Vol. 179: 41–54, 1999 | MARINE ECOLOGY PROGRESS SERIES Mar Ecol Prog Ser | Published April 15

Sources of urea in arctic seas: zooplankton metabolism

R. J. Conover[1,2,*], K. R. Gustavson[1,]**

[1]Department of Biology, Dalhousie University, Halifax, Nova Scotia B3H 4J1, Canada

[2]Department of Fisheries and Oceans, Biological Sciences Branch, Bedford Institute of Oceanography, PO Box 1006, Dartmouth, Nova Scotia B2Y 4A2, Canada

ABSTRACT: The copepod *Pseudocalanus acuspes* in the high arctic frequently occurs under fast ice in April and May, where it apparently feeds on ice algae. Excretion measurements for this species in summer from Barrow Strait, Northwest Territories, Canada, showed that urea frequently exceeded ammonia as the primary nitrogenous excretory product. Subsequent experiments in different seasons, also including observations on several other species of arctic zooplankton, notably the copepods *Calanus hyperboreus*, *C. glacialis* and *Metridia longa*, similarly demonstrated high levels of urea excretion. The *Calanus* spp. showed strong correlation between size (dry weight) and metabolic rates for oxygen consumption and for ammonia excretion, and, for *C. glacialis*, between size and urea excretion as well. No size-related metabolic relationships were found for *P. acuspes*, probably because of the small size range of the experimental specimens used. However, *P. acuspes* and both *Calanus* species demonstrated striking changes in weight-specific metabolism with season. For all species examined, weight-specific estimates of respiration and excretion frequently yielded O:N ratios less than 20, and occasionally less than 10, when urea and ammonia excretion were combined in the calculations.

KEY WORDS: Urea · Ammonia · Nitrate · Total dissolved nitrogen · Zooplankton · Excretion · Respiration · O:N ratio · Arctic · Fast ice

INTRODUCTION

Urea concentrations, sometimes greater than 2 µmol l^{-1}, accounted for >50% of dissolved nitrogen at most stations in the upper mixed layer of northern Baffin Bay and eastern Lancaster Sound (Canadian Arctic) in the late summer of 1980, but decreased with depth in the euphotic zone and with distance from land (Harrison et al. 1985). At that time, urea was already recognized as a potentially important source of recycled nitrogen in tropical, sub-tropical and temperate marine environments, both coastal and offshore (e.g. Remsen 1971, Remsen et al. 1974, Harvey & Caperon 1976, Horrigan & McCarthy 1981, Kristiansen 1983), but no earlier observations on the role of urea in the nitrogen cycle of high latitude seas were available. Since the work of Harrison et al. (1985), similar studies in the Barents Sea and its associated ice environments (Kristiansen & Lund 1989, Kristiansen & Farbrot 1991), and also in the Weddell Sea (Kristiansen et al. 1992) and near the ice edge in the Bellingshausen Sea (Bury et al. 1995) in the Southern Ocean, have examined urea as a nitrogen source for primary production in cold waters. Recent papers by Sapozhnikov & Propp (1990) regarding the Antarctic seas and by Kavakina & Sapozhnikov (1994) regarding the Bering Sea have substantially increased information on the distribution of urea in both polar oceans. In the pycnocline of the Bering Sea, values from 4 to 5.5 µmol l^{-1} were found while, away from the shelf edge in water 500 to 1000 m deep, urea concentrations sometimes decreased to 1–2.5 µmol l^{-1} as nitrate concentrations increased. They concluded that the high concentrations of urea, and also ammonia, originated on the Bering Shelf, perhaps being related to low temperatures (<0 to 2°C) which retarded development of and mineralization by bacteria, nano- and microheterotrophs.

Present addresses:
*7415 McAllister Road, RR2, Bewdley, Ontario K0L 1E0, Canada. E-mail: rconover@eagle.ca
**Department of Geography, University of Victoria, PO Box 3050, Victoria, British Columbia V8W 3P5, Canada

Still, the sources of high concentrations of urea in remote marine environments with low mean temperatures are not at all clear. A wide variety of poikilothermal animals, both vertebrate and invertebrate, are known to excrete urea in varying proportions with other nitrogenous waste products (Conover 1978), but apparently only Lomstein et al. (1989) have examined the role of benthic macrofauna in urea cycling at high latitudes by direct excretion experiments, fortuitously also on the Bering Shelf. They demonstrated there that the quality and quantity of organic matter available for degradation was highly correlated with 3 factors of gross production of urea, high concentrations of both urea and ammonia, and high sediment-water exchange rates favoring hydrolysis of urea to ammonia.

The importance of urea on the Bering Shelf has been further elaborated as part of the Inner Shelf Transfer and Recycling (ISHTAR) program by Walsh et al. (1989). The bacteria-microplankton loop utilized more than half the primary production along the relatively impoverished Alaskan coast but only 8% in the richer Anadyr Water that invades the Chukchi Sea on the Russian side of Bering Strait, where more than 75% of the phytoplankton input sinks to form a large detrital pool. Concomitantly remineralization is proportionately greater in the water column off Alaska while the liquid excretions by macrobenthos are predominant in the Anadyr Water. However the picture becomes even more complicated in winter, when these rich sediments become anoxic and sulfate-utilizing bacteria become increasingly important in the remineralization process. There is good evidence for urea production by bacteria in marine sediments (Pedersen et al. 1993a,b, Therkildsen & Lomstein 1994) though not as yet from an ice-influenced environment.

As for the role of crustacean zooplankton in processes controlling the flux of recycled nitrogen in the sea, urea was generally a small fraction of total nitrogen released (Corner & Newell 1967, Jawed 1969, Le Borgne 1977, Dagg et al. 1980 [2 of 3 species], Mitamura & Saijo 1980, Smith & Whitledge 1982, Conover & Cota 1985), but there were exceptions (Eppley et al. 1973, Smith 1978a,b, Dagg et al. 1980 [1 of 3 species], Båmstedt 1985) all in relatively low or mid-latitude environments.

The O:N ratio, the proportion of atoms of oxygen respired to atoms of nitrogen excreted in metabolism, has often been recorded as 'high' (>100) in arctic animals, especially during winter, when little food is available and the animals largely depend on stored lipid that contains no nitrogen to meet metabolic demands, in contrast with protein metabolism (O:N = 3 to 30 or so) found for short-lived zooplankton from temperate or tropical locations that excrete primarily ammonia (Conover & Corner 1968, Mayzaud & Conover 1988).

In the summer of 1990 and the spring and summer of 1991 a series of respiration and excretion experiments were carried out at Resolute, Northwest Territories, Canada, with the common neritic copepod *Pseudocalanus acuspes*, which was found to excrete urea at the same or higher rates than it excreted ammonia whether or not in the presence of ice (Gustavson 1992). While these results were unique, and rather unexpected, only a single copepod species had been studied during a relatively brief period of the arctic year. Hence, when a group of government agencies and universities in Canada initiated POLAR PRO, a 12 mo, multi-disciplinary study of the oceanography of Resolute Passage and Barrow Strait in January 1993, it was decided to include seasonal studies of respiration and nitrogen excretion as part of a year-round investigation of the physiology and population dynamics of the dominant zooplankton species in the area. Here Gustavson's unpublished observations are combined with those from POLAR PRO to present a more complete seasonal analysis of excretory and respiratory metabolism for several common arctic zooplankton species.

MATERIALS AND METHODS

Sites and seasons. All experiments were carried out in Resolute Passage and adjacent waters (Fig. 1). In May 1991 and between January 16 and June 25, 1993, sampling was carried out through a hole in the ice at a station protected by a portable structure equipped with a diesel generator, and either an electrically powered or a hydraulically driven winch, about 5 km off Sight Point in about 100 m of water. In winter, transportation to the site was by snowmobile (1991) or with a Bombardier tracked vehicle (1993) which also supplied hydraulic power to operate the winch. During August 1990, sampling was carried out from RV 'Ogac', a fully equipped research vessel owned by Fisheries and Oceans Canada, and, in August 1991 and July 27 to September 16, 1993, from a gasoline-driven, inboard-outboard, 7 m aluminum boat equipped with a hydraulic winch, usually in the vicinity of the earlier ice station. After fall freeze-up, sampling was again carried out through the ice, initially in October 1993 in Resolute Bay in approximately 35 m of water, and in November through December 1993 in Allen Bay (Fig. 1) in about 80 m of water. Early in the fall, when the ice was still relatively thin, a hole was generally cut at the site with a gasoline-driven auger. Later a portable 'ice shack' was installed at the site as was done in the previous winter except that sampling was then carried out with a portable electric winch driven

by a portable generator, both mounted on a sled and pulled to the sampling location by a snowmobile.

Sampling. Plankton were usually captured by vertical tow with 0.5 m ring nets of 102, 202 or 780 µm mesh, either over the entire water column or over a selected portion determined by closing the net at a selected depth using a messenger-operated, single-release throttling system. Samples to be preserved were concentrated, if necessary, using a plankton-net cod end with a screened window of the same mesh as the plankton net, and preserved in 0.5 l polyethylene containers with 2% reagent grade formaldehyde. Samples to be used for experimental work were copiously diluted with sea water at *in situ* temperature and returned to the laboratory in wide-mouth, 8 l carboys, where they were kept in an aquarium receiving sea water at *in situ* temperature pumped directly from 7 to 8 m depth from Resolute Bay. Sorting for experimental purposes was normally carried out within 24 h unless otherwise dictated by the experimental design.

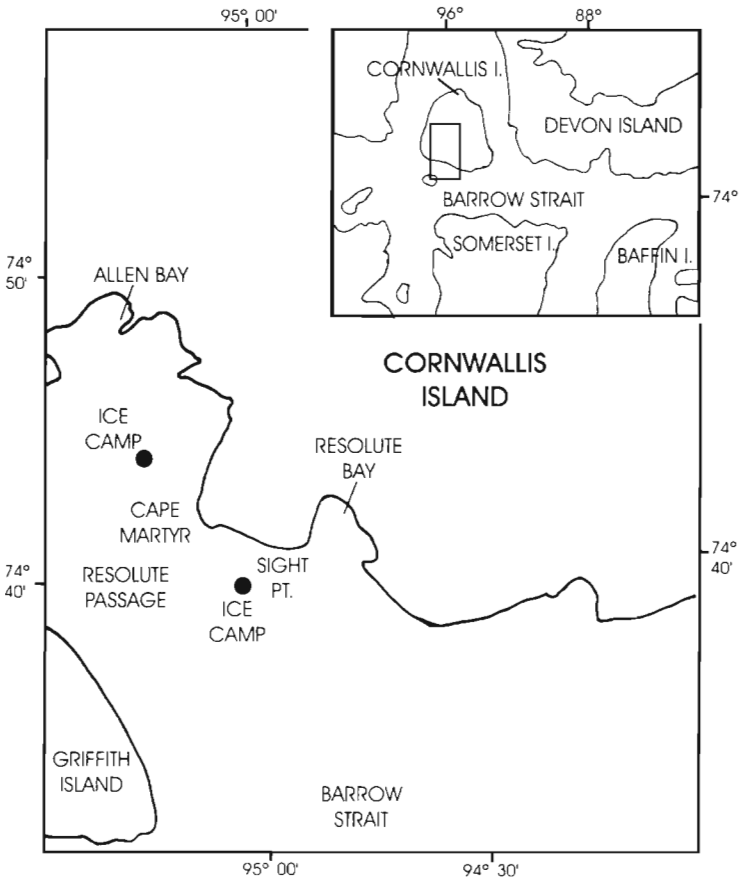

Fig. 1 Approximate location of the winter ice camp off Resolute Bay, Northwest Territories, Canada; the general area of sampling in 1990, 1991 and 1993 during the open water season is shown. Samples were also taken in Resolute Bay (site not shown) and in Allen Bay just after freeze-up in the fall of 1993

Experimental protocol. A number of zooplankton species were used in respiration and excretion experiments, depending in part on their availability. Because of the considerable difference in size among them, the numbers of zooplankton included in the pre-conditioned, glass-stoppered, 300 ml experimental bottles varied from 5 to several hundred, but otherwise the methods were virtually identical. Experimental zooplankton were selected, using a dissecting microscope when necessary, usually by wide-mouth pipette from a recently captured assemblage or from zooplankton previously sorted in the laboratory and maintained in the sea water aquarium subject to several types of pretreatment, and then added to 50 or 100 ml beakers containing experimental water, larger beakers being used for larger numbers of zooplankton. Finally zooplankton were added, either by pouring or transferring by pipette, if of a size that such handling was more efficient, to the experimental bottles. The water into which the zooplankton were sorted initially was then removed, using a siphon covered with a bolting cloth screen, taking care that the zooplankton were never left completely dry. The experimental material was then flushed, again using siphons, 3 times with the experimental water, which had been previously prepared by gravity filtration through 30 µm bolting cloth and allowed to stand overnight to assure oxygen equilibration. Bottles were then wrapped in aluminum foil, black cloth or black plastic, together with 3 to 5 similarly prepared controls, and incubated at *in situ* temperature, generally −1.0 to −1.8°C, for 36 to 48 h. The long incubations enabled the use of somewhat lower concentrations of zooplankton at the low experimental temperatures while achieving a significant oxygen and/or nitrogen change.

Following the incubations, a siphon was again used to remove water for oxygen and nitrogen analyses into two or three 60 ml glass-stoppered bottles for oxygen determination and into a single acid-washed polyethylene bottle for ammonia and urea determination. The zooplankton remaining in the bottles were transferred to a small sorting dish (salt dish) from which they were removed by fine pipette or watchmakers' forceps to the surface of a pad of Schleicher and Schul sharkskin filter paper. The zooplankton were carefully blotted to remove the adhering sea water, counted and then transferred to tared aluminum boats and dried at 60°C for 48 h prior to weighing on a Cahn microbalance. They were not rinsed with distilled water.

Table 1 *Pseudocalanus acuspes*. Forms of nitrogen in soluble excretory products and their effect on the O:N ratio (by atoms) of zooplankton studied in the Resolute area in 1990 and 1991

Stage	Date	No. of experiments	Mean O:N ratio			SD (summed)
			Ammonia alone	Urea alone	Summed	
Mixed (Stages 4, 5, 6 female)	20–21 Aug 1990	9	45.21	10.44	8.19	3.23
Mixed (Stages 4, 5, 6 female)	21–22 Aug 1990	6	37.57	11.97	8.64	3.01
Mixed (Stages 4, 5, 6 female)	21–23 Aug 1990	8	23.93	26.33	11.94	2.42
Mixed (Stages 5, 6 male and 6 female)	9–10 May 1991	5	42.78	127.23	40.48	7.03
Mixed (Stages 5, 6 male and 6 female)	17–19 May 1991	2	21.43	15.64	8.53	3.63
Mixed (Stages 5, 6 male and 6 female)	18–19 May 1991	3	16.35	28.93	10.33	5.55
Stage 6 female	3–4 Aug 1991	6	81.40	37.63	25.19	8.38
Stage 4	12–14 Aug 1991	5	37.16	18.04	11.40	3.29
Stage 6 female	12–14 Aug 1991	3	40.92	16.99	11.36	4.42

Analyses. Oxygen analyses were carried out using the whole-bottle method described by Levy et al. (1977), but using 60 ml glass-stoppered bottles instead of 125 ml bottles, also making slight adjustments in the reagent concentrations. In our experiments, the usual change in oxygen was between 3 and 20% with a mean titration error of 0.5%. Ammonia was determined by the Solórzano (1969) method and by a slight modification of the method of McCarthy (1970) for urea analyses as in Harrison et al. (1985). The analytical errors for ammonia and urea were considerably greater than for oxygen, generally between 5 and 15%, but change in nitrogen concentrations in the experimental bottles was usually 30 to 60% and often exceeded 100%, especially when the background (control) concentration was low. Chlorophyll, used in certain experiments designed to test the effects of food on zooplankton metabolism, was determined by fluorescence after Yentsch & Menzel (1963).

RESULTS

Metabolic experiments, 1990 and 1991

The relatively small and numerically dominant copepod in the Resolute area, *Pseudocalanus acuspes*, responds physiologically quite rapidly to a sudden increase in its food supply even in a cold-water environment (Conover et al. 1986, 1988, Bedo et al. 1990), and it was therefore selected to study the effects of the arctic summer phytoplankton bloom on respiration and nitrogen excretion, presumably ammonia, during August 1990 and 1991 in comparison with conditions in May 1991, when there was little food in the water column. There were no clear seasonal differences in the resulting respiration rates (not shown), but unexpectedly high urea excretion rates, together with the anticipated ammonia waste, resulted in unusually low O:N ratios during both spring and summer environmental conditions (Table 1).

Effects of laboratory conditions

At the start of POLAR PRO (January 1993), the South Camp laboratory at Resolute, with its excellent wet lab and aquarium facility, enabled the maintenance of a variety of organisms, including zooplankton, at near natural conditions. Populations of mixed zooplankton and individual species were kept in apparently excellent condition for up to several months but, as a form of verification, we carried out several experiments to examine the potential impact of laboratory conditions on copepod metabolism. In the first of these experiments, we examined the respiration of small populations of several species on 2 dates 4 d apart and found no detectable change in metabolic response (Table 2). In a similar experiment we compared respiratory physiology of zooplankton kept in the laboratory overnight with another population maintained on the water table for 8 d and again found no significant difference. These experiments were carried out in mid-winter, when many species showed no evidence of feeding or development, but analogous experiments carried out in fall, with some zooplankton having been in the laboratory for several months, gave similar results for respiration and for ammonia excretion, although urea excretion increased significantly with time in the laboratory for the later stages of *Calanus hyperboreus* and *C. glacialis*.

In the previous series of experiments there was no attempt made to alter the environment by feeding the zooplankton and therefore presumably their physiological behavior would have been primarily a response to conditions prevailing in the natural sea water flowing through the aquarium system. We then set up an experiment (June 7, 1993) in which sixteen 1 l bottles

Table 2. *Pseudocalanus acuspes* and *Calanus glacialis*. Effect of 4 d in the laboratory on respiration. Based on the Wilcoxen matched-pairs signed-ranks test, the null hypothesis that the respiration rate on the 2 dates did not differ cannot be rejected (Siegel 1956). dw: dry weight

Species and stage	Mean dry weight (mg ind.$^{-1}$)	15 Feb 1993			19 Feb 1993		
		Number of individuals	Duration of experiment (h)	Respiration (µl O_2 mg dw^{-1} d^{-1})	Number of individuals	Duration of experiment (h)	Respiration (µl O_2 mg dw^{-1} d^{-1})
Calanus glacialis	1.200	20	41.3	6.579	20	37.5	6.203
(Stage 5)	1.103	20	41.3	6.262	18	37.4	5.710
	1.152	20	41.2	6.986	18	37.5	7.365
Calanus glacialis	1.024	15	41.1	11.264	15	37.4	10.208
(Stage 6 female)	1.053	15	41.1	10.262	14	37.3	9.282
	1.038	15	41.0	9.693	14	37.3	8.728
Calanus glacialis (Stage 6 male)	1.163	15	40.9	8.748	15	37.2	8.110
Pseudocalanus acuspes	0.034	87	40.9	7.203	58	37.2	8.228
(mixed)	0.034	144	40.8	6.662	84	37.0	10.906

Table 3. Effects on metabolism of feeding ice algae to several zooplankton species for 2 wk (June 9 to 24, 1993). Only urea excretion for low food zooplankton changed significantly. All other null hypotheses were confirmed (Siegel 1956)

Species and stage	Initial: 7–9 June 1993			Final: 22–24 June 1993		
	Respiration	Excretion		Respiration	Excretion	
		Ammonia	Urea		Ammonia	Urea
	(µl O_2 mg dw^{-1} d^{-1})	(ng-at. N mg dw^{-1} d^{-1})	(ng-at. N mg dw^{-1} d^{-1})	(µl O_2 mg dw^{-1} d^{-1})	(ng-at. N mg dw^{-1} d^{-1})	(ng-at. N mg dw^{-1} d^{-1})
High food						
Pseudocalanus acuspes	54.574	91.146	64.740	45.499	3.021	37.344
(mixed)	54.471	56.684	71.123	36.500	18.824	53.155
Calanus glacialis	17.544	11.238	20.646	25.412	3.223	22.015
(Stage 4)	15.682	4.904	22.084	20.921	7.289	26.325
Calanus glacialis	16.536	5.794	4.732	16.763	7.736	10.759
(Stage 6 female)	16.872	7.018	7.130	18.871	10.533	4.274
Calanus hyperboreus	7.387	0	2.412	8.321	2.537	1.930
(Stage 6 female)	7.355	0	4.821	8.448	3.170	0.840
Low food						
Pseudocalanus acuspes	52.941	45.874	36.250	23.894	31.958	23.217
(mixed)	47.144	59.038	44.872	25.289	50.128	34.615
Calanus glacialis	21.850	9.577	24.382	24.962	12.846	10.000
(Stage 4)	23.768	10.265	60.959	24.254	15.827	12.010
Calanus glacialis	18.308	6.711	14.828	16.500	6.601	6.919
(Stage 6 female)	19.250	6.970	7.676	17.904	7.679	5.015
Calanus hyperboreus	8.317	4.864	6.304	8.183	4.093	2.036
(Stage 6 female)	9.153	0	5.486	8.455	2.399	2.002

containing zooplankton and 4 similar controls were divided into equal numbers of replicates, half receiving enough ice algae, collected with a suction corer (Welch et al. 1988), to yield a concentration of about 3 µg l^{-1} of total chlorophyll-derived pigment (chlorophyll *a* plus pheopigments) and the remainder receiving otherwise untreated, 30 µm filtered sea water (mean chloro-pigment, 0.36 µg l^{-1}) as a control, which was approximately half the chlorophyll concentration in the totally untreated water at the time. Both groups were maintained under the light conditions prevailing in the laboratory, i.e. overhead fluorescent lights superimposed on the natural cycle of continuous daylight outside at this season in the high arctic. The metabolism of the experimental zooplankton was measured before they were transferred to 1 l chambers and after 2 wk in them, first transferring them to fresh 30 µm filtered water again. Water (and food where appropriate) was changed every 3 to 5 d and chloropigment 'consumption' was determined over the final 2 feeding intervals by comparing the pigment samples containing zooplankton with the 'initial' sample of stock culture for the next feeding interval.

The experiment was primarily designed to examine the effect of additional food on the respiration and excretion of the zooplankton and should not be considered as a 'grazing' experiment; urea excretion by the copepods receiving 30 µm filtered water showed a significant reduction, but ammonia excretion and respiration were unaffected by either treatment (Table 3). An important point here may be that respiration rates in June for *Calanus glacialis* females, and particularly for *Pseudocalanus acuspes*, are already substantially higher at the start of the feeding experiment than those for the same species and stage in February (Table 2). In that season, the concentration of chlorophyll *a* was generally less than 0.1 µg l^{-1} (Conover et al. 1999, in this issue), so it would appear that a substantial 'seasonal' increase in metabolism had already occurred before our feeding experiment was initiated.

Interpretation of the feeding portion of this experiment was not entirely straightforward either. Over the 2 feeding intervals about half the containers showed a significant reduction in total chloro-pigments, but all those containing zooplankton showed a marked decrease in the chlorophyll/pheopigment ratios (Table 4). A shift in the chlorophyll/pheopigment ratio would be anticipated if copepod grazing had occurred (Mackas & Bohrer 1976), although loss of pigment during grazing is also recognized (Helling & Baars 1985) and enzymatic breakdown of the chlorophyll *a* molecule can also occur *in vitro* (Owens & Falkowski 1982). While this experiment did not yield a clear answer for the question asked, there was no evidence that prolonged exposure of wild zooplankton to conditions in the South Camp wet lab was seriously detrimental to them.

Effect of size on metabolism

For the last 4 developmental stages of *Calanus hyperboreus*, there was a strong correlation between $\log_{10}$ of dry weight and that of respiration (Fig. 2), yielding a highly significant (R^2 = 0.794) regression constant (b = 0.839 ± 0.050), similar to that found for 'boreal' zooplankton (b = 0.830) by Ikeda (1970). For the second *Calanus* species, *C. glacialis*, a highly significant exponent (b = 0.842 ± 0.057, R^2 = 0.742) was also found for just 3 developmental stages, but there seemed to be no relationship between respiration and size in *Pseudocalanus acuspes* (b = −0.347 ± 0.287, R^2 = 0.055), probably because the experimental population was a heterogeneous mix of sizes and stages.

Similar analysis was carried out using $\log_{10}$ transformations of ammonia and urea excretion rates as dependent variables for the same 3 copepod species

Table 4. Effects of the presence of zooplankton on the concentration and composition of chlorophyll-derived pigments from feeding-metabolism experiments. C/P: chlorophyll *a* (chl *a*)/phaeopigments (pheo.)

Species and stage	13–19 June 1993				19–22 June 1993			
	Chl *a* (µg l^{-1})	Pheo. (µg l^{-1})	Total (µg l^{-1})	C/P	Chl *a* (µg l^{-1})	Pheo. (µg l^{-1})	Total (µg l^{-1})	C/P
Initial/control:	2.24	0.74	2.98	3.03	2.06	0.96	3.02	2.15
Pseudocalanus acuspes	0.37	1.49	1.86	0.25	1.00	1.48	2.48	0.68
(mixed)	0.38	2.18	2.56	0.17	0.77	2.41	3.18	0.32
Calanus glacialis	1.20	2.43	3.63	0.49	0.90	2.02	2.92	0.45
(Stage 4)	0.65	2.04	2.69	0.32	0.73	2.54	3.27	0.29
Calanus glacialis	0.38	2.56	2.94	0.15	0.26	2.70	2.96	0.10
(Stage 6 female)	0.28	2.00	2.28	0.14	0.23	2.30	2.53	0.10
Calanus hyperboreus	0.42	1.78	2.20	0.24	0.34	2.21	2.55	0.15
(Stage 6 female)	2.88	3.34	6.22	0.86	1.74	3.28	5.02	0.53

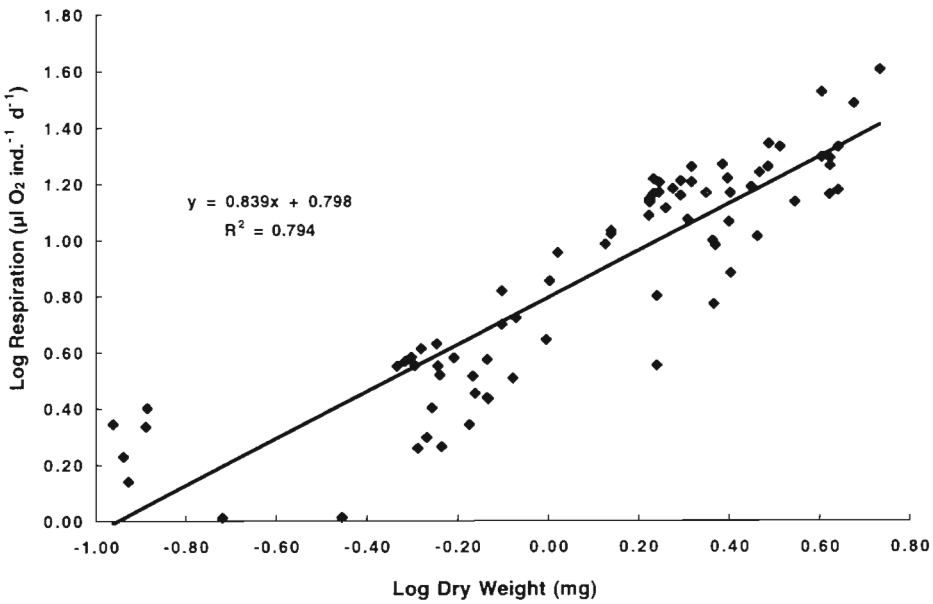

Fig. 2. *Calanus hyperboreus*. Log-log plot of the relationship between respiration and dry body weight

(Table 5). For *Pseudocalanus acuspes* the relations were not significant or, in the case of that for ammonia and dry weight, just barely so. *Calanus hyperboreus* showed no relationship between ammonia excretion and size, but a good correlation of urea excretion with dry weight, although the regression coefficient (0.584) is more characteristic of a tropical, ammoniotelic species than a boreal one (Ikeda 1974). Both regression coefficients were significant for *C. glacialis*, that for ammonia metabolism (0.766) being similar to that for northern forms (0.790) studied by Ikeda (1974). The regression coefficient for urea excretion (0.306) would seem low; however, to our knowledge there have been no previously published results relating urea excretion and size for zooplankton from any environment.

Seasonal variations in metabolism

Although there was no clear and consistent relationship for all the species examined between size and the several manifestations of metabolism, most that were continuously available over the year showed some form of seasonal change in respiration and excretion. For example, *Pseudocalanus acuspes*, which was generally the most abundant species in 100 µm tows, showed a clear peak in respiration in early June for mixed late developmental stages (Fig. 3A, Table 3). A somewhat similar trend was shown in the excretion measurements (Fig. 3B), even though the early spring information is lacking because of problems with the spectrophotometer. Note however that urea excretion exceeded that for ammonia, particularly in spring and again in fall.

The peak in late spring-early summer metabolic activity was definitely not related to temperature because the ocean was still frozen at this latitude until mid-July and water temperatures were well below zero (~−1.7°C). There was a downward trend in mean weight for this size category of *Pseudocalanus acuspes* between late February and late April but no further change through the summer and fall, even though

Table 5. Relationship between $\log_{10}$ dry weight (X) and $\log_{10}$ nitrogen excretion rate (ammonia [Y_a] or urea [Y_u]) for 3 of the zooplankton species studied. ns: not significant

Species	No. of observations	Regression equations	R^2	F	Significance of F
Calanus hyperboreus	36	$Y_a = 0.430 + 0.374X_a$	0.099	3.747	0.061 ns
	37	$Y_u = 0.657 + 0.584X_u$	0.345	18.452	0.00013
Calanus glacialis	57	$Y_a = 0.863 + 0.766X_a$	0.357	30.507	9.35×10^{-7}
	54	$Y_u = 0.787 + 0.306X_u$	0.127	7.572	0.00814
Pseudocalanus acuspes	15	$Y_a = -3.83 + -2.00X_a$	0.273	4.871	0.046
	16	$Y_u = -1.04 + -0.35X_u$	0.151	2.486	0.137 ns

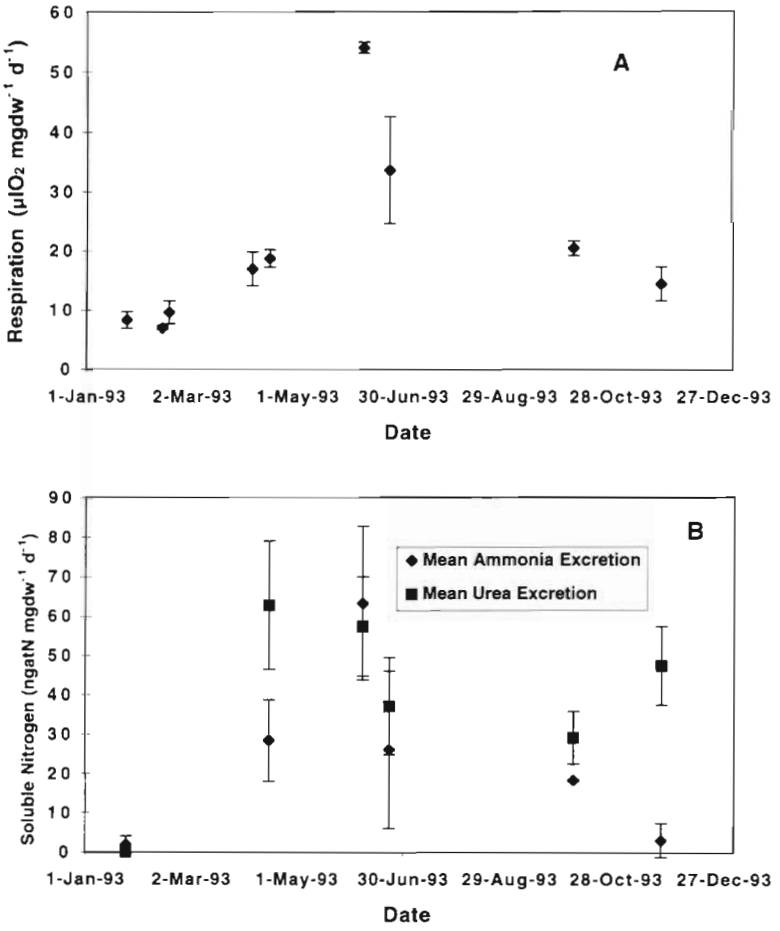

Fig. 3. *Pseudocalanus acuspes*. Seasonal distribution of weight-specific (A) respiration and (B) excretion of ammonia and urea nitrogen for mixed late stages. Vertical bars represent 1 SD

there was an increase in metabolism per individual organism. The peak correlates well with increasing light, occurring about the time of annual maximum irradiance at the ice surface. There might also be a relationship between *P. acuspes* and its food supply as this species can feed on ice algae (Conover et al. 1986), and there was a modest peak in total chlorophyll (chlorophyll and pheophorbide), averaging 1.2 µg l^{-1}, in the upper 10 m of the water column on 8 June 1993. Laboratory feeding experiments did not induce a metabolic increase (Table 3), but the initial level of respiration in those experiments was the highest observed for the entire year.

Calanus hyperboreus, which is larger than *Pseudocalanus* spp. by 10^3 times in individual biomass, showed similar metabolic cycles (Fig. 4A,B), but slightly different explanations might apply here. As in the case of *P. acuspes*, this large *Calanus* species also lost weight during the spring, especially in the adult females which can reproduce without feeding; however, gravid females also respired more rapidly than immature individuals (Conover 1962), which probably contributed to the increased metabolism in April and May. By June, most females were spent or newly molted, but their weight-corrected respiration and excretion rates were still quite high, although excretion decresed more rapidly than respiration later in the month (Fig. 4A,B). Comparable measurements on copepodid Stage 4 of *C. hyperboreus* showed similar seasonal patterns to those of the females except that the peaks were later, after the phytoplankton bloom in the ice-free water column had become established, or, in the case of excretion, had even begun to sink (Fig. 5A,B) (Conover et al. 1999).

Effect on O:N ratios

As shown in Table 1, the O:N ratio for *Pseudocalanus acuspes* based on ammonia excretion alone was in the range 16 to 81, suggesting protein- or mixed protein and lipid-based metabolism, but the inclusion of urea excretion dramatically reduced the ratio. However, the excretion of urea in high latitude environments has rarely been measured and included in the O:N calculations. In the case of *P. acuspes*, the O:N ratio also showed a spring increase (Fig. 6) that closely paralleled the other measures of metabolism (Fig. 3A,B), indicating that the increase in respiration was proportionately greater than that for nitrogen excretion, and thus net nitrogen incorporation could be occurring, which is an important consideration! A similar, parallel pattern of decrease in the O:N ratio occurred in October and November. Only in January, when nitrogen excretion was minimal, were O:N ratios greater than 90 observed.

In Table 6 are shown O:N measurements for several so-called 'herbivorous' arctic copepods of the genus *Calanus*, which are believed to depend on lipid-based metabolism to survive long periods of deprivation when darkness prevents primary production. Yet over most of the year in the water near Resolute their O:N ratios generally followed a pattern of fluctuation similar to that of respiration and nitrogen excretion, although these ratios were often greater than those observed for *Pseudocalanus acuspes*, therefore also suggesting a positive nitrogen balance. That *Metridia longa*, a long-recognized omnivore, and *Euchaeta gra-*

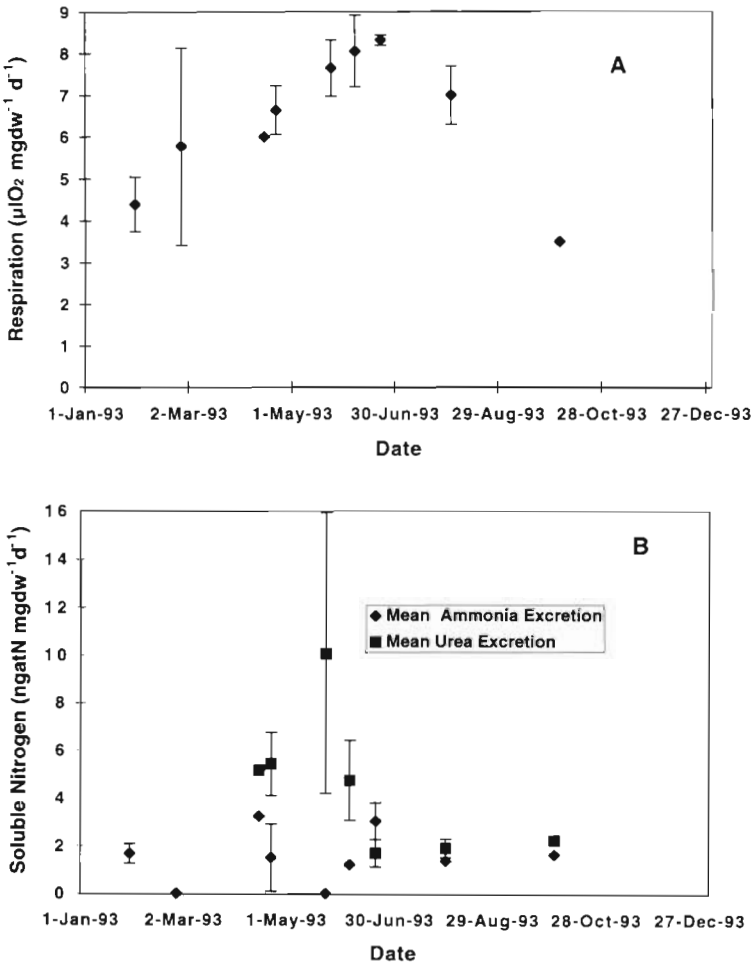

Fig. 4. *Calanus hyperboreus*. Seasonal distribution of weight-specific (A) respiration and (B) excretion of ammonia and urea nitrogen for adult females. Vertical bars represent 1 SD

cilis, an obligate carnivore, have low O:N ratios is not surprising (Ikeda 1977), but all the herbivores apparently depended to a considerable degree on 'protein' metabolism in most seasons of the year when their O:N ratios also included urea excretion, which in retrospect is perhaps not too surprising either.

DISCUSSION

Our use of a sea water aquarium for physiological experiments with zooplankton in the high arctic was generally successful: zooplankton in captivity did not differ greatly in their metabolic responses from freshly captured individuals, nor did increasing the available food to a concentration of chlorophyll considerably above ambient concentrations have a consistent influence on either respiration or excretion. However, there was generally a statistically significant relationship between dry weight and respiration in the *Calanus* species (Fig. 2) and a similar relationship with urea excretion, and also with ammonia excretion, but only for *C. glacialis*. For *Pseudocalanus acuspes* there were no significant size/metabolism relationships, most probably because of the narrow range of sizes and mixed developmental stages used in the experiments.

We have pointed out the general similarities among our size-related regression co-

Table 6. Forms of nitrogen in zooplankton excretory products and their effect on the O:N ratio (by atoms)

Species and stage	Number of experiments	Mean O:N ratio Ammonia alone	Urea alone	Summed	SD (summed)
Calanus hyperboreus (Stage 6 female)	20	174.58	66.00	47.89	16.07
C. hyperboreus (Stage 6 male)	4	266.92	263.24	132.53	18.94
C. hyperboreus (Stage 5)	11	46.15	118.92	32.25	9.79
C. hyperboreus (Stage 4)	18	45.78	51.33	24.20	5.92
C. hyperboreus (Stage 3)	6	39.57	50.36	22.16	21.11
C. glacialis (Stage 6 female)	15	39.50	72.82	25.61	7.45
C. glacialis (Stage 6 male)	6	60.70	40.55	24.31	4.61
C. glacialis (Stage 5)	13	43.38	67.76	26.45	12.09
C. glacialis (Stage 4)	25	45.03	26.11	16.53	6.54
Pseudocalanus acuspes (mixed stages)	19	38.68	25.27	15.29	14.18
Metridia longa (mixed stages)	6	23.60	23.75	11.84	3.78
M. longa (Stage 6 female)	1	11.72	25.21	8.00	–
Euchaeta gracilis (Stage 6 female)	3	22.12	44.45	14.77	7.77
E. gracilis (Stage 5)	2	26.11	54.06	17.61	4.23
Xanthocalanus borealis (mixed stages)	1	17.84	39.94	12.33	–

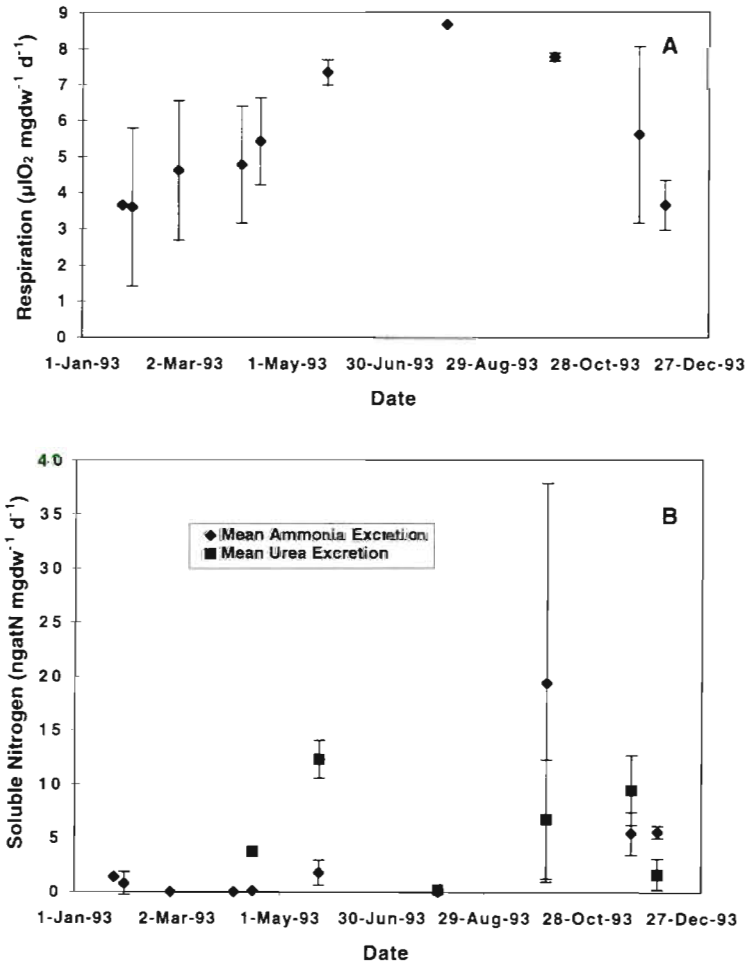

Fig. 5. *Calanus hyperboreus*. Seasonal distribution of weight-specific (A) respiration and (B) excretion of ammonia and urea nitrogen for copepodid Stage 4. Vertical bars represent 1 SD

efficients for respiration and ammonia excretion for several species of arctic copepods and those reported by Ikeda (1970, 1974) for 'boreal' zooplankton species. We did not use the geometric mean regression (GM) model recommended by Ricker (1973) to describe our data, nor did Ikeda. However, Vidal & Whitledge (1982) have also examined metabolism of zooplankton from several latitudes and compared their results with those of Ikeda (1970, 1974) using the traditional least-squares regression model and the GM model and, unlike Ikeda, found no significant differences in the slopes of allometric relationships between tropical and boreal zooplankton populations. They also found the regression coefficients for the GM regression to be slightly, but generally less than 5%, higher than those for the usual least-squares regressions. As we mentioned earlier, the exponential slopes describing the relationship between dry body weight and urea excretion for the *Calanus* species from the Resolute area did not compare well with the respiration and ammonia excretion coefficients from the same area, nor with those given by Ikeda (1970, 1974) and Vidal & Whitledge (1982) for high latitude populations. At this time we do not have an explanation for this discrepancy, but, if urea excretion is a response to environmental change such as temperature or, more likely, osmotic conditions, physiological or biochemical change would seem to have precedence over size-related allometry.

For the *Calanus* species and for *Pseudocalanus acuspes* there were important seasonal changes in metabolism. There were peaks in both respiration and excretion, and also O:N ratio, during the spring-summer period which varied with species, and, where sufficient data were available, also with stage. Temperature was probably not a factor as the seasonal changes in the high arctic water column were about 1°C over the year.

Off Resolute, a spring bloom of ice algae was initiated in early April, which was apparently heavily grazed by *Pseudocalanus acuspes* (Conover et al. 1986), thus contributing to high weight-specific excretion of urea at that time to be followed by peak respiration and ammonia excretion in early June (Fig. 3A,B) as ice algal populations were starting to melt off the ice. On first consideration, our failure to induce further metabolic increase by feeding would appear to contradict these observations, until it is recognized that the data for *P. acuspes* in Table 3 are the same as the June data in Fig. 3. Indeed our timing was bad; a further increase in metabolic activity in the presence of food would not have been anticipated at or near the metabolic peak for the year. Nonetheless, these seasonal changes in metabolism would appear directly comparable to striking changes in respiration and excretion (ammonia only), accompanied by statistically significant increases in surface chlorophyll and in gut pigment observed between April 25 and May 30, 1986, at Resolute (Bedo et al. 1990). However, both sets of observations are in marked contrast with the generally unchanging bulk metabolism measurements on Resolute Bay zooplankton made by Welch et al. (1997).

For female *Calanus hyperboreus* maximum weight-specific urea excretion was in late May followed again by respiration and ammonia excretion peaks in June (Fig. 4A,B), although there was no clear evidence of feeding by the zooplankton. Similarly, for *C. hyperboreus* Stage 4, peak urea production also occurred in

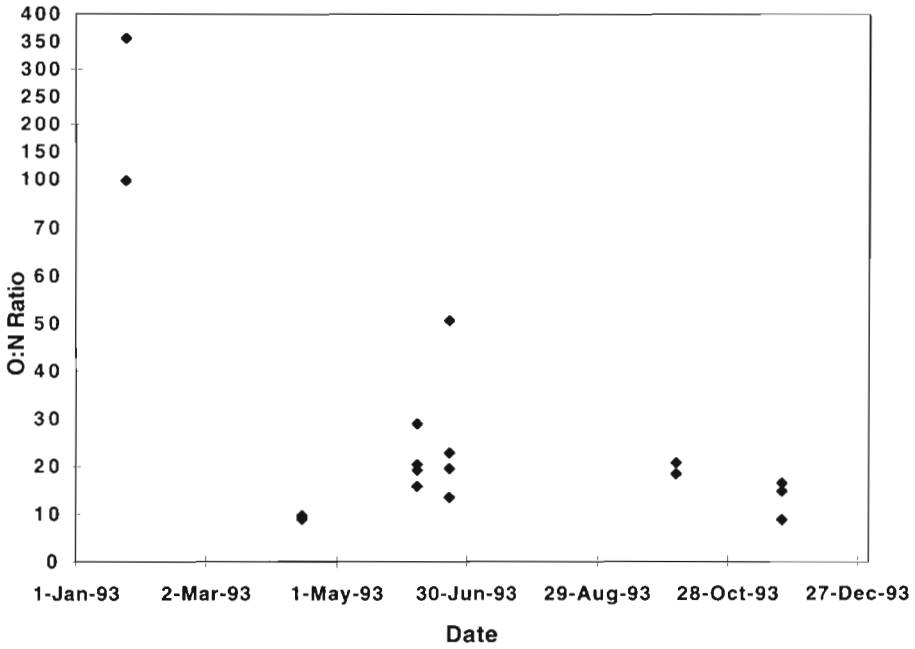

Fig. 6. *Pseudocalanus acuspes*. Seasonal distribution of the O:N ratio based on the same data used to construct Fig. 3

May, but that for respiration was in August, when there was plenty of phytoplankton in the water, and that for ammonia excretion in October, when particulate carbon was still quite high (Fig. 5A,B). Similar seasonal cycles in metabolism (but also without urea measurements) were observed for *Calanus hyperboreus*, and also for *Metridia longa* and several other species from the Gulf of Maine, USA, except that peaks were earlier in the year, apparently coinciding with the annual 'spring' bloom in March (Conover & Corner 1968). There, laboratory feeding studies regularly showed higher metabolic rates in the presence of food, and statistical analysis of field populations demonstrated that highest metabolic rates were associated with low dry weight:body N ratios, low fat, and probably high growth rates in response to increased chlorophyll in the water column.

Regardless of seasonal effects, the most interesting and unique aspect of this study was the unexpectedly large amount of urea excreted by almost all the copepods examined at all seasons, resulting in considerably reduced O:N ratios (Table 6). The exceptions were male *Calanus hyperboreus*, which do not feed and are short-lived, surviving almost entirely on stored lipid. While the number of observations of nitrogen excretion with boreal or arctic species, which have also included measurement of urea production, are few, several examples have been examined. Conover & Cota (1985) looked at soluble nitrogen excretion for 4 stages of *C. hyperboreus* and late stages of *C. glacialis* from northern Baffin Bay and Lancaster Sound, Canada, during open water season, finding a range of urea excreted as percentage of ammonia excreted between 5.4 and 13.4. Urea excretion rates were somewhat higher, but still less than in the Canadian Arctic, for *C. hyperboreus* and *Metridia longa* from Kosterfjorden (58° 52' N, 11° 06' E) on the west coast of Sweden, which ranged from 12.5 to 23.4% and 14.7 to 35.9% respectively as a fraction of ammonia excretion, with some of the variance attributable to seasonal change (Båmstedt 1985).

An important question to ask now is whether the differences observed between environments and, more importantly, between the same species in different environments, is genetic or in some way related to differences in environmental conditions that might or might not be seasonally induced. A second question would be to what extent these genetic or environmental influences affect the amount and distribution of urea in marine environments, especially in high latitudes. Here we will suggest some alternative answers to the first of these questions; possible answers to the second question are examined in the accompanying paper (Conover et al. 1999).

Urea is among the simpler organic compounds containing both carbon and nitrogen, and biochemically would seem quite easy for living systems to synthesize if water is plentiful in the environment. In mammals and a few fish, the ornithine-urea cycle (OUC) generally prevails, and is usually identified by the enzymes catalyzing the several reactions. Thus arginase causes the breakdown of the essential amino acid arginine to form urea and ornithine; this reacts with carbamoyl phosphate under the influence of ornithine-carbomoyl

transferase to yield citrulline, which combines with aspartate and ATP to form arginino-succinate catalyzed by arginino-succinate synthetase; arginino-succinate lyase breaks down arginino-succinate to arginine and fumarate, thus completing the cycle (Regnault 1987). However, in some organisms, including the Crustacea, the necessary enzymes to regenerate arginine are not available internally, in which case the OUC may not be functional.

That crustaceans are ammonotelic is not in dispute here but it has long been recognized that most species excrete some urea. The most common alternative mechanism for urea synthesis would seem to be the uricolytic system, by means of which nucleic acids are degraded through the purine bases to uric acid and thence to allantoin, allantoic acid, and urea plus glyoxylic acid under the influence of the enzymes carbamoyl phosphate synthetase (CPS type 2), uricase, allantoinase, and allantoicase respectively (Wood 1993). Such a mechanism could account for the usual estimates for urea excretion by crustaceans of 1 to 5% (Regnault 1987).

Indeed very little more is known about the physiology and biochemistry of urea excretion in the Class Crustacea. Apparently osmotic stress can significantly increase urea excretion in the crayfish *Orconectes rusticus* (Sharma 1966, 1968). Although these experiments were not performed in the arctic, they might suggest an alternative approach for the study of certain crustacean species living at the ice-water interface which regularly undergo osmotic stress during the melt season, and respond by increasing ammonia excretion (Aarset 1991). Apparently urea excretion was not examined by Aarset.

Another example might be provided by data on urea distribution and excretion in the Kuruma shrimp *Penaeus japonicus*, a species much used in aquaculture in the Far East where the environment may be considerably affected by high concentrations of ammonia and nitrite. Concentrations of 5.166 mg l^{-1} or greater of ambient ammonia in the environment induced a switch from ammonotelism to ureotelism in this species within 24 h, but there was no information on the pathway (Chen & Cheng 1993). High concentrations of nitrite caused an increase of both ammonia and urea in the haemolymph of the same shrimp, but no increase in urea excretion (Chen & Cheng 1995). Note that these ammonia concentrations are several orders of magnitude greater than those found in the Canadian arctic (Conover et al. 1999).

While all cartilaginous fishes have the ornithine-urea enzymes, very few bony fishes utilize the OUC pathway and accordingly most excrete less than 25% of their nitrogenous waste as urea (Wood 1993). The exceptions are usually freshwater fishes which inhabit highly alkaline environments such as the tilapia species *Oreochromis alcalicus grahami* from Lake Magadi, Kenya (pH = 10), which has the OUC enzymes and must be considered ureotelic (Randall et al. 1989, Wood et al. 1989, 1994). However, there is no evidence that OUC enzymes can be induced by subjecting a fish belonging to the same genus, and originally from the same continent, *O. niloticus*, to conditions favorable to the generation of excess ammonia. When subjected to 1 mM NH_4Cl solution or freshwater at pH = 10 for 7 d, urea excretion increased by 3- to 5-fold but only the uricolytic enzyme allantoicase activity significantly increased (Wright 1993).

At this time we know nothing about the mechanism for urea production in the crustacean zooplankton of the high arctic ocean or the causes, but there are several environmental conditions common to the arctic which, by analogy with observations on other crustaceans and fish, might induce urea production by 1 or more enzyme pathways. We have already mentioned the potentially severe osmotic stress on animals and plants living at the ice-water interface occurring during the annual melt and de-salinization, which may persist for 6 or more weeks prior to break-up. At freeze-up, the accompanying brine rejection, re-salinization and the formation of the cold halocline (Aagaard et al. 1981) may well generate a reverse osmolytic stress, the effects of which on nitrogen cycling are not presently clear to us. On the other hand, the extremely concentrated community of sympagic organisms, both plant and animal, living on the under-ice surface would presumably have the potential of regenerating considerable, potentially toxic ammonia if no mechanism, such as conversion to urea, were available. Determination of the enzyme pathways for urea production in the sympagic zone, in the planktonic communities, and perhaps also in the arctic cod should be given high priority in planning future polar research.

Acknowledgements. We are very grateful to Harold (Buster) Welch and the other participants in POLAR PRO, particularly to Cathy Welch, who kept us from starvation, and to Tim Siferd, Henry Landry, Philip Bruecker, Peter Amarualik and Shawn MacKenzie, who made the field work possible. The Polar Continental Shelf Project (Energy Mines and Resources Canada) provided valuable logistical support, and the 'comforts of home', during part of the year. One of us (K.R.G.) received support from a NSERC Post Graduated Scholarship (PGS1 and 2), a NSERC Operating Grant to R.J.C. (#OGP0002979) and a Department of Indian Affairs and Northern Development, Northern Scientific Training Grant.

LITERATURE CITED

Aagaard K, Coachman LK, Carmack EC (1981) On the halocline of the Arctic Ocean. Deep-Sea Res 28A:529–545

Aarset AV (1991) The ecophysiology of under-ice fauna. In: Sakshaug E, Hopkins CCE, Øritsland NA (eds) Proc Pro Mare Symp Polar Mar Ecol, Trondheim, 12–16 May 1990. Polar Res 10(1):309–324

Båmstedt U (1985) Seasonal excretion rates of macrozooplankton from the Swedish west coast. Limnol Oceanogr 30:607–617

Bedo AW, Head EJH, Conover RJ, Horne EPW, Harris LR (1990) Physiological adaptations of an under-ice population of *Pseudocalanus* in Barrow Strait (N.W.T.) to increasing food supply in spring. Polar Biol 10:561–570

Bury SJ, Owens NJP, Preston T (1995) ^{13}C and ^{15}N uptake by phytoplankton in the marginal ice zone of the Bellingshausen Sea. Deep-Sea Res II 42:1225–1252

Chen JCh, Cheng ShY (1993) Urea excretion by *Penaeus japonicus* Bate exposed to different concentrations of ambient ammonia. J Exp Mar Biol Ecol 173:1–9

Chen JCh, Cheng ShY (1995) Accumulation of urea in the haemolymph and ammonia excretion of *Penaeus japonicus* exposed to ambient nitrite. Comp Biochem Physiol 110C:1–6

Conover RJ (1962) Metabolism and growth in *Calanus hyperboreus* in relation to its life cycle. Rapp PV Cons Int Explor Mer 153:190–197

Conover RJ (1978) Transformation of organic matter. In: Kinne O (ed) Marine ecology, Vol IV. Dynamics. John Wiley & Sons Ltd, Chichester, p 221–499

Conover RJ, Corner EDS (1968) Respiration and nitrogen excretion by some marine zooplankton in relation to their life cycles. J Mar Biol Assoc UK 48:49–75

Conover RJ, Cota GF (1985) Balance experiments with arctic zooplankton. In: Gray JS, Christiansen ME (eds) Marine biology of polar regions and effects of stress on marine organisms. John Wiley & Sons Ltd, Chichester, p 217–236

Conover RJ, Herman AW, Prinsenberg SJ, Harris LR (1986) Distribution of and feeding by the copepod *Pseudocalanus* under fast ice during the arctic spring. Science 232: 1245–1247

Conover RJ, Bedo AW, Spry JA (1988) Arctic zooplankton prefer living ice algae: a caution for zooplankton excretion measurements. J Plankton Res 10:267–282

Conover RJ, Mumm N, Bruecker P, MacKenzie S (1999) Sources of urea in arctic seas: seasonal fast ice? Mar Ecol Prog Ser 179:55–69

Corner EDS, Newell BS (1967) On the nutrition and metabolism of zooplankton. IV. The forms of nitrogen excreted by *Calanus*. J Mar Biol Assoc UK 47:113–120

Dagg M, Cowles T, Whitledge T, Smith S, Howe S, Judkins D (1980) Grazing and excretion by zooplankton in the Peru upwelling system during April 1977. Deep-Sea Res 27A: 43–59

Eppley RW, Renger EH, Venrick EL, Mullin MM (1973) A study of plankton dynamics and nutrient cycling in the Central Gyre of the North Pacific Ocean. Limnol Oceanogr 18:534–551

Gustavson KR (1992) Nutrition and distribution of the arctic calanoid copepod *Pseudocalanus acuspes* during spring and summer in Resolute Passage and Barrow Strait, N.W.T., Canada. MSc thesis, Dalhousie University, Halifax

Harrison WP, Head EJH, Conover RJ, Longhurst AR, Sameoto DD (1985) The distribution and metabolism of urea in the eastern Canadian Arctic. Deep-Sea Res 32:23–42

Harvey WA, Caperon J (1976) The rate of utilization of urea, ammonium, and nitrate by natural populations of marine phytoplankton in a eutrophic environment. Pac Sci 30: 329–340

Helling GR, Baars MA (1985) Changes of the concentrations of chlorophyll and phaeopigment in grazing experiments. Hydrobiol Bull 19:41–48

Horrigan SG, McCarthy JJ (1981) Urea uptake by phytoplankton at various stages of nutrient depletion. J Plankton Res 3:403–414

Ikeda T (1970) Relationship between respiration rate and body size in marine plankton animals as a function of the temperature of habitat. Bull Fac Fish Hokkaido Univ 21:91–112

Ikeda T (1974) Nutritional ecology of marine zooplankton. Mem Fac Fish Hokkaido Univ 22:1–97

Ikeda T (1977) The effect of laboratory conditions on the extrapolation of experimental measurements to the ecology of marine zooplankton IV. Changes in respiration and excretion rates of boreal zooplankton species maintained under fed and starved conditions. Mar Biol 41:241–252

Jawed M (1969) Body nitrogen and nitrogenous excretion in *Neomysis rayii* Murdoch and *Euphausia pacifica* Hansen. Limnol Oceanogr 14:748–754

Kavakina SV, Sapozhnikov VV (1994) Major patterns of urea distribution in the Bering Sea and its role in the nitrogen cycle. Oceanology 33:768–773

Kristiansen S (1983) Urea as a nitrogen source for phytoplankton in Oslofjord. Mar Biol 74:17–24

Kristiansen S, Farbrot T (1991) Nitrogen uptake rates in phytoplankton and ice algae in the Barents Sea. In: Sakshaug E, Hopkins CCE, Øritsland NA (eds) Proc Pro Mare Symp Polar Mar Ecol, Trondheim, 12–16 May 1990. Polar Res 10(1):187–192

Kristiansen S, Lund BAa (1989) Nitrogen cycling in the Barents Sea—I. Uptake of nitrogen in the water column. Deep-Sea Res 36:255–268

Kristiansen S, Syvertsen EE, Farbrot T (1992) Nitrogen uptake in the Weddell Sea during late winter and spring. Polar Biol 12:245–251

Le Borgne R (1977) Étude de la production pélagique de la zone équatoriale de l'Atlantic a 4° W. III. Respiration et excrétion d'azote et de phosphore du zooplancton. Cah ORSTOM Sér Océanogr 15:349–362

Levy EM, Cunningham CC, Conrad CDW, Moffat JD (1977) The determination of dissolved oxygen in sea water. Bedford Institute of Oceanography Report Series BI-R-77-9

Lomstein BAa, Blackburn TH, Henriksen K (1989) Aspects of nitrogen and carbon cycling in the northern Bering Shelf sediment. I. The significance of urea turnover in the mineralization of NH_4^+. Mar Ecol Prog Ser 57:237–247

Mackas D, Bohrer R (1976) Fluorescence analysis of zooplankton gut contents and an investigation of diel feeding patterns. J Exp Mar Biol Ecol 25:77–85

Mayzaud P, Conover RJ (1988) O:N atomic ratio as a tool to describe zooplankton metabolism. Mar Ecol Prog Ser 45: 289–302

McCarthy JJ (1970) A urease method for urea in sea water. Limnol Oceanogr 15:309–313

Mitamura O, Saijo Y (1980) Urea supply from decomposition and excretion of zooplankton. J Oceanogr Soc Jpn 36: 121–125

Owens TG, Falkowski PG (1982) Enzymatic degradation of chlorophyll *a* by marine phytoplankton *in vitro*. Phytochemistry 21:979–984

Pedersen H, Lomstein BAa, Blackburn TH (1993a) Evidence for bacterial urea production in marine sediments. FEMS Microbiol Ecol 12:51–59

Pedersen H, Lomstein BAa, Isaksen MF, Blackburn TH (1993b) Urea production by *Thiosphaera pantotropha* and by anaerobic enrichment cultures from marine sediments. FEMS Microbiol Ecol 13:31–36

Randall DJ, Wood CM, Perry SF, Bergman HL, Maloiy GMO, Mommsen TP, Wright PA (1989) Urea excretion as a strategy for survival in a fish living in a very alkaline environment. Nature 337:165–166

Regnault M (1987) Nitrogen excretion in marine and freshwater Crustacea. Biol Rev 62:1–24

Remsen CC (1971) The distribution of urea in coastal and oceanic waters. Limnol Oceanogr 16:732–740

Remsen CC, Carpenter EJ, Schroeder BW (1974) The role of urea in marine microbial ecology. In: Colwell RR, Morita RY (eds) Effect of the ocean environment on microbial activities. University Park Press, Baltimore, p 286–304

Ricker WE (1973) Linear regressions in fisheries research. J Fish Res Board Can 30:409–434

Sapozhnikov VV, Propp LN (1990) Major patterns of urea distribution in Antarctic waters. Oceanology 30:144–147

Sharma M (1966) Studies on the changes in the pattern of nitrogenous excretion of *Orconectes rusticus* under osmotic stress. Comp Biochem Physiol 19:681–690

Sharma M (1968) Studies on the sources and mechanisms of increased urea production by *Orconectes rusticus* under osmotic stress. Comp Biochem Physiol 24:55–60

Siegel S (1956) Nonparametric statistics for the behavioral sciences. McGraw-Hill, New York

Smith SL (1978a) Nutrient regeneration by zooplankton during a red tide off Peru, with notes on biomass and species composition of the zooplankton. Mar Biol 49: 125–132

Smith SL (1978b) The role of zooplankton in the nitrogen dynamics of a shallow estuary. Estuar Coast Mar Sci 7: 555–565

Smith S, Whitledge T (1982) Regeneration of nutrients by zooplankton and fish off northwest Africa. Rapp PV Réun Cons Int Explor Mer 180:206–208

Solórzano L (1969) Determination of ammonia in natural waters by the phenolhypochlorite method. Limnol Oceanogr 14:799–801

Therkildsen MS, Lomstein BAa (1994) Seasonal variation in sediment urea turnover in a shallow estuary. Mar Ecol Prog Ser 109:77–82

Vidal J, Whitledge TE (1982) Rates of metabolism of planktonic crustaceans as related to body weight and temperature of habitat. J Plankton Res 4:77–84

Walsh JJ, McRoy CP, Coachman LK, Goering JJ, Nihoul JJ, Whitledge TE, Blackburn TH, Parker PL, Wirick CD, Shuert PG, Grebmeier JM, Springer AM, Tripp RD, Hansell DA, Djenidi S, Deleersnijder E, Henriksen K, Lund BA, Andersen P, Müller-Karger FE, Dean K (1989) Carbon and nitrogen cycling within the Bering/Chukchi Seas: source regions for organic matter effecting AOU demands of the Arctic Ocean. Prog Oceanogr 22:277–359

Welch HE, Bergmann MA, Jorgenson JK, Burton W (1988) A subice suction corer for sampling epontic ice algae. Can J Fish Aquat Sci 45:562–568

Welch HE, Siferd TD, Bruecker P (1997) Marine zooplanktonic and benthic community respiration rates at Resolute, Canadian high Arctic. Can J Fish Aquat Sci 54:999–1005

Wood CM (1993) Ammonia and urea metabolism and excretion. In: Evans DH (ed) The physiology of fishes. CRC Press, Boca Raton, p 379–425

Wood CM, Perry SF, Wright PA, Bergman HL, Randall DJ (1989) Ammonia and urea dynamics in the Lake Magadi tilapia, a ureotelic teleost fish adapted to an extremely alkaline environment. Respir Physiol 77:1–20

Wood CM, Bergman HL, Laurent P, Maina JN, Narahara A, Walsh PJ (1994) Urea production, acid-base regulation and their interactions in the Lake Magadi tilapia, a unique teleost adapted to a highly alkaline environment. J Exp Biol 189:13–36

Wright PA (1993) Nitrogen excretion and enzyme pathways for ureagenesis in freshwater tilapia (*Oreochromis niloticus*). Physiol Zool 66:881–901

Yentsch CS, Menzel DW (1963) A method for the determination of phytoplankton chlorophyll and phaeophytin by fluorescence. Deep-Sea Res 10:221–231

Editorial responsibility: Otto Kinne (Editor), Oldendorf/Luhe, Germany

Submitted: November 28, 1997; Accepted: November 18, 1998
Proofs received from author(s): March 31, 1999