

## Respiration in marine zooplankton—the other side of the coin: CO<sub>2</sub> production

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### Abstract

We measured respiratory release rates of CO<sub>2</sub> from various taxonomic groups of zooplankton during three cruises in winter, spring, and summer in the North Atlantic Ocean. Zooplankton species collected comprised different species of thaliacea (salps), mollusc thecosomes, amphipod hyperiids, copepods, decapods, and euphausiids. Hourly, individual rates ranged from a minimum of 0.02 μl h<sup>-1</sup> for the smallest copepods (*Oncaea* sp. and *Acartia*) to a maximum of 90.6 μl CO<sub>2</sub> for the largest euphausiids (*Meganctiphanes norvegica*), corresponding to a range of weight-corrected rates of 0.1 μl CO<sub>2</sub> for the thecosome *Cymbulia peronii* and 5.6 μl CO<sub>2</sub> for the smallest copepods. Size was the major factor controlling the recorded rates. Allometric coefficients varied between 0.6 and 0.7 for weight and 1.5 and 2.2 for length, which is in agreement with the theoretical values and the values recorded for oxygen consumption. RQ values (CO<sub>2</sub> released/O<sub>2</sub> consumed) were computed for each group from simultaneous measurements of both respiratory processes. Model II regressions yielded mean RQs of 0.87 ± 0.40 for copepods, 0.94 ± 0.40 for thecosomes, and 1.35 ± 0.08 for large crustacean and salp species. We propose that the increase in RQ value from copepod to large crustacean species is related to the development of the muscular mass and activity rather than a simple shift in respiratory metabolic substrate. Realistic RQ values, taking species differences into account, should be used to derive population and community CO<sub>2</sub> release rates from simpler oxygen consumption measurements.

Respiration rate is a fundamental measure of biological activity. It is known to be related to most other physiological rates in cells, including growth, excretion (Banse 1982; Ikeda 1985), size (Ikeda 1970), and external trophic and physical descriptors, such as food supply and temperature (Ikeda 1974). From a general point of view, respiration is a two-phase process, with an intake of oxygen or external respiration and an output of carbon dioxide or internal respiration (Ikeda et al. 2000). From a metabolic standpoint, respiration occurs in three stages: (1) organic fuel molecules (glucose, fatty acids, and some amino acids) are oxidized to yield two carbon fragments in the form of acetyl coenzyme A (acetyl CoA); (2) these acetyl groups are fed into the citric acid cycle, which enzymatically oxidize them to CO<sub>2</sub> to produce reduced electron carriers; and (3) these electron carriers are oxidized to produce electrons. These electrons are transported along the respiratory chain to oxygen, which they reduce to form H<sub>2</sub>O (see Lehninger et al. 1993). In the study of respiration of marine organisms, respiratory rates have been almost always synonymous with oxygen consumption rates because of methodological limitations and the key interest in energy in the form of adenosine triphos-

phate (ATP)–producing processes (Mayzaud and Conover 1988).

Few attempts have been made to quantify the rates of CO<sub>2</sub> production in marine plankton (Raymont and Krishnaswamy 1968; Rakusa-Suszczewski et al. 1976; Kremer, 1977) because of the difficulty of accurately determining carbon dioxide in seawater. The RQ values measured (RQ = carbon dioxide produced: oxygen consumed) varied from low values (0.61 ± 0.06 for the ctenophore *Mnemiopsis leidyi* at 15°C, 0.5–0.8 for female *Neomysis integer*) to very low values (0.44 ± 0.06 for female *Rhincalanus gigas*, 0.2–0.4 for female *N. integer*), suggesting an underestimation of CO<sub>2</sub> production in most cases. As a result, most calculations requiring a value for the respiratory quotient are based on theoretical considerations (Omori and Ikeda 1984) rather than actual measurements. Hence, the rate of remineralization of primary production or, more generally, of the particulate organic matter by zooplankton consumers remains tentative despite the importance of the biological recycling processes leading to increased CO<sub>2</sub> in the water column. Recently, Mayzaud et al. (2002) showed that Antarctic copepod communities present in the first 200 m produced a mean value of 4 to 4.5 mmol CO<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup> in spring, representing 30% to 40% of the daily integrated primary production. Their calculation was based on a single theoretical RQ value of 0.95, which might not apply to all taxonomic groups when entire zooplankton communities are considered.

In this study, we used a more sensitive coulometric total carbon dioxide technique to measure the CO<sub>2</sub> release by various species of zooplankton from the Atlantic Ocean.

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Table 1. Dates and locations of sampling sites.

|        | Winter cruise |                    | Spring cruise |                    | Summer cruise |                    |
|--------|---------------|--------------------|---------------|--------------------|---------------|--------------------|
|        | Date          | Position           | Date          | Position           | Date          | Position           |
| Site 1 | 2–3 Mar 01    | 40°07'N<br>18°46'W | 19–20 Apr 01  | 39°45'N<br>19°47'W | 20–21 Sept 01 | 40°06'N<br>19°23'W |
| Site 2 | 6–7 Mar 01    | 41°01'N<br>18°39'W | 23–24 Apr 01  | 41°44'N<br>19°44'W | 24/25 Sept 01 | 42°18'N<br>19°52'W |
| Site 3 | 11–12 Mar 01  | 41°46'N<br>19°14'W | 27–28 Apr 01  | 41°57'N<br>17°28'W | 28–29 Sept 01 | 41°31'N<br>21°55'W |
| Site 4 | 15–16 Mar 01  | 43°12'N<br>17°20'W | 2–3 May 01    | 43°21'N<br>18°57'W | 03 Oct 01     | 42°22'N<br>17°51'W |

## Materials and methods

Zooplankton was collected during the POMME cruises (Programme Ocean Multidisciplinaire Meso Echelle) at three periods in the year (winter, spring, and late summer) with either a WP II net (200- $\mu\text{m}$  mesh size) or a BIONESS multinet (500- $\mu\text{m}$  mesh size). WP II hauls were made vertically from 200 m to the surface, whereas the BIONESS was operated obliquely over nine different strata between 250 m and the surface. All hauls were made at night. Small-sized zooplankton originated from the WP II, whereas large zooplankton was collected from the BIONESS samples. Dates of collection and position of the sampling sites is indicated in Table 1.

The zooplankton collected were immediately diluted in a large-volume plastic cooler filled with surface seawater and brought back in a cold room at seawater temperature. Individuals were sorted immediately under a dissecting microscope to species and stage and isolated in 1-liter beakers filled with filtered seawater (0.45  $\mu\text{m}$ ) for <1 h. Groups of individuals of a given species were then transferred to experimental chambers filled with filtered seawater saturated in oxygen. Volume of the chambers and animal densities varied with the size of the animals. Copepods were usually placed in flasks (50 or 100 ml) at densities ranging from 80 (adult *Euchirella*) to 400 (small *Oithona*) individuals per liter. Larger species, such as euphausiids or hyperiids, were placed in volumes ranging from 250 ml to 1 liter at densities ranging from 1 to 12 per liter. Pteropods were placed in 100-ml flasks at densities ranging from 20 to 50 individuals (ind)  $\text{L}^{-1}$ . Salps were placed in 500 ml at densities ranging from 2 to 20 ind  $\text{L}^{-1}$ . Animals were incubated in darkness at seawater temperature (13–14°C for winter–spring; 17–18°C for fall) for 10–15 h. Control bottles without zooplankton were incubated under the same conditions to monitor possible oxygen changes.

Oxygen concentration was monitored with a Strathkelvin oxygen meter equipped with a Clark-type electrode. Aliquots of incubation seawater were withdrawn with a 2-ml syringe for oxygen concentration. Then, 0.2 ml of mercuric chloride (saturated) was added to stop all reaction prior to  $\text{CO}_2$  measurements. Carbon dioxide concentrations were analyzed by coulometric titration (Johnson et al. 1987, DOE 1994). Analysis of seawater  $\text{TCO}_2$  (reference materials certified at  $2,006.21 \pm 0.66 \mu\text{mol kg}^{-1}$ ,  $n = 12$ ; A.G. Dickson) throughout the cruises (mean =  $2,005.9 \pm 1.11$ ,  $n = 105$ ) provided

quality assessment of the precision and accuracy of the  $\text{TCO}_2$  measurements.

Oxygen consumption and  $\text{CO}_2$  production were computed by difference between beginning and end of the incubations and corrected for possible changes in control bottles. Experimental animals were preserved in 5% neutralized formaldehyde for length measurements and confirmation of species identification. Dry weight and length per species were obtained from individuals sorted at the same time but immediately rinsed with ammonium formate and deep frozen (–80°C). Dry weight was measured after drying at 60°C until constant weight, and length was measured with an image analyzer mounted on a dissecting microscope. Length measurements vary with the group considered. For copepod, cephalosome length was used. For euphausiid and decapods, standard length I was considered (Mauchline 1980). Total length was measured for amphipods and salps, whereas shell length was used for thecosomes. When dry weights were not available for a given species, values were computed from relationships between size and length.

The allometric relationships between dry weight or length and metabolic rates were computed after log–log transformation and model I regression (Sokal and Rohlf 1981). Comparisons of regression lines were made by covariance analyses to test the equality of both slopes and intercepts ( $H_0$  tested is equality of slopes or intercept). The respiratory quotient was calculated as  $\text{RQ} = \Delta\text{CO}_2 / -\Delta\text{O}_2$ . The ratio was first calculated for each experience before computing the geometric means for each species. Because geometric means are back-transformed means of log-transformed variables (Sokal and Rohlf 1981), the same computation was followed with standard deviation to obtain a statistic of dispersion. RQs for the different taxonomic group were computed with model II ( $y = u + vx$ ) or reduced major axis regressions (Sokal and Rohlf 1981), in which the slope (RQ) is the ratio of two standard deviations ( $v_{y,x} = s_y/s_x$ ). When the intercept of the regression line was not significantly different from zero, the intercept term was deleted, but the regressions were not forced through the origin. Analyses were made with Statgraphics 5.1 software.

## Results

The different mean values of carbon dioxide production and RQs are summarized in Table 2. Although the RQ values include respiration rates, details about values of oxygen con-

Table 2. Experimental data on body weight, body size, temperature of incubation, carbon dioxide production, and RQs for different species of pelagic zooplankton. *n*, number of replicates. Values are means ± SD.

| Species  | <i>n</i> | Temp<br>(°C) | Length<br>(mm) | Dry wt<br>(mg) | CO <sub>2</sub> produced                |   | RQ*<br>CO <sub>2</sub> :O <sub>2</sub> |
|--|----------|--------------|----------------|----------------|---|---|--|
|  |          |              |                |                | (μl ind <sup>-1</sup> h <sup>-1</sup> ) | (μl [mg dry wt] <sup>-1</sup> h <sup>-1</sup> ) |  |
| Thaliacea, salps                                     |          |              |                |                |   |   |  |
| <i>Pegea</i> sp.                                     | 6        | 13.9         | 49.3±2.5       | —              | 40.3±10.5                               | —   | 1.07±0.12                              |
| <i>Thalia</i> sp.                                    | 3        | 13.9         | 17.0±0.4       | —              | 2.84±0.68                               | —   | 0.78±0.02                              |
| Mollusc thecosome                                    |          |              |                |                |   |   |  |
| <i>Euclio pyramidata</i>                             | 2        | 13.9         | 9.2±3.2        | 3.0±1.1        | 4.08±4.44                               | 1.36±1.04                                       | 0.80±0.09                              |
| <i>Limacina helicoides</i>                           | 2        | 13.9         | 3.5±0.4        | 1.7±0.1        | 0.55±0.12                               | 0.32±0.04                                       | 0.94±0.19                              |
| <i>Diacria trispinosa</i>                            | 3        | 13.9         | 9.2±0.2        | 15.2±0.8       | 4.79±0.99                               | 0.31±0.04                                       | 0.86±0.05                              |
| <i>Cavolinia inflexa</i>                             | 3        | 17.8         | 4.3±0.3        | 1.3±0.4        | 1.44±0.71                               | 1.11±0.14                                       | 1.31±0.05                              |
| <i>Cymbulia peronii</i>                              | 6        | 13.9         | 46.5±2.8       | 333±88         | 35.7±5.7                                | 0.11±0.03                                       | 0.97±0.15                              |
| Crustacean Hyperiid                                  |          |              |                |                |   |   |  |
| <i>Themisto</i> sp.                                  | 8        | 13.9         | 20.6±1.0       | 12.1±2.1       | 43.7±9.7                                | 3.7±0.8   | 1.58±0.34                              |
|  | 4        | 13.9         | 14.5±0.6       | 3.9±0.5        | 18.9±1.2                                | 4.9±0.5   | 0.97±0.04                              |
| <i>Phronima sedentaria</i> isolated (without barrel) | 2        | 13.9         | 34.9±3.9       | 39.7           | 83.7±4.3                                | 2.0   | 1.07±0.03                              |
| <i>Phronima sedentaria</i> (in barrel)               | 2        | 13.9         | 31.0±2.7       | —              | 86.8±5.2                                | —   | 1.10±0.04                              |
| Euphausiids  |          |              |                |                |   |   |  |
| <i>Meganyctiphanes norvegica</i>                     | 3        | 12.5         | 29.5±2.2       | 38.6±11.6      | 90.6±21.2                               | 2.3±0.8   | 1.29±0.10                              |
|  | 6        | 17.8         | 26.5±2.0       | 28.4±10.6      | 60.7±16.3                               | 2.1±0.4   | 1.62±0.23                              |
| Decapods   |          |              |                |                |   |   |  |
| Sergestidae  |          |              |                |                |   |   |  |
| <i>Sergestes</i> sp.                                 | 1        | 13.9         | 36.1           | 68.8           | 111.8                                   | 1.6   | 1.4                                    |
| Caridae  |          |              |                |                |   |   |  |
| <i>Systellaspis debilis</i>                          | 1        | 12.5         | 30.5           | 48.0           | 70.4                                    | 1.5   | 1.3                                    |
| Copepods   |          |              |                |                |   |   |  |
| <i>Euchirella rostrata</i>                           | 10       | 13.9         | 2.73±0.11      | 0.68±0.06      | 1.22±0.26                               | 1.77±0.29                                       | 0.85±0.17                              |
|  | 4        | 17.8         | 2.82±0.27      | 0.75±0.17      | 0.53±0.21                               | 0.70±0.13                                       | 0.80±0.16                              |
| <i>Calanus gracilis</i>                              | 11       | 17.8         | 2.47±0.17      | 0.54±0.08      | 0.56±0.21                               | 0.99±0.28                                       | 0.95±0.14                              |
| <i>Pleuromamma gracilis</i>                          | 4        | 17.8         | 2.15±0.20      | 0.40±0.09      | 0.46±0.13                               | 1.16±0.15                                       | 0.75±0.17                              |
| <i>Euchaeta</i> spp.                                 | 5        | 17.8         | 2.89±0.56      | 0.81±0.34      | 0.71±0.27                               | 0.90±0.16                                       | 0.81±0.04                              |
| <i>Candacia</i> spp.                                 | 4        | 17.8         | 1.76±0.09      | 0.25±0.03      | 0.45±0.12                               | 1.87±0.55                                       | 0.84±0.20                              |
| <i>Calanus</i> sp.                                   | 2        | 14.4         | 3.91±0.13      | 1.58±0.12      | 0.93±0.17                               | 0.60±0.15                                       | 0.61±0.14                              |
| <i>Oncaea</i> sp.                                    | 3        | 17.8         | 0.58±0.02      | 0.004±0.0001   | 0.023±0.012                             | 5.61±2.62                                       | 0.88±0.10                              |
| <i>Acartia clausi</i>                                | 2        | 17.8         | 0.70±0.01      | 0.005±0.0001   | 0.015±0.007                             | 3.10±1.55                                       | 0.66±0.23                              |

\* Geometric means.

sumption will be presented elsewhere. The zooplankters considered in this study belonged to zoological groups with different morphological, physiological, and ecological characteristics. The individuals used in the incubation corresponded to three different taxonomic classes (i.e., Thaliacea, Mollusca, and Crustacea). Thaliacea comprised two species of salps with different size characteristics. Mollusca belonged to the thecosome order, with five different species of different size, all feeding on microparticles. Crustacea comprised two species of hyperiid amphipod, eight species of copepod, two species of decapod, and one species of euphausiid, corresponding to different trophic types.

Because of the variety of species, size, and seasons, the rates of CO<sub>2</sub> production recorded were highly variable. On a per individual basis, the hourly rates ranged from 0.01 μl CO<sub>2</sub> ind<sup>-1</sup> for the smallest copepods (*Oncaea*, *Acartia*) to 80–90 μl CO<sub>2</sub> ind<sup>-1</sup> for the largest hyperiids and euphausiids (*Phronima sedentaria*, *Meganyctiphanes norvegica*). Metab-

olism is known to be strongly related to size or weight according to the relationship:  $R = aW^b$ , where  $R$  is the metabolic rate,  $W$  the size or weight,  $b$  is the exponential constant, and  $a$  is a constant of proportionality. The log plot of CO<sub>2</sub> released per individual versus size is presented in Fig. 1 for the three taxonomic groups considered. The comparison of the regression lines by covariance analysis showed that the copepod and large crustacean regressions are not significantly different (slopes  $F_{1,71} = 1.41$ ,  $p = 0.240$ ; intercept  $F_{1,72} = 1.43$ ,  $p = 0.235$ ), whereas the thecosome regressions were significantly different from the other two taxonomic categories (slope  $F_{1,62} = 17.27$ ,  $p = 0.0001$ ; intercept  $F_{1,63} = 5.88$ ,  $p = 0.01$ ). Weight-corrected hourly rates varied inversely from 0.1 μl CO<sub>2</sub> (mg dry wt)<sup>-1</sup> for the largest species of thecosome (*Cymbulia peronii*) to >5 μl CO<sub>2</sub> mg dry wt<sup>-1</sup> for the small *Oncaea* sp. Log plots of weight-corrected CO<sub>2</sub> release versus log of dry weight are presented in Fig. 2 for the same three groups. Regression lines for thecosomes and

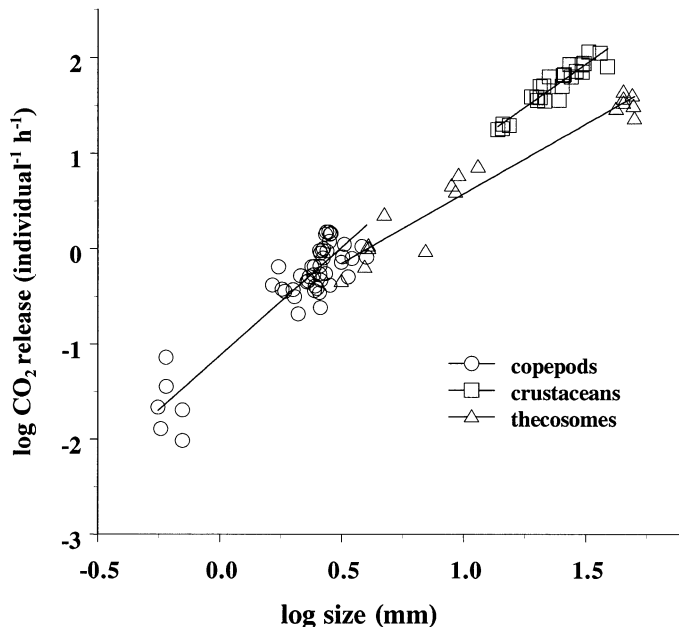


Fig. 1. Log-log relationship between the individual hourly CO<sub>2</sub> release rates and the mean size (length) of the zooplankton species.

copepods were not significantly different (slope  $F_{1,62} = 0.38$ ,  $p = 0.54$ ; intercept  $F_{1,63} = 0.17$ ,  $p = 0.67$ ) and thus can be pooled, whereas the regression for large crustaceans was significantly different from the other two with a different mean ( $F_{1,87} = 370$ ,  $p = 0.00001$ ) but a similar slope ( $F_{1,86} = 0.63$ ,  $p = 0.43$ ). The regression equations and correlation coefficients are presented in Table 3. Size or weight appeared to be the main factor explaining the changes in metabolic rates, with  $r^2$  ranging from 0.82 for copepods to 0.99 for thecosomes. The influence of temperature on these regressions is difficult to ascertain because not all taxonomic groups were sampled at all seasons. Indeed, large crustaceans and thecosomes were mostly sampled in winter/spring under a similar temperature regime, whereas copepods were the only group sampled at all three seasons. Hence, the influence of temperature was tested only for copepods and was significant (Student's  $t = 4.21$ ,  $p > 0.0001$ ). The resulting multiple regression including size and temperature enhanced the  $r^2$  to 0.892 (ANOVA:  $F_{2,48} = 189.7$ ,  $p > 0.0001$ ,  $s_{y,x} = 0.17$ ), yielding a more complete relationship:  $\log \text{CO}_2 = 1.089 + 0.624 \log W - 0.069 T$  (°C).

The RQ values showed a limited range of variability, with values between 0.6 (*Calanus* sp.) and 1.6 (*M. norvegica*,

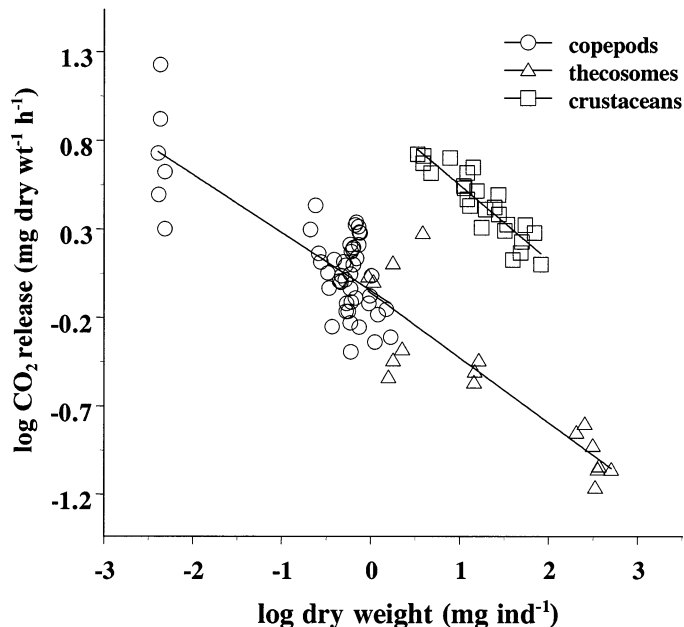


Fig. 2. Log-log relationship between the weight-corrected hourly CO<sub>2</sub> release rates and the mean dry weight of the zooplankton species.

*Themisto* sp.). When all data are combined, a multiple regression analysis indicated that RQ changes are related to size ( $F_{1,89} = 14.8$ ,  $p > 0.01$ ,  $r^2 = 0.236$ ) but not to dry weight ( $p = 0.63$ ) or temperature ( $p = 0.74$ ). The slope of the regression between CO<sub>2</sub> production and oxygen consumption represents the mean value of RQ for a given taxonomic group. To evaluate the possibility that differences in the physiological activity of the different taxa will influence the actual value of RQ, we considered separately four groups: copepods, large crustaceans, thaliacea, and thecosomes. Regressions for large crustaceans and salps were not significantly different (slopes:  $F_{1,31} = 0.10$ ,  $p = 0.75$ ). All regressions computed failed to show intercepts different from 0 ( $p > 0.05$ ) and followed the relationship.

$$\text{Respiration (CO}_2 \text{ released)} = b \times \text{Oxygen consumed}$$

Details on the statistics of the regressions are given in Table 4. As shown Fig. 3, regression for the larger crustaceans and salps showed a mean slope or RQ of  $1.36 \pm 0.11$  and  $1.16 \pm 0.07$ , respectively. The RQ values recorded for the other two groups decreased to  $0.94 \pm 0.04$  for thecosomes and

Table 3. Regression statistics of the log CO<sub>2</sub> respiration rates ( $\mu\text{l CO}_2 \text{ ind}^{-1} \text{ h}^{-1}$ ) on log size (length and dry weight,  $\log \text{CO}_2 = \log a + b \log X$ ) for all three cruises. MSE, mean square error.

| Zooplankton group | df | a     | b     | X      | $r^2$ | $S_{y,x}$ MSE | F     | p      |
|-------------------|----|-------|-------|--------|-------|---------------|-------|--------|
| Copepods          | 48 | -0.04 | 0.672 | Weight | 0.847 | 0.207         | 266.9 | 0.0001 |
|                   | 48 | -1.11 | 2.21  | Length | 0.822 | 0.218         | 222.6 | 0.0001 |
| Thecosomes        | 16 | -0.06 | 0.632 | Weight | 0.903 | 0.221         | 150.5 | 0.0001 |
|                   | 16 | -0.88 | 1.46  | Length | 0.939 | 0.173         | 245.5 | 0.0001 |
| Large crustaceans | 24 | 0.98  | 0.570 | Weight | 0.892 | 0.078         | 200.6 | 0.0001 |
|                   | 24 | -0.77 | 1.81  | Length | 0.877 | 0.083         | 172.4 | 0.0001 |

Table 4. Model II regression statistics of CO<sub>2</sub> respiration rates ( $\mu\text{l CO}_2 \text{ ind}^{-1} \text{ h}^{-1}$ ) on O<sub>2</sub> consumption rates ( $\mu\text{l O}_2 \text{ ind}^{-1} \text{ h}^{-1}$ ) for the definition of mean RQ values for different taxonomic groups using reduced major axis regression. NS, not significant.

| Zooplankton group | df | a  | b = RQ | $\pm$ SD | $r^2$ | F   | p      |
|-------------------|----|----|--------|----------|-------|-----|--------|
| Large crustaceans | 22 | NS | 1.36   | 0.11     | 0.849 | 125 | 0.0001 |
| Salps             | 8  | NS | 1.16   | 0.07     | 0.971 | 276 | 0.0001 |
| Thecosomes        | 15 | NS | 0.94   | 0.04     | 0.960 | 358 | 0.0001 |
| Copepods          | 48 | NS | 0.87   | 0.04     | 0.853 | 279 | 0.0001 |

0.87  $\pm$  0.04 for copepods (Table 4), with correlation coefficients exceeding 0.93 ( $r^2 > 0.85$ ).

Discussion

Respiratory CO<sub>2</sub> rate measurements in seawater have usually been considered laborious and complex, especially when small changes had to be monitored. The initial Warburg manometric technique was well suited for tissue prepara-

tions, but the need for measurements on larger marine animals have prompted an adaptation of the method that uses the manometric blood gas apparatus developed by Van Slike (1927), which yielded CO<sub>2</sub> respiration rates for various benthic marine organisms (Bosworth et al. 1963). Although the RQ values observed were well in the range of theoretical values, the method was complex, fairly insensitive, and required incubation over several days. More recently, measurements of carbon dioxide production was obtained from

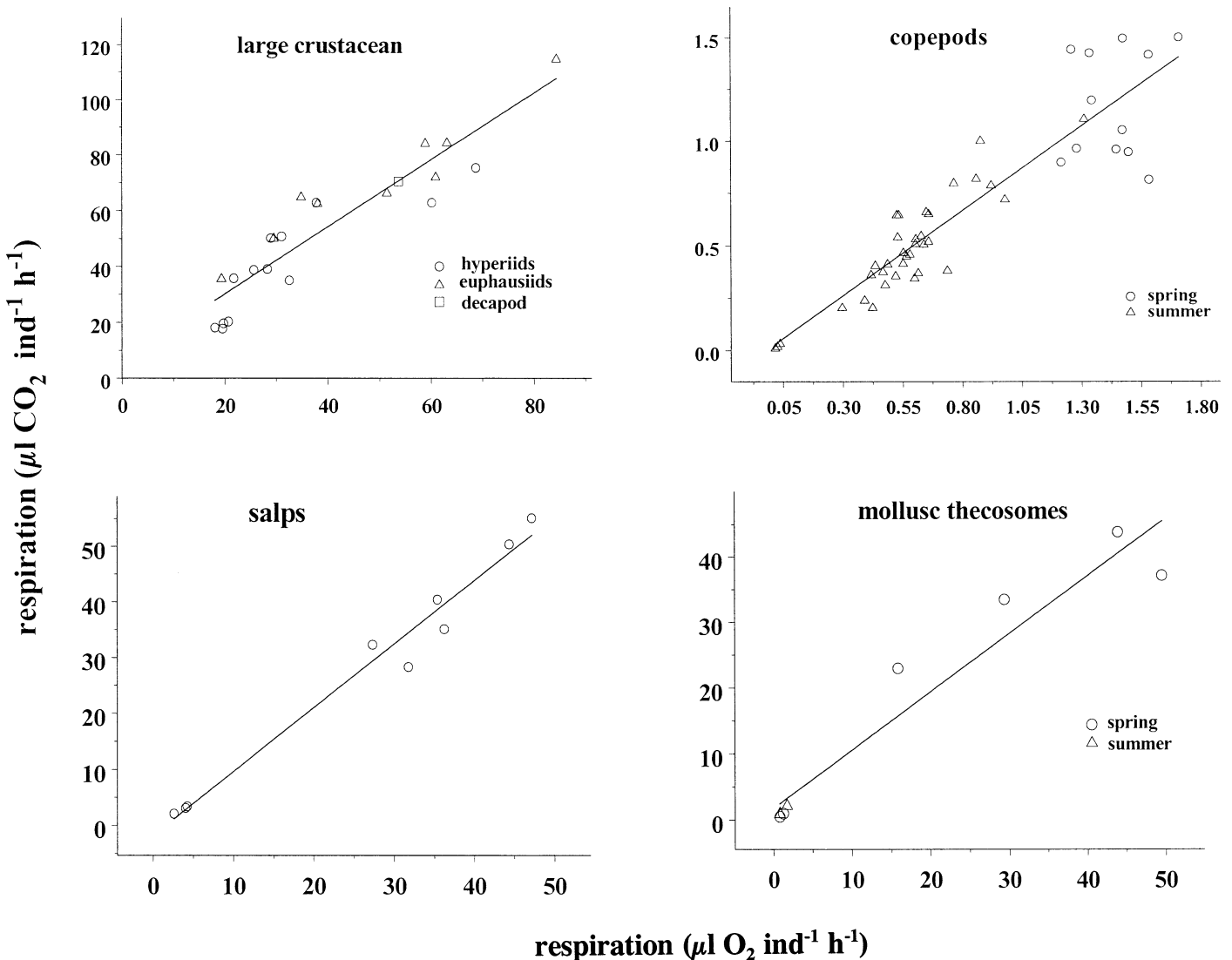


Fig. 3. Model II regression between the CO<sub>2</sub> release rates and the oxygen consumption rates.

the change in pH in the incubation chamber (Lyman 1961; Smith and Key 1975) and applied in experiments with bivalves (Mori 1968, 1975; Barber and Blake 1985), gastropods (Hatcher 1989), ascidians (Hatcher 1989, 1991), cephalopods (Boucher-Rodoni and Boucher 1993), and corals (Gattuso et al. 1993). The resulting RQs are quite variable, and unexplained variations outside theoretical limits have been noted, causing problems in interpretation. Total alkalinity and pH determinations remain difficult with zooplankton, mainly when oxygen consumption must be measured simultaneously with low experimental density and reasonable incubation times. The introduction of other techniques, such as coulometric determination of total CO<sub>2</sub> (Johnson et al. 1993), reduced these limitations and allowed measurements of CO<sub>2</sub> with an accuracy of 1 μmol kg<sup>-1</sup>, therefore allowing the measurement of respiration on low-density experimental populations of zooplankton.

Because of the paucity of data on CO<sub>2</sub> production by zooplankton and related RQ, comparisons with existing data are extremely limited. No information is available on the influence of environmental and biological factors such as temperature and size on CO<sub>2</sub> respiration rates. We could only assume that the rules recorded for oxygen consumption rates apply to the present case. Because of the seasonal changes in composition of the zooplankton communities, temperature could only be tested for the copepod group, which could be sampled at all three cruises. Over the relatively small range observed, it showed a small influence compared with the size constraints. Allometric relationships linking individual CO<sub>2</sub> production to either size or weight showed mean exponent values ranging from 0.6 to 0.7 for weight and from 1.5 to 2.2 for length. Such ranges of values fit well with the theoretical values of 0.66 and 2, respectively, for the weight exponent and length exponent of oxygen consumption rates (von Bertalanffy 1951) and with the data reported by Conover (1959) for marine copepods (length exponent: 1.6–2.7; weight exponent: 0.50–0.91), by Jawed (1973) for marine mysids (weight exponent: 0.62–0.70), by Sameoto (1972) for chaetognaths (weight exponent: 0.68–0.79 between 10°C and 15°C), and by Ikeda (1970, 1974) for temperate and subtropical species of zooplankton (weight exponent: 0.66–0.75).

Direct comparison of rates is limited to few cases. A CO<sub>2</sub> respiration rate of  $1.35 \pm 0.17 \mu\text{l CO}_2 \text{ ind}^{-1} \text{ h}^{-1}$  ( $0.21 \pm 0.02 \mu\text{l CO}_2 (\text{mg dry wt})^{-1} \text{ h}^{-1}$ ) could be computed for the Antarctic copepod *R. gigas* at an experimental temperature of  $-1.8^\circ\text{C}$  with the mean RQ of  $0.44 \pm 0.06$  reported by Rakusa-Suszczewski et al. (1976). For the mysid *N. integer* (female stage), Raymont and Krishnaswami (1968) reported a mean rate of  $5.2 \pm 1.3 \mu\text{l CO}_2 \text{ ind}^{-1} \text{ h}^{-1}$  and a mean RQ value of  $0.49 \pm 0.18$  under conditions of simultaneous determinations of O<sub>2</sub> and CO<sub>2</sub> respiration and an experimental temperature of 18°C. Although, *R. gigas* is larger (dry weights of adult females  $6.6 \pm 0.7 \text{ mg}$ ) than any of the copepod species sampled during this study (see Table 2), the CO<sub>2</sub> production rate seems low compared with the present data, even when considering the difference in temperature of incubation ( $\Delta t > 15^\circ\text{C}$ ). With individuals ranging between 10 and 15 mm in size and 3 mg mean dry weight (Roast et al. 2000), the rates recorded for *N. integer* are also below

our range of values for similar size and experimental temperature. Kremer (1977) reported for the ctenophore *M. leidyi*, a CO<sub>2</sub> production rate of  $1.8 \mu\text{mol (g dry wt)}^{-1} \text{ h}^{-1}$  (equivalent to  $1.7 \mu\text{l CO}_2 \text{ ind}^{-1} \text{ h}^{-1}$  for a mean weight of 42 mg ind<sup>-12</sup>) corresponding to an RQ of 0.61 for a temperature of 15°C. Direct comparison with our data is not possible because dry weight of gelatinous animals were not measured and could be misleading. In all studies, the surprising results were the low RQ values (0.4–0.6), which fell below the theoretical minimum of 0.71 (Richardson 1929).

The interpretation of RQ ratios raised several questions. Classically, changes in RQ are related to the catabolic substrate used in respiration. Catabolism of protein and lipid result in values of 0.8 and 0.7, respectively. If carbohydrates are oxidized, equal amounts of carbon dioxide and oxygen are involved, and a value of 1.0 is found (Richardson 1929). With a slightly different approach, Gnaiger (1983) proposed that the values resulting from protein catabolism vary with the end products. An RQ of 0.83 for urea-oriented animals and 0.95 for ammonia-oriented animals is proposed. RQ > 1 indicates conversion of carbohydrates to lipids (Mori 1968, 1975; Gabbott 1976). However, these theoretical values are mostly concerned with tissues catabolizing various metabolic substrates and simply might not apply to entire organisms. As indicated by Hatcher (1989), because the potential for differential storage of respiratory gases exists, there is no reason to assume that the RQ of the whole animal is the same as the RQ within the tissue. In addition, the degree of coupling between CO<sub>2</sub> production and O<sub>2</sub> consumption rates in intact animals will also be a function of the differential diffusion rates of CO<sub>2</sub> and O<sub>2</sub> between the animal and the water and the differential controls on CO<sub>2</sub> production and O<sub>2</sub> consumption determined by the physiological makeup of the species (Hatcher 1989). This probably explains the discrepancies in the interpretation of RQ experimental values, which are considered reliable indicators of catabolism for some organisms (Barber and Blake 1985; Gattuso and Jaubert 1990; Boucher-Rodoni and Boucher 1993) but not for others (Hatcher 1989, 1991).

The questions of differential storage and diffusion are unlikely to be crucial with zooplankton organisms since gas exchange is known to proceed through the body surface or through preferential channels at the body surface (Prosser 1973). However, the differential controls on CO<sub>2</sub> production and O<sub>2</sub> consumption is an open question, which relates to the steady state between the two processes. Hatcher (1989), using successively longer incubation times, showed that initial short-term changes were strongly influenced by animal conditions, with strong decoupling between the two respiratory processes within the first 2 h. Stabilization was observed after 4 h, and coupling remained effective for up to 12 h, suggesting that longer term incubations should be preferred to short-term ones. However, in a closed system with unfed individuals, substrate switching during catabolism is likely to occur over time, and conclusions about which class of substrate was being used under natural feeding conditions should be viewed with extreme caution. In this study, we used incubation times of 10–15 h to avoid the influence of initial conditions, and the lack of exceedingly high values (>2) is indicative of reasonable coupling between the two

aspects of respiration. The increasing values from copepods to large crustaceans suggest that the RQ recorded might be related to the activity of the animals as well as the catabolic substrate used. The mean RQs recorded for copepods and mollusc thecosomes (0.87 and 0.94, respectively) fell within the theoretical range of values and suggest a protein-based catabolism (ammonia-oriented species, according to Gnaiger 1983). The value computed for large crustaceans and salps exceeded 1 (1.36 and 1.16) but could hardly reflect conversion of carbohydrates to lipids because additional measurements on atomic O:N ratios (Mayzaud et al. unpubl. data) confirmed the protein-based catabolism of these species. More likely, these values are indicative of the growing importance of muscle tissues associated with a higher level of swimming activity characteristic of most large crustaceans. Hence, muscle catabolism probably induced a different coupling or balance between the various metabolic pathways controlling CO<sub>2</sub> production and O<sub>2</sub> consumption. Indeed, like most zooplankters, large crustaceans cannot rely on a large supply of glycogen (Raymont et al. 1968) and must fuel the catabolic processes either from protein (free amino acid pool) or lipid oxidative pathways. As shown by Saether and Mohr (1987), euphausiid abdominal mass showed a free amino acid pool dominated by proline. Rapid mobilization of proline might derive from the Krebs cycle intermediates (i.e., the conversion from  $\alpha$ -ketoglutarate to glutamate and proline; Lehninger et al. 1993), which would result in an increased production of CO<sub>2</sub> without corresponding oxygen demand. Alternatively, working muscles are known to present transitory anaerobic phases of catabolism. If so, increasing CO<sub>2</sub> production could originate from partial anaerobic decarboxylation during activity (Hochachka 1973). Whatever process is actually at work, it is worth noticing that all species that showed RQ > 1 present an important muscular development: abdominal tail muscles in euphausiids, hyperiids, and decapods and muscle bands in salps. None of the other groups analyzed at the same time showed such departure from theoretical limits. If the present observations are confirmed, we could anticipate that other groups of planktonic organisms with high swimming activity will fall in the same category: chaetognaths and amphipods other than hyperiids. However, more information on the importance of changing physiological activity is needed to evaluate the influence of feeding and reproduction on the RQ values. Data from benthic organisms suggested that gonad development is an important factor resulting in high RQ values (Barber and Blake 1985), probably because of enhanced lipid synthesis.

In the context of a renewed interest in the recycling processes of the particulate organic carbon at sea, the importance of zooplankton communities remains largely unknown. Routine measurements of CO<sub>2</sub> production rates at sea are feasible but remain more complex than the simple polarographic determination of oxygen consumption rates. Hence, proper conversion factors (RQ) from oxygen consumption to CO<sub>2</sub> production rates are needed, and a global estimate, which would pool data from many different taxa, is potentially misleading. Indeed, grouping all data would yield an RQ of 1.31, which would result in an overestimation for many of the dominant zooplankton taxa. The attempt made

by Mayzaud et al. (2002) to estimate the community rate of carbon recycling for an Antarctic copepod community used a single RQ of 0.95, derived from Gnaiger (1983), and assumed that it applied to all organisms of the community. If the theoretical values applied to the copepod population, our results suggest that they might not be the same for communities comprising significant proportions of large crustaceans or thaliaceans. Further results are obviously needed to generalize such conclusions, but this work provides a more realistic base for such calculations.

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