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A preliminary study on the effects of temperature variability on the metabolic rates of Antarctic Krill under laboratory conditions

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ABSTRACT

The slow but steady increase in temperature in the Antarctic ecosystem has been a cause of concern and raises questions on the adaptability of polar marine ectotherms to the variation in the environmental factors. In this study, metabolic rates of Antarctic Krill were determined in the austral summer of 2009 in the Prydz Bay region of east Antarctica during the 28th Indian Scientific Expedition to Antarctica. Live individuals of *Euphausia crystallorophias* were maintained onboard under controlled laboratory conditions between a temperature range of 0 to 6 °C and the effects of temperature (T) on oxygen consumption (O_2), ammonia excretion (NH_3) and phosphate excretion (PO_4) rates were quantified. Body composition of caught krill was expressed as water (percentage wet weight = 79.4 ± 5.1), Carbon (percentage dry weight= 44.0 ± 0.26), Nitrogen (percentage dry weight = 9.22 ± 0.41), Phosphorus (percentage dry weight = 1.44 ± 0.14 and ash (percentage dry weight = 13.6 ± 2.2). Oxygen consumption increased linearly with temperature from 5.54 ± 0.13 to 7.92 ± 0.30 µl O_2 $indiv^{-1}$ h^{-1} ($r^2 = 0.86$, O_2 rate = 0.5392t + 4.9845). Similar trend was observed with ammonia excretion (0.28 ± 0.03 to 0.61 ± 0.03 µg N $indiv^{-1}$ h^{-1}, NH_3-rate = 0.0686t + 0.2176, $r^2 = 0.88$) and phosphate excretion (0.40 ± 0.02 to 0.73 ± 0.13 µg P $indiv^{-1}$ h^{-1}, PO_4 = 0.0724t + 0.3055, $r^2 = 0.73$) rates. Mean percentage daily losses of body carbon, nitrogen and phosphorus were 1.06 ± 0.15 %, 0.55 ± 0.19 % and 2.1 ± 1.25 % implying active feeding by the krill during the experiments. The effect of temperature changes on the metabolic quotients (O: N, N: P and O: P ratios) was found to be insignificant for the experimental individuals. Our results suggest a significant increase in metabolic activity with a gradual rise in water temperature. However, there was close to 45-50% mortality observed at higher temperature ranges (5-6 °C). The data set obtained from present study enables a preliminary understanding of the physiological responses of Antarctic krill towards a possible rise in sea water temperature in the future.

Keywords: Antarctic Krill; Metabolic rates; Temperature

1. INTRODUCTION

Polar Regions are under great pressure from the changing world climate. The slow but steady increase in the temperatures in the Antarctic ecosystem has been a cause of concern. Polar ecosystems are characterized by low seasonal variation in temperatures, yet they are the fastest warming regions on the planet (Vaughan et al. 2003).

These ecosystems also provide case studies of how marine organisms are affected by the variation in the environmental factors. Polar marine ectotherms are able to survive only at low temperatures and within small temperature ranges (Somero 1998; Peck and Conway 2000). Metabolic rates in ectotherms vary with ambient temperature. Recent studies in Antarctic marine invertebrates show that as temperature rises, oxygen consumption and heartbeat rate increases while blood oxygen content declines (Peck 1989; Pörtner et al. 1999; Peck et al. 2002; Pörtner 2002).

Antarctic krill is the keystone species of the Antarctic ecosystem and exemplifies the potential sensitivity to climate change. This key species is stenothermal, and completion of its life cycle depends on blooms at critical times of the year (Quetin and Clarke 1994; Quetin and Ross 2001).Experimental approaches have revealed how environmental variability affects krill physiology (Ikeda 1985; Quetin and Clarke 1994). Krill represents an important link in the ecology of Antarctica and is a primary component of the food chain. Any major changes in its abundance will in turn negatively affect the large fauna dependant on it as it is the major food source of whales, seals and penguins.

This study was aimed at evaluating the effects of increasing temperature on the physiology of Antarctic krill. The change in the rates of oxygen consumption, ammonia excretion and phosphate excretion were measured with a controlled change in the water temperature. Metabolic rates of Antarctic krill at ambient temperatures have been studied previously by several authors (Ikeda and Mitchell 1982; Quentin and Ross 1989; Ikeda and Kirkwood 1989; Huntley and Nordhausen 1995; Huntley et al 1994; Swadling et al. 2005). Temperature is an environmental factor that has a substantial effect on the body functioning. Investigations on the body responses of krill towards temperature have shown interesting aspects of its biology. Poleck and Denys (1982) studied the effect of temperature on moulting, growth and maturation under lab conditions while some studies also gave useful insights on its effects on metabolic rates and development times of early larval stages of *Euphausia superba* (Ross et al. 1988; Quentin and Ross 1989). Most of these studies have focussed on the measurement of metabolic rates at ambient temperatures. However knowledge of krill's

metabolic rate in a range of temperature is also important as it will provide a basic indication of its metabolic requirements in different environments.

In this study we sampled the nearshore habitant of Antarctic continent, *Euphausia crystallorophias* Holt and Tattersall (1906) which is an important food source for upper trophic level predators, including fishes (Hopkins 1987; Hubold 1991) and studied the effect of a range of temperatures on its metabolic activity. In the wake of globally rising temperatures, more studies on *E. crystallorophias* have become imperative as it's biology is still poorly known (Daly and Zimmerman 2004).

2. MATERIALS AND METHODS

a. Collection of specimens

A 1m diameter ring net, 300 cm in length equipped with 3 mm mesh and a 5 litre protected cod end (PVC cylinder) was typically deployed through an ice-free area alongside the ship. Weights of about 1.5 kg were attached to the base of the cylinder. Small battery-operated lights were attached to the mouth of the net to attract the swarms. Oblique tows from a depth of approximately 0-5 m below surface were made slowly to minimize damage to the organisms. Each tow was made for 15-20 minutes while the speed of ship was maintained between 1-2 knots. Specimens were immediately transferred to 13-litre transparent PVC buckets filled with filtered seawater maintained at ambient seawater temperature (0 to 1 °C). Surface seawater from the sampling sites was collected and used in experiments. All the experiments were started within 12 hours of the catch.

b. Experimental setup

The animals were sorted out into two size classes on the basis of their body length viz. adult krill (AK) and juvenile krill (JK) by simple observation. The animals were divided into two different setups. Each setup had 2 buckets each of 5000 ml and 2500 ml and 4 buckets of 1000 ml each i.e. a total of 8 buckets. The adult and juvenile animals were then placed in buckets as given in Table 1. Control buckets were prepared separately for adult (CA) and juvenile (CJ) for assessment of the normal metabolic rate of the krill while test buckets were used for measuring the same in experimental temperature conditions. Buckets without krill were also prepared concurrently.

All the buckets were covered by black plastic to keep the experimental conditions dark as the natural conditions of krills. In order to minimize contamination, the animals were washed before being transferred to the experimental buckets by the method given by Ikeda and Mitchell (1982).

All glassware were washed in 10% HCl and then thoroughly rinsed with deionized water prior to use in experiments. Preliminary experiments were conducted to determine the appropriate combination of stocking density, container size, and experimental duration that would yield reproducible results and minimize experimental errors.

The temperature variability was studied for the six temperatures i.e. 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 °C (± 0.2 °C). The first two temperature ranges were easily maintained at ambient temperature while last four were achieved manually. Aquarium heaters were used to raise the temperature as required after which the animals were introduced in the containers. These buckets were then immediately transferred to 4°C freezer present on the ship. Each temperature range was maintained for more than 24 hours and all the parameters (dissolved oxygen, ammonia and phosphate) were measured twice at 12 hour intervals. Each experiment was performed in duplicate. When the experiments were completed krill were removed from the experimental buckets, placed on a filter paper to remove excess water, their length measured and immediately frozen at -20 °C for later morphometric and biochemical analyses in laboratory. Similar experiments were performed with the control buckets.

Experiments were continued for 12 days. Any dead krill were removed and fresh individuals were added subsequently from the stock kept at 0 °C.

c. Metabolic rate measurements

Chemical analyses were conducted within a few hours after each experiment. Dissolved oxygen, phosphate and ammonium concentrations were determined using molybdate and phenol-hypochlorite methods, respectively (Strickland and Parsons 1972). Standards were prepared daily.

After incubation, duplicate 50, 10 and 10 ml water samples were siphoned out for duplicate measurements of dissolved oxygen, ammonia and phosphate, respectively. Initial samples were taken in duplicate from each container at the beginning of an experiment, as were final samples after 12 hours of incubation. Blank containers (without krill) were treated in the same fashion.

3. RESULTS

Live specimens of *E. crystallorophias* were collected in Prydz Bay, East Antarctica in the austral summer of 2009 during the 28th Indian Scientific Expedition to Antarctica. A majority of the specimens were collected between midnight of 2nd February to 6th February when the weather

was completely cloudy. Table 1 summarizes the date of catch, number of animals caught, coordinates of the sampling sites and surface temperature at the collection site.

Table 1 Capture details of the Antarctic Krill Euphausia crystallorophias caught from Prydz Bay.

Date of Catch (dd/mm/yyyy)	Number of individuals	Coordinates	Sea Surface Temperature (°C)
2/2/2009	122	69°08'59"S ; 76°09'25"E	0.3
5/2/2009	145	69°12'31"S ; 76°08'10"E	0.7
6/2/2009	109	69°01'41"S ; 76°02'51"E	1

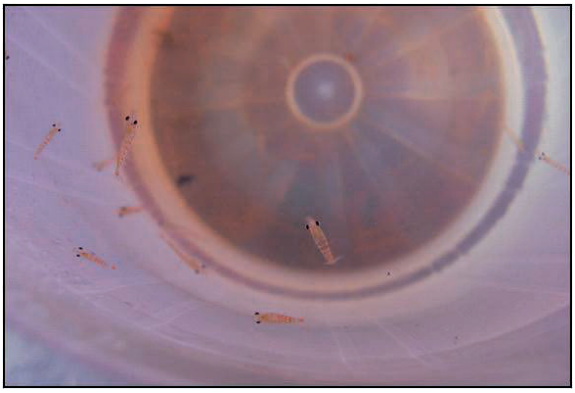

Figure 1a Antarctic Krill Euphausia crystallorophias

Table 2 Details of experimental setup for the test buckets

Bucket Type (Test)	Volume of buckets (L)	Number of buckets	No of Individuals/ bucket	Total number of individuals
Adult	5	2	10	20
Krill	2.5	2	5	10
	1	4	2	8
Juvenile	5	2	10	20
Krill	2.5	2	5	10
	1	4	2	8
Total experimental individuals				76*

The same numbers of animals were used for control buckets

Figure 1b Antarctic Krill captured at Prydz Bay kept in experimental buckets

The data on body composition, metabolic rates, metabolic quotients and daily metabolic losses is summarised in Table 3. Body size of adult *E. crystallorophias* ranged from 5.6 to 28.3 mg in terms of dry weight. The length composition was dominated by krill of 21 mm modal size. Metabolic rates were measured over a range of temperatures from 1.2 C to 5.3 °C (see Figure 2a-c). Temperature had a linear effect on the oxygen consumption of the krill. The O_2 consumption ranged from 5.54 ± 0.13 to 7.92 ± 0.30 µl O_2 indiv^{-1} h^{-1}. The regression graph (Figure 2a) shows a strong effect of temperature on O2 consumption ($y = 0.5392x + 4.9845$, $r^2 = 0.86$). NH_3 excretion rates (Figure 2b) were also strongly affected with a rise in temperature ($y = 0.0686x + 0.2176$, $r^2 = 0.88$), increasing linearly from 0.28 ± 0.03 to 0.61 ± 0.03 µg N indiv^{-1} h^{-1}. PO_4 excretion (Figure 2c) ranged from 0.40 ± 0.02 to 0.73 ± 0.13 µg P indiv^{-1} h^{-1} with temperature affecting it significantly ($y = 0.0724x + 0.3055$, $r^2 = 0.73$).

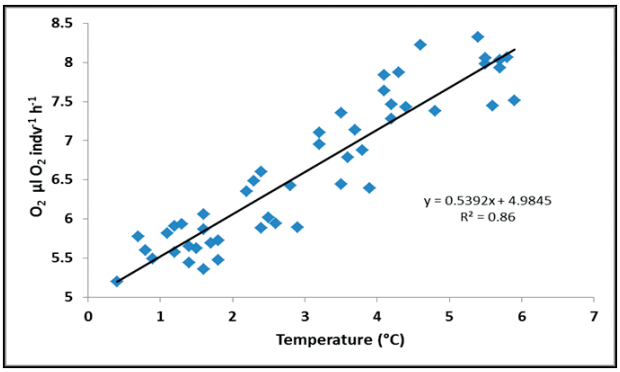

Figure 2a O_2 consumption by Antarctic krill under varying experimental temperature stress

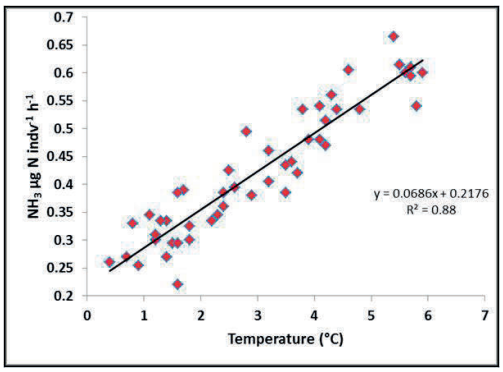

Figure 2b NH_3 excretion by Antarctic krill under varying experimental temperature stress

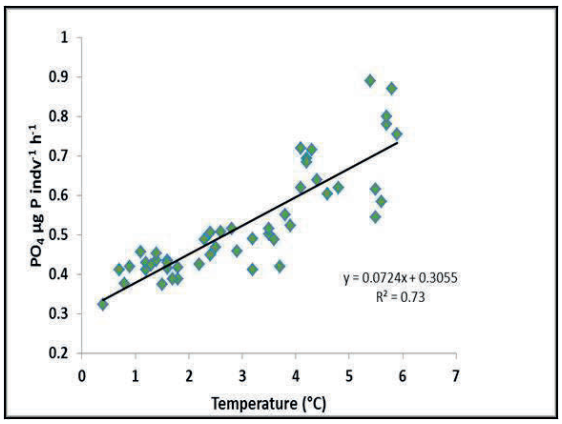

Figure 2c PO_4 excretion by Antarctic krill under varying experimental temperature stress

Metabolic rate data was combined with body composition of the krill to estimate the daily losses of body carbon, nitrogen and phosphorus (Ikeda and Kirkwood 1989). Water content was expressed as percent wet weight (% ww), while ash, carbon, nitrogen and phosphorus were calculated as percent dry weight (% dw).

Table 3 Body composition, metabolic rates and daily metabolic elemental loss of Antarctic Krill under laboratory conditions

Measurements	This study	Ikeda and Kirkwood 1989	Ikeda and Bruce 1986
Mean body weight	16.65 ± 4.35 (65)	17.69 ± 6.85 (14)	17.09 ± 6.33 (13)

Metabolic rates			
O_2	8.69 ± 1.07 (42)	8.76 ± 3.01 (14)	9.20 ± 2.87 (13)
NH_3	0.62 ± 0.15 (28)	0.63 ± 0.44 (14)	0.36 ± 0.25 (13)
PO_4	0.95 ± 0.28 (33)	0.23 ± 0.14 (14)	ND
Body composition			
Water	79.4 ± 5.1 (62)	77.8 ± 1.1 (14)	77.9 ± 1.8 (13)
Ash	13.6 ± 2.2 (62)	13.4 ± 0.0 (2)	11.8 ± 0.4 (2)
Carbon	44.0 ± 0.26 (62)	44.97 ± 0.04 (2)	45.32 ± 0.24 (2)
Nitrogen	9.22 ± 0.41 (62)	12.35 ± 0.11 (2)	11.01 ± 0.04 (2)
Phosphorus	1.44 ± 0.14 (62)	1.24 ± 0.08 (2)	ND
Daily metabolic loss			
Body Carbon	1.06 ± 0.15 (41)	1.40 ± 0.21 (14)	1.52 ± 0.17 (13)
Body Nitrogen	0.55 ± 0.19 (39)	0.69 ± 0.34 (14)	0.43 ± 0.15 (13)
Body Phosphorus	2.1 ± 1.25 (41)	2.50 ±1.06 (14)	ND

Both O:N and O:P ratios were correlated negatively with experimental temperature while N:P ratio had a slightly positive correlation with it (Table 4). Temperature had a significant effect on O:N and O:P ratios but not on the N:P ratio. This indicates that metabolic quotients are largely independent of temperature in Antarctic krill.

Table 4 Regression statistics for metabolic rates of Antarctic Krill with experimental temperature

Metabolic Quotient	N	Y = bx + c		R^2
		b	c	
O:N	28	-1.3202	20.235	0.61**
O:P	33	-0.6054	14.823	0.30**
N:P	28	0.0255	0.736	0.1

**** Statistically significant**

4. DISCUSSION

Many marine crustaceans are regulators, and are able to maintain a constant respiration rate until the oxygen content of the water decreases to a low level. In fact metabolic regulation is the general rule in highly active animals like krill and adult euphausiids are known to regulate their oxygen consumption rates. Adult krill rely on external gills, which greatly increase their surface area to volume ratio, and active blood pigments to increase

their ability to remove oxygen from the water (Quetin and Ross 1989). Carbon content (44% of DW) was low in the collection period in accordance with the late spring data from previous studies on krill (Meyer et al. 2010) reflecting a used-up body lipid store. Protein content in the experimental krill was low as exhibited by the significantly low body Nitrogen (9% of DW). The individuals were not classified into males and females and thus no difference can be stated in terms of body composition. Oxygen consumption and ammonia excretion rates displayed higher correlation with the increasing temperature than phosphate excretion. Similar observations are given by Ikeda (1985) where oxygen uptake rates were significantly affected by temperature although the ammonia and phosphate excretion rates were not that significant. Oxygen uptake is correlated with body mass and temperature more closely than are ammonia and phosphate excretion. The results can be explained by the fact that oxygen-uptake represents the total metabolism of an animal in contrast to partial representation of nutrient excretion (ammonia and phosphate). Live zooplankton require oxygen, but do not necessarily excrete ammonia or phosphate.

Antarctic krill are keystone species of southern ocean ecosystem. Our study was first attempt in Indian Antarctic expeditions to study krill under laboratory conditions; the results are preliminary and need further experimentation under a better controlled facility to gain in-depth understanding of the krill physiology. We strongly suggest development of a laboratory facility at Indian Antarctic Station BHARATI to maintain krill under experimental conditions.

5. ACKNOWLEDGEMENT

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