cycles through remineralization of biodeposits (feces and pseudofeces) and through direct excretion of large amounts of ammonium (Gardner et al. 1995; Arnott and Vanni 1996; Lavrentyev et al. 2000). Other nutrient cycles can also be affected by invasive species; previous studies have highlighted the modifications in benthic silicate fluxes caused by *C. fornicata* proliferation in the Bay of Brest (Chauvaud et al. 2000; Ragueneau et al. 2002). Invasive mollusks significantly influence carbon dioxide (CO_2) concentration in seawater through their respiration and calcification processes (see Chauvaud et al. 2003). Respiration induces CO_2 release by aerobic oxidation of organic carbon, whereas calcification mainly results in shifts in the seawater carbonate equilibrium through the

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generation of dissolved CO_2 (Copin Montégut and Copin Montégut 1999). The increased benthic respiration can also decrease dissolved-oxygen concentration in the affected areas (Caraco et al. 2000). By their shell synthesis, invasive mollusks mainly affect carbonate budgets related to calcium carbonate (CaCO_3) precipitation and dissolution. Mollusks are considered among the major benthic producers of CaCO_3 in marine ecosystems, and, together with algae, corals, and foraminiferans (Beukema 1980), they are considered the most important CaCO_3 producers in temperate benthic systems (Beukema 1980, 1982; Chauvaud et al. 2003). CaCO_3 precipitation has been extensively investigated in tropical marine ecosystems (see Gattuso et al. 1998), whereas temperate environments have received less attention.

Despite the rapid proliferation of *C. fornicata* in Europe, few measurements have been made of the organism's metabolic rates. This information is essential, however, for accurate estimates of annual carbon, carbonate, and nitrogen budgets in colonized areas. Since the laboratory studies of Newell and Kofoed (1977a,b) and Newell and Branch (1980) on *C. fornicata* respiration and filtration rates, no subsequent physiologic study has been conducted, and no information is available on in situ respiration, calcification, and excretion. The respiration, calcification, and excretion rates of other temperate mollusks are known to vary according to internal factors such as body size (Smaal et al. 1997; Sukhotin and Portner 2001) and external factors such as temperature and food availability (Haure et al. 1998; Pilditch and Grant 1999). Temperature is the most important exogenous factor that regulates mollusk metabolism, and its seasonal influence on *C. fornicata* metabolism has already been reported (Newell and Kofoed 1977a,b; Newell and Branch 1980).

We hypothesized that the respiration, calcification, and excretion processes of *C. fornicata* would directly affect carbon and nitrogen cycling in the Bay of Brest ecosystem and have major effects on ecosystem functioning through an increase of carbon and ammonium regeneration in the system. The purpose of the present study was then (1) to assess the physiologic functions of *C. fornicata* that influence carbon and nitrogen concentrations in seawater, taking into consideration both seasonal variations and individual size, and (2) to estimate the potential impact of this exotic species on annual fluxes of carbon, carbonate, and nitrogen both in highly colonized areas and in the overall ecosystem of the Bay of Brest.

Material and methods

Study site—*C. fornicata* respiration and calcification were assessed in situ in the Bay of Brest, a 180-km² semienclosed area located along the western coast of France (Fig. 1). The hydrology of the bay is controlled by tidal forcing and, to a lesser extent, by moderate river inputs. Temperature, salinity, and chlorophyll *a* (Chl *a*) concentrations in surface waters were provided by the automated MAREL Iroise Station (IUEM-UBO, Observatoire du Domaine Côtier), located near the experimental site. In this area, lowest water temperature occurred in

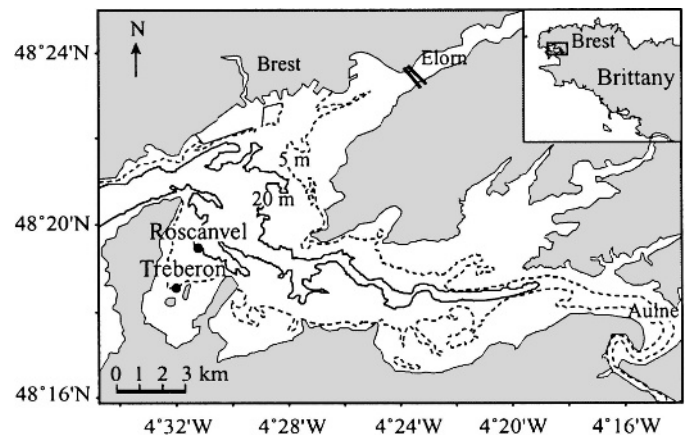


Fig. 1. Locations of the sampling station (Roscanvel) and the experimental site (Treberon) in the Bay of Brest.

March (8.5°C) and highest water temperature occurred in August (18.7°C) (Fig. 2). Salinity (in the Practical Salinity Scale) ranged from 33.3 in winter to 35.6 in summer. Chl *a* concentrations reached maxima in late April 2003 (3.9 $\mu\text{g L}^{-1}$) and May 2004 (1.7 $\mu\text{g L}^{-1}$) and were minimal in January 2004 (0.4 $\mu\text{g L}^{-1}$).

Population structure and dynamics—During March, May, July, and October 2000, samplings were carried out at Roscanvel Station, a highly colonized zone (Fig. 1), to assess the population structure and dynamics of *C. fornicata*. Three 0.125-m² samples were collected by

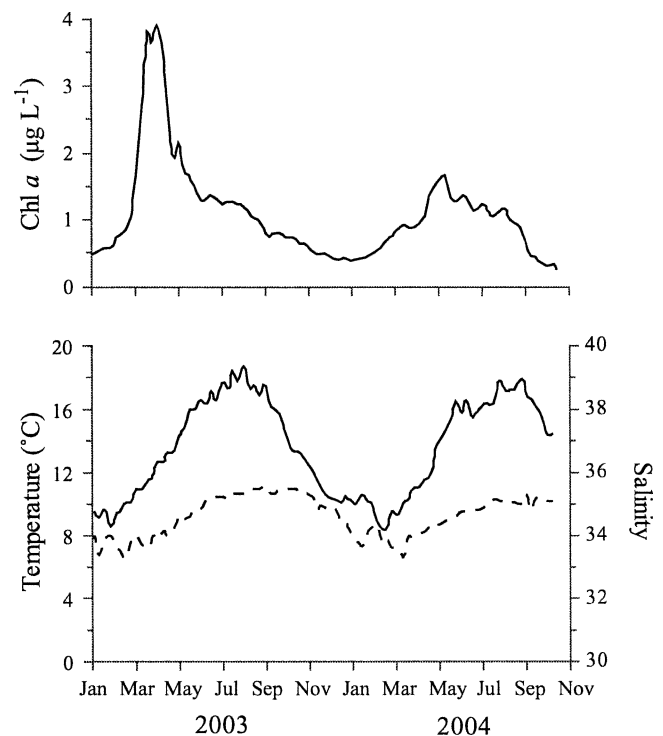


Fig. 2. Evolution of mean values of Chl *a* ($\mu\text{g L}^{-1}$), temperature ($^{\circ}\text{C}$), and salinity in surface waters of the Bay of Brest during the period 2003–2004.

SCUBA divers, who used an airlift suction sampler, and then sieved through a 1-mm mesh screen. Size, based on shell straight length (longest anteroposterior length, L), was measured to the nearest mm, and population size structure was assessed through size-frequency histograms (2-mm intervals).

Individual treatment—The slipper limpets accumulate into chains of several individuals, and each individual adheres to the dorsal surface of the shell of the subjacent partner in the chain. Samples of 300 chains of 5 to 10 individual *C. fornicata* were seasonally collected by SCUBA divers at Roscanvel Station. Epibionts were gently removed from the shells in the laboratory. Subchains were assigned to three size groups according to individual shell length: small (< 20 mm), medium (20–40 mm), and large (> 40 mm). Animals were then immersed at the experimental site to acclimatize for more than a week before metabolic experiments were conducted.

Incubation protocol—Incubations were carried out from the RV *Côtes de la Manche* near Treberon Island (Fig. 1) at a depth of 5–10 m, according to the tide. Measurements were performed seasonally in July 2003 (summer), October 2003 (autumn), March 2004 (winter), and May 2004 (spring). Chains of small, medium, and large *C. fornicata* were separately incubated in three 34.8-liter chambers composed of opaque acrylic hemispheres fixed to PVC plates. Incubations were carried out for 1.5 h and replicated two times a day. The enclosures were opened for 30 min between successive incubations to restore ambient conditions. Adjustable submersible pumps connected to waterproof batteries mixed the water inside the enclosures. As metabolic responses depend on hydrodynamics (Forja and Gomez-Parra 1998), water flow in each enclosure was adjusted to the minimum value (2 L min⁻¹) to allow stable measurements. Incubations without mollusks were performed as a reference treatment.

Respiration, calcification, and excretion measurements—O₂ concentrations (mg L⁻¹), salinity, temperature, and depth were recorded every minute inside each chamber by use of multiparameter probes (YSI 6920). Water samples were taken with 450-mL syringes at the beginning and at the end of the incubations for ammonium concentration, pH, and total alkalinity (TA) measurements. The pH (in total scale) was measured immediately on board by use of a pH meter (PHM 240, Radiometer) standardized with TRIS/HCl and 2-aminopyridine/HCl buffer solutions (DOE 1994). Ammonium concentrations were determined by use of the phenolphthalein method (Solórzano 1969). Water samples were transferred in 100-mL glass flasks, and reagents were added immediately. Flasks were stored in the dark, pending analysis. TA samples were passed through GF/F filters, poisoned with mercuric chloride (DOE 1994), and stored in a cool dark place, pending analysis. TA was determined in the laboratory on 20-mL subsamples by the automatic potentiometric method, which utilized a titrator (Titralab TIM 865, Radiometer). The concentration of dissolved inorganic carbon (DIC) was calculated from pH,

total alkalinity, temperature, and salinity according to the method of Lewis and Wallace (1998).

Data treatment—The chains of *C. fornicata* enclosed in the chambers were collected at the end of the experiments. Individuals were carefully separated and their shell lengths (L) were measured. Soft body tissues were extracted from shells and their total dry weight (dry wt) and ash-free dry weight (AF dry wt, W) were measured with a precision of 1 mg, after drying at 100°C for 48 h and combustion at 450°C for 4 h. Total dry weight of shells (W') was also measured.

Calcification and partitioning of organic and inorganic carbon respiration were computed by application of the alkalinity-anomaly technique (Smith and Key 1975; Chisholm and Gattuso 1991). Respiration, calcification, and excretion rates were calculated as follows:

$$R_{O_2} = \frac{\Delta O_2 \times v}{W \times \Delta t} \quad R_{CO_2} = \frac{\Delta DIC \times v}{W \times \Delta t} - G$$

$$G = \frac{\Delta TA \times v}{2 \times W \times \Delta t} \quad E = \frac{\Delta NH_4^+ \times v}{W \times \Delta t}$$

where R is respiration ($\mu\text{mol O}_2$ or $\text{CO}_2 \text{ g}^{-1}$ AF dry wt h^{-1}); G is calcification ($\mu\text{mol CaCO}_3 \text{ g}^{-1}$ AF dry wt h^{-1}); E is excretion ($\mu\text{mol NH}_4^+ \text{ g}^{-1}$ AF dry wt h^{-1}); ΔO_2 is the change in dissolved O₂ concentration during the incubation ($\mu\text{mol L}^{-1}$); ΔDIC is the change in total inorganic carbon ($\mu\text{mol L}^{-1}$); ΔTA is the change in total alkalinity ($\mu\text{eq L}^{-1}$); ΔNH_4^+ is the change in ammonium concentration ($\mu\text{mol L}^{-1}$); v is the chamber volume (L); W is the soft body tissue AF dry wt in the chambers (g); and Δt is the incubation time (h). Respiration, calcification, and excretion rates were corrected for the O₂, CO₂, CaCO₃, and NH₄⁺ fluxes obtained from the reference incubations.

The relations between L , W , and W' were described by the general length–weight allometric equation W (or W') = aL^b . Mean individual soft body-tissue weight (W) and physiologic rates (Y) were related by use of the allometric equation rate $Y = aW^b$, where a and b are coefficients. The a coefficient describes the physiologic rate of an individual of 1 g AF dry wt, and the b coefficient indicates the rate at which metabolism changes with size. Allometric relations were log₁₀ transformed ($\log Y = \log a + b \times \log W$) to linearize the relations. Covariance analyses (ANCOVAs) were performed to test heterogeneity of a and b coefficients among seasons. Pairwise ANCOVAs were conducted to test significant differences between pairs of seasons. Data were checked for normality and equal variances. The respiratory quotient (RQ) was calculated as $\text{RQ} = |\Delta \text{CO}_2 / \Delta \text{O}_2|$ by application of a functional regression (Ricker 1973).

The data on seasonal variations of the size structure of the *C. fornicata* population were used together with the allometric relations aL^b and aW^b for CO₂, CaCO₃, and NH₄⁺ fluxes to infer seasonal and annual fluxes of carbon, carbonate, and nitrogen in areas highly colonized by *C. fornicata*, on the basis of sampling at Roscanvel Station. A mean value of 1,700 individuals m⁻² was considered in sites that displayed high densities of *C. fornicata* (Ragueneau et

al. 2002). To extrapolate our results to carbon, carbonate, and nitrogen fluxes in the overall ecosystem of the Bay of Brest, we used the mean density of 260 (SD 619) individuals m^{-2} measured in the year 2000 for the entire benthic surface area of the bay (Guérin unpubl. data). Means and standard deviations for fluxes were estimated by Monte Carlo simulations in which the parameters of aL^b and aW^b were sampled randomly (1,000 replicate runs).

Results

Length–weight allometric relations—The mean shell lengths of the *C. fornicata* size groups were 18.1 ± 1.9 mm for small, 35.8 ± 1.7 mm for medium, and 46.8 ± 2.3 mm for large, and their mean individual soft body-tissue weights were 0.02 ± 0.00 g AF dry wt for small, 0.14 ± 0.03 g AF dry wt for medium, and 0.33 ± 0.06 g AF dry wt for large. The total biomass per chamber was 6.00 ± 1.26 g AF dry wt for small, 14.47 ± 4.53 g AF dry wt for medium, and 19.84 ± 3.6 g AF dry wt for large specimens, which corresponded to 335 ± 56 , 99 ± 22 , and 61 ± 11 individuals, respectively. The b coefficients of the aL^b relations between soft-body weight (W , g AF dry wt) and shell length (L , mm) did not differ significantly among seasons (ANCOVA, $F_{3,69} = 1.82$, $p = 0.152$) and averaged 3.04 (Fig. 3). Conversely, the a coefficients were significantly different among seasons (ANCOVA, $F_{3,69} = 18.94$, $p < 0.001$). Pairwise comparisons of the a coefficients showed that the highest values occurred in summer ($a = 3.62 \times 10^{-6}$) and the lowest occurred in winter ($a = 2.09 \times 10^{-6}$). The a coefficients in spring and autumn did not differ significantly (ANCOVA, $F_{1,43} = 0.03$, $p = 0.869$), with an overall mean of 3.01×10^{-6} . The allometric relations between shell weight (W' , g dry wt) and shell length (L , mm) did not differ significantly among seasons (ANCOVAs, $F_{3,45} = 1.11$, $p = 0.340$ and $F_{3,45} = 0.52$, $p = 0.598$ for a and b coefficients, respectively). Shell weight was, therefore, related to shell length by $W' = 3.44 \times 10^{-5} L^{3.24}$.

Respiration—The temporal evolution of oxygen uptake was always linear during the incubations ($r^2 > 0.99$). Oxygen saturation in the enclosures was always above 90% at the end of the experiments, which, thus, indicated the absence of hypoxic stress in the chambers. Oxygen consumption showed large variations according to size and season (Fig. 4), from 6 to $63 \mu\text{mol O}_2 \text{ g}^{-1}$ AF dry wt h^{-1} , which corresponded to 4 to $45 \mu\text{mol O}_2 \text{ g}^{-1}$ dry wt h^{-1} . The size of individuals explained between 72% and 91% of oxygen-uptake variability. Carbon dioxide release varied from 2 to $44 \mu\text{mol CO}_2 \text{ g}^{-1}$ AF dry wt h^{-1} (r^2 varied from 0.23 to 0.86). The b coefficients did not differ significantly among seasons both for O_2 (ANCOVA, $F_{3,55} = 2.77$, $p = 0.051$) and for CO_2 (ANCOVA, $F_{3,50} = 0.75$, $p = 0.528$). Accordingly, the overall mean b coefficient for the four seasons was -0.31 (SD 0.07) for O_2 and -0.22 (SD 0.13) for CO_2 . Conversely, the a coefficients were significantly different for O_2 (ANCOVA, $F_{3,55} = 92.41$, $p < 0.001$) and CO_2 (ANCOVA, $F_{3,50} = 24.27$, $p < 0.001$). Pairwise comparisons of the a coefficients showed that the

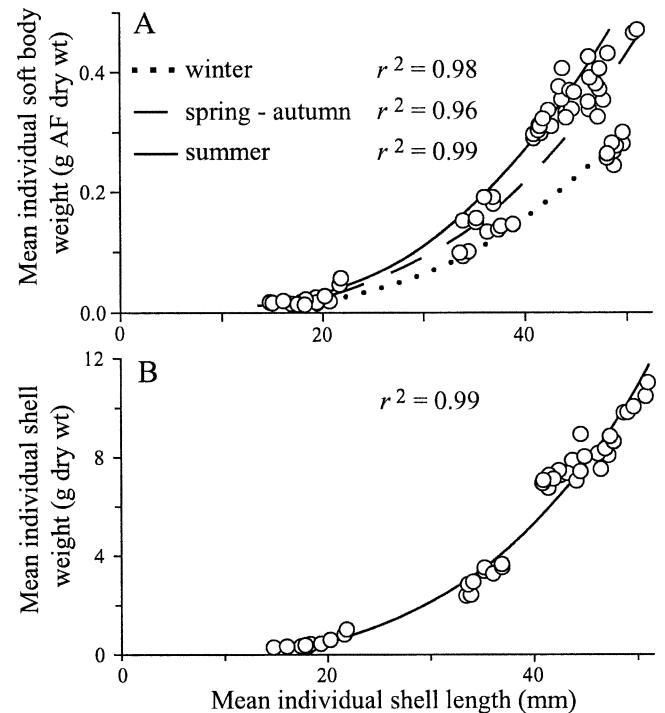


Fig. 3. Allometric relations (aL^b) between shell length (L) and (A) mean individual soft body-tissue ash-free dry weight (W) and (B) mean individual shell dry weight (W').

highest values occurred in summer and spring, and the lowest values occurred in winter (Table 1).

The RQ was 0.72 (SD 0.05; $r^2 = 0.76$). The intercept given by the functional regression (-0.0002 , SD 0.0000) was not significantly different from 0 (Z-test, $Z = 0.00$; $p = 0.99$).

Calcification— CaCO_3 fluxes varied from -44 to $4 \mu\text{mol CaCO}_3 \text{ g}^{-1}$ AF dry wt h^{-1} (Fig. 5). Fluxes were negative in summer, autumn, and spring, which reflected CaCO_3 precipitation by *C. fornicata* calcification; conversely, fluxes were positive or null in winter, which reflected CaCO_3 dissolution or no calcification. The size of individuals explained more than 63% of variation in CaCO_3 flux. The a and b coefficients differed significantly among seasons (ANCOVAs, $F_{3,50} = 73.14$, $p < 0.001$ and $F_{3,50} = 33.91$, $p < 0.001$, respectively). Pairwise comparisons of the a coefficients showed that the highest values occurred in summer, the intermediate values occurred in spring and autumn, and the lowest values occurred in winter (Table 1).

Excretion and O:N ratios—Ammonium fluxes varied from 0.7 to $3.1 \mu\text{mol NH}_4^+ \text{ g}^{-1}$ AF dry wt h^{-1} (Fig. 6), which corresponded to 0.5 to $2.3 \mu\text{mol NH}_4^+ \text{ g}^{-1}$ dry wt h^{-1} . The size of individuals explained more than 53% of the variation. The a and b coefficients were significantly different among seasons (ANCOVAs, $F_{3,53} = 20.44$, $p < 0.001$ and $F_{3,53} = 5.81$, $p < 0.01$, respectively). Pairwise comparisons of the a coefficients showed that the highest values occurred in spring (Table 1).

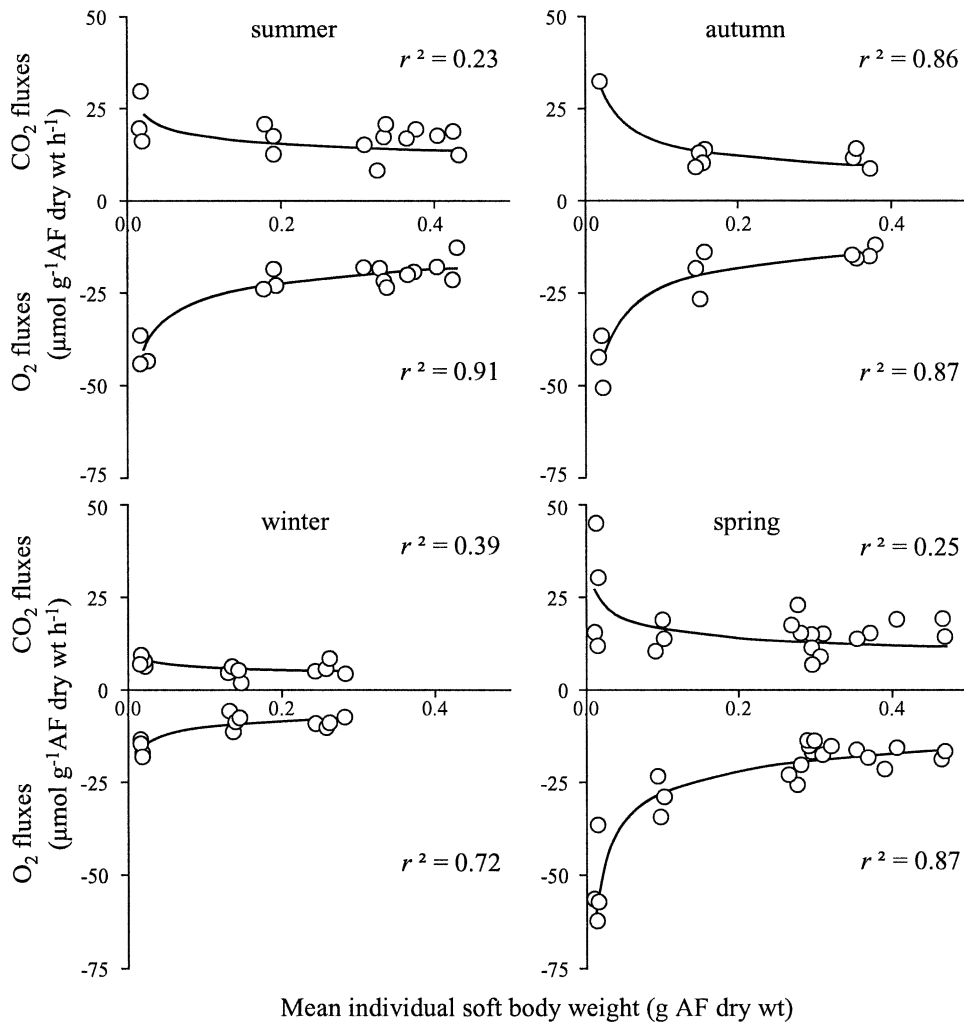


Fig. 4. Allometric relations (aW^b) between mean individual soft body-tissue ash-free dry weight (W) and respiration rates (O_2 consumption and CO_2 release) in summer, autumn, winter, and spring.

Table 1. Rates of respiration, calcification, and excretion versus body weight (W , g AF dry wt), expressed as aW^b .

Rate	Season	n	a	SD	b	SD
O_2 consumption ($\mu\text{mol g}^{-1}$ AF dry wt h^{-1})	Summer	15	14.74 ^{ab}	0.95	-0.26 nd	0.02
	Autumn	11	9.75 ^a	1.76	-0.38 nd	0.05
	Winter	12	5.74 ^c	0.93	-0.24 nd	0.05
	Spring	22	12.60 ^b	1.26	-0.34 nd	0.03
CO_2 release ($\mu\text{mol g}^{-1}$ AF dry wt h^{-1})	Summer	15	14.51 ^a	1.72	-0.10 nd	0.05
	Autumn	8	6.02 ^b	1.10	-0.41 nd	0.06
	Winter	12	4.01 ^c	0.86	-0.17 nd	0.07
	Spring	20	11.54 ^{ab}	2.25	-0.18 nd	0.07
$CaCO_3$ precipitation ($\mu\text{mol g}^{-1}$ AF dry wt h^{-1})	Summer	15	2.37 ^a	1.15	-0.62 ^a	0.13
	Autumn	9	0.99 ^b	0.55	-0.47 ^b	0.17
	Winter	12	-0.11 ^c	0.12	-0.82 ^c	0.28
	Spring	19	0.82 ^b	0.46	-0.63 ^b	0.14
NH_4^+ release ($\mu\text{mol g}^{-1}$ AF dry wt h^{-1})	Summer	15	0.92 ^{ab}	0.09	-0.15 ^a	0.04
	Autumn	9	0.50 ^b	0.07	-0.43 ^b	0.04
	Winter	12	0.49 ^a	0.06	-0.37 ^b	0.03
	Spring	22	1.17 ^c	0.10	-0.21 ^a	0.03

Superscripted letters a, b, and c on values indicate significant differences between seasons after pairwise comparisons (ANCOVA; $p < 0.05$); nd indicates no difference. The a and b coefficients are presented with SD; n is the number of observations.

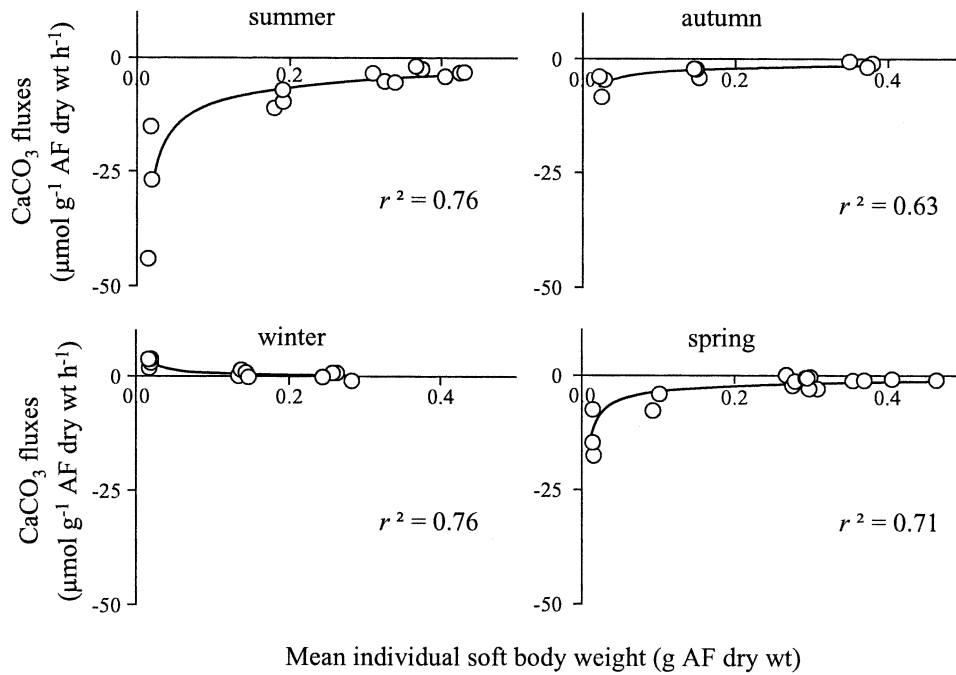


Fig. 5. Allometric relation (aW^b) between mean individual soft body-tissue ash-free dry weight (W) and calcification rates in summer, autumn, winter, and spring.

Atomic ratios of oxygen consumption to nitrogen excretion (O:N) ranged from 10.9 to 54.6. No significant relations occurred between the O:N ratio and mean individual soft body-tissue ash-free dry weight (linear regression, $p > 0.05$), except in spring (linear regression, $r^2 = 0.32$, $p = 0.011$). Because of the lack of strong relations between O:N ratios and mean individual soft

body-tissue ash-free dry weight, we used ANOVA instead of ANCOVA to test for seasonal heterogeneity. The ratios differed significantly across seasons (ANOVA, $F_{3,51} = 20.76$, $p < 0.001$), with the highest values in summer (40.0, SD 9.3) and autumn (37.8, SD 8.7), intermediate values in spring (28.7 SD 7.8), and minimum values in winter (17.1, SD 3.3; Tukey's HSD test, $p < 0.05$).

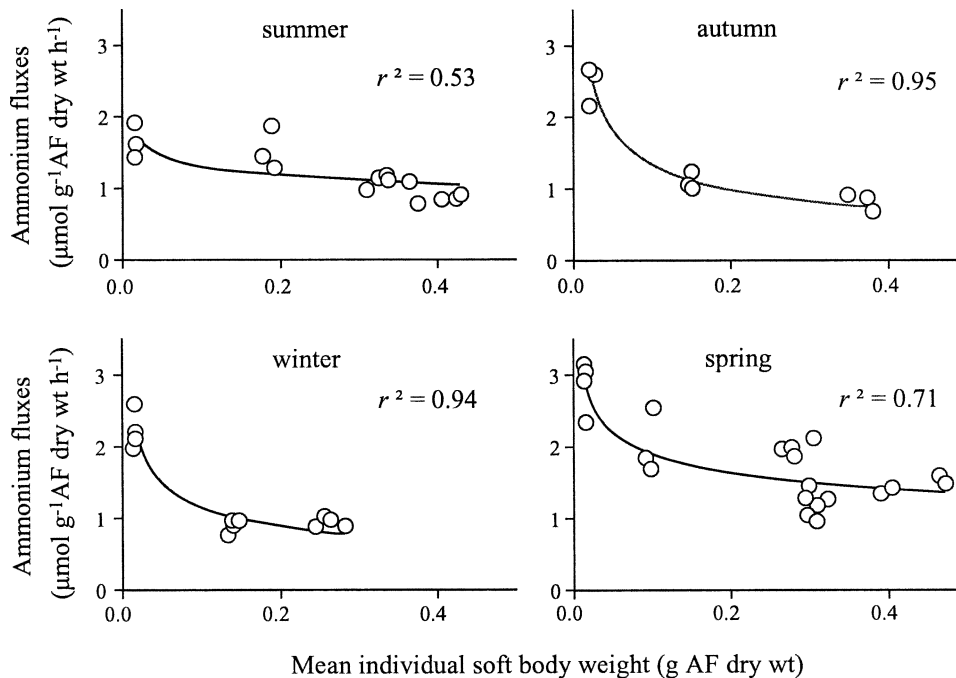


Fig. 6. Allometric relation (aW^b) between mean individual soft body-tissue ash-free dry weight (W) and excretion rates in summer, autumn, winter, and spring.

Carbon, carbonate, and nitrogen budgets—The *C. fornicata* population exhibited temporal changes in size-frequency distributions between winter and autumn 2000 at Roscanvel Station (Fig. 7). The proportion of small (< 20 mm) individuals was highest in March (49%) and lowest in October (32%). Relative frequency of intermediate-length individuals (20–40 mm) was maximum in May (47%) and roughly stable at the other seasons (~40%). The maximum proportion of large individuals (> 40 mm) was observed in October (26%), and the minimum proportion was observed in July and May (~10%). Seasonal changes of carbon, carbonate, and nitrogen fluxes were, thus, estimated according to these seasonal size-frequency variations.

For sites with high densities of *C. fornicata* at Roscanvel Station, carbon released by respiration was estimated to be 248 (SD 88) g C m⁻² yr⁻¹ (Table 2). The ratio (ψ) of CO₂ released to CaCO₃ precipitated during calcification, which depends on temperature and salinity (Frankignoulle et al. 1994), varied seasonally between 0.65 and 0.72 (Table 3). Carbonate production calculated from CaCO₃ fluxes was 515 (SD 578) g CaCO₃ m⁻² yr⁻¹ and corresponded to a release of 41 (SD 47) g C m⁻² yr⁻¹. Therefore, total C release by *C. fornicata* in highly colonized sites in the Bay of Brest was 289 (SD 135) g C m⁻² yr⁻¹. Nitrogen production by ammonium excretion was estimated at 26 (SD 6) g N m⁻² yr⁻¹.

Considering the overall ecosystem of the Bay of Brest, the potential CO₂ release by *C. fornicata* respiration (38 g C m⁻² yr⁻¹; SD 13) and calcification (6 g C m⁻² yr⁻¹; SD 7) averaged 44 (SD 21) g C m⁻² yr⁻¹. Potential nitrogen release by *C. fornicata* excretion was estimated at 4 (SD 1) g N m⁻² yr⁻¹.

Discussion

Respiration—Few studies have been conducted on *C. fornicata* respiration rates (Newell and Kofoed 1977a,b; Newell and Branch 1980), and our measurements are the first ever performed in situ. The respiration rates measured in this study are similar to those previously reported for laboratory experiments (13 to 44 μ mol O₂ g⁻¹ dry wt h⁻¹) (Newell and Kofoed 1977b). *C. fornicata* respiration rates are also similar to those reported for another gastropod,

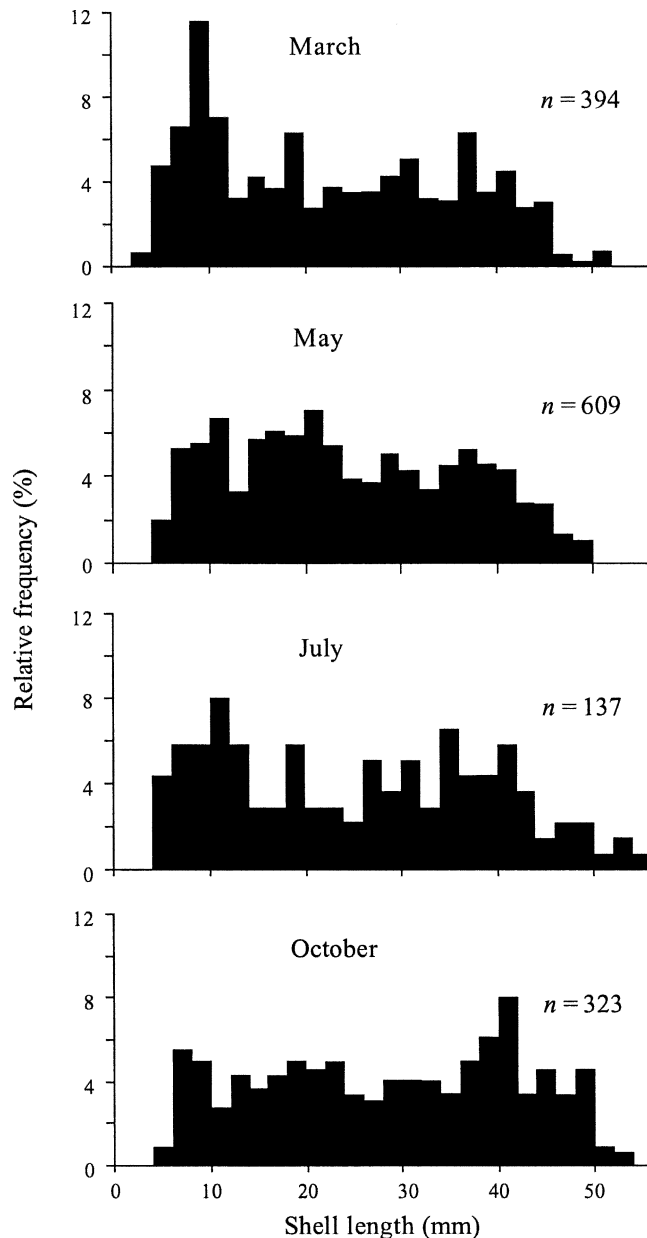


Fig. 7. Seasonal size-frequency distributions of *C. fornicata* at Roscanvel Station in the year 2000.

Table 2. Mean seasonal and annual carbon, carbonate, and nitrogen fluxes (SD in parentheses) for high densities of *C. fornicata* (~1,700 individuals m⁻²).

Season	Respiration	Calcification		Excretion	Total		
	CO ₂ release (mmol m ⁻² h ⁻¹)	CaCO ₃ precipitation (mmol m ⁻² h ⁻¹)	CO ₂ release (mmol m ⁻² h ⁻¹)	NH ₄ ⁺ release (mmol m ⁻² h ⁻¹)	Total CO ₂ release (g m ⁻² d ⁻¹)	Total CaCO ₃ precipitation (g m ⁻² d ⁻¹)	Total N release (g m ⁻² d ⁻¹)
Summer	3.98 (1.36)	1.59 (1.13)	1.04 (0.73)	0.27 (0.08)	1.44 (0.60)	3.83 (2.71)	0.09 (0.02)
Autumn	2.57 (0.84)	0.50 (0.42)	0.35 (0.29)	0.22 (0.06)	0.84 (0.32)	1.21 (1.01)	0.07 (0.02)
Winter	0.56 (0.23)	-0.25 (0.66)	-0.18 (0.47)	0.11 (0.03)	0.11 (0.20)	-0.61 (1.58)	0.04 (0.01)
Spring	2.33 (0.91)	0.51 (0.43)	0.35 (0.30)	0.25 (0.07)	0.77 (0.35)	1.21 (1.04)	0.08 (0.02)
Mean	2.36 (0.83)	0.59 (0.66)	0.39 (0.45)	0.21 (0.06)	0.79 (0.37)	1.41 (1.58)	0.07 (0.02)
Total (g m ⁻² yr ⁻¹)					289 (135)	515 (578)	26 (6)

Table 3. Mean surface-water temperature and salinity in the experimental site and molar ratio of CO₂ released versus CaCO₃ precipitated (ψ) at the four seasons studied.

Season	Average water temperature T (°C)	Average water salinity S	ψ
Summer	17.44	35.33	0.65
Autumn	13.11	35.23	0.69
Winter	9.99	34.22	0.72
Spring	13.18	34.27	0.69

The value ψ is calculated according to the following relations: $\psi = 0.8 - 8.3 \times 10^{-3}T$ (for S = 35) and $\psi = 0.949 - 7.9 \times 10^{-3}S$ (at T = 15°C) (Frankignoulle et al. 1994).

Haliotis fulgens (5–44 $\mu\text{mol g}^{-1}$ dry wt h^{-1}) (Farias et al. 2003), and are in the range of those recently reviewed for suspension-feeding mollusks (5–50 $\mu\text{mol g}^{-1}$ dry wt h^{-1}) (Pilditch and Grant 1999; James 2001; Huang and Newell 2002). Most respiration rates given for mollusks are based on O₂ measurements, whereas CO₂ release is poorly documented. Measurements of CO₂ by the pH/alkalinity method and calculation of ΔDIC difference at the beginning and at the end of the experiments are more difficult and less accurate than continuous oxygen measurements. Determination of carbon released by mollusk respiration often is estimated from O₂ consumption and RQ (Hawkins and Bayne 1985). However, RQs are extremely variable, and unexplained variations outside theoretical limits have been noted, which caused problems in interpretation for mollusks (Barber and Blake 1985; Hatcher 1989). The RQ calculated in the present study ranged between 0.7 and 0.8, which suggests that lipids as well as proteins could be used as metabolic substrates. Theoretically, RQ is about 0.7 for lipid oxidation, 0.8 for protein oxidation, and 1 for aerobic oxidation of carbohydrates (Gnaiger 1983). An apparent RQ lower than 1 could also partly result from direct use of some respired CO₂ for calcification (Tanaka et al. 1986).

Biomass-specific oxygen requirements are maximal for small *C. fornicata* compared with individuals of larger size, as previously reported for other mollusks (Smaal et al. 1997; Sukhotin and Portner 2001). This result may be explained by the difference in surface/mass exchange relation and by the decrease of growth rates during animal life (Bougrier et al. 1995). Respiration rates of *C. fornicata* also showed strong seasonal changes, with the highest values in summer and the lowest values in winter. Numerous variables influence mollusk respiration rates and, in natural conditions, these factors act in synergy. On one hand, temperature, salinity, oxygen concentration, water flow, and trophic relations have been suggested to affect respiration (Jorgensen et al. 1986; Patterson et al. 1991; Riisgard and Larsen 2001). Most of these factors varied during our experiments. Harris et al. (1999) estimated that O₂ consumption for the abalone *Haliotis laeigata* was independent of ambient O₂ concentration down to 6.3 mg L⁻¹ (80% oxygen saturation at ~18°C). The percentage of O₂ saturation was always superior to 90% at the end of our incubations and, thus, should not affect *C. fornicata* physiologic rates. Salinity only varied

from 34.2 in winter to 35.3 in summer in the experimental zone, and water flow was constant during the incubations. Therefore, temperature would be the major factor that explains why respiration rates increased significantly from winter to summer. Its influence on oxygen consumption by temperate species of marine mollusks has been studied extensively (e.g., Haure et al. 1998; Pilditch and Grant 1999; Huang and Newell 2002). The effects of temperature on *C. fornicata* respiration has been previously observed (Newell and Kofoed 1977b; Newell and Branch 1980), with a 3.5-fold increase of oxygen consumption from 5°C to 30°C. Comparatively, during our study, we observed a twofold increase in oxygen consumption from 9°C to 17°C. Food probably enhanced respiration rates in spring when phytoplankton abundance, as indicated by pigment concentrations, was highest. Phytoplankton blooms typically occur in early May in the bay. On the other hand, a close relation has been described between oxygen consumption and gametogenesis in marine mollusks (Bayne and Widdows 1978; Navarro and Torrijos 1994). In our study, high oxygen consumption in spring may be related to active gametogenesis by *C. fornicata* during this period in the Bay of Brest (Richard et al. in press).

Calcification—Calcification processes can be assessed in two major ways. The calcimass method (carbonate standing stock) is the most frequently used (Migné et al. 1998; Medernach et al. 2000; Chauvaud et al. 2003). However, the alkalinity-anomaly technique (Smith and Key 1975; Chisholm and Gattuso 1991) based on measurement of total alkalinity changes in a closed circuit is considered the most convenient for short-duration experiments on isolated organisms (Gattuso et al. 1999). Our results demonstrate a pronounced size and season dependence in CaCO₃ production. Variations in CaCO₃ production rates are controlled by both environmental factors and physiologic constraints and depend on the balance between calcification and dissolution. The importance of age and temperature is well documented (Kennish and Olsson 1975; Berta 1976). In this study, small individuals precipitated more CaCO₃ than did medium and large specimens, and CaCO₃ production rates were maximal in summer but nil or negative in winter. This pattern is in agreement with shell-growth scope for temperate species (Gruffydd 1981). Negative CaCO₃ production rates observed for *C. fornicata* in winter can be explained by a preponderance of shell dissolution at this time. Furthermore, calcification processes are sensitive to seawater pH, and a decrease of pH caused by respiration in the enclosures promotes the dissolution of CaCO₃ (Rhoads and Lutz 1980). This pattern observed for *C. fornicata* during winter experiments probably occurred in the other seasons when respiration rates were higher; consequently, measurements of calcification in this study could be underestimates.

Published information on *C. fornicata* shell growth is scarce (Coum 1979; Deslous-Paoli 1985), and the interpretation of growth marks on the shell surface is difficult. Surface erosion and epibiont colonization, as well as the intermittent deposition of marks associated with

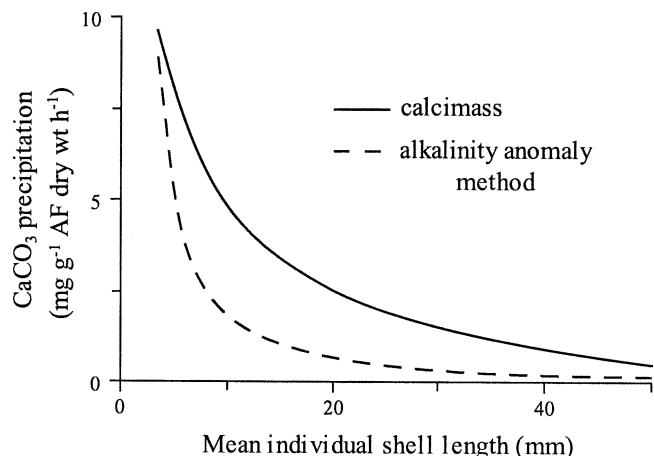


Fig. 8. Relations between mean individual CaCO_3 production and shell length calculated by the calcimass method and alkalinity-anomaly technique.

disturbances, alter the external structure of *C. fornicata* shells. The close relation between mean individual length and weight allows reliable estimation of CaCO_3 production as a function of individual shell length (Fig. 8). Coum (1979), by use of the Von Bertalanffy model $L = 66.4 (1 - e^{-0.18(t - 0.37)})$, reported a shell-growth rate of about 10 mm yr^{-1} during the first 3 years, and then a decrease down to 2 mm yr^{-1} for the oldest individuals. CaCO_3 precipitation measured by use of the alkalinity-anomaly method accounted for 23% to 90% of CaCO_3 precipitation measured by use of the calcimass method. Shell-forming carbonates in mollusks are not only taken up from DIC in seawater but also may originate from respiration products (McConnaughey et al. 1997). Tanaka et al. (1986) found that 35% to 85% of the shell-calcite carbon came from respiratory CO_2 in the bivalves *Mytilus edulis* and *Mya arenaria*. This figure is in agreement with the RQ (<1) measured in this study; the latter would partly result from the use of respired carbon for calcification. Moreover, underestimation of CaCO_3 precipitation by *C. fornicata* by use of the alkalinity-anomaly technique can be attributed to potential shell dissolution that results from pH decrease in the chambers.

Excretion—This study is, to our knowledge, the first to report measurements of ammonium excretion for *C. fornicata*. The excretion rates measured are similar to those previously reported for other mollusks (see Arnott and Vanni 1996). Excretion did not appear to be correlated seasonally with respiration, because maximum excretion rates were observed in spring. Therefore, the seasonal cycle of ammonium excretion is not mainly influenced by temperature, but, instead, factors such as food availability and quality (e.g., C:N ratio) may play a major role on the physiologic responses of *C. fornicata*. Ammonium excretion was highest during spring phytoplankton blooms, which is also the period when chlorophyll biomass in surface sediments is the highest at Roscanvel Station (Sagan and Thouzeau 1998).

Mollusks catabolize different biochemical substrates during the year (Huang and Newell 2002). Ratios of O_2 consumption to N excretion (O:N) have been used to determine the proportion of proteins relative to lipids and carbohydrates catabolized for energy metabolism (Kreeger 1993), which gives information on the physiologic status of the organisms (Hatcher et al. 1997). The O:N ratios for *C. fornicata* are comparable to those reported in the literature for other suspension-feeding mollusks that range from 10 to 60 (Kreeger 1993; Hatcher et al. 1997; Huang and Newell 2002). The O:N ratios measured in our study varied seasonally, with the lowest ratios in winter and spring and the highest ratios in summer and autumn. Low ratios during winter have been previously reported for *M. edulis*, which indicates a preferential utilization of proteins as catabolic substrate when mussels were stressed by low food availability (Bayne and Widdows 1978). Low O:N values in spring may partly result from reproduction activity; Bayne and Widdows (1978) and Barber and Blake (1985) reported that proteins are highly catabolized during gametogenesis. The highest O:N ratios in summer and autumn would be characteristic of high food availability (Kreeger 1993). Seasonal variations in O:N ratios can also be explained by a metabolic adjustment to the composition in C and N of the food available (Huang and Newell 2002). Low O:N ratios may be indicative of a high-quality food source with a low C:N ratio (Hatcher et al. 1997). Thus, the low O:N ratio measured in spring probably results from a catabolism adapted to a rich phytoplanktonic or phytobenthic food source, or both, with a lower C:N ratio than that of seston available at the other seasons.

Carbon, carbonate, and nitrogen budgets—Total carbon production as CO_2 was mainly influenced by respiration in this study. CO_2 release by shell calcification represented only one-sixth of total CO_2 production. However, total CO_2 production and the ratio between CO_2 released by respiration and calcification differ according to species. Total CO_2 production reported by Chauvaud et al. (2003) for *Potamocorbula amurensis* in San Francisco Bay was $55 \text{ g C m}^{-2} \text{ yr}^{-1}$, including $18 \text{ g C m}^{-2} \text{ yr}^{-1}$ ($\sim 30\%$) released by CaCO_3 production. *Ophiotrix fragilis* respiration releases $635 \text{ g C m}^{-2} \text{ yr}^{-1}$, whereas calcification releases $58 \text{ g C m}^{-2} \text{ yr}^{-1}$ ($\sim 10\%$) (Migné et al. 1998). Our result for total carbon release in the *C. fornicata* high-density areas ($\sim 250 \text{ g C m}^{-2} \text{ yr}^{-1}$) is higher than those reported for other invasive mollusks in temperate systems (Chauvaud et al. 2003) and lower than carbon release estimated on coral reefs ($\sim 6,000 \text{ g C m}^{-2} \text{ yr}^{-1}$) (Gattuso et al. 1998). Annual CaCO_3 production for *C. fornicata* in high-density areas averaged $500 \text{ g CaCO}_3 \text{ m}^{-2} \text{ yr}^{-1}$ and might reach $\sim 2,000 \text{ g CaCO}_3 \text{ m}^{-2} \text{ yr}^{-1}$ when calculated by use of the calcimass method. These results are within the range measured for other temperate mollusks. Chauvaud et al. (2003) estimated carbonate-production rates of the invasive *P. amurensis* between 7 and $1,100 \text{ g CaCO}_3 \text{ m}^{-2} \text{ yr}^{-1}$. The mean annual value was $118 \text{ g CaCO}_3 \text{ m}^{-2} \text{ yr}^{-1}$ for *Cerastoderma edule* (Beukema 1982) but only $13 \text{ g CaCO}_3 \text{ m}^{-2} \text{ yr}^{-1}$ for *Macoma balthica* (Beukema 1980). In tropical marine systems, mollusk CaCO_3 pro-

duction reaches $300 \text{ g CaCO}_3 \text{ m}^{-2} \text{ yr}^{-1}$ (Moore 1972) and up to $2,000 \text{ g CaCO}_3 \text{ m}^{-2} \text{ yr}^{-1}$ in highly productive systems such as coral reefs (Gattuso et al. 1998). The results found in this study are close to those reported for tropical systems; they emphasize the significance of biogenic carbonate production in temperate coastal systems.

Considering the entire Bay of Brest ecosystem, the annual CO_2 production by *C. fornicata* respiration is about one-third of uptake by phytoplanktonic production ($148 \text{ g C m}^{-2} \text{ yr}^{-1}$) (Del Amo 1996). Thus, considering its respiration, *C. fornicata* can consume a significant part of the pelagic carbon production of the bay and can divert carbon and nitrogen primary production from planktonic to benthic food webs. However, the genus *Crepidula* exhibits dual feeding modes of suspension feeding and grazing of the substrate (Chaparro et al. 2002). Suspended particles are not the only food source for *C. fornicata*, especially for motile individuals (i.e., small individuals, juveniles, and adult males) that graze the biofilm on top of the substrate. In opposition to other invasive suspension feeders that may remove large amounts of particles from the water column, the filtration capacity of *C. fornicata* is low ($1 \text{ to } 2 \text{ mL mg}^{-1} \text{ AF dry wt h}^{-1}$) (Newell and Kofoed 1977) and about 10-fold lower than that of invasive zebra mussels (Lavrentyev et al. 1995). The effects of *C. fornicata* filtration on primary production in the water column are probably moderate because the chlorophyll biomass has remained constant, despite the proliferation of this invasive species during the past 10 years (Del Amo et al. 1997). By contrast, *C. fornicata* probably has a major influence on microorganisms at the water-sediment interface and may change species composition and dynamics in overlying water and in the sediments by selecting the particles according to size and quality (Chaparro et al. 2002). As reported for other suspension feeders (Lavrentyev et al. 1995; Lavrentyev and Yang 2000), *C. fornicata* may remove a large proportion of protozoans and phytoplankton compared with bacteria, which affect the microbial food web and associated carbon and nitrogen cycling processes by increasing bacterial abundances and activities. *C. fornicata* biodeposition also affects the microbial food web at the water-sediment interface by increasing sediment organic-matter content. In 1995, annual biodeposit production by *C. fornicata* was estimated to be 11,000 tons of dry organic matter (Chauvaud et al. 2000), and this value should be higher now when the ongoing biomass increase is taken into consideration (Guérin unpubl. data). In addition to their direct excretion and respiration activities, *C. fornicata* has an indirect effect on the respiration and ammonium excretion of the community by stimulating the bacterial-decomposition processes (degradation of organic material generates carbon and ammonium). *C. fornicata* direct respiration and excretion accounted, respectively, for 55% and 85% of the benthic-community respiration and excretion measured from benthic-chamber experiments in slipper limpet high-density areas of the southern basin of the Bay of Brest (Martin unpubl. data). Thus, *C. fornicata* metabolism dominates the nitrogen-regeneration and carbon-release processes at the water-sediment interface in

highly colonized areas. Respiration and excretion by *C. fornicata* may be considered as the major factors that regulate carbon and ammonium fluxes of the benthic community, as previously reported for other invasive suspension feeders (Gardner et al. 1995).

In seawater, the different forms of carbon are abundant, but nitrogen may be limiting for primary production in shallow coastal waters. Consequently, ammonium regeneration in coastal sediments is an important mechanism that may regulate primary production and secondary productivity. Ammonium concentrations in the Bay of Brest vary from $0.8 \text{ to } 2.2 \mu\text{mol L}^{-1}$, according to temporal variations of river inputs, losses (by phytoplankton uptake and marine water renewal), and regeneration processes (by excretion and remineralization). Nitrate concentrations vary from $< 0.05 \text{ to } 83.4 \mu\text{mol L}^{-1}$ in the bay (Del Amo et al. 1997). Minimum ammonium and nitrate concentrations are observed from April to September during the most productive phytoplankton period. The ratio between phytoplanktonic C and N production in the Bay of Brest is highly variable (Daniel 1995) but ranges around the Redfield C:N ratio (6.6) (Redfield et al. 1963). Accordingly, annual nitrogen production by phytoplankton can be estimated at $\sim 25 \text{ g N m}^{-2} \text{ yr}^{-1}$ in the bay. This value is about 6.5-fold higher than nitrogen release by *C. fornicata* ammonium excretion. However, maximal benthic nitrogen release by *C. fornicata* in spring and summer might support phytoplanktonic blooms when ammonium and nitrate concentrations become limiting. If *C. fornicata* continues to spread in the Bay of Brest, the nitrogen loading that will result from its excretion could potentially increase eutrophication and promote phytoplankton growth, organic-matter sedimentation, microbial decomposition, and hypoxic events. The significant excretion of nitrogen may also alter nutrient ratios, such as Si:N, and cause a shift in phytoplankton-community composition from a dominance of diatoms to flagellates and dinoflagellates (Cloern 2001). However, *C. fornicata* also modifies silicate fluxes by favoring biogenic silica retention in the sediments and by secondary remineralization (Ragueneau et al. 2002), and the balance between these two phenomena needs to be clarified for an understanding of the effects on nutrient limitation and ecosystem functioning.

As a first step, we extrapolated the results obtained at Roscanvel Station to the entire Bay of Brest ecosystem, on the assumption that environmental parameters, *C. fornicata* physiology, and size-frequency distribution were similar across the entire bay. Changes in spatial distribution of *C. fornicata* size frequency have been highlighted (Guérin unpubl. data). However, we do not have any information on the spatial variability of *C. fornicata* physiology. Variations in salinity, particularly in the vicinity of the two river estuaries (Aulne and Elorn), could also modify carbon fluxes that result from changes of molar ratio of CO_2 released versus CaCO_3 precipitated (ψ), even if salinity variations in bottom water are limited in *C. fornicata* high-density areas. Further investigations on the spatial variability of these parameters are required to extrapolate our data to the entire Bay of Brest ecosystem or to other affected ecosystems.

The effects of exotic-species introductions in ecosystems simultaneously subjected to other large-scale modifications, such as eutrophication, are often difficult to estimate. These experiments on *C. fornicata* isolated from the whole benthic assemblage provide an understanding of the potential effects of this exotic species on a temperate shallow ecosystem. Its effects on carbon and nitrogen fluxes through respiration, calcification, and excretion make *C. fornicata* a key species in the functioning of the entire Bay of Brest ecosystem. This species continues to spread along the European coastline and high-density (> 1,000 individuals m⁻²) areas are reported for numerous ecosystems along the coasts of France, England, and the Netherlands (Thieltges et al. 2003). Accordingly, the extension of *C. fornicata* along European coasts, as well as the worldwide spread of other invasive mollusks, must be considered as a potential source of carbon and nitrogen that may have large implications for ecosystem processes.

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