

NOAA Technical Memorandum GLERL-120

RESPIRATION, NITROGEN EXCRETION, AND O:N RATIOS OF THE GREAT LAKES AMPHIPOD *DIPOREIA* SP.

Michael A. Quigley, Peter F. Landrum, Wayne S. Gardner,
Christina R. Stubblefield, and Wendy M. Gordon

NOAA, Great Lakes Environmental Research Laboratory, Ann Arbor, MI 48105

Great Lakes Environmental Research Laboratory
Ann Arbor, Michigan
March 2002



UNITED STATES
DEPARTMENT OF COMMERCE

Donald Evans
Secretary

NATIONAL OCEANIC AND
ATMOSPHERIC ADMINISTRATION

Conrad C. Lautenbacher, Jr.
Administrator

NOTICE

Mention of a commercial company or product does not constitute an endorsement by the NOAA Environmental Research Laboratories. Use of information from this publication concerning proprietary products or the tests of such products for publicity or advertising purposes is not authorized. This is GLERL Contribution No. 704.

This publication is available as a PDF file and can be downloaded from GLERL's web site: www.glerl.noaa.gov. Hard copies can be requested from: GLERL Information Services, 2205 Commonwealth Blvd., Ann Arbor, MI 48105. pubs.glerl@noaa.gov.

Table of Contents

ABSTRACT	4
INTRODUCTION	4
MATERIALS AND METHODS	5
Presence/Absence of Sediment Substrate (Exp. No. 5)	6
Animal Acclimation in Test Vessels (Exp. No. 6)	6
Effects of Decreased Oxygen Availability on Respiration	6
RESULTS	7
O:N Experiments (Nos. 1-3) O ₂ consumption (Exp. No. 5)	7
Presence/Absence of Sediment Substrate (Exp. No. 5)	7
Animal Acclimation in Test Vessels (Exp. No. 6)	7
Effects of Decreased Oxygen Availability on Respiration	7
DISCUSSION	9
Oxygen Consumption	9
Ammonia Excretion	11
O:N Ratios	11
Acknowledgements	13
REFERENCES	13

Figures

Figure 1. Effects of acclimation on <i>Diporeia</i> sp. oxygen consumption (mg O ₂ mg ⁻¹ DW day ⁻¹)	8
Figure 2. Eadie-Hofstee plot of regression line calculated for <i>Diporeia</i> sp. oxygen consumption	8
Figure 3. <i>Diporeia</i> sp. O ₂ clearance (mL H ₂ O cleared of oxygen g ⁻¹ DW h ⁻¹) versus ambient oxygen concentration (mg O ₂ L ⁻¹)	9
Figure 4. <i>Diporeia</i> sp. oxygen consumption (ng-atom O mg ⁻¹ DW h ⁻¹) versus N excretion (ng-atom N mg ⁻¹ DW h ⁻¹)	12

Tables

Table 1. Mean <i>Diporeia</i> sp. oxygen consumption, ammonia-N excretion [\pm (SE)] measured in six 1986-1989 experiments	7
---	---

Respiration, Nitrogen Excretion, and O:N Ratios of the Great Lakes Amphipod *Diporeia* sp.

Michael A. Quigley
Peter F. Landrum
Wayne S. Gardner
Christina R. Stubblefield
Wendy M. Gordon

ABSTRACT. *Diporeia* sp. (formerly *Pontoporeia hoyi*) Amphipoda, Crustacea obtained from a 24-29 m deep site in southeastern Lake Michigan were held in stoppered 60 mL BOD bottles in darkness and at 4°C during 24-hour intervals. Change in dissolved oxygen concentration of filtered lakewater within test vessels indicated that mean *Diporeia* sp. oxygen consumption ranged from 13.9 to 33.9 mg O₂ g⁻¹ DW d⁻¹. Test vessel acclimation studies revealed a minor increase in oxygen uptake early in the incubation interval that was not reflected in overall oxygen consumption measured over the entire 24-hour period. Mean oxygen consumption of amphipods provided with a coarse, ignited sand substrate was not significantly different (P<0.05) from O₂ consumption of animals in test vessels where no substrate was provided. In a series of 10 other experiments, where initial ambient oxygen concentration was varied (from 95 to 23% saturation), Michaelis-Menten kinetics analysis indicated that *Diporeia* sp. was able to maintain a relatively uniform level of oxygen consumption over the upper portion of oxygen concentrations tested. V_{max} (predicted O₂ consumption at 100% saturation) was 25.5 mg O₂ g⁻¹ DW d⁻¹ and fell within the 13.9-33.9 mg O₂ g⁻¹ DW d⁻¹ range noted in 24-hour oxygen consumption experiments at near-saturation O₂ levels. K_m (the predicted O₂ concentration where oxygen consumption had declined to 50% of the V_{max} value) was 2.2 mg L⁻¹. *Diporeia* sp. nitrogen (ammonia) excretion, simultaneously measured during three of the oxygen consumption experiments, ranged from 0.45 to 0.77 ng-atom N mg⁻¹ DW h⁻¹. Calculation of O:N ratios (atoms of oxygen consumed per atom of N excreted) implied that substrates catabolized by *Diporeia* sp. consisted primarily of lipids since most (62%) O:N values were greater than 60. These results complement previously observed high lipid content of *Diporeia* sp. and further suggest that the economy of lipids is a prominent aspect of the amphipod's life history.

INTRODUCTION

The amphipod *Diporeia* sp. is a prominent macroinvertebrate throughout benthic communities of the Great Lakes. This organism was previously classified as *Pontoporeia hoyi*, a species that was believed to have been evolved from populations of the (sub) arctic marine species *Pontoporeia femorata* that were isolated in North American lakes following the retreat of Pleistocene glaciers. However, recent morphological and electrophoretic studies (Bousfield 1989, Vainola and Varvio 1989) have suggested that *Diporeia* sp. is more distantly related to *P. femorata* and, accordingly, *Pontoporeia hoyi* has now been assigned to a new genus--*Diporeia* (Bousfield 1989). Moreover, the identification of distinctly different mature male forms of *Diporeia* in North American lakes (Seegerstrale 1971) implies that numerous

species may exist, and at least four may occur in Lake Michigan alone (E.L. Bousfield, National Museums of Canada, P.O. Box 3443, Station D, Ottawa, Ontario, Canada K1P 6P4). In the absence of further taxonomic clarification of North American *Diporeia* species, we refer, herein, to our Lake Michigan test organisms as *Diporeia* sp.

In Lake Michigan, *Diporeia* sp. constitutes up to 65% of the resident benthic macroinvertebrate biomass (Nalepa 1989). Its high abundance in Lake Michigan [a mean abundance of ca. 7000 individuals m⁻² (Nalepa et al. 1985)] and its high lipid (and energy) content [up to 50% lipid (dry weight basis) or ca. 5000 cal gm⁻¹, (Green 1971, Gardner et al. 1985)] suggest that further documentation of *Diporeia* sp. energetics would provide a clearer understanding of the amphipod's role in coupling of pelagic and benthic production. *Diporeia* sp. provides an important food base for a variety of Great Lakes commercial and sportfish species (Van Oosten and Deason 1938, Wells and Beeton 1963, Anderson and Smith 1971, Wells 1980). In addition, organic contaminant uptake studies (Landrum 1982, 1988, Landrum et al. 1985) have shown that *Diporeia* sp. accumulates lipophilic organic contaminants from surrounding sediments and porewaters and thus may represent an important route in the cycling of such contaminants within Great Lakes food webs.

At present, considerable evidence indicates that *Diporeia* sp. production is intimately linked with primary production of pelagic Lake Michigan algal communities (Gardner et al. 1990). Diatom blooms (and subsequently rich inputs of organic matter to sediments) have recently been associated with increases in *Diporeia* sp. feeding intensity (Quigley 1988, Evans et al. 1990) and the subsequent and rapid accumulation of storage lipids (triacylglycerols) (Gardner et al. 1985, Quigley et al. 1989). A similar linkage between primary production and *Diporeia* production has also been noted for Lake Ontario (Dermott and Corning 1988).

Little detailed information is available on *Diporeia* sp.'s digestive or metabolic physiology. In particular, the biochemical identity of substrates assimilated and metabolized by *Diporeia* sp. to support routine energy demands of growth and reproduction are not presently known. The objective of our series of studies was to estimate both oxygen consumption and ammonia excretion in *Diporeia* sp. and to compare these two rates within an O:N ratio (atoms of oxygen consumed per atoms of nitrogen excreted). O:N ratios have been used extensively to study the underlying metabolism of a variety of crustacean zooplankton species (see review of Mayzaud and Conover 1988). In general, low O:N ratios (3-16) imply almost exclusive reliance on proteins as metabolic substrates. Ratios between 16 and 60 are typical for metabolic use of a mixture of proteins, and lipids and/or carbohydrates, while ratios above 60 reflect a primary dependence on lipids and/or carbohydrates. For amphipods, investigation of O:N ratios is presently limited to two published studies outlining the metabolism of (1) two brackish-marine gammarid species (*G. duebeni* and *G. oceanicus*, Tedengren et al. 1988), and (2) an arctic sympagic gammarid (*G. wilkitzkii*; Aarset and Aunaas 1990).

MATERIALS AND METHODS

Lake Michigan *Diporeia* sp., collected with a PONAR grab at depths of 24-29 m from a nearshore area, 4.8 km west of Grand Haven, Michigan, were transported to the laboratory where they were maintained at 4°C, in constant darkness, in plexiglas aquaria containing lakewater and a 5-cm layer of sediment collected from the site.

In three (O:N) experiments (Experiments Nos. 1-3), both respiration (oxygen consumption) and ammonia-nitrogen excretion rates were determined by maintaining two juvenile animals (mean individual dry weight = 1.6 mg) within a 60-mL BOD bottle containing filtered (1 μm glass filter) lakewater, in darkness at 4°C. In a fourth experiment (No. 4), only oxygen consumption was measured. Decline in oxygen concentration ($\text{mgO}_2 \text{ L}^{-1}$ over 24 hours) in (7-10 replicate) bottles containing test animals was adjusted to include any changes noted in blank (animal-free) bottles. Changes in ammonium concentration were likewise adjusted to blanks. Following 24 hours of incubation, animals were removed from test bottles, blotted dry, and weighed. Wet weights were then multiplied by 0.269 to obtain dry weight (dry weight percentage determined for *Diporeia* at study site; Landrum 1988) or dry weight of animals was directly determined after 24-hour drying at 60°C. Oxygen concentrations were determined by Winkler titration (Grasshoff 1983) while ammonium concentrations were determined with a micro-fluorometric method (Gardner 1978).

In three other experiments, we examined potential effects of conditions in test vessels on *Diporeia* sp. respiration. These included the influence of (a) the presence/absence of a sediment substrate on respiration rate (Experiment No. 5), (b) the amount of time provided for animals to acclimate to the test vessels prior to vessel closure and the subsequent oxygen consumption determination (Experiment No. 6), and (c) the decline in availability of ambient dissolved oxygen in test vessel water occurring during the oxygen consumption determination interval (Experiment No. 7).

Presence/Absence of Sediment Substrate (Experiment No. 5)

We evaluated possible effects of the presence/absence of sediments on *Diporeia* sp. respiration by providing a 1-cm deep layer of pre-combusted (550°C for 2 hours) 500-550 μm sand to one group of the test animals while another group's oxygen consumption was measured without any substrate at the bottom of test vessels. Previous studies (Eriksen 1963, 1968, Vernberg et al. 1977) have indicated that oxygen consumption of benthic animals may be significantly affected by the presence and nature of a substrate into which animals can burrow.

Animal Acclimation in Test Vessels (Experiment No. 6)

A sixth experiment focused on identifying possible changes in O_2 consumption resulting from handling stress and/or increased activity of animals following introduction to test bottles. Oxygen consumption of pre-acclimated animals (animals held, under flow-through conditions, in test vessels for 24 hours prior to the experiment) was compared with respiration estimates of freshly-introduced animals during respiration measurement intervals of 3.9, 7.9, 24.4, or 48.8 hours.

Effects of Decreased Oxygen Availability on Respiration

Because we relied on closed-vessel respirometry to determine *Diporeia* sp. O_2 consumption rates, we examined potential effects of declining oxygen concentration during the 24-hour incubation period on corresponding *Diporeia* sp. respiration. To test for this effect, we measured *Diporeia* sp. O_2 uptake in a series of 10 experiments where ambient oxygen concentration at the start of the incubation interval was reduced to approximately 25-75% of control (near-saturation) values by partial stripping with nitrogen. The ability of test animals to take up and use available oxygen under such O_2 -limited conditions was then evaluated in terms of both respiration rate ($\text{mg O}_2 \text{ g}^{-1}\text{DW day}^{-1}$) and corresponding oxygen clearance rate ($\text{mL of H}_2\text{O cleared of oxygen mg}^{-1}\text{DW h}^{-1}$). Oxygen clearance rates estimated the total volume of water animals were required to "clear" of oxygen to satisfy required metabolic demands thus providing an assessment of energetic costs imposed on test animals by low dissolved oxygen concentrations.

RESULTS

O:N Experiments (Nos. 1 - 3), O₂ Consumption (Experiment No. 4)

Mean oxygen consumption rates measured in the three O:N experiments (1-3) ranged from 13.9 to 31.3 mgO₂ g⁻¹DW d⁻¹, while ammonia-nitrogen was 0.45 - 0.77 ng-atom N mg⁻¹hr⁻¹ (Tables 1, 2). Corresponding mean O:N ratios ranged from 61.6 to 230.0. Oxygen consumption measured alone in Experiment No. 4 was 33.9 ± 0.37 (SE).

Presence/Absence of Sediment Substrate (Experiment No. 5)

In experiment #5, a t-test (Zar 1984) comparing means of O₂ consumption of animals with, and without, a sand substrate indicated that oxygen consumption rates were not significantly different (P<0.05). Mean O₂ consumption of both substrate and non-substrate groups was 13.9 mg O₂ g⁻¹DW day⁻¹ and consequently, the two groups were pooled to give an overall mean of 13.9 ± 1.5 (SE) mg O₂ g⁻¹DW day⁻¹.

Animal Acclimation in Test Vessels (Experiment No. 6)

In Experiment #6, animals held in test vessels under flow-through conditions for 24 hours prior to vessel closure initially exhibited respiration rates that were significantly lower (T test (P<0.05), Zar 1984) than rates of non-acclimated animals. Although differences in mean O₂ consumption were apparent between pre- and non-acclimated groups after 4.1 and 7.9 hours (Figure 1), the two treatments did not differ after a 24.4-hour interval. The initial elevation of oxygen consumption rates may have been caused by increased activity as the newly-introduced, non-acclimated animals explored the test container (e.g. Sutcliffe 1984), but subsequently this activity decreased with no discernible differences in mean oxygen consumption rates existing over 24 hours. Thus, results of both experiments were pooled within an overall mean of 17.2 ± 0.8 (SE) mg O₂ g⁻¹DW day⁻¹.

Effects of Decreased Ambient O₂ on Respiration (Experiment No. 7)

Comparison of *Diporeia* sp. oxygen consumption versus initial ambient oxygen concentration indicated that O₂ consumption rates remained nearly constant over an interval of 12.4 - 7.5 mg O₂ L⁻¹ (or a range of 95 - 57% O₂ saturation at 4°C) and gradually declined at lower ambient O₂ levels. This trend has been

Experiment No.	Date	Objective	DW (mg)	N Excretion ng-atom N mg ⁻¹ DW h ⁻¹	O ₂ Consumption	O:N Ratio
1	4/25/86	O:N	2.4±0.3 (10)	0.47±0.10 (8)	31.3±1.5 (10)	230.0±56.1 (8)
2	5/8/86	O:N	3.0±0.2 (10)	0.95±0.13 (7)	17.2±1.4 (7)	55.8±10.5 (7)
3	9/22/88	Substrate/O:N	1.6±0.1 (10)	0.45±0.08 (7)	13.9±1.5 (10)	92.23±19.8 (7)
4	3/19/86 4/25/86	O ₂ Consumption	1.9±0.2 (21)		33.9±0.37 (20)	
5	5/8/86	Test vessel Acclimation	1.5±0.1 (10)		17.2±0.8 (10)	
6	10/89 11/89	Decreased O ₂	2.2±0.1 (n=48)		25.5 (Vmax)	

Table 1. Mean *Diporeia* sp. oxygen consumption, ammonia-N excretion [± SE n)] measured in six 1986-1989 experiments.

Figure 1. Effects of acclimation on *Diporeia* sp. oxygen consumption ($\text{mg O}_2 \text{ mg}^{-1} \text{ DW day}^{-1}$). Striped bars denote respiration rates of animals pre-acclimated in test vessels for 24 hours, under flow-through conditions, while cross-hatched bars represent respiration of non-acclimated animals in experiments where determination of oxygen consumption began immediately following animal introduction to test vessels. Error bars are \pm SE around a mean.

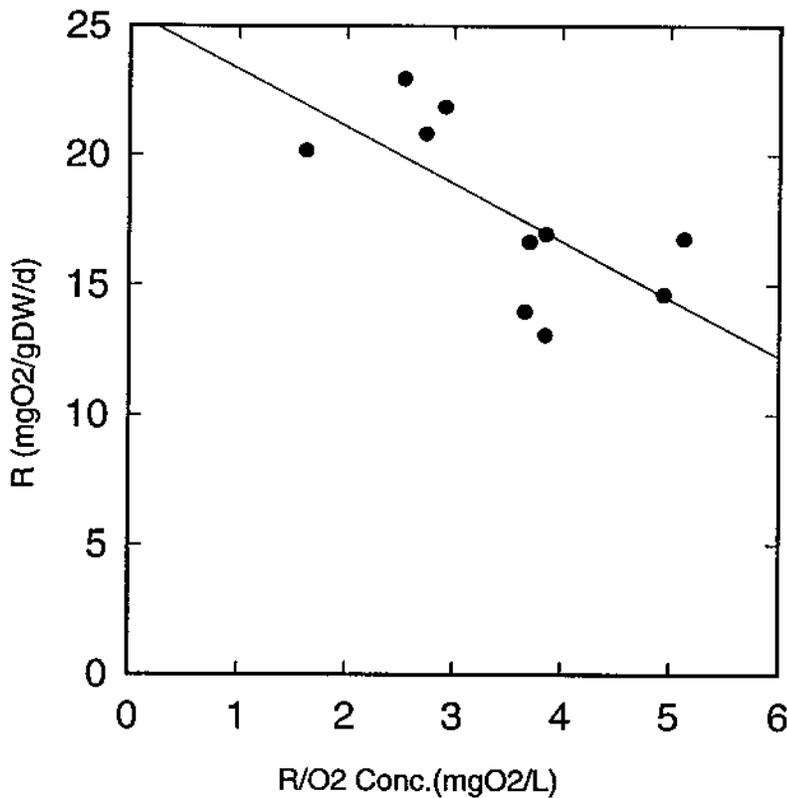
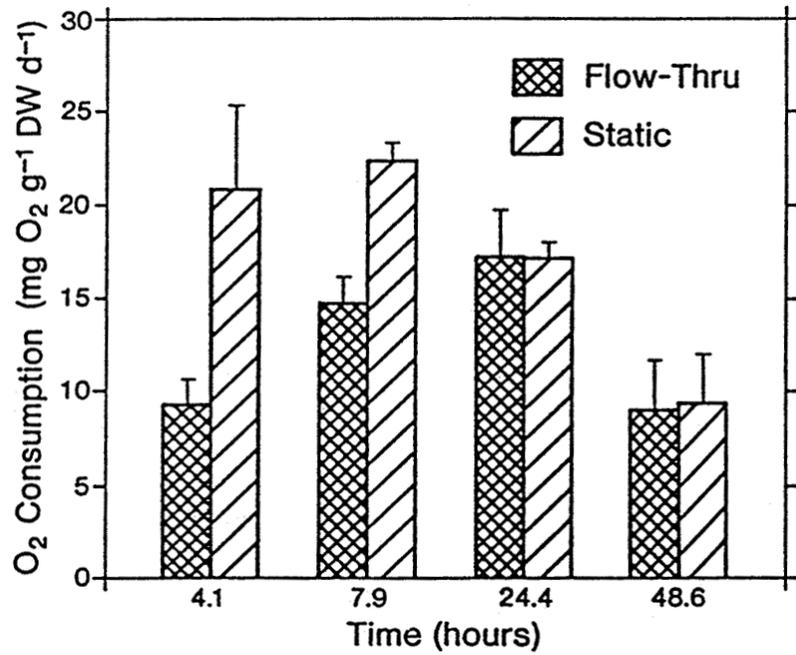


Figure 2. Eadie-Hofstee plot of regression line calculated for *Diporeia* sp. oxygen consumption (R) ($\text{mg O}_2 \text{ g}^{-1} \text{ DW d}^{-1}$) versus R divided by ambient oxygen concentration (D.O.) ($\text{mg O}_2 \text{ L}^{-1}$). $R = -2.1939 (R/D.O.) + 25.4247$.

previously observed in other amphipod species (*Gammarus* spp.; Sutcliffe 1984). Since the relationship between dissolved oxygen concentration and O_2 consumption approximated that seen for common single-substrate enzyme reactions, we replotted the data in Eadie-Hofstee form of the Michaelis-Menten equation (Lehninger 1975) (Figure 2). This transformed the data to linear form and a regression of O_2 consumption (dependent variable) versus oxygen consumption divided by ambient O_2 concentration indicated significant linear correlation ($P < 0.05$, $r = 0.69$, $n = 10$). V_{max} , the predicted oxygen consumption rate at 100% O_2 saturation was $25.5 \text{ mg } O_2 \text{ g}^{-1} \text{ DW d}^{-1}$ and K_m the corresponding ambient O_2 concentration where oxygen consumption declined to 50% of the V_{max} was $2.2 \text{ mg } O_2 \text{ L}^{-1}$.

A plot of oxygen clearance rates versus ambient O_2 concentration (Figure 3) showed that animals responded to decreased oxygen availability by processing progressively larger volumes of water to satisfy metabolic demands.

DISCUSSION

Oxygen Consumption

Diporeia sp. mean oxygen consumption estimated in our four experiments ranged from 13.9 to 33.9 $\text{mg } O_2 \text{ mg}^{-1} \text{ DW day}^{-1}$. To compare these rates with data from existing published data on *Diporeia* sp. respiration (Johnson 1970; Johnson and Brinkhurst 1971; Bay of Quinte, Lake Ontario, Johannsson et al. 1985) we entered the test temperature (4°C) and the overall mean dry weight (1.6 mg) of our test animals into a regression equation used to describe O_2 consumption of juvenile Lake Ontario *Diporeia* sp. where;

$\ln R = 1.48 + 0.014 T - 0.3 \ln W$ (Johnson and Brinkhurst 1971) and;

T = Temperature ($^\circ\text{C}$)

W = Ash-free dry weight (AFDW)(mg) = $0.87 \times \text{dry weight}$ (Landrum 1988)

R = Respiration ($\mu\text{g } O_2 \text{ mg}^{-1} \text{ AFDW day}^{-1}$)

When dry weight of our test animals was converted to AFDW by multiplying by 0.87 (Landrum 1988) for a temperature (T) of 4°C , this equation gave a respiration rate R (corrected back to a dry weight

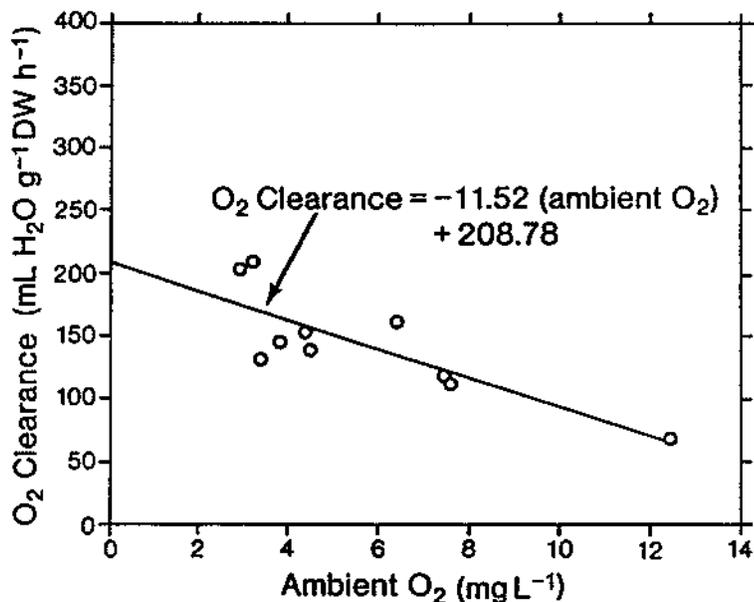


Figure 3. *Diporeia* sp. O_2 clearance ($\text{mL H}_2\text{O}$ cleared of oxygen $\text{g}^{-1} \text{ DW h}^{-1}$) versus ambient oxygen concentration ($\text{mg } O_2 \text{ L}^{-1}$). Solid line denotes calculated regression line where $r = 0.82$ (significant ($P < 0.05$, $n = 10$)) and O_2 Clearance = -11.51 (Ambient O_2) + 208.78.

basis) of $4.0 \text{ mg O}_2 \text{ g}^{-1} \text{ DW day}^{-1}$ that was only 12 to 29% of our values. Johnson and Brinkhurst (1971) measured oxygen consumption of juvenile animals using closed vessels, but measured oxygen with an electrode equipped with an oscillating stirrer. One would expect, *a priori*, that O_2 consumption might be elevated if the constant agitation used, produced increased activity and higher oxygen uptake in test animals. In subsequent studies of Lake Ontario macroinvertebrates, Chua and Brinkhurst (1973) and Brinkhurst et al. (1972) showed that oligochaete oxygen consumption (also measured by Johnson and Brinkhurst in the 1971 study) was higher when measured with the electrode/oscillating stirrer device compared to an electrode/rotary stirrer. Brinkhurst et al. (1972) suggested that excessive vibration produced by the oscillating stirrer may have led to higher activity and elevated O_2 consumption of test animals. If *Diporeia* sp. respiration measured in the 1971 study was similarly affected, O_2 consumption would be expected to be larger than rates measured with our Winkler method where no water movement or disturbance occurred.

Sutcliffe (1984) extensively reviewed a variety of factors influencing oxygen uptake in both freshwater and brackish-marine Gammarus amphipod species and concluded that among numerous studies, estimates of active metabolism (O_2 consumption measured in animals held in constantly-stirred water) are generally 2-4 times the standard or resting rates (where O_2 consumption was measured in unstirred, undisturbed water). Given such results, Johnson and Brinkhurst's data should reflect higher activity and oxygen consumption compared to our estimates. However, our estimates were greater than rates predicted by the Lake Ontario equation for animals with weights comparable to those tested in our study. Aside from the minor increase in activity we noted following introduction of animals to test vessels (see results) *Diporeia* sp. activity was likely not higher than the activity level of Lake Ontario animals. Cederwall (1979) noted increased activity and higher oxygen consumption associated with swimming in overlying water, during dark portions of a laboratory photoperiod in two Baltic pontoporeiids, and such differences in activity and respiration rates might occur in *Diporeia* sp. We did not evaluate such possible diurnal changes in activity and oxygen consumption. However, our observations of *Diporeia* sp. during periods when animals were held in sediments and constant darkness have indicated that animals generally adopt a consistent level of activity under such conditions, and we believe that such activity may approximate normal levels for individuals in natural conditions.

A second study of *Diporeia* sp. respiration at three Lake Ontario locations (Johannsson et al. 1985) provided a regression equation where;

$$R = \exp [-7.19 + 0.036 (T) - 0.219 \ln (W)] \text{ and;}$$

R = Respiration ($\text{mg O}_2 \text{ mg}^{-1} \text{ DW hr}^{-1}$)
T = Temperature ($^{\circ}\text{C}$)
W = Dry Weight (mg)

At 4°C and a mean dry weight of 1.6 mg this equation predicts O_2 consumption of $18.9 \text{ mg O}_2 \text{ g}^{-1} \text{ DW d}^{-1}$. This value was toward the lower end of our Lake Michigan range of $13.9 - 33.9 \text{ mg O}_2 \text{ g}^{-1} \text{ DW d}^{-1}$ and the calculated Michaelis-Menten V_{max} of $25.5 \text{ mg O}_2 \text{ g}^{-1} \text{ DW d}^{-1}$.

Overall, our estimates of Lake Michigan *Diporeia* sp. respiration rates appear to be equal to, or greater than, rates noted for Lake Ontario animals. It is possible that marked physiological differences exist between *Diporeia* sp. populations of the two lakes. Although the underlying reasons for such differences are not presently known, the two populations may possess distinct and separate life history strategies in response to differing environments of the two lakes. Moreover, given the apparent existence of numer-

ous *Diporeia* species in the Great Lakes and other large lakes of North America (see Introduction), the oxygen consumption differences we noted between our Lake Michigan data and Lake Ontario results may be due to species differences between resident populations of the two lakes.

Results of Michaelis-Menten analysis of *Diporeia* sp. oxygen consumption at decreased ambient O₂ concentrations demonstrated that the amphipod is able to maintain a fairly uniform uptake of O₂, despite declines in available oxygen. Thus, Lake Michigan *Diporeia* sp. are partial respiratory “regulators” and have at least some capability of tolerating low-oxygen concentrations. In addition, these results suggest that the approximate 10% drop in dissolved O₂ concentrations that occurred during our 24-hour incubations had minimal influence on the corresponding *Diporeia* sp. oxygen consumption rates. The 24-hour incubation interval was also sufficiently long to offset effects of increased O₂ consumption noted during the early portion of the incubation period. Thus, our 24-hour measurement interval was sufficiently long to ensure reliable O₂ consumption estimates, yet short enough to avoid effects of decreased oxygen availability occurring in test vessels.

We were not able to compare our Michaelis-Menten / oxygen clearance results from Lake Michigan *Diporeia* sp. directly with either of the two Lake Ontario studies because effects of decreased ambient O₂ concentrations were not examined. Moreover, Michaelis-Menten analysis of data for two Baltic Sea pontoporeiids *Monoporeia affinis* (formerly *Pontoporeia affinis*) and *Pontoporeia femorata* did not show any significant linear relationship (P<0.05) existing for a Eadie-Hofstee transform (O₂ consumption versus O₂ consumption divided by O₂ concentration) (raw data provided by Hans Cederwall, Dept. Systems Ecology, Univ. Stockholm, S-106 91, Stockholm, Sweden).

Ammonia Excretion

Mean ammonia excretion determined for *Diporeia* sp. in the present study varied between 0.45 and 0.77 ng-atom N mg⁻¹ DW hr⁻¹. This range was lower than a previously reported 3-5 ng-atom N mg⁻¹ DW hr⁻¹ range for Lake Michigan *Diporeia* sp. obtained at 45 and 100 m depths and held at 4°C (Gardner et al. 1987), but overlapped with a 0.5-1.3 ng-atom N mg⁻¹ DW hr⁻¹ range recently reported for animals collected at our 29 m Lake Michigan station (4°C test temperature; Gauvin et al. 1989). Nitrogen excretion rates for *Diporeia* sp. are generally lower than published rates of other amphipod species. Nitrogen excretion of two Baltic brackish-marine gammarid species (10°C) *G. duebeni* and *G. oceanicus* ranged from 2.6-2.8 ng-atom N mg⁻¹ DW h⁻¹ (Tedengren et al. 1988) and mean nitrogen excretion of the marine amphipod *Corophium volutator* was 5.7 ng-atom N mg⁻¹ DW h⁻¹ at 22° C (Hawkins and Keizer 1982). Although the rates of these species are higher than those we noted for *Diporeia* sp., this difference may be partly explained by differences in experimental temperatures employed.

O:N Ratios

O:N ratios (atoms of oxygen consumed per atom of nitrogen excreted) (Figure 4) were generally greater than 60 (i.e. 62% of all data points exceeded this value). These results suggest that most of the animals in the experiments were relying primarily on lipids as metabolic substrates with protein catabolism playing a secondary role. These conclusions are based on a recent, extensive review of the use of O:N atomic ratios to assess nutritional status and underlying metabolism (anabolism/catabolism) in crustacean zooplankton populations (Mayzaud and Conover 1988). In general, zooplankton O:N ratios were characterized as being strongly influenced by the disposition of an animal’s free amino acid pool with regard to tissue growth (anabolism) or tissue degradation (catabolism). Depending on an animal’s internal energy requirements, nutritional status and trophic history, and quantity and quality of food supply, free amino acids may be incorporated within tissues as protein, or catabolized to produce energy

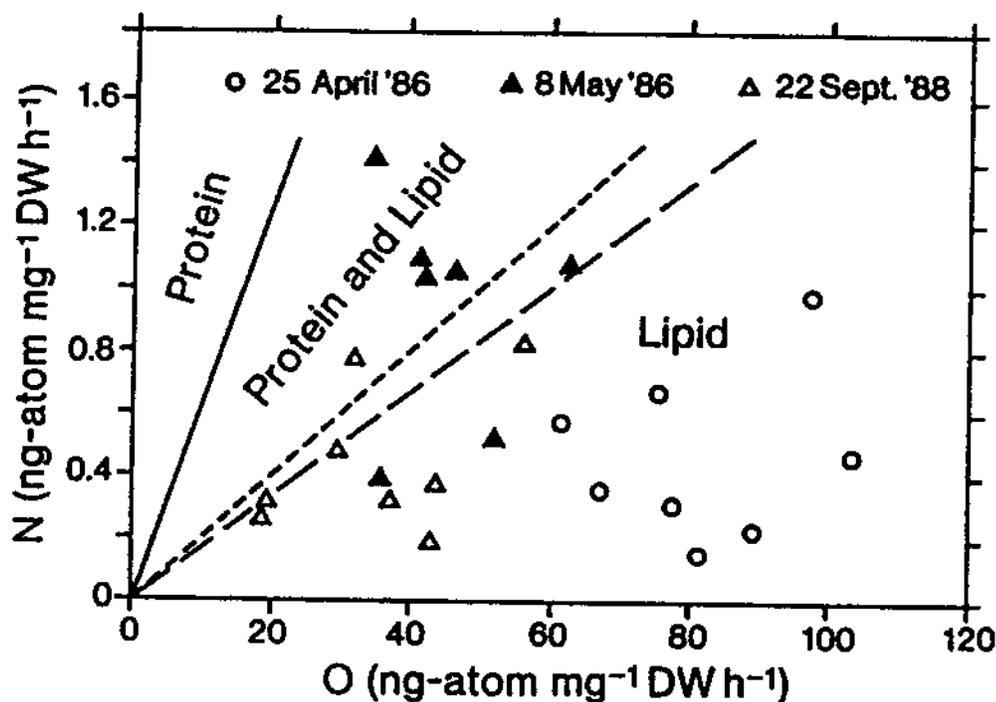


Figure 4. *Diporeia* sp. oxygen consumption (ng-atom O mg⁻¹ DW h⁻¹) versus N excretion (ng-atom N mg⁻¹ DW h⁻¹). Regions in figure correspond to (1) Exclusive catabolism of protein (O:N<16), (2) Catabolism dominated by protein (16 >O:N<50), (3) Catabolism of equal amounts of protein and lipid (50>O:N<60), and (4) Catabolism dominated by lipids (O:N>60).

(primarily through transdeamination via the glutamate dehydrogenase pathway (GDH) in crustaceans). Oxidative deamination of amino acids through the GDH pathway, in turn, produces ammonia which is subsequently excreted. When a variety of amino acids and possible catabolic pathways were considered with respect to NH₃ excretion and oxygen consumption, Mayzaud and Conover concluded that animals catabolizing protein exclusively would exhibit O:N ratios between 3 and 16. When theoretical considerations of both protein and lipids were considered, catabolism of approximately equal amounts of these two substrates were expected to produce O:N ratios between 50 and 60. Above 60, O:N ratios were interpreted as reflecting a lipid-dominant catabolism. Overall, the apparent lipid-dominant catabolism we observed in *Diporeia* sp. was far different than results noted in the two other existing studies of O:N ratios of amphipods. Tedengren et al. (1988) found a protein-dominant catabolism (O:N ratios of 33-38) in two brackish marine gammarids and Aarset and Aunaas (1990) noted utilization of a protein/lipid substrate by an arctic sympagic gammarid (O:N ratio of 21). In two other related studies, however, measurement of the respiratory quotient (CO₂ produced/O₂ consumed) indicated lipid-dominant catabolism (R.Q. of 0.72-0.85) in *Gammarus pulex* (Wright and Wright 1976) and catabolism of both lipid and protein in *G. fossarum* (R.Q.=0.64) (Orzechowski 1974).

While the above results indicate that the nature of the substrates catabolized by different amphipod species may vary, other indirect evidence suggests that *Diporeia* sp. has a lipid-dominant catabolism, given its biochemical composition and life history. Total carbon content (more specifically lipid content) is closely associated with variation in the O:N ratio of aquatic invertebrates (Mayzaud and Conover 1988). The unusually high lipid content of *Diporeia* sp. is well-known (Green 1971, Gardner et al. 1985). Lipids can constitute up to half of the amphipod's dry weight (Gardner et al 1985) and lipid content steadily increases with size throughout juvenile life (Quigley et al. 1989). Thus, it is not surprising that the O:N ratios we observed show a catabolism that is largely lipid-dominant.

Diporeia sp.'s ability to store and use lipids appears to be part of an energetic strategy that allows the organism to rapidly exploit food (primarily phytoplankton or detritus generated from the Lake Michigan

spring diatom bloom) that is only seasonally available (Gardner et al. 1990). This strategy is similar to one typical of crustacean zooplankton species which store large lipid supplies, enabling them to maintain metabolic homeostasis for considerable periods when food is limited or unavailable. For such species, O:N ratios will primarily reflect seasonal changes in biochemical composition associated with storage and use of energy reserves (Mayzaud and Conover 1988). Given *Diporeia* sp.'s high lipid content (most notably triacylglycerols; Gardner et al. 1985, Quigley et al. 1989), this amphipod should also show a close relationship between O:N ratio and biochemical composition. Though *Diporeia* sp. lipid content is known to change seasonally (Gardner et al. 1985), its relationship to O:N ratios has not yet been examined. Although we did not document lipid content of test animals in our study, the clumping of September O:N data points in the upper oxygen consumption range (Figure 4) suggests some seasonal difference, compared to April and May results. Additional assessment of such trends relative to *Diporeia* sp. biochemical composition is clearly needed and may yield further detailed understanding of the energetic and biochemical changes occurring throughout the animal's life history.

ACKNOWLEDGMENTS. We thank Catherine Hill, Tom Nalepa, and Henry Vanderploeg for the valuable comments they provided in review of our manuscript. We also extend special thanks to Hans Cederwall for graciously providing his raw data and for his review of the manuscript.

REFERENCES

- Aarset, A.V., and T. Aunaas. Effects of osmotic stress on oxygen consumption and ammonia excretion of the Arctic sympagic amphipod *Gammarus wilkitzkii*. *Marine Ecology Progress Series* 58:217-224 (1990).
- Anderson, E.D., and L.L. Smith. A synoptic study of food habits of 30 fish species from western Lake Superior. Univ. Minn. Agric. Exp. Stn. Tech. Bull. no. 279 (1971).
- Bousfield, E.L. Revised morphological relationships within the amphipod genera *Pontoporeia* and *Gammaracanthus* and the "glacial relict" significance of their postglacial distributions. *Canadian Journal of Fisheries and Aquatic Sciences* 46:1714-1725 (1989).
- Brinkhurst, R.O., K.I. Chua, and N.K. Kaushik. Interspecific interactions and selective feeding by tubificid oligochaetes. *Limnology Oceanography* 17:122-133 (1972).
- Cederwall, H. Diurnal oxygen consumption and activity of two *Pontoporeia* (Amphipoda, Crustacea) Species, In *Cyclic Phenomena in Marine Plants and Animals 13th European Marine Biology Symposium*, E. Naylor and R. G Hartnoll, Eds. Pergamon, Oxford, England, pp. 309-316 (1979).
- Chua, K.E., and R.O. Brinkhurst. Evidence of interspecific interactions in the respiration of tubificid oligochaetes. *Journal of Fisheries Research Board of Canada* 30:617-622 (1973).
- Dermott, R., and K. Corning. Seasonal Ingestion Rates of *Pontoporeia hoyi* (Amphipoda) in Lake Ontario. *Canadian Journal of Fisheries and Aquatic Sciences* 45:1886-1895 (1988).
- Eriksen, C.H. The relation of oxygen consumption to substrate particle size in two burrowing mayflies. *Journal of Exp. Biology* 40:447-453 (1963).

- Eriksen, C.H. Ecological significance of respiration and substrate for burrowing Ephemeroptera. *Canadian Journal of Zoology* 46:93-103 (1968).
- Evans, M.S., M.A. Quigley, and J.A. Wojcik. Comparative ecology of *Pontoporeia hoyi* populations in southern Lake Michigan: the profundal versus the slope and shelf regions. *Journal of Great Lakes Research* 16:27-40 (1990).
- Gardner, W.S. Microfluorometric method to measure ammonium in natural waters. *Limnology Oceanography* 23:1069-1072 (1978).
- Gardner, W.S., T.F. Nalepa, W.A. Frez, E.A. Cichocki, and P.F. Landrum. Seasonal patterns in lipid content of Lake Michigan macroinvertebrates. *Canadian Journal of Fisheries and Aquatic Sciences* 42:1827-1832 (1985).
- Gardner, W.S. , T.F. Nalepa, and J.M. Malczyk. Nitrogen mineralization and denitrification in Lake Michigan sediments. *Limnology Oceanography* 32:1226-1238 (1987).
- Gardner, W.S. , M.A. Quigley, G.L. Fahnenstiel, D. Scavia, and W.A. Frez. *Pontoporeia hoyi* an apparent direct link between spring diatoms and fish in Lake Michigan. In *Functional and Structural Properties of Large Lakes*, M. Tilzer, Ed. Heidelberg, Springer-Verlag, pp 632-644 (1990).
- Gauvin, J.M., W.S. Gardner, and M.A. Quigley. Effects of food-removal on nutrient-release rates, lipid content and survival time of Lake Michigan *Pontoporeia hoyi*. *Canadian Journal of Fisheries and Aquatic Sciences* 46:1125-1130 (1989).
- Grasshoff, K. Determination of oxygen. In *Methods of Seawater Analysis*. K. Grasshoff, M. Ehrhardt, and K. Kremling, Eds. Verlag Chemie, Weinheim, Federal Republic of Germany, Chapter 4, pp. 61-72 (1983).
- Green, R.H. Lipid and caloric contents of the relict amphipod *Pontoporeia affinis* in Cayuga Lake, New York. *Journal of Fisheries Research Board of Canada* 28:776-777 (1971).
- Hawkins, C.M., and P.D. Keizer. Ammonia excretion in *Corophium volutator*: Using an automated method. *Canadian Journal of Fisheries and Aquatic Sciences* 39:640-643 (1982).
- Hill, C., and R. Elmgren. Vertical distribution in the sediment in the co-occurring benthic amphipods *Pontoporeia affinis* and *P. femorata*. *Oikos* 49:221-229 (1987).
- Johannsson, O.E., R.M. Dermott, R. Feldkamp, and J.E. Moore. Lake Ontario Long Term Biological Monitoring Program. Report for 1981 and 1982. Can. Tech. Rep. Fish. Aquat. Sci. No. 1414 (1985).
- Johnson, M.G. Production, energy flow and structure in benthic macroinvertebrate communities of Lake Ontario. Doctoral Dissertation, Univ. Toronto, Ontario, Canada (1970).
- Johnson, M.G., and R.O. Brinkhurst. Production of benthic macroinvertebrates of Bay of Quinte and Lake Ontario. *Journal of Fisheries Research Board of Canada* 28:1699-1714 (1971).

- Landrum, P.F. Uptake, depuration and biotransformation of anthracene by the scud, *Pontoporeia hoyi*. *Chemosphere* 11:1049-1057 (1982).
- Landrum, P.F. Toxicokinetics of organic xenobiotics in the amphipod, *Pontoporeia hoyi*: Role of physiological and environmental variables. *Aquatic Toxicology* 12:245-271 (1988).
- Landrum, P.F., M.D. Reinhold, S.R. Nihart, and B.J. Eadie. Predicting the bioavailability of organic xenobiotics to *Pontoporeia hoyi* in the presence of humic and fulvic materials and natural dissolved organic matter. *Environmental Toxicology and Chemistry* 4:459-467 (1985).
- Lehninger, A.L. *Biochemistry*. New York: Worth Publishers Inc. (1975).
- Mayzaud, P., and R.J. Conover, R.J. O:N atomic ratio as a tool to describe zooplankton metabolism. *Marine Ecology Progress Series* 45:289-302 (1988).
- Nalepa, T.F. Estimates of macroinvertebrate biomass in Lake Michigan. *Journal of Great Lakes Research* 15:437-443 (1989).
- Nalepa, T.F., M.A. Quigley, K. Childs, J.M. Gauvin, T.S. Heatlie, M. Parker, and L. Vanover. *The macrobenthos of southern Lake Michigan*. NOAA Data Report ERL GLERL-28, Great Lakes Environmental Research Laboratory, Ann Arbor, MI (1975).
- Orzechowski, B. The correlation between the respiration metabolism of *Gammarus fossarum* (Koch) and the presence of some ammonium salts in water. *Acta Universitatis Nicolai Copernici, Nauki Matematyczno-Przyrodnicze, Zeszyt.* 34:1-16 (1974).
- Quigley, M.A. Gut fullness of the deposit-feeding amphipod, *Pontoporeia hoyi*, in southeastern Lake Michigan. *Journal of Great Lakes Research* 14:178-187 (1988).
- Quigley, M.A., J.F. Cavaletto, and W.S. Gardner. Lipid composition related to and size and maturity of the amphipod *Pontoporeia hoyi*. *Journal of Great Lakes Research* 15:601-610 (1989).
- Segestrale, S.G. The distribution and morphology of *Pontoporeia affinis* Lindstrom f. *brevicornis* (Crustacea: Amphipoda) inhabiting North American lakes, with a description of a new aberrant form from the area. *Comment. Biol. Soc. Sci. Fennica* 32:3-19 (1971).
- Sutcliffe, D.W. 1984. Quantitative aspects of oxygen uptake by *Gammarus* (Crustacea, Amphipoda): a critical review. *Freshwater Biology* 14:443-489 (1984).
- Tedengren, M., M. Arner, and N. Kautsky. Ecophysiology and stress response of marine and brackish water *Gammarus* species (Crustacea, Amphipoda) to changes in salinity and exposure to cadmium and diesel-oil. *Marine Ecology Progress Series* 47:107-116 (1988).
- Vainola, R., and S. Varvio. Molecular divergence and evolutionary relationships in *Pontoporeia* (Crustacea: Amphipoda). *Canadian Journal of Fisheries and Aquatic Sciences* 46:1705-1713 (1989).

Van Oosten, J., and H.J. Deason. The food of the lake trout (*Cristivomer namaycush namaycush*) and of the lawyer (*Lota maculosa*) of Lake Michigan. *Transaction of the American Fisheries Society* 67:155-177 (1938).

Vernberg, W.B., B.C. Coull, and D.D. Jorgensen. Reliability of laboratory metabolic measurements of meiofauna. *Journal of Fisheries Research Board of Canada* 34:164-167 (1977).

Wells, L. Food of alewives, yellow perch, spottail shiners, trout-perch, and slimy and four-horn sculpins in southeastern Lake Michigan. U.S. Fish. and Wild. Serv. Tech. Pap. 98 (1980).

Well, L., and A.M. Beeton. Food of the bloater, *Coregonus hoyi* in Lake Michigan. *Transactions of the American Fisheries Society* 92:245-255 (1963).

Wright, E.A., A.A. and Wright. The respiratory quotient of *Gammarus pulex* L. *Comp. Biochem. Physiol.* 53A:45-46 (1976).

Zar, J.H. 1984. *Biostatistical Analysis*. Englewood Cliffs, NJ: Prentice-Hall Inc. (1984).