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NOTE

High dissolved C : P excretion ratios for large benthic marine invertebrates

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ABSTRACT: CO_2 respiration and P excretion were measured in 13 species of large benthic invertebrates. Wet weight, dry weight, and C, N, P content for each species was determined. CO_2 respiration per dry weight and P excretion per dry weight decreased as animal size increased. A closer relation was found between CO_2 respiration and P excretion than between either CO_2 respiration or P excretion and dry weight: $P = 1.13\ CO_2^{1.23}$ (P in nmoles $gDW^{-1}\ h^{-1}$ and CO_2 in μmoles $gDW^{-1}\ h^{-1}$). The mean C : P excretion ratio, 609 : 1, is higher than O : P ratios for zooplankton, probably reflecting detritus or macrophyte food sources which have substantially higher C : P ratios than phytoplankton or bacteria.

In the surface oceans nutrient excretion by zooplankton is a major source of inorganic nutrients for phytoplankton growth. Consequently the nutrient excretion rates of zooplankton are studied in relation to animal size, food availability, feeding activity, environmental conditions, and respiration (Corner and Davies, 1971). Nutrient excretion rates, usually N or P, and respiration rates, as measured by C production or O consumption, are ratioed to one another. Redfield et al. (1963) showed that the net removal of dissolved N and P from the surface oceans matched the average N : P composition of plankton, and the decomposition of these organisms in deep water resulted in the production of N : P in the same ratio. It has been a common working hypothesis that, because zooplankton generally feed on other plankton, zooplankton might metabolize O, C, N and P in the Redfield ratio. However, planktonic animals tend to excrete N and P relative to O consumption or C production in a range of ratios that usually only approximate the Redfield ratio. The variability in these ratios has been shown to be a function of food sources, activity, and environmental conditions. Changes in zooplankton nutrient release ratios can change the relative flux of nutrients to phytoplankton, thus altering their growth characteristics. Therefore the dependence of phytoplankton growth on 'new' or recycled nutrients apparently must change depending on nutrient excretion characteristics.

The relation between 'new' and recycled or 'old', nutrients in benthic systems is extremely variable. Some confined systems rely heavily on recycled nutrients (Smith and Atkinson, 1983), while other more open systems appear completely flushed by 'new' nutrients (Teal, 1982). Benthic plant composition, and consequently nutrient requirements for benthic ecosystems, deviate markedly from the Redfield ratio. Benthic plants have C : P composition ratios nearly 5 times higher than the Redfield ratio and N : P ratios nearly twice as high (C : N : P = 550 : 30 : 1, Atkinson and Smith, 1983). Macrophytes and macrophytic detritus are notoriously low in protein, making them a poor food source (Mann, 1972). In the case of N, N_2-fixation in the gut of some invertebrates and bacterial enrichment (C : N ratios are near 5 : 1) are sources of N which enable grazers to survive on high C : N (low protein) material (Guerinot, 1979, Fong and Mann, 1978, Mann, 1982). If invertebrates are feeding on macrophytic material but metabolizing bacterial food sources, then their C : P excretion ratios should be low (bacteria are rich in P, C : P near 50 : 1). On the other hand, if they are metabolizing predominantly macrophytic material their C : P excretion ratios should be high. We hypothesized that larger benthic marine invertebrates which generally graze on benthic material should have C : P ratios of excretion much higher than the Redfield ratio (106 : 1), otherwise they must have another significant source of P. Some values in the literature indicate that benthic animals indeed have higher O : P release ratios than plankton (Satomi and Pomeroy, 1965, Johannes, 1964), although there are no published C : P excretion values. A knowledge of benthic excretion ratios would help in estimating nutrient flux from

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benthic animals, and thus evaluate 'new' *vs.* 'old' nutrients in benthic systems. In this study we measured respiration and P excretion in a variety of benthic marine invertebrates, and determined the body composition of these elements.

Animals were collected from intertidal limestone reefs north of Perth, Western Australia and at Rottnest Island, Western Australia (32°00' S, 115°30' E). Animals were immediately cleaned of encrusting organisms and placed into five litre glass aquaria which were used as incubation chambers. Within one hour after the animals were collected, CO_2 respiration rate was measured by monitoring changes in pH of the incubation water for 30 min; then the aquarium was aerated with an aquarium pump and air stone, and P excretion was determined by measuring changes in P for the next 4 to 12 h; immediately after termination of the P excretion incubation, the air stone was removed and CO_2 respiration was again measured for 30 min. The second measurement of CO_2 respiration rate provided a good check on the health of the animals after the P incubation.

The number of animals and the water volume in the aquaria were varied to get a similar change in magnitude of pH and P; this was done to ensure similar analytical errors between the incubations of different species. The number of individuals per incubation varied from 1 to 300 while the water volume varied from 1 to 6 l. Different incubations for each species had roughly equal numbers of individuals. The temperature of the incubation water was held at the *in situ* temperature which ranged from 15 to 20 °C.

CO_2 respiration rate was determined as an increase in total CO_2 of the incubation seawater. Total CO_2 was calculated from pH assuming a constant total akalinity throughout each incubation (Skirrow, 1975; Smith and Kinsey, 1978). pH was measured using an Orion Combination Ross electrode and a custom made pH meter, giving a precision of 0.003 pH units. Total akalinities were measured on water samples taken at the beginning and end of each incubation by pH change after acid addition (Smith and Kinsey, 1978). These akalinities were not different within the error of the technique thus validating the assumption of constant alkalinity throughout the incubation. Given a pH measurement error of 0.003 pH units and the assumption of constant alkalinity the maximum error in total CO_2 was 2 μmoles C l^{-1}. Exchange of CO_2 with the atmosphere proceeds tens to hundreds of times slower than O_2 diffusion (Smith and Kinsey, 1978). Using a diffusion coefficient of 0.3 md^{-1} (data compiled by Smith and Atkinson, 1983) under the experimental conditions the rate of CO_2 gas diffusion out of the incubation water was estimated to be only 1 to 2 % of the respiration rates. Respiration rates were calculated from regressions of total CO_2 *vs.* time. All regressions included 9 to 13 data points and had r^2 values greater than 0.90. The 2 estimates of respiration rate for each P incubation (before and after the P incubation) were never significantly different, therefore a mean of the 2 rates was used as the respiration rate of the animals during the P incubation. If it assumed that all respiration was aerobic (1 mole CO_2 respired consumes 1 mole of O_2) then in all incubations for the determination of CO_2 respiration rate, the O_2 consumption was less than 2 ppm, and the incubation water O_2 content never dropped below about 4 ppm. The incubation aquaria were aerated during the P incubations to maintain saturated O_2 levels. Because of the vigorous aeration during the P incubations, by the beginning of the second determination of the respiration rate (at the end of P incubation) the incubation seawater was brought back to a CO_2 equilibrium pH, of about 8.2.

To determine inorganic P excretion rate 10 water samples were taken throughout each P incubation. These samples were analysed for inorganic P (reactive) using an acid molybdate, ascorbic acid mixed reagent (Strickland and Parsons, 1968). Additional samples were taken to determine total P excretion, measured as inorganic P after oxidation of the dissolved organic matter by persulphate (Menzel and Corwin, 1965). To retain as much water in the incubations as possible, water for total P analysis was taken twice at the beginning and several times towards the end of each incubation. The P excretion rate was determined by regressions of inorganic P vs. time. All regressions were linear with r^2 values greater than 0.50.

At the completion of each incubation, each animal was weighed. Subsamples of the animals were dried at 70 °C and reweighed to determine dry to wet weight conversions. These subsamples were then ground to a fine powder for C, N and P analysis. Organic C was determined as 40 % of the weight loss after ashing for 2 h at 550 °C ($CH_2O + O_2 = CO_2 + H_2O$, C/CH_2O = 0.40); inorganic C was determined as 27 % of weight loss after ashing for a further 2 h at 1,000 °C ($CaCO3 = CaO + CO_2$, C/CO_2 = 0.27). Total N was determined by the Kjeldahl method (American Public Health Association, 1971). Total P content was determined by shaking a sample, which had been ashed for 2 h at 550 °C, in 50 ml 1N HCl for 16 h, and then measuring the centrifuged supernatent for inorganic P (Aspila et al., 1976). The regression statistics for respiration and excretion rate were type 1 (assumes only 1 variable has error), all others were type 2, geometric mean (assumes both variables have error, Ricker, 1973).

The respiration rates for the 13 species studied ranged from 1 to 30 μmole CO_2 gDW^{-1} h^{-1} and the inorganic P excretion rates ranged from 2 to 140 nmoles P gDW^{-1} h^{-1} (Table 1). Total P excretion was

Table 1. Summary of incubations in which measurements of CO_2 respiration and inorganic P excretion rates were made. Total number of animals is the number of animals used in all incubations. Mean dry weight for each species was calculated as mean dry weight of the total number of animals determined from regressions of wet weights *vs.* dry weight (Table 2). Respiration and excretion rates were calculated from regressions of total CO_2 *vs.* time and P *vs.* time. Each C:P release ratio is the mean respiration rate divided by mean P excretion rate for each species

Species	Number of incubations	Total number of animals	Mean dry weight (g)	Respiration rate mean (± SE) (μmoles CO_2 gDW^{-1} h^{-1})	Excretion rate mean (± SE) (nmoles P gDW^{-1} h^{-1})	Release C:P molar
Mollusca						
Patelloida alticostata	2	49	0.32	28.6 (7.1)	139.0 (82.8)	206
Patallanax laticostata	2	9	15.82	4.4 (0.4)	14.0 (11.0)	314
Pyrene bidentata	3	959	0.05	16.6 (3.5)	44.6 (14.2)	372
Thais orbita	1	21	1.61	16.3	18.3	891
Charonia lampas rubicunda	2	5	15.94	8.4 (2.6)	15.7 (11.6)	535
Cypraea caputserpentis	2	196	0.67	8.8 (5.0)	8.6 (5.7)	1023
Turbo intercostralis	2	60	7.02	5.3 (0.7)	8.6 (5.7)	616
Aplysia sp.	3	109	1.26	29.6 (17.2)	42.3 (27.1)	700
Mytilus edulis	1	720	0.34	6.7	15.7	427
Echinodermata						
Echinometra mathaei	10	160	35.22	1.3 (0.1)	2.0 (0.7)	650
Tripneustes gratilla	5	57	28.27	3.8 (0.8)	5.3 (4.1)	717
Stichopus mollis	2	3	30.84	5.3 (0.6)	6.4 (2.3)	828
Patiriella gunnii	3	44	3.42	3.1 (0.7)	4.9 (1.9)	633

Table 2. Summary of regression equations of the form DW = a + bWW relating dry weight (DW) to wet weight (WW), in g. a and b = intercept and slope, respectively; r^2 = coefficient of determination; n = number of animals for which both dry and wet weights were measured

Species	a	b	r^2	n
Mollusca				
Patelloida alticostata	0.11	0.02	0.85	22
Patallanax laticostata	2.53	0.07	0.80	9
Pyrene bidentata	0.03	0.08	0.80	50
Thais orbita	0.14	0.04	0.66	21
Charonia lampas rubicunda		0.04		2
Cypraea caputserpentis	−0.15	0.08	0.95	20
Turbo intercostralis	1.31	0.05	0.99	20
Aplysia sp.	0.08	0.04	0.90	20
Mytilus edulis	0.02	0.06	0.96	20
Echinodermata				
Echinometra mathaei	0.65	0.48	0.97	20
Tripneustes gratilla	0.51	0.22	0.95	20
Stichopus mollis	0.00	0.11	0.91	3
Patiriella gunnii	0.11	0.32	0.89	25

linearly related to inorganic P excretion (TP = 0.71 + 1.40 P, n = 256, r^2 = 0.71). Therefore, on average, 29 % of P was excreted as organic P and 71 % as inorganic P. Animal wet weight to dry weight conversions used in the calculations of dry weight are shown in Table 2.

As expected, both the weight specific respiration and excretion rates decreased as body size increased (CO_2 = 3.60 $DW^{-1.00}$, n = 13, r^2 = −.25; P = 3.16 $DW^{-0.53}$, n = 13, r^2 = −.50). P excretion and respiration were more highly correlated than either were with body weight: P excretion was related to respiration by a power function (P = 1.13 $CO_2^{1.23}$, n = 13, r^2 = 0.84, CO_2 in units of μmoles gDW^{-1} h^{-1} and P in units of nmoles gDW^{-1} h^{-1}; Fig. 1). The exponent 1.23 indicates that as weight specific respiration rate increases the weight specific P excretion rate increases proportionately more, or the C : P ratio decreases as respiration rate increases. This decrease in C : P ratio with increasing respiration is consistent with earlier O : P work on smaller marine animals (Johannes, 1964). Thus the C : P ratio of excretion ranges from 885 for an animal respiring at 1 umole CO_2 gDW^{-1} h^{-1} to 360 for one respiring at 50 umole CO_2 gDW^{-1} h^{-1}. Using this equation an estimate of the C : P excretion ratio of zooplankton with a respiration rate of 1,000 μmoles CO_2 gDW^{-1} h^{-1} gives a C : P excretion ratio of 180 : 1, a value much closer to the O : P ratios of zooplankton found in the literature than the values for the larger benthic invertebrates. Even though the power function is the best estimate of P excretion *vs.* respiration, the exponent 1.23, is not significantly different from 1 (modified t-test, $p < 0.05$, Clarke, 1980) indicating that the C : P ratio of excretion might have been constant over the entire respiration range. We therefore limit our discussion to explanations for the relatively high mean C : P excretion ratio. The mean C : P excretion ratio was 609 : 1.

Table 3 is a summary of the C, N, P composition of the animals. The compositional ratios average 239 : 60 : 1 with a C : P range from 116 : 1 to 337 : 1,

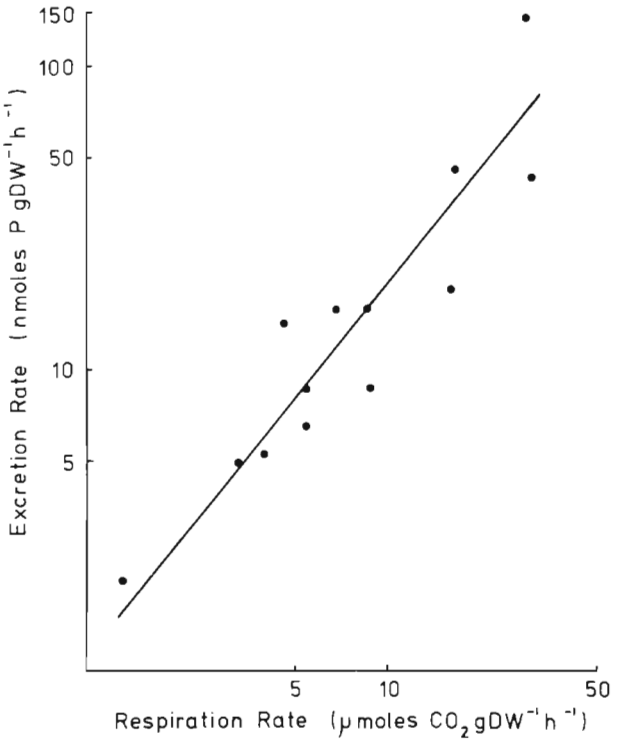

Fig. 1. P excretion rate g^{-1} dry weight *vs.* respiration rate g^{-1} dry weight, for species of large benthic invertebrates (see Table 1 for species and rates). Each point represents a single species

N : P range from 19 : 1 to 108 : 1, and a C : N range from 3.0 to 6.2.

In this study the molar C : P excretion ratios for all the benthic animals studied were higher than previously reported molar O : P ratios for zooplankton. There are virtually no direct measurements of C : P excretion for zooplankton (although there are O : P, C : N, and O : N ratios); however, if a R.Q. (CO_2/O_2) of approximately 1.0 is assumed for zooplankton, then the C : P excretion ratios of these large benthic invertebrates are 5 to 6 times higher than the estimated C : P excretion ratios for zooplankton.

There are several possible explanations for the high dissolved C : P excretion ratios. The first explanation is that there is a large amount of P excreted that we did not measure, either as dissolved organic P or as faeces. Satomi and Pomeroy (1965) showed that the ratio of total P excretion to inorganic P excretion for three populations of zooplankton was 1.56. In this study, the total P excretion to inorganic P excretion ratio was 1.40 (or only 29 % of P was excreted as organic P); thus both the benthic animals in this study and the zooplankton in a previous study excreted approximately the same ratio of organic to inorganic P. We have no data to verify P enrichment of faeces, and in fact, except for sea urchins and holothurians, we observed very little faecal production during the course of the incubations.

The second explanation for the high C : P excretion ratios is that there is a large source of C relative to P. A suggestion by Miller and Mann (1973) is that animals which feed predominantly on carbohydrate (high C : N : P ratios such as those found in benthic plants) must either release soluble organic C or directly respire more C to retain N. The composition C : N ratios of these invertebrates were much lower than benthic plants (Table 3, and Atkinson and Smith, 1983), indicating that the animals were relatively rich in protein compared to that food source. To maintain the reported C : N and C : P composition ratios (Table 3) while feeding on benthic plants, or material derived from benthic plants, these animals must release proportionately more C than either N or P, or,

Table 3. Summary of organic and inorganic C, total N and total P composition of a sample of animals from each respiration and excretion incubation. C : N : P ratios are mean organic C composition divided by either mean total N or mean total P composition

Species	Organic C, mean (± SE) (mmoles gDW^{-1})	Inorganic C, mean (± SE) (mmoles gDW^{-1})	Total N, mean (± SE) (mmoles gDW^{-1})	Total P, mean (± SE) (μmoles gDW^{-1})	C:N:P	C:N
Mollusca						
Patelloida alticostata	28.9 (0.6)	1.7 (0.1)	8.3 (0.2)	189.0 (73.3)	153: 44:1	3.48
Patallanax laticostata	30.2 (0.2)	1.1 (0.03)	10.0 (0.1)	90.5 (1.2)	334:107:1	3.02
Thais orbita	29.2 (0.2)	3.5 (2.3)	7.8 (0.9)	100.9 (13.3)	289: 77:1	3.74
Charonia lampas rubicunda	27.3 (0.03)	2.2 (0.01)	8.8 (0.7)	80.9 (0.04)	337:108:1	3.10
Cypraea caputserpentis	25.1 (0.1)	3.1 (0.02)	7.2 (0.1)	112.4 (9.9)	223: 64:1	3.49
Turbo intercostralis	27.9 (0.3)	1.5 (0.1)	7.5 (0.1)	99.5 (15.4)	280: 75:1	3.72
Aplysia sp.	25.6 (0.4)	2.6 (0.1)	4.8 (0.2)	112.0 (6.4)	229: 42:1	5.33
Mytilus edulis	27.7	2.0	7.2	122.9	225: 58:1	3.85
Echinodermata						
Echinometra mathaei	6.2 (0.2)	7.7 (0.1)	1.0 (0.2)	53.0 (13.5)	116: 19:1	6.20
Tripneustes gratilla	6.3 (0.2)	8.0 (0.1)	1.1 (0.1)	35.1 (2.9)	180. 30:1	5.73
Stichopus mollis	21.2 (2.0)	3.7 (0.5)	4.2 (0.1)	74.1 (4.5)	287: 56:1	5.05
Patiriella gunnii	11.5 (0.2)	6.2 (0.1)	2.2 (0.2)	53.8 (1.9)	214: 41:1	5.23

they must have another significant source of N or P. The other possibility is that these animals generally feed on low C : P food (phytoplankton or bacteria, C : P = 50–150) and gain the extra source of C through absorption of dissolved organic material. This mechanism although possible is unlikely in that the animals would require 4 to 5 times more C by absorption than by direct feeding to achieve the observed C : P excretion ratio. This study was not designed to distinguish these alternative mechanisms, but it seems likely that the high C : P excretion ratios of these benthic invertebrates reflect a high carbohydrate diet.

The consequence of these high ratios of dissolved C : P excretion is that in benthic systems the flux of P from these animals into moving water may not be enough to affect either the plankton community or the benthic community. As an example we make the following calculation. The urchin *Echinometra mathaei* is a dominant grazing organism on Radar Reef at Rottnest Island, Western Australia (Black et al., 1984). From the typical C respiration and P excretion rates (Table 1) we can estimate the contribution of urchins to community respiration and P recycling. On Radar Reef the mean density of urchins is approximately 50 m^{-2} (1.76 kg DW m^{-2}); using a mean respiration rate of 1.3 µmoles C gDW^{-1} h^{-1} and a P excretion rate of 2 nmoles P gDW^{-1} h^{-1} (C : P = 650 : 1, Table 1) it is calculated that the urchins respire approximately 55 mmoles C m^{-2} d^{-1} and excrete 85 µmoles P m^{-2} d^{-1}. Assuming a typical reef flat respiration rate of 500 mmole C m^{-2} d^{-1} (Kinsey, 1979) the urchins represent a significant proportion of the community respiration (11 %). We estimate the total flux of P onto the reef from moving water (1 m deep × 0.3 mmole P m^{-3} [15 min]$^{-1}$ water residence time) to be on the order of 29 mmoles P m^{-2} d^{-1}; thus the P excretion from the urchins is only 0.3 % of the total P delivery. Even though the urchins are the major grazers on the reef and are significant proportion of community respiration they are negligible source of P compared to the flux of P in the water.

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