

RESPIRATION

T.T. Packard¹, C. Joiris², P. Lasserre³, H.J. Minas⁴
M. Pamatmat⁵, A.R. Skjoldal⁶, R.E. Ulanowicz⁷,
J.H. Vosjan⁸, R.M. Warwick⁹, and P.J. Le B. Williams¹⁰

1. Bigelow Laboratory for Ocean Sciences, West Boothbay Harbour, Maine 04575, U.S.A.
2. University of Brussels, Fac. Wetenschappen, Lab. voor Ekologie en Systematik, Pleinlaan 2, 1050 Brussel Belgium
3. Station Biologique d'Arcachon, Universite de Bordeaux 2 rue du Professeur-Jolyet, 3312 Arcachon, France
4. Laboratoire d'Océanographie, Faculte des Sciences de Luminy, Case 902, 13288 Marseille Cedex 2, France
5. Tiburon Center for Environmental Studies, San Francisco State University, PO Box 855 Tiburon Calif 94920, U.S.A.
6. Institute of Marine Research, PO Box 1870, N5011 Nordnes, Bergen, Norway
7. Chesapeake Biological Laboratory, University of Maryland, Box 36, Solomons, Maryland 20688, U.S.A.
8. Netherlands Institute for Sea Research, PO Box 59 Texel, The Netherlands
9. Institute for Marine Environmental Research, Prospect Place, The Hoe, Plymouth, Devon PL1 2PB, U.K.
10. Department of Oceanography, The University, Southampton Hampshire, SO9 5NH, U.K.

The respiration working group conducted nine discussions. Each was led by a working group member after presenting his recent ideas and results. The discussions focused on topics that facilitated achieving the following objectives:

1. to develop a fundamental definition of respiration that is accurate and descriptive at all levels of biological organization.
2. to review the limitations and strengths of current methods of measuring respiration.
3. to identify areas for the potential application of new technology.
4. to identify "user interest" in respiration measurements (i.e., the modeling community) and how dimensions, time-scale, and space-scale effects the "usefulness" of a respiration measurement.
5. to determine the feasibility of developing a unifying model of respiration that can be applied to different organisms, communities, and ecosystems.
6. to assess the compatibility of the results of respiration studies with the results of other process studies.
7. to summarize the state of knowledge of respiration, to identify the limitations of that knowledge, and to recommend research for the next decade.

Progress was made on items one, two, four and five, but little progress was made on the remainder. In the following report is: (1) a summary of each member's informal contribution; and (2) the chairman's impressions of the progress made in achieving the above objectives.

SUMMARY OF INFORMAL CONTRIBUTIONS

In the first discussion, Ulanowicz explained how modelers view respiration. He presented a simple conceptual model in which (1) respiration represents the difference between the total input of a component in an ecosystem and the sum of the export out of the ecosystem and the throughput to the next compartment (within the ecosystem) and (2) respiration is guaranteed to be positive by the second law of thermodynamics (Ulanowicz and Kemp, 1979). Throughout his presentation he stressed the quest of modelers for fundamental relationships that would mathematically describe biological processes and would also be consistent with thermodynamics.

Later, Lasserre explained how both microcalorimetry (Wagensberg et al., 1978) and the cartesian diver (Zeuthen, 1943; Price and

Warwick, 1980) could be used to determine respiration rates in small planktonic and benthonic organisms. Both methods appear to offer absolute direct measurements of respiration at low levels but they are beset by the effect of starvation and isolation and by the inconvenience of the lengthy incubation time.

Skjoldal suggested that in certain situations the indirect biochemical methods for respiration determinations may be preferred. He presented an analysis of the errors associated with the ETS method and showed that in assessing the respiration rate of mixed zooplankton populations the ETS method is accurate to $\pm 30\%$ of the true rate. Furthermore, he showed by statistical analysis that the error is reduced when species diversity is high and suggested that calibration in the field would also reduce the error.

Joiris presented the results of a Belgian study of the ecology of the southern bight of the North Sea (Joiris *et al.*, 1982). The results clearly showed the importance of the microbial heterotrophs in the water column in utilizing the primary productivity. These organisms appear much more important than the zooplankton in respiration and remineralization. Joiris also showed that the local primary productivity could not sustain the local respiratory demands of the planktonic community and argued that either the ^{14}C -method for productivity underestimated true productivity or advection served to supply organic matter to the region of study.

Williams presented more evidence that bacteria and other microheterotrophs contribute greatly to total community respiration of the water column. He argued that in the water column, bacterial biomass, growth rates and respiration are much higher than previously thought. In agreement with Joiris, he suggested microheterotrophs may be more important in cycling organic matter and inorganic nutrient salts than the zooplankton. He also briefly described his new microprocessor-controlled Winkler titration system for respiration and productivity measurements (Williams and Jenkinson, 1982) and presented data showing that microbial growth in and on the incubation chamber does not interfere with the measurement.

Minas explained how one can use hydrographic and nutrient data in mixing models to calculate cumulative respiration rates in the oceanic water column below the euphotic zone. Using Mediterranean data, T-S diagrams, O_2 -S diagrams and nutrient-salinity diagrams, he demonstrated the effect of deep-ocean respiration on the distribution of oxygen, nitrate and phosphate.

Pamatmat discussed the literature on sediment respiration in relation to new data and concluded that present fractionation techniques of community respiration, involving the separation of infauna from the sediment, yield questionable results. Heat-flow measurements indicate the coupled metabolism of microbes and meiofauna,

making it extremely difficult to separate them without disturbing the metabolic activity of both. He suggested that the fractionation approach might be improved if we understood the physiological ecology of the component species in the community and paid more attention to their natural life habits. He argued that calorimetry of intact sediment measures benthic energy flow, which includes undisturbed community respiration. He proposed that the accuracy of the sum of fractionated benthic respiration can be estimated by such measurement (Pamatmat, 1982a and b). Oxygen uptake ignores anaerobic metabolism, which varies in relation to total metabolism in different benthic communities.

Vosjan reminded the group of the diversity of anaerobic types of respiration and their contribution to the carbon flux through benthic communities. He pointed out that most investigators of benthic catabolism have focused on sulfate reduction and that we should learn more about other types of respiration such as, fermentation, denitrification, etc. He pointed out that new microelectrodes offer great promise in facilitating future studies and argued that the indirect biochemical methods (ATP, ETS, etc) are still very useful in survey work where temporal and spatial coverage is required.

Warwick made six points in his discussion of respiration in the microbenthos. (1) In contrast to the plankton, the benthos have a size-frequency particle distribution that is not uniform. There are three peaks corresponding to the bacteria, microfauna, and macrofauna with two minima at 8 and 500 μ between the three peaks. (2) The relative importance of the three peaks has never been determined at one site. The relative importance of microfauna to total catabolism is highly variable and cannot be explained. (3) There are synergistic interactions between the three benthic components such that macrofaunal and meiofaunal presence stimulates respiration and remineralization in the bacterial component. (4) Recent evidence that the size dependence of macro- and meio- fauna respiration cannot be described by the same allometric equation, suggests that modelers will predict erroneous respiration rates if they persist in using a common allometric equation for all sizes of organisms. (5) Anaerobiosis in the meiofauna may explain much of the imbalance that often occurs in benthic respiration budgets. (6) Evidence that Q_{10} for meiofauna respiration varies from 2 to 4 suggest that modelers using a constant Q_{10} throughout the size spectrum will generate erroneous respiration rates.

PROGRESS

After the above series of presentations and discussions the group was able to conclude with the following statements and recommendations.

On the Definition of Respiration

Respiration is an energy yielding process in living systems that degrades organic matter beyond the point of immediate biological utility. The energy released during the degradation is used by the living system to achieve the goals of its survival strategy. The type of organic matter degraded, the degree to which it is oxidized, and the amount of useable energy extracted from the process varies with both the biological system and its environment.

Fermentation is a special case of respiration in which the terminal electron acceptor is an organic compound. Denitrification and sulfate reduction are special cases of respiration in which nitrate, nitrite, nitrous oxide and sulfate serve as electron acceptors in the process. Carbon dioxide production occurs during most but not all types of respiration. Heat production occurs in all cells as organic matter is degraded and as mechanical work is performed. Heat production can be enhanced when respiration is uncoupled from oxidative phosphorylation (ATP production) and it can be reduced when respiration is coupled to extensive growth and biosynthesis. However, under steady state situations, heat production accurately reflects the energy released during respiration.

On Reporting Respiration Data

Measurements of respiration are often converted from their primary units to more conventional ones such as $\mu\text{l O}_2 \text{ h}^{-1}$ per mg dry weight. This conversion requires a conversion factor which contributes an error. The working group concluded that the primary measurement as well as the conversion factors should always be reported. If joule sec^{-1} were measured in a calorimeter then these units should be reported. If millielectron equivalents were measured with an ETS assay then these units should be reported. Only after this primary measurement is reported should conversion factors be employed to report the respiration in units of oxygen, carbon or calories. Furthermore, to enable measurements to be used by as many scientists as possible, reports of respiration in marine organisms, populations or ecosystems should not only give the biomass, volume or area-specific rate (i.e. $\mu\text{l O}_2 \text{ h}^{-1} \text{ m}^{-3}$); but also the volume and areal distribution of the biomass and the depth over which the rates have been integrated. Also, since respiration rates may have a diel wave function they should only be reported for the time unit over which the primary measurement was made.

On the Calibration Problem

As with any other analytical measurement, respiration measurements are only as good as their calibration. Unlike chemical or biochemical measurements, physiological measurements require two levels

of calibration. The first and most obvious is the degree of transducer response to changes in reactants or products of respiration (i.e., CO₂, O₂, heat, etc.). The second level of calibration is more subtle; it requires definition of the relationship between the in situ respiration rate and the apparent one as measured. This requirement arises because of the physiological changes that often occur when an organism is removed from its natural environment and is maintained in a respiration chamber. Because of these changes the measured respiration may not be representative of the in situ respiration. Since there is no way to measure the in situ respiration without some disturbance to the organism a true calibration between the measured and in situ rates cannot be made. Nevertheless, investigators can attempt evaluation of the in situ rate for a few measurements by running time course experiments. The working group recommends that such experiments be conducted in future experimental programs.

On Recommending Methods

In making recommendations of methods to be used in respiration research, so much depends on the objectives of the researcher. On one hand, geochemists may be interested in the respiration rate in the deep-sea and its effect on the chemistry of seawater over hundreds of years. On the other hand, a benthic ecologist may be interested in the diel respiratory cycle of a certain species of protozoa. In between these two extremes are the plankton ecologists who may be interested in the respiration of entire plankton communities in a water column, or the benthic ecologists who may be interested in community metabolism of the benthos. Needless to say, there is no one method that can satisfy such a spectrum of scientific users. For ecological and physiological studies with individual microorganisms the most promising methods appear to be the cartesian diver techniques of Seuthen (1943) as recently employed by Klekowski (1977); Klekowski et al. (1980), and the microcalorimetry techniques (Wagensberg et al., 1978; Castell et al., 1981) as described by Lasserre (this volume).

For studies of community respiration in the benthos, direct calorimetry as recently described by Pamatamat (1982 a and b; in press) appears to be the best method. It reflects all types of respiratory metabolism, whether it be aerobiosis, denitrification, sulfate reduction or fermentation. The major problem is the long incubation time (6-8 hr) each measurement requires. Thus only 3-4 samples a day can be run per calorimeter. This precludes the method from being used in surveys in which temporal and spatial distribution must be resolved. Also, calorimetry does not reflect the heat production of extracellular chemical reactions that, at times, may be significant and may lead to an over-estimation of respiration. For water column respiration, the best method has a similar limitation. The direct determination of oxygen changes by the new microprocessor-controlled Winkler titration of Williams and Jenkinson (1982) is currently the

best way to measure water column respiration in oceanic surface waters. However, it has limitations similar to those of the ^{14}C -method for productivity that limit its use for temporal and spatial distribution work. In both the benthos and in the plankton, the use of a metabolic index, such as ETS activity (Christensen and Packard, 1977; Olańczuk-Neyman and Vosjan, 1977; Skjoldal and Lännergren, 1978; Packard, 1979), in combination with calorimetry and/or Winkler measurements can make such temporal and spatial respiration surveys feasible.

For geochemical work one can use the helium-dating method (Jenkins, 1980), the advection-diffusion model (Craig, 1971) and the ETS method (Packard *et al.*, 1977 and in press). The He dating method should only be used above 1000 m and the Craig method should only be used below 1000 m. The ETS method can be used throughout the water column. In all three methods, assumptions and constants are used that detract from their accuracy.

On Formulating a "Unified Field Theory" for Respiration

The development of a unifying mathematical description of respiration is an objective rarely attempted, but it should be encouraged because such a description would represent a significant thermodynamic hypothesis and would greatly improve our ability to use respiration in ecosystems modeling. Achieving such an objective would represent a "quantum jump" in our understanding of the respiratory process. From the diversity of the above discussions it is easy to understand why so little progress has been made towards this objective. The allometric equation (Bertalanffy, 1964) was offered as a unifying model many years ago and in spite of its many limitations is still accepted and widely used by theoreticians and modelers. One line of research tries to explain the allometric equation on the basis of energy transfers across membrane surfaces (Schmidt-Nielsen, 1970). Another line of research ignores the allometric equation and its dependence on size and instead focuses on the chemical basis of the physiological process (Atkinson, 1968 and 1977; Packard, 1971; Jacobus *et al.*, 1982). Its objective is to develop equations based on enzyme kinetics that will describe respiration. To date neither approach has succeeded in developing the equivalent of the unified field theory or even the equivalent of the perfect gas law for respiration.

Acknowledgements

This report was supported by NATO and by ONR Contract N000 14-76-C-0271 to T.T. Packard. It is contribution number 82021 from the Bigelow Laboratory for Ocean Sciences.

REFERENCES

- Atkinson, D.E., 1968, Regulation of enzyme function, *Ann. Rev. Micro Biol.*, 23: 47.
- Atkinson, D.E. 1977, "Cellular energy metabolism and its regulation" Academic Press, New York.
- Bertalanffy, L.V. 1964, Basic concepts in quantitative biology of metabolism, *Helgol. Wiss. Meeresunters.*, 9: 5
- Castell, C., Wagensberg, J., Tejero, A., and Vallespinós, F., 1981, Identificación de las fases metabólicas en termogramas de cultivos bacterianos, *Inv. Pesq.*, 45:291
- Christensen, J.P., and Packard, T.T., 1977, Sediment metabolism from the northwest African upwelling system, *Deep-Sea Res.*, 24:331.
- Craig, H., 1971, The deep metabolism: oxygen consumption in abyssal ocean water, *J. Geophys. Res.*, 76:5078
- Jacobus, W.E., Moreadith, R.W., and Vandegaer, K.M., 1982, Mitochondrial respiratory control, *J. Biol. Chem.*, 257:2397
- Jenkins, W.J., 1980, Tritium and ^3He in the Sargasso Sea, *J. Mar. Res.*, 38:533.
- Joiris, C., Billen, G., Lancelot, C., Daro, K.H. Mommaerts, J.P., Hecq, J.H., Bertels, A., Bossicart, M., and Nijs, J., 1982, A budget of carbon cycling in the Belgian coastal zone: Relative roles of zooplankton, bacterioplankton and benthos in the utilization of primary production, *Neth. J. Sea Res.*, 16:260.
- Klekowski, R.Z., 1977, Microrespirometer for shipboard measurements of metabolic rate of microzooplankton, *Pol. Arch. Hydrobiol.*, 24-Suppl.:455.
- Klekowski, T.Z., Kukina, I.V., Tumanseva, N.I. 1977, Respiration in the microplankton of the equatorial upwellings in the eastern Pacific Ocean, *Pol. Arch. Hydrobiol.*, 24-Suppl.:467
- Laybourn-Parry, J., Baldock, B., and Kingmill-Robinson, C., 1980, Respiratory studies on two small freshwater amoebae, *Microb. Ecol.*, 6:20-9216.
- Olánczuk-Neyman, K.M., and Vosjan, J.H., 1977, Measuring respiratory electron-transport system activity in marine sediment, *Neth. J. Sea Res.*, 11:1
- Packard, T.T., 1971, The measurement of respiratory electron transport activity in marine phytoplankton, *J. Mar. Res.*, 29:235
- Packard, T.T., 1979, Respiration and respiratory electron transport activity in plankton from the Northwest African upwelling area, *J. Mar. Res.*, 37:711.
- Packard, T.T., Minas, H.J., Owens, T., and Devol, A., 1977, Deep-sea metabolism in the eastern tropical north Pacific Ocean, in: "Oceanic Sound Scattering Predictions" N.R. Andersen and B.J. Zahuranec, eds., Plenum, New York.
- Packard, T.T., Garfield, P.C., and Codispoti, L.A., In press, Oxygen consumption and denitrification below the Peruvian upwelling, in: "Coastal Upwelling: It's Sediment Record," E. Suess, ed., Plenum, New York.

- Pamatmat, M.M., 1982a, Heat production by sediment: Ecological significance, Science, 215:395
- Pamatmat, M.M., 1982b, Direct calorimetry in benthos and geochemical research, in: "The Dynamic Environment of the Ocean Floor," K.S. Fanning and F. Manheim, eds., Lexington Books, Toronto.
- Pamatmat, M.M., In press, Simultaneous direct and indirect calorimetry, in: "Handbook on polarographic oxygen sensors: aquatic and physiological applications," E. Graiger, H. Forstner, eds., Springer, Berlin.
- Price, R., and Warwick, R.M., 1980, The effects of temperature on the respiration rate of meiofauna, Oecologia (Berl.), 44:145
- Schmidt-Nielsen, K., 1970, Energy metabolism, body size and problems of scaling, Fed. Proc., 29-1524.
- Skjoldal, H.R., and L nnergren, C., 1978, The spring phytoplankton bloom in Lindaspollene, a land-locked Norwegian fjord. II. Biomass and activity of net and nanoplankton, Mar. Biol., 47:313.
- Ulanowicz, R.E., and Kemp, W.M., 1979, Towards canonical trophic aggregations, Amer. Naturalist, 114:871.
- Wagensberg, J., Castell, C., Torra, V., Rodellar, J., and Vallespin s 1978, Estudio microcalorim trico del metabolismo de bacterias marinas: detecci n de procesos r tmicos, Inv. Presq., 42:179.
- Williams, P.J. LeB., and Jenkinson, J.W., 1982, A transportable micro-processor-controlled precise Winkler titration suitable for field station and shipboard use, Limnol. Oceanogr., 27:576.
- Zeuthen, E., 1943, A cartesian diver micro-respirometer with a gas volume of 0.1 μ l, Compt.-rend. Lab. Carlsberg S r. Chim. 24:479.