Seasonal variations in the rates of aquatic and aerial respiration and ammonium excretion of the ribbed mussel, *Geukensia demissa* (Dillwyn)

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Abstract

Aquatic and aerial rates of oxygen consumption and ammonium excretion of ribbed mussels, *Geukensia demissa* (Dillwyn), collected from the mid-intertidal zone of a mid-Atlantic salt marsh, were measured under ambient conditions of food, temperature, and salinity over five seasons. Rates of aquatic respiration covaried with body size and season, as the rates were high and strongly related to mussel tissue weight in spring and summer but low and weight independent in winter. There was a significant interannual difference between summer of 1995 and 1996. Rates of aerial respiration also varied seasonally, with high rates of oxygen consumption in summer and low rates in winter. The magnitude of these seasonal variations were greater than those for aquatic respiration, and as a result, the ratio of aerial to aquatic respiration was higher in summer (0.61 and 0.52) than in winter (0.11). This indicates that *G. demissa* was able to actively regulate aerial respiration, thereby permitting high aerobic metabolism during prolonged periods of air exposure in summer. We hypothesize that such high rates of aerial respiration, during the seasons of high metabolic activity, are required to provide sufficient energy for mussels to facilitate food digestion during air exposure at low tide. Rates of ammonium excretion varied seasonally and increased with mussel weight in all seasons. The atomic ratio of oxygen to nitrogen (O:N), calculated from aquatic respiration vs. ammonium excretion, was significantly lower in autumn (26) than in other seasons (46–60) among which the O:N did not vary significantly. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Ribbed mussels, *Geukensia demissa* (Dillwyn), are dominant benthic macrofauna inhabiting salt marshes on the Atlantic coast of North America and can exert a profound influence on ecological processes of salt marshes (Kuenzler, 1961; Jordan and Valiela, 1982; Bertness and Grosholz, 1985; Kreeger and Newell, 2000). Shells of these mussels are relatively thin, which makes them vulnerable to predation in the subtidal zone (Seed, 1980; Lin, 1990); consequently, ribbed mussels are restricted to living in the intertidal zone and of all bivalve species, appear uniquely adapted physiologically to this extreme environment (Gillmor, 1982). In many salt marshes, the ribbed mussels are exposed to air for up to 70% of the tidal cycle (Stiven and Gardner, 1992). This long period of exposure exerts a severe stress on the mussels because of the limited time available for them to perform essential physiological functions, such as feeding and defecation.

Desiccation is a major stress to bivalves associated with aerial exposure. The typical response of most bivalves exposed to air is closure of shell valves to prevent desiccation (Newell, 1979). As a result, the oxygen supply is cut off and the animal has to rely on anaerobic pathways for the supply of energy (De Zwaan and Wijsman, 1976; Pamatmat, 1979; Widdows et al., 1979; Shick et al., 1988). Unusually, ribbed mussels have a pronounced capability for maintaining high rates of aerobic respiration even during aerial exposure through "shell gaping" behavior, in which the mussels respire atmospheric oxygen by opening their valves slightly to allow gas exchange (Widdows et al., 1979). The ribbed mussel's aerial rate of oxygen consumption can be as high as ∼ 60% of the magnitude of aquatic respiration (Booth and Mangum, 1978; Widdows et al., 1979). This aerobic capacity is facilitated by the mussels' microhabitat in the marshes, where it attaches with byssal threads to the rhizomes of cordgrass plants and lives almost completely buried in mud. Because this mud is poorly drained, the high interstitial water content can be used to restore any water lost from the mantle cavity through evaporation even during periods of aerial exposure. Ribbed mussels possess other physiological adaptations in addition to a high capacity for aerial respiration to enable them to cope with living in the intertidal zone. For example, ribbed mussels living high in the intertidal zone exhibit higher feeding rates (Charles and Newell, 1997) and assimilate their food with greater efficiency (Kreeger et al., 1990) than those from the mid and low intertidal zone.

Temperature is the most important exogenous factor regulating the metabolism of ectothermic organisms, and its influence on the rates of oxygen consumption, feeding, and nitrogen excretion in temperate species of marine bivalves has been studied extensively (see reviews by Newell, 1979; Newell and Branch, 1980; Bayne and Newell, 1983). These studies show that physiological rate functions are positively correlated with seasonal variations in temperature (Newell and Bayne, 1980; Bayne and Newell, 1983). Super-imposed on the seasonal metabolic cycle associated with temperature are variations related to endogenous factors, such as nutritive status and reproductive condition. Although the metabolic rate of ribbed mussels at different temperatures has been determined previously (Kuenzler, 1961; Read, 1962; Hilbish, 1987), those studies have focused mainly on the response of mussels to experimentally manipulated abrupt rates of temperature change and not the incremental rates of seasonal temperature change experienced by ribbed mussels living their natural habits. For example, Kuenzler (1961), in his study of the energy flow of a

mussel population, measured aquatic respiration rates of ribbed mussels subject to acute temperature changes over a matter of hours in March. Kuenzler (1961) then combined these data collected in March with aerial respiration rates measured in July to estimate seasonal metabolic demands of the mussel population. Because of the artificial nature of these earlier studies, there is a lack of reliable information on the seasonal metabolic rates of ribbed mussel populations on the Atlantic coast of North America, which is necessary for an accurate estimation of annual carbon and nitrogen budgets. The overall objective of our study was to determine the seasonal changes in aquatic and aerial rates of oxygen consumption and rates of ammonium excretion in relation to mussel size. These data will then be used in future publications as a component of an ongoing project to construct energy budgets and quantify trophic transfers for a natural population of ribbed mussels in Canary Creek salt marsh, Delaware, USA (Kreeger and Newell, 2000; Huang et al. in press (a),(b)).

2. Material and methods

2.1. Collection of mussels

We conducted experiments over five seasons starting in early August 1995 (summer 1995), late October 1995 (autumn 1995), early March 1996 (winter 1996), late May 1996 (spring 1996), and finished in early August 1996 (summer 1996). The reason we did our winter experiment in March, rather than January or February, was because ribbed mussels remain inactive during the winter on mid-Atlantic coasts when water temperatures approach freezing point. From the perspective of mussel physiology, we delayed the winter sampling until March 1996, at which time the water temperature was at ~ 5 °C (Huang et al., in press (a)), the minimal level at which ribbed mussels start to become active (Wilbur and Hilbish, 1989).

Each season, we collected 25 ribbed mussels ranging between 3 and 8 cm in shell length from the Canary Creek salt marsh near Lewes, DE, USA (38°47.203′ N, 75°09.044′ W). Located near the mouth of Delaware Bay, this marsh is dominated by the input of estuarine water from the bay (Roman and Daiber, 1989; Huang et al. in press (a)). Ribbed mussels live tightly attached to the rhizomes of smooth cordgrass, *Spartina alterniflora*, the dominant species of marsh plant on the creek bank. The collected mussels were returned to the laboratory, scrubbed clean, and individually marked on the shell. They were then held for 1–2 days in an aquarium system with natural marsh water and maintained under a simulated tidal cycle of 6 h submerged and 6 h emerged. During the entire acclimatization period, mussels were held in an environmental chamber maintained at the ambient water temperature at the time of collection (i.e., air temperature during the emersion period was the same as the water temperature).

2.2. Physiological measurements

Aquatic rates of oxygen consumption (ml O_2 h^{-1}) of mussels ($n = 22–25$; Table 1) were measured in respirometer chambers containing natural marsh water near full oxygen saturation (Bayne et al., 1985). The chambers were immersed in a temperature-controlled

water bath to maintain constant ambient water temperatures (± 0.1 °C). To accommodate different sizes of mussels, chamber volumes varied from 0.28 l for mussels < 4 cm in shell length to 1.3 l for mussels > 8 cm. A mussel was placed on a perforated, elevated platform in the center of the chamber and underneath a magnetic stir bar mixed water in the chamber continuously. Partial pressure of oxygen (pO_2) was measured with a Radiometer oxygen electrode inserted into the top center of the chamber. The sensor was connected to a Radiometer blood-gas analyzer (PHM72 Mk2) and pO_2 readings were recorded continuously on a chart recorder. The rate of decline of pO_2 was determined by the slope of pO_2 against time once the mussels had acclimatized in the chamber and pO_2 steadily decreased. Chambers without mussels were used to calculate background rates of microbial metabolism in the unfiltered marsh water; these background rates were negligible over the time course of our measurements.

Aerial rates of oxygen consumption (ml O_2 h^{-1}) were measured using a Gilson Differential respirometer at temperatures corresponding to ambient water temperature. Due to the limited size of the respirometer chamber, only mussels with shell lengths < 5.5 cm could be measured. Each season, only $\sim 50\%$ of the mussels that we had used to measure aquatic respiration rates were small enough to be used to measure aerial respiration (Table 1). The humidity in the flask was kept high by the addition of 1 ml of 0.22-μm filtered seawater. Mussels were allowed to acclimatize for 30 min before the chambers were sealed and the first reading taken. The volume of oxygen consumed by each mussel was recorded every 15 min for 2 h, except for the winter when readings were taken every 30 min for 3 h. Flasks without mussels served as controls.

Rates of ammonium excretion (μg NH_3-N h^{-1}) were measured for the same individual mussels ($n = 19-24$; Table 1) that we had used to measure rates of aquatic and aerial respiration. Individual mussels were placed in 500-ml beakers filled with either 200 or 400 ml of 0.22-μm filtered ambient marsh water depending on mussel size. The mussels were allowed to excrete for 2 h in the beakers maintained at ambient water temperature. Water samples were taken for the determination of ammonium concentrations using the phenolhypochlorite method (Solozano, 1969) at the beginning and the end of the 2-h incubation period. We calculated the increase in ammonium concentration in each beaker relative to other control beakers without mussels.

Finally, we measured the shell length of each mussel by determining the maximum shell dimension using calipers. Mussels were then dissected and soft tissues placed onto preweighed aluminum pans. Dry tissue weights were determined after the soft tissue was dried at 60 °C until constant weight (~ 24 h).

2.3. Statistical analysis

We modeled the relationships between rates of physiological functions (aquatic and aerial respiration and ammonium excretion) and dry tissue weight using the allometric equation: rate $= a \times W^b$, where constant b is the weight exponent and a is the rate for a mussel of 1 g dry tissue weight. The relationships between shell length (L) and dry tissue weight (W) were modeled as $W = a \times L^b$. All the parameters were $\log_{10}$ transformed and SIGMASTAT 2.0 (Jandel Scientific Software) was used to perform linear regressions as well as calculate slopes (b) and intercepts ($\log_{10} a$). ANCOVA was performed to test the

heterogeneity of b and a coefficients of the physiological parameters among seasons using BIOMstat 3.2 (Applied Biostatistics), complementary software to Biometry (Sokal and Rohlf, 1981). If b coefficients significantly differed among seasons, pairwise ANCOVA was conducted to compare different pairs of seasons. When b coefficients were homogeneous, a common b value was fitted to the pooled data and this is used to estimate new a values for each season.

In previous publications (Kreeger and Newell, 2001), we have provided data on other physiological activities, such as clearance rate and assimilation efficiency, for mussels of a ''standard'' tissue weight of 0.27 g collected from the same population. In order to facilitate comparison with those data, we calculated the rates of aquatic and aerial respiration and ammonium excretion for mussels of 0.27 g dry tissue weight.

3. Results

3.1. Aquatic and aerial respiration

The metabolic rates of different size mussels varied seasonally in complex ways (Table 1) Rates of aquatic respiration were positively related to dry tissue weight in most seasons

Table 1
Regression coefficients for rates of respiration and ammonia excretion vs. dry tissue weight (g), expressed as rate $= a \times W^b$, for *G. demissa*. The b coefficients differed significantly among seasons for aquatic respiration, but not for aerial respiration and ammonia excretion (ANCOVA; $p = 0.05$). Different superscripted letters on regression coefficients indicate significant differences between seasons (pairwise comparisons; $p = 0.05$); nd = no difference. Water temperature: summer, 26 °C; autumn, 15 °C; winter, 5 °C; spring, 22 °C. Ranges shown are dry tissue weight and shell lengths of mussels measured for each function

Rate	Season	n	a	b	r^2	F	p	Tissue weight (g)	Shell length (cm)
Aquatic respiration (ml O_2 h^{-1})	Summer 1995	23	1.528	1.004^a	0.96	562.6	<0.001	$0.08-0.63$	$3.3-7.5$
	Autumn 1995	23	0.291	0.393^c	0.45	17.24	<0.001	$0.11-1.12$	$3.5-8.1$
	Winter 1995	22	0.138	0.212^c	0.07	1.40	0.252	$0.08-0.87$	$2.8-6.8$
	Spring 1996	25	0.806	0.696^b	0.84	123.6	<0.001	$0.18-1.65$	$3.5-7.8$
	Summer 1996	25	0.715	0.662^b	0.85	132.0	<0.001	$0.23-2.00$	$3.9-10.0$
Aerial respiration (ml O_2 h^{-1})	Summer 1995	14	0.563^a	0.763^{nd}	0.86	71.94	<0.001	$0.08-0.28$	$3.3-5.1$
	Autumn 1995	11	0.067^c	0.196^{nd}	0.03	0.29	0.603	$0.16-0.26$	$4.0-5.0$
	Winter 1995	11	0.041^c	0.658^{nd}	0.31	4.11	0.074	$0.08-0.42$	$3.1-5.6$
	Spring 1996	14	0.265^b	0.580^{nd}	0.78	42.02	<0.001	$0.18-0.66$	$3.5-5.6$
	Summer 1996	11	0.551^a	0.895^{nd}	0.43	6.71	0.029	$0.23-0.40$	$3.9-4.7$
Ammonium excretion (μg NH_3-N h^{-1})	Summer 1995	19	27.24^a	0.700^{nd}	0.66	32.65	<0.001	$0.08-0.63$	$3.3-7.5$
	Autumn 1995	22	17.50^b	0.549^{nd}	0.60	30.36	<0.001	$0.11-1.12$	$3.5-8.1$
	Winter 1995	22	6.52^c	0.609^{nd}	0.56	25.73	<0.001	$0.08-0.87$	$2.8-6.8$
	Spring 1996	24	17.03^b	0.639^{nd}	0.50	22.36	<0.001	$0.18-1.65$	$3.5-7.8$
	Summer 1996	21	18.66^{ab}	0.424^{nd}	0.55	23.37	<0.001	$0.23-1.94$	$3.9-9.0$

($p < 0.001$), except in winter 1995 when the linear regression between the $\log_{10}$ trans-formed rates and dry tissue weights was not significant. Although rates of aquatic respiration increased with mussel weight for the other four seasons, the b coefficients were significantly heterogeneous (ANCOVA, $F = 12.8$, $p < 0.001$). Pairwise comparisons of the b coefficients showed that the highest slope occurred in summer 1995, the intermediate slopes in spring 1996 and summer 1996, and the lowest in winter 1995 (Table 1). In spring and summer 1996, both the slopes and intercepts did not differ significantly. We calculated a common b coefficient of 0.678 and a common a coefficient of 0.76 ml O_2 g^{-1} h^{-1} for these two seasons. Rates of aerial respiration increased significantly with body weight (Table 1) in three of the five seasons, including summer 1995 ($b = 0.763$), spring 1996 ($b = 0.580$), and summer 1996 ($b = 0.895$). ANCOVA on rates of aerial respiration showed that the b coefficients for the five seasons did not differ significantly ($F = 0.39$, $p = 0.81$) but the a coefficients differed significantly ($F = 190$, $p < 0.001$).

We measured both aerial and aquatic respiration for the same individual mussels. This enabled us to calculate the ratio of aerial to aquatic respiration for individual mussels instead of deriving the ratio from the seasonal regression equations, which could lead to accumulation of statistical errors. Regression of the aerial/aquatic respiration ratio against $\log_{10}$-transformed dry weight showed that the ratio was not related to mussel weight in any season ($p > 0.05$). ANOVA showed that there were significant differences in the ratio of aerial to aquatic respiration among seasons ($F = 61.8$, $p < 0.001$). The average ratio (Fig. 1) was highest in summer 1995 (0.61) and lowest in winter 1995 (0.11). Summer 1996 had
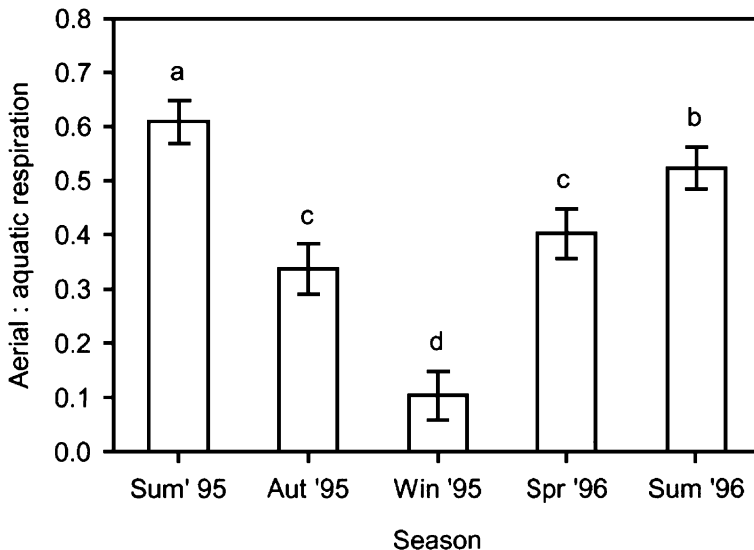

Fig. 1. Seasonal means ($\pm$ 95% confidence limits) of aerial to aquatic respiration ratios for *G. demissa* ($n = 11$–14) from autumn 1995 to summer 1996. Different letters above each bar indicate significant differences ($p = 0.05$; SNK test). Water temperature: summer, 26 °C; autumn, 15 °C; winter, 5 °C; and spring, 22 °C.

Table 2
Regression coefficients for atomic O:N ratio vs. dry tissue weight (g), expressed as $O:N = a \times W^b$ for *G. demissa*

Season	n	a	b	r^2	F	p
Summer 1995	19	74.9	40.5	0.26	6.12	0.024
Autumn 1995	22	23.0	− 7.6	0.05	1.13	0.303
Winter 1995	18	39.0	− 32.7	0.05	0.84	0.373
Spring 1996	22	72.0	27.2	0.04	0.80	0.381
Summer 1996	21	52.8	27.0	0.22	5.41	0.031

the second highest ratio (0.52) but significantly lower than summer 1995 (SNK test, $p = 0.05$). Autumn and spring had intermediate ratios of 0.34 and 0.40, respectively, and these two seasons did not differ significantly (SNK test, $p = 0.05$).

3.2. Ammonium excretion and O/N ratio

The rate of ammonium excretion increased with dry tissue weight in all seasons (Table 1). The b coefficients varied between 0.424 and 0.700 over the five seasons, but were not significantly heterogeneous (ANCOVA, $F = 0.58$, $p = 0.681$). Conversely, ANCOVA showed that a coefficients of the five regressions were significantly different (ANCOVA, $F = 32.1$, $p < 0.001$). A common b coefficient of 0.595 was calculated from the ANCOVA and the a coefficients for the five seasons were recalculated as 23.3 (summer 1995), 18.3
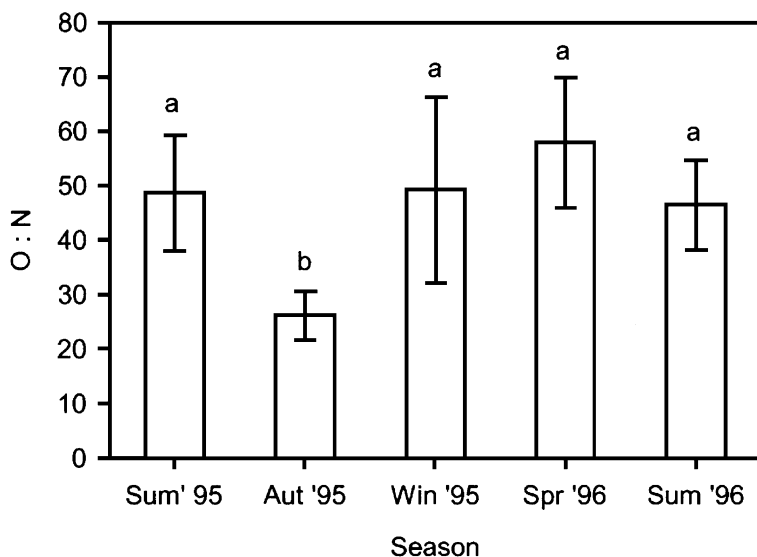

Fig. 2. Seasonal means ($\pm 95\%$ confidence limits) of atomic O:N ratios for *G. demissa* ($n = 19–24$) from autumn 1995 to summer 1996. Different letters above each bar indicate significant differences ($p = 0.05$; SNK test). Water temperature: summer, 26 °C; autumn, 15 °C; winter, 5 °C; and spring, 22 °C.

(autumn 1995), 5.12 (winter 1995), 16.5 (spring 1996), and 20.5 mg $NH_3-N\ h^{-1}$ (summer 1996).

Atomic ratios of oxygen consumption to nitrogen excretion (O:N) were calculated (Bayne et al., 1985) from the data for individual mussels. The regressions of the O:N ratio to $\log_{10}$-transformed weight in the five seasons exhibited a positive relationship both in summer 1995 ($F = 6.12$, $p = 0.024$) and summer 1996 ($F = 5.41$, $p = 0.031$), but there was not a significant relationship in the other three seasons (Table 2). The overall lack of a strong relationship between O:N and mussel weight allowed us to use ANOVA instead of ANCOVA to test the differences among seasons for O:N ratio, which was found to be significant ($F = 7.51$, $p < 0.001$). A SNK multiple comparison test indicated that the difference among seasons was solely attributable to autumn 1995, which exhibited a lower O:N ratio (26) than the other four seasons, which had means ranging from 46 to 58 (Fig. 2).

3.3. Seasonal allometry of length and weight

The regressions of log-transformed dry weight against log-transformed shell length in *G. demissa* were significant in all five seasons (Table 3), with *b* coefficients ranging between 2.375 in summer 1996 and 2.824 in autumn 1995. There were significant differences among the *b* coefficients for all five seasons (ANCOVA, $F = 2.64$, $p = 0.038$). Pairwise comparisons between seasons by ANCOVA showed that the *b* coefficient in autumn 1995 was significantly greater than the *b* coefficients in summer 1995 ($p = 0.021$) and summer 1996 ($p = 0.006$), and the remaining seasonal pairs did not differ significantly ($p > 0.05$). Since the *b* coefficients were not different for the seasons of summer 1995, winter 1995, spring 1996, and summer 1996 (Table 3), we tested differences between the *a* coefficients of these four seasons. The *a* coefficients were lowest in summer 1995 and highest in spring 1996 and summer 1996. These latter two seasons did not differ significantly. The absence of any difference between spring and summer 1996 in both *b* coefficients and *a* coefficients indicated that *G. demissa* populations were homogeneous at these time in terms of allometry between tissue weight and shell length.

Table 3

Regression coefficients for dry tissue weight (*W*, g) vs. shell length (*L*, cm), expressed as $W = a \times L^b$, for *G. demissa*. Differences in *b* coefficients among seasons were significant (ANCOVA, $p < 0.01$). Different superscripted letters on regression coefficients indicate significant differences between seasons (pairwise comparisons; $p = 0.05$). Ranges shown are dry tissue weight and shell lengths of mussels measured for each function

Season	*n*	*a*	*b*	r^2	*F*	*p*	Tissue weight (g)	Shell length (cm)
Summer 1995	23	0.00504	2.415[b]	0.94	341.3	<0.001	0.08–0.63	3.3–7.5
Autumn 1995	23	0.00303	2.824[a]	0.97	717.6	<0.001	0.11–1.12	3.5–8.1
Winter 1995	26	0.00552	2.560[ab]	0.95	480.6	<0.001	0.08–0.87	2.8–6.8
Spring 1996	27	0.00597	2.715[ab]	0.94	401.6	<0.001	0.18–1.65	3.5–7.8
Summer 1996	25	0.00974	2.375[b]	0.96	504.7	<0.001	0.23–2.00	3.9–10.0

4. Discussion

4.1. Aerial and aquatic respiration

There were significant seasonal variations in the rates of aquatic respiration for *G. demissa* except for spring 1996 and summer 1996, when they were homogeneous even though the ambient water temperature increased from 22 °C in spring 1996 to 26 °C in summer 1996 (Table 1). The recalculated *a* coefficient for these two seasons (0.76 ml O^2 g^{-1} h^{-1}) was higher than reported for mussels held at similar water temperature in earlier studies [e.g., 0.63 ml O_2 g^{-1} h^{-1} at 22 °C (Read, 1962) and 0.38 ml O_2 g^{-1} h^{-1} at 20 °C (Kuenzler, 1961)]. Our measured rates are double those obtained by Kuenzler (1961), which is likely due to the fact that the mussels in our study were freshly collected from the salt marsh. Furthermore, all of our measurements were performed on mussels fed on the natural seston complement of marsh water and at seasonal ambient water temperatures. In contrast, Kuenzler (1961) performed all his measurement on mussels that were collected in March and then subjected to acute temperature changes.

In addition to the seasonal changes in weight-specific rates of aquatic respiration that we observed, which primarily resulted from temperature changes, the differences in *b* coefficients reveal a variable seasonal response between different sized mussels (Table 1). Large individuals (ca. 1 g dry tissue weight) exhibited greater seasonal variations in aquatic respiration rate than small individuals (Fig. 3). As a result, the *b* coefficient for rate of aquatic respiration vs. dry tissue weight varied seasonally, with a tendency for a steeper slope during warmer summer (26 °C) and spring (22 °C) and a shallower slope in the cooler season (autumn, 15 °C and winter, 5 °C). We performed a correlation test between *b* coefficients and temperatures of the corresponding seasons and found they were positively correlated ($r = 0.91$, $n = 5$). The *b* coefficient for *G. demissa* reported by Kuenzler (1961) varied among
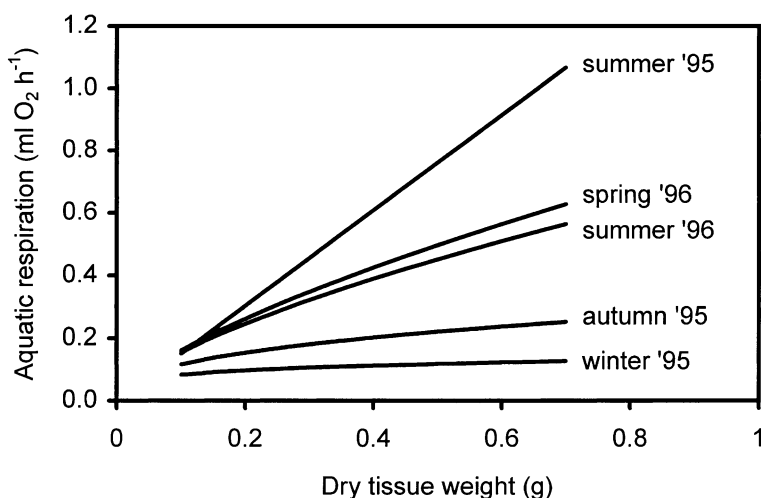

Fig. 3. Seasonal rates of aquatic respiration plotted against dry tissue weight (rate = $a \times W^b$) for *G. demissa*. Lines are derived from the regression parameters listed in Table 1.

different temperatures, but there was no clear seasonal pattern. Smaal et al. (1997) reported that the *b* coefficient for the blue mussel *Mytilus edulis* varied between 0.409 and 0.857 over a 2-year period, with the variability appearing random with the *b* coefficient as high as 0.837, even when water temperature was low in March.

Though *b* coefficients of aquatic respiration could be influenced by water temperature in most seasons, temperature alone cannot explain the high *b* coefficient of 1.004 in summer 1995 compared to 0.662 in summer 1996 because water temperatures were the same in both years. Bayne and Newell (1983) calculated an average *b* coefficient of 0.7 for the relationship between metabolic rate and body weight in marine molluscs. In our study, spring and summer 1996 were closest to this value. It is not clear why the *b* coefficient in summer 1995 was so much higher than that in summer 1996. This was not due to the lack of large mussels in the groups we measured in summer 1995 (maximum, 0.63 g) compared to summer 1996 (maximum, 2.0 g), because when we reanalyzed these data with the large individuals in summer 1996 removed, the *b* coefficient did not change significantly. Thus, the observed variation in *b* coefficients is likely associated with differences in mussel tissue condition between the two summers because mussels of 7-cm shell length weighed 45% less in summer 1995 than summer 1996 (Fig. 4). The relatively light tissue weight of mussels in summer 1995 may have been caused by lower food supply in summer 1995 than in summer 1996. Huang et al. (in press (a)) reported that in the same location from which these mussels were collected, phytoplankton biomass, indicated by chlorophyll *a* concentration, was about three times higher in summer 1996 (9.2 μg l^{-1}) than in summer 1995 (2.7 μg l^{-1}). However, it is unlikely that mussels would sustain an elevated metabolic rate while losing weight due to starvation. An alternative explanation for the difference in weight is that the ribbed mussels we collected in summer 1995 had recently spawned, resulting in a relatively
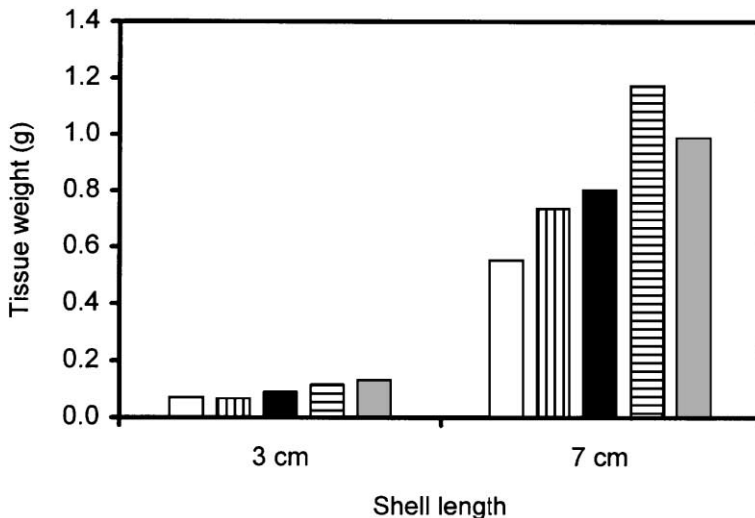

Fig. 4. Seasonal variation in dry tissue weight for 3 and 7 cm shell length *G. demissa* calculated from the regression parameters listed in Table 3. White bar: summer 1995; vertical stripe bar: autumn 1995; black bar: winter 1996; horizontal stripe bar: spring 1996; gray bar: summer 1996.

light tissue weight per unit shell size for the large mussels. The high metabolic activity we measured may stem from high activity, such as feeding and digestion, necessary to rebuild nutrient reserves.

Aerial respiration was also related to the tissue weight of mussels and our results showed significant b coefficients in some seasons (Table 1), with the strongest relationship between aerial respiration and body size being in summer 1995 ($b = 0.763$, $p < 0.001$) and spring 1996 ($b = 0.580$, $p < 0.001$). This size-dependent rate of aerial respiration has previously been reported by Kuenzler (1961). The absence of a significant relationship in autumn 1995 and the weak relationship in summer 1996 were most likely an experimental artifact caused by the limited size range of mussels that we could accommodate in the aerial respiration chambers. Consequently, it is not appropriate to compare the b coefficient directly to those calculated for other metabolic parameters, such as aquatic respiration and ammonium excretion.

The seasonal variation in the aerial to aquatic respiration ratio, being high in summer (0.61 and 0.52) and low (0.11) in winter (Fig. 1), was caused by the proportionally larger changes in rates of aerial respiration than in aquatic respiration (Fig. 5). The rate of aerial respiration for a mussel of 0.27 g dry weight was 10 times higher in summer 1996 (0.17 ml O_2 h^{-1}) than in winter 1995 (0.017 ml O_2 h^{-1}). On the other hand, the rate of aquatic respiration was only three times higher in summer 1996 (0.30 ml O_2 h^{-1}) than in winter
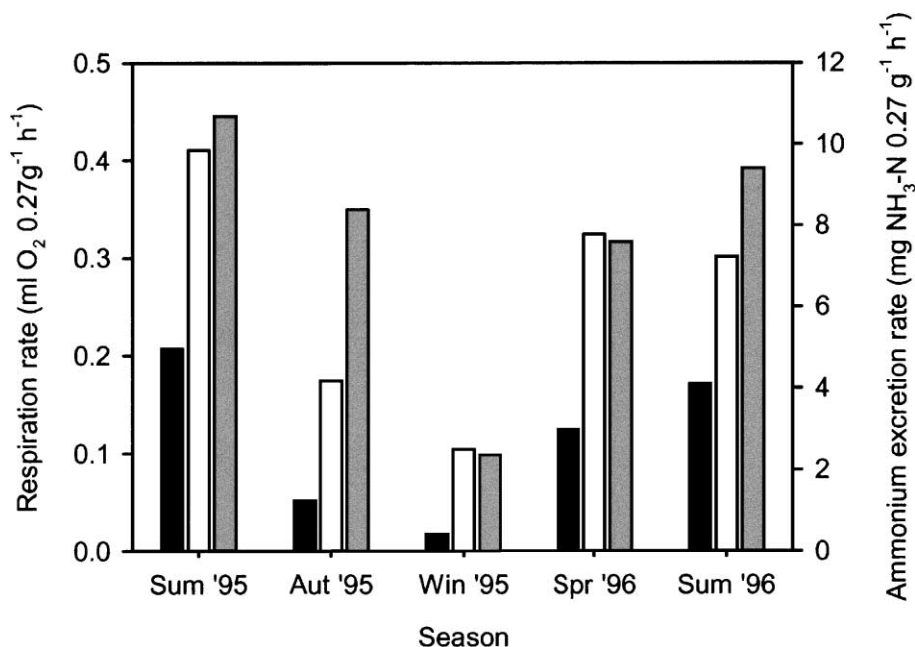

Fig. 5. Seasonal variation in the rate of aerial and aquatic respiration and ammonium excretion for 0.27 g dry tissue weight *G. demissa* calculated from the regression parameters listed in Table 1. Black bar: aerial respiration; white bar: aquatic respiration; gray bar: ammonium excretion.

1995 (0.10 ml O_2 h^{-1}). This dramatic seasonal variation in aerial respiration indicates that aerial metabolism is not controlled solely by exogenous factors such as temperature. Instead, our study indicates that ribbed mussels are capable of actively regulating aerial respiration on a seasonal basis that results in exceptionally high rates in summer.

Maintaining a high metabolic rate through aerobic respiration at a time when feeding is precluded during aerial exposure would appear to be energetically disadvantageous. Charles and Newell (1997) reported, however, that ribbed mussels feed actively during immersion and that ingested material is subject to digestion during the subsequent emersion period. Because the "Specific Dynamic Action" (e.g., enzyme synthesis, food uptake, formation of digestive tubules, etc.) requires a large amount of energy (Bayne et al., 1988; Widdows and Hawkins, 1989), it is likely that ribbed mussels can only satisfy these high-energy requirements by maintaining active aerobic respiration during aerial exposure. Thus, we hypothesize that aerial respiration is required to provide sufficient energy for the mussels to maintain an active metabolism during the prolonged period of aerial exposure that for mussels high in the intertidal zone can be up to 70% of the tidal cycle.

4.2. Ammonium excretion and O/N ratio

There have been few measurements of seasonal rates of ammonium excretion for *G. demissa*. Jordan and Valiela (1982) studied a *G. demissa* population in a New England salt marsh and found the rates to be 13.8 μg NH_3–N h^{-1} g^{-1} (spring), 33.7 μg NH_3–N h^{-1} g^{-1} (summer), and 11.8 μg NH_3–N h^{-1} g^{-1} (autumn). The rates of ammonium excretion for large mussels (1 g dry tissue weight) in our study are comparable to their results (spring = 16.5 μg NH_3–N h^{-1} g^{-1}, summer = 20.5 μg NH_3–N h^{-1} g^{-1}, and autumn = 18.3 μg NH_3–N h^{-1} g^{-1}). However, the common regression *b* coefficient (0.595) in our study was higher than the *b* coefficients reported by Jordan and Valiela (1982), which ranged from 0.4 to 0.473. Nevertheless, given the highly variable nature of ammonium excretion in marine bivalves (Bayne and Newell, 1983) and in our study (between 0.424 and 0.7), the differences between the two studies are unlikely to be significant. The lack of seasonal variation in *b* coefficients in our study, and their similarity to those reported by Jordan and Valiela (1982), indicate that the seasonal changes in the rate of ammonium excretion in *G. demissa* does not interact with body size, i.e., both large and small individuals change their rates of ammonium excretion in the same proportion seasonally. This pattern differs from that observed in other bivalve species. For example, the *b* coefficient for ammonium excretion by the blue mussel, *M. edulis*, varies greatly seasonally (Bayne and Scullard, 1977), ranging from 0.48 in November to 1.48 in May.

Wilbur and Hilbish (1989) studied thermal acclimation in *G. demissa* and found that ammonium excretion rates doubled from ca. 1.0–2.0 μg NH_3–N h^{-1} for 0.126 g dry tissue weight mussel a week after transferring mussels from 15 to 25 °C. The rate of ammonium excretion then decreased to 1.3 μg NH_3–N h^{-1} after 3 weeks, exhibiting the effects of thermal acclimation. At a comparable temperature in our study (autumn, 15 °C and summer, 26 °C), the excretion rates for 0.126 g mussels were 5.3 and 6.7 μg NH_3–N h^{-1}, respectively, which is appreciably higher than values reported by Wilbur and Hilbish (1989). The higher rates observed in our measurements of mussels freshly collected from the field compared with the laboratory held mussels studied by Wilbur and Hilbish (1989)

most likely reflects greater ammonium excretion from the catabolism of high levels of nitrogen-containing compounds present in the marsh seston (Huang et al., in press (b)).

The O:N ratio measured by Wilbur and Hilbish (1989) for ribbed mussels, ranging from 62 to 118, is greater than our values, which were generally between 45 and 60, with the exception of the low O:N ratio of 26 in autumn 1995 (Fig. 2). The range of O:N ratios we observed indicates a general utilization of carbon-based substrates, such as carbohydrate and lipid, as the primary catabolic substrate by the mussels. We measured the seasonal changes in food supply available to mussels at the salt marsh sampling site and found that the seston C:N ratio (varying between 9 and 11) was fairly uniform (Huang et al., in press (b)). Therefore, the constant O:N ratio we measured for ribbed mussels in all seasons, except autumn, reflects this stability in nitrogen content of their natural diet.

A low O:N ratio (below 20) has been used as an indicator of nutritional stress for marine bivalves (Bayne et al., 1985) as it shows an increased reliance on protein as a catabolic substrate rather than carbohydrate and lipid. Seasonal variations in O:N ratio, however, do not always stem from physiological stress but often reflect the metabolic adjustments to the composition (i.e., C:N) of the available diet, as well as to the demand of nutrients necessary to sustain gametogenesis (Hawkins and Bayne, 1985; Hawkins et al., 1985; Kreeger, 1993). Autumn is the season when many species of marine bivalves store carbon-based energy, such as glycogen and lipid, for utilization in winter and early spring (Newell and Bayne, 1980; Bayne and Newell, 1983). Jordan and Valiela (1982) measured the seasonal cycle of relative carbon and nitrogen tissue content for the *G. demissa* population in a New England salt marsh. The nitrogen content was greatest when the mussels were reproductively ripe in summer and, conversely, carbon content was highest from autumn to early spring. Kreeger (1993) suggested that the low O:N ratio for the mussel *Mytilus trossulus* between spawning in summer and late autumn is characterized by low assimilation efficiency for dietary protein, high catabolism of assimilated protein, and increasing tissue carbohydrate content. Similarly, the low O:N ratio of 26, exhibited by ribbed mussels during autumn in our study, was likely indicative of the greater demand for dietary carbon than nitrogen in this season. We suggest that relatively high rates of protein catabolism in autumn are not caused by stress or malnutrition, but stemmed from the ecophysiological adaptation by *G. demissa* to preserve carbon-based energy for winter when food resources are low.

Acknowledgements

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