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The Effects of Temperature on Metabolic Rates of Different Life Stages of *Calanus glacialis* in the Barents Sea

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Summary. The effects of temperature on rates of respiration, excretion and gut evacuation were examined for copepodite stages and adult female *Calanus glacialis* collected in areas close to the ice-edge during the arctic summer in the Barents Sea. The various life history stages responded differently to acute temperature changes above the in situ temperature (ca. −1.7 °C). Respiration rates of early copepodite stages (C I to C IV) were very variable whereas excretion rates declined with increasing temperature in the range from −1.7 °C to +5 °C. Rate of oxygen consumption of adult females were independent of temperature between −1.7° and +5 °C, but increased as temperature increased from +5° to 10 °C. Rates of excretion of copepodite stage V and adult females were independent of temperature in the range from −1.7° to +2 °C, whereas excretion of copepodite stages III and IV was negatively related to temperature in the range from −1.7° to +5 °C. In C IV, C V and adult females the instantaneous rate of gut evacuation increased with increasing temperature. The different response patterns of metabolic rates of small copepodite stages, copepodite stage V and adult females *C. glacialis* to acute temperature changes suggest that the capacity for adjustment of ammonia excretion is better developed in C V's and adult females than in the younger life stages.

Introduction

Most of our knowledge about the metabolic rates of animals from Polar regions stems from work designed to compare temperature-dependent respiration rates of warm, temperate and cold water species (see for instance Scholander et al. 1953). The prevailing conclusion from these studies is that species living in Polar regions have evolved a temperature-compensated metabolism with respect to their warm-water and temperate counterparts (Wohlschlag 1960, 1964). However, arguments against the emperical concept of metabolic cold adaptation have been emphasised by Everson (1977) and Clark (1980, 1983).

Much of the extensive literature of the effects of temperature on individual rate functions of marine organisms derives from studies of temperate species (see for instance Kinne 1970) and, consequently, the acute responses of any particular physiological process to changes in temperature have usually been monitored over a large temperature range intermeasurement intervals (>5 °C). The seasonal variation in sea temperature in arctic waters of the Barents Sea is very restricted (Tantiura 1959), with maximal annual variations in sea surface temperatures of only 3°–4 °C. Thus, the thermal environment of planktonic copepods inhabiting the Barents Sea is relatively stable but virtually nothing is known about how temperature influences the rates of metabolism of the copepods. Since arctic poikilotherms are usually subject to only minor variations in temperature, an examination of an integrated metabolic response pattern should be conducted using narrow temperature intervals within the seasonal temperature variation present in the natural habitat.

The present study was conducted in order to get basic information of in situ rates of respiration, excretion and gut evacuation for copepodite stages and adult females *Calanus glacialis* during spring bloom conditions in the Barents Sea. Data relating to the metabolic response to acute temperature changes are also presented.

Materials and Methods

The results presented in this paper all stem from shipboard experiments conducted on copepodite stages and adult females of *Calanus glacialis* sampled in arctic waters or in polar front areas of the Barents Sea (Fig. 1). Cruises were made in 1983, 1984, and 1986. The area of sampling during the cruise with *RV Lance* in 1983 is described in detail in Tande and Båmstedt (1985) and Båmstedt and Tande (1985).

The first cruise in 1984, with *RV G.O. Sars*, lasted from May 28 to June 18, and the station positions are given in Fig. 1. Most of the sam-

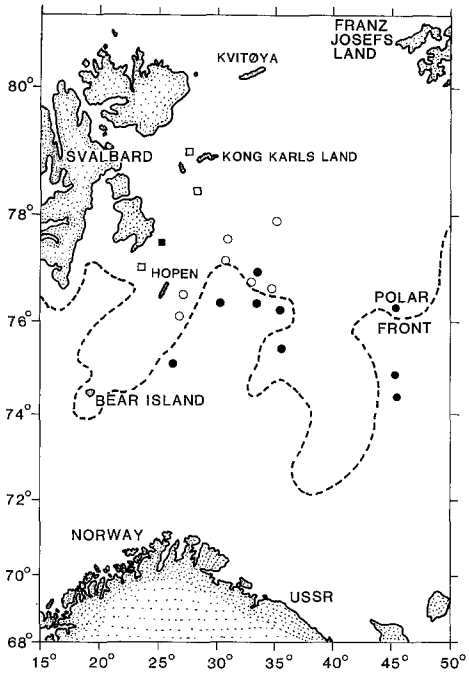

Fig. 1. The area of the Barents Sea covered during the cruises in 1983, 1984 and 1986. Dashed lines define the Polar Front area. Symbols: ○ = cruise from May 23 to June 16 in 1983; ● = cruise from May 28 to June 18 in 1984; □ = cruise from July 18 to August 15 in 1984; ■ = cruise from May 21 to June 10 in 1986

pling was conducted in Polar Front areas in central parts of the Barents Sea between 75° and 77 °N.

The second cruise in 1984, with *RV Lance*, lasted from July 18 to August 15, and sampling was concentrated in areas of Arctic waters of the east coast off Svalbard from ca. 77° to ca. 81 °N. A few measurements on respiration of adult females were conducted onboard *RV Lance* from May 21 to June 10, 1986. Animals were sampled north to the ice edge in Arctic waters. The hydrography and seasonal variations in the sea ice conditions in these areas of the Barents Sea are given in Tantiura (1959) and Loeng (1979), respectively.

Collection and Handling of Animals

Zooplankton samples were collected using a WP-2 plankton net (0.5 m opening diameter, 200 µm mesh size) connected with a large non-filtering cod end (ca. 30 l) made of PVC. Animals for use in the experiments were always subsampled from hauls taken from 50–0 m of depth. Copepodite stages were gently sorted into beakers of 200 µm screened seawater at in situ temperatures.

Determination of Respiration and Ammonia Excretion Rates

The rates of respiration, excretion and gut evacuation of copepodite stages and adult females of *Calanus glacialis* were measured on individuals sampled from different localities during spring bloom conditions in areas around the ice-edge. In situ metabolic rates were measured immediately after sampling for individuals taken in localities where the sea temperature was < -1.5 °C. The metabolic rates at +2° and 5 °C, here defined as responses to acute changes in temperature, were measured within a maximum of 1 h after sampling. A decline in respiration rate with time after capture have been found in *C. glacialis* (Båmstedt and Tande 1985). The measurements of respiration and excretion have been standardized such that the results represent rates integrated over a period from 6 h to 12 h after capture. Animals used for these measurements were taken from localities where sea temperature and salinity varied from −1° to +2 °C and 34.0‰ to 35.0‰, respectively, over the depth range 0–100 m. Finally, a few respiration measurements were conducted on animals sampled to the north of the ice-edge during a cruise in Arctic waters of the Barents Sea from May 21 to June 10 in 1986.

Respiration measurements of *Calanus glacialis* in the Arctic spring 1983 were obtained using a polarographic technique. Ammonia excretion was determined by a method described by Grasshoff (1976). The procedures have been described in detail by Båmstedt and Tande (1985).

In 1984, respiration rates of *Calanus glacialis* were measured in closed chambers using a micro Winkler technique. The oxygen level in blank and experimental bottles were measured by subsampling 3 ml of water with a 5 ml disposable plastic syringe (Luer type, sabre). Winkler solutes A and B (30 µl of each) were added separately. The syringe was stoppered using a finntip blocked with wax, and the reagents were carefully mixed with the sample by vigorously shaking the syringe. The hydroxide complexes formed were dissolved in 30 µl of 6N sulphuric acid. Optical density was measured on a Vitatron digital concentration photometer at 450 nm wavelength within 30 min. The OD readings were converted to oxygen concentration using a standard curve (ml $O_2\,l^{-1} = 5.84 \times A_{450} - 0.736$; $r^2 = 0.979$; $n = 15$) were derived by relating oxygen concentration (Winkler titration) to photometric measurements (micro Winkler) from replicate subsamples of sets of seawater samples containing different oxygen concentrations. Experiments were conducted by incubating either 1–3 adult females, 4–7 copepodite stage IV or V in glass bottles (ca. 16 ml of volume), or 6–15 copepodite stages I–III in 11 ml glass bottles filled with 200 µm screened seawater adjusted to preferred temperatures. All experiments were run in thermostatted water baths at selected temperatures and experiments were run for a maximum of 12 h.

Rates of oxygen consumption and ammonium excretion were monitored simultaneously with a 3 ml water sample being used for oxygen determinations and a 2 ml sample being used for ammonia analysis. Ammonia was determined by analytical methods described by Grasshoff (1976) with samples being read in a Vitatron digital concentration photometer using a 2 cm flowcuvette. A more detailed description of methodology and the procedure for dry weight determinations is given in Båmstedt and Tande (1985).

Determination of Gastric Evacuation Rate

The gastric evacuation rates of copepodite stages IV and V *Calanus glacialis* were estimated in experiments carried out onboard *RV Lance*. A planktion sample from 50–0 m depth was gently concentrated by filtering through plankton gauze (mesh size 200 µm) held under water. The copepods were then gently washed in GFC filtered seawater and transferred to beakers containing 1 l GFC filtered seawater which had been incubated at predetermined temperatures in thermostated water baths. The gastric evacuation rate was determined by sampling periodically for analysis of gut contents. A detailed description of the analytical procedure for measuring gut fluorescence in copepods is given in Tande and Båmstedt (1985).

Results

Respiration and Excretion Rates

Experiments conducted at +1 °C revealed that the respiration rate decreased with increasing weight (i.e., with stage) in this species (Table 1). Although fewer life stages were examined the same trend was also apparent at −1.7 °C. The mean oxygen consumption rate in C III's and C IV's when measured at three different temperatures varied between 1.09–1.57 and 0.92–1.40 µl O_2 mg dw^{-1} h^{-1}, respectively (Table 1). A Mann-Whitney U-test showed that the respiration rate was not significantly different between −1.7° and +5 °C neither in C III ($P = 0.194$) nor in C IV ($P = 0.129$).

Table 1. *Calanus glacialis.* Respiration rate (µl O_2 mg dw^{-1} h^{-1}) in various life stages at three different temperatures. Values are quoted as mean +95% confidence interval, with number of measurements in brackets. Data for C V's and adult females at −1.7 °C are from Båmstedt and Tande (1985)

Copepodite stages	Temperature (°C)		
	−1.7	+1	+5
I/II		1.98+0.22 (6)	
II			1.10 (2)
II/III		2.33+0.24 (7)	
III	1.38+0.29 (12)	1.57+0.38 (8)	1.09+0.20 (3)
IV	0.92+0.07 (27)	1.40+0.36 (5)	1.09+0.54 (4)
V	0.57+0.08 (21)	0.93+0.21 (7)	
Adult females	0.60+0.08 (26)	0.90+0.25 (6)	

Table 2. *Calanus glacialis.* Excretion rate (nmol NH_4 mg dw^{-1} h^{-1}) in various life stages at three different temperatures. Values are quoted as mean +95% confidence interval, with number of measurements in brackets. Data for C V's and adult females at −1.7 °C are from Båmstedt and Tande (1985)

Copepodite stage	Temperature (°C)		
	−1.7	+1	+5
I/II	8.22 (1)	5.25+2.22 (6)	
II			3.95+1.94 (6)
II/III	5.66 (2)	6.18+1.34 (6)	
III	7.63+3.48 (3)	4.71+2.20 (6)	2.57+1.26 (5)
IV	7.26+1.61 (16)	3.50+0.99 (5)	1.94+1.75 (6)
V	4.75+1.00 (28)	3.42+0.94 (10)	
Adult female	4.73+1.26 (28)	4.72+1.68 (8)	

Respiration rate of adult females *Calanus glacialis* sampled in prebloom waters of −1.5 °C in June 1986 varied between 0.58 and 1.40 µl O_2 mg dw^{-1} h^{-1} at in situ temperature (Fig. 2). The acute response in respiration rate measured at different temperatures above the acclimation temperature of −1.5 °C, showed that the metabolic rate appeared to be unaffected by the temperature in the range of −1.7° to +5 °C. However, respiration rate increased at higher temperatures.

Ammonia excretion rate decreased with increasing body weight, both at −1.7° and +1 °C (Table 2). The effect of acute temperature on excretion rate were measured on C III's and C IV's at three different temperatures and indicated that excretion rate was negatively correlated to temperature from −1.7° to +5 °C. A Mann-Whitney U-test showed that the excretion rate at −1.7 °C was significantly higher than at +5 °C both in C III's ($P = 0.014$) and C IV's ($P = 0.001$). Information from the same temperature range for the other copepodite stages and adult females was not obtained in the present study.

Gastric Evacuation Rate

A combined plot of the instantaneous rate of gut evacuation (R) for copepodite stages IV, and V and adult females *Calanus glacialis* suggests evacuation rate increases with increasing temperature in the range −1.8° to +5 °C (Fig. 3). Further observations, are, however, needed before definite conclusions can be made concerning the acute effect of temperature on the gut evacuation rate of *C. glacialis.*

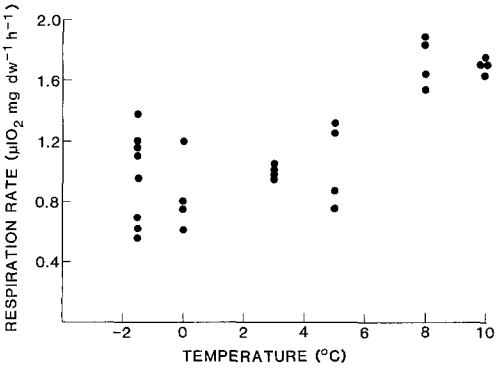

Fig. 2. *Calanus glacialis.* The effect of acute temperature changes on respiration rate in adult females

Discussion

In the present study the majority of the measurements of metabolic rates in *Calanus glacialis* were made in ice edge areas during phytoplankton bloom conditions in May and June. The feeding activity and the nutritional status of the species under study are considered to be important factors determining the levels of metabolic rates (Conover 1962). In this study some of the variability in the metabolic rates was almost certainly caused by differences in nutritional status between animals. Nevertheless, the rates measured at −1.7 °C should be considered as being representative of in situ metabolic rates of *C. glacialis* during the productive season. The response of metabolic rates to acute temperature increases above this temperature present complicated patterns which differ between copepodite stages and adult females of *C. glacialis.*

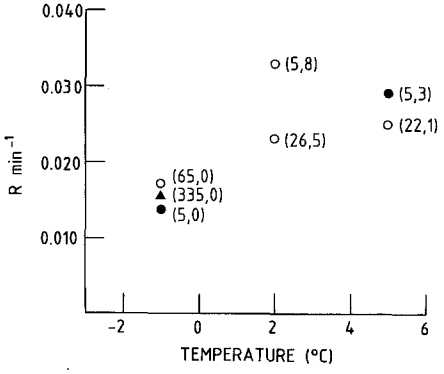

Fig. 3. *Calanus glacialis.* The relationship between temperature and the instantaneous rate of gut evacuation (R). Symbols: ● = CIV; ○ = CV; ▲ = adult females. *Values in brackets* denote initial gut pigment content in ng chl equiv. $anim^{-1}$

It was unexpected that rates of ammonia excretion should be negatively correlated to temperature in copepodite stage I to IV. The biochemical processes that result in ammonia excretion are those concerned with deamination of amino acids. The amino acid carbon chains are used either for the anabolic process of chain elongation in wax ester synthesis (Sargent et al. 1977) or for metabolic fuel. The basis for these processes are biochemical reactions involving cellular and enzymic mechanisms, which in turn define the relationship between excretion rates and temperature. The biochemical mechanisms underlying the different enzyme systems in species living at low temperatures have been reviewed by Somero (1969a) and Hochachka and Somero (1971), and the existence of different enzyme variants with different properties at low and high temperatures have been suggested (Somero 1969a, b). The negative correlation between ammonia excretion rate and temperature in small copepodite stages of *Calanus glacialis* could indicate that the enzyme systems governing these metabolic processes are not functioning at the same level of efficiency at higher temperatures.

Respiration rates of adult females at −1.7 °C were similar when measured either for animals sampled in productive water around the ice edge or for adult females sampled during pre-bloom conditions c. 50 n miles north of the ice edge (Table 1 and Fig. 2). The effects of acute temperature increase on respiration rate measured on groups of adult females sampled at one locality, revealed that the rate of oxygen consumption appeared to be independent of temperature between −1.7° and +5 °C, although rates increased as temperature was increased from 5° to 10 °C. Similar findings have been reported for the antarctic planktonic species *Euphausia superba* collected at −1.27° to +2 °C (McWhinnie 1964). This suggests that there may be a considerable degree of metabolic independence for adults of these two herbivorous zooplankters from polar regions.

Ammonia excretion rates in copepodite stage V and adult females appeared to be independent of temperature between −1.7° and +1 °C. Although a certain amount of scatter is apparent at in situ temperatures, ca. 80% of the measurements fell within 2 and 6 nmole NH_4 mg dw^{-1} h^{-1} which is within the range of all measurements obtained at +1 °C. Ammonia excretion rates have been correlated to food consumption in many species (Jobling 1981), but this has not been demonstrated in herbivorous zooplankton. Although there was considerable variation in excretion rates of *Calanus glacialis* at in situ temperatures, Båmstedt and Tande (1985) concluded that food supply was not a major regulating factor of metabolic activities of this copepod species. Ammonia excretion rates of copepodite stage V and adult females appeared to be temperature independent and this differs from the situation found for small copepodite stages (C I – C IV). The differences in temperature-dependent response patterns between small copepodite stages and copepodite stage V and adult females *C. glacialis* suggest age-specific differences with capacity for adjustment of ammonia excretion being better developed in C V's and adult females than in young life stages such as C I to C IV.

The estimations of the instantaneous gut evacuation rate in *Calanus glacialis* at three different temperatures suggest that the potential for energy intake increased with increasing temperature. A positive relationship between the instantaneous rate of gut evacuation and temperature has also been observed in the copepods *Neocalanus plumchrus* and *N. cristatus* (Dagg and Wyman 1983) and in trout *Salmo trutta* (Elliot 1972) measured in the temperature range from 4° to 9 °C and 5° to 15 °C, respectively. The present data indicated no increase in R between 2° and 5 °C. Defeacation rate is probably proportional to the amount of food in the gut (Holling 1966; Slagstad and Tande 1981). The estimates of R made at 2° and 5 °C were conducted on animals sampled in water masses with ambient chlorophyll concentrations from 0.5–2 μg chl l^{-1}. Gut passage rates of *Neocalanus plumchrus* were found to decrease when the animals were exposed to chlorophyll concentrations in this range (Dagg and Walser 1987), so gut content could have been responsible for the apparent lack of a temperature effect on R, between 2° and 5 °C, seen in the present study with *C. glacialis*.

The rate of ammonia excretion, which reflects deamination of amino acids, could be considered to be indicative of the rate at which metabolic systems are digesting, assimilating and processing nitrogenous substrates. Thus, a decrease in excretion rate with increasing temperature in the copepodite stages of *Calanus glacialis* suggest a decrease in digesting and assimilation efficiencies at higher temperatures. The rate of gut evacuation increases with increasing temperature suggesting that the consumption rate is positively correlated with temperature between −1.7° and +2 °C. In a review on the influence of temperature on metabolism, Newell and Branch (1980) presented evidence for the ability of intertidal animals to make differential adjustments in either energy intake or metabolic energy expenditure in order to maintain positive energy balance. Thus, the different response patterns in respiration, excretion and gut evacuation rates of *C. glacialis* should be viewed as responses of integrated metabolic systems, but there appears to be age-specific differences with respect to the responses shown to different temperature regimes.

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