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ORIGINAL PAPER

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The influence of acclimation and substratum on the metabolism of the Antarctic amphipods *Waldeckia obesa* (Chevreux 1905) and *Bovallia gigantea* (Pfeffer 1888)

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Abstract Respiration rates in the Antarctic amphipods *Waldeckia obesa* (Chevreux 1905) and *Bovallia gigantea* (Pfeffer 1888) were measured in relation to the presence or absence of a substratum to attach to, and the amount of time spent in a respirometer. During the first 4 h after placing animals in respirometers oxygen consumption in *W. obesa* was reduced by factors between 1.2 and 3.6 times by the presence of a nylon mesh net substratum. Oxygen consumption over the first 12 h after being placed in respirometers was reduced by factors of between 1.1 and 3.9 times for *B. gigantea* by the presence of pieces of corrugated plastic pipe. The effects on oxygen consumption of acclimating animals to respirometers were only assessed for *W. obesa*. Rates during the first 12 h after placing animals in chambers were 3.6 times higher than rates between 12 and 30 h after the start of trials. Standard metabolic rates were measured in *W. obesa* in the presence of a mesh substratum and following a 12 h acclimation period after 60 days of starvation. Under these conditions oxygen consumption was 2.5 μl O_2 h^{-1} for a specimen of 0.113 g dry mass. This was 3–5 times lower than routine metabolic rates previously reported for *W. obesa* and 2.4–18 times lower than routine rates for other Antarctic gammaridean amphipods.

Introduction

Early studies of metabolism in polar ectotherms tended to show higher rates of oxygen consumption than would have been predicted by extrapolation from data on respiration rates of temperate organisms. This phenomenon of a raised metabolic rate was called "metabolic cold adaptation", or MCA (Wohlschlag 1964), and was first described in fish. This concept, which was based mainly on the work of two authors (Scholander et al. 1953; Wohlschlag 1957, 1960, 1964), spread rapidly and was widely cited (e.g. Fry 1958; Dunbar 1968). Later, however, Holeton (1974), in a careful study, showed that some of the earlier results had probably been overestimates caused in part by raised stress levels in experimental animals due to the holding conditions or experimental protocols used.

During this period the concept of MCA had been supported by studies on euphausiids (McWinnie 1964) and especially amphipods (Armitage 1962; Klekowski et al. 1973; Rakusa-Suszczewski and Klekowski 1973). However, as it had been for fish, the validity of the concept was questioned for invertebrates (Clarke 1980), and later works found low metabolic rates in a wide range of organisms, including decapods (Maxwell 1976), isopods (White 1975; Maxwell 1976; Luxmoore 1984), gastropods (Ralph and Maxwell 1977a), bivalves (Ralph and Maxwell 1977b), nemerteans (Clarke 1983) and brachiopods (Peck et al. 1987). The early works showing high metabolic rates in cold-water amphipods, however, remained the only studies on this phylum, and they, therefore, appeared to be one of the few groups still unequivocally supporting the idea of MCA (Opalinski 1982).

Numerous factors are known to affect oxygen consumption in marine invertebrates (Luxmoore 1984). These include both exogenous parameters such as temperature, salinity and photoperiod which can be carefully controlled, and endogenous factors including activity, reproductive and feeding state, and stress which are more difficult to control. The aim of this study was to measure oxygen consumption in Antarctic amphipods held under stringently controlled conditions, and to quantify the effects of two potentially stressful factors: the presence or absence of a substratum to attach to, and the amount of time spent acclimating to a respirometer.

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In this study the exogenous factors of temperature, salinity and light regime were rigorously controlled, while variations due to endogenous factors were reduced as much as possible by measuring standard metabolic rates. Standard metabolism has been defined by Bayne et al. (1976) as the metabolic rate under physiologically steady state conditions in the absence of food. It approximates to the sum of the metabolic activities needed to keep an organism alive (basal metabolism) plus the instantaneous contributions from any growth or gametogenesis taking place during the period of the experiment.

The amphipod species used in this study were *Waldeckia obesa* and *Bovallia gigantea*. Both are circumantarctic species and hardy in captivity. Their ecology, reproductive biology and animal husbandry techniques are also being studied by one of the authors in aquaria in Belgium (G.C.). These are all data which proved useful in the present investigation. The main part of the study was carried out on the lysianassoid amphipod *W. obesa*, which is an important member of the guild of necrophagous animals (Arnaud et al. 1986; Presler 1986; De Broyer and Klages 1990). It has a wide bathymetric range, having been recorded at littoral sites in the West Antarctic and from samples as deep as 660 m in the Eastern Weddell Sea (Nagata 1986; De Broyer and Klages 1990). *W. obesa* is generally inactive compared to closely related species, as it seldom swims or walks on the seabed, but it does congregate in large numbers on dead animal remains.

B. gigantea is a larger predatory species and was used in some of the experiments for comparison with the data collected on *W. obesa*. It lives in shallower habitats, and is found between the littoral level and 90 m depth throughout the West Antarctic (Lowry and Bullock 1976; De Broyer and Jazdzewski 1993). It is predominantly a sit-and-wait predator, although diatoms have been noted as a small part of the diet in some animals in summer (Bone 1972). *B. gigantea* is usually found hidden in macroalgae or under stones waiting for copepods, amphipods or other prey items to pass nearby (Bone 1972; Scailteur and De Broyer unpublished data). In nature several specimens can often be found sharing the same shelter, although holding large numbers together in aquaria can lead to cannibalism, especially after moulting. The tendencies of both *W. obesa* and *B. gigantea* to spend long periods of time in an inactive state make them suitable species to hold in experimental systems, and especially so for investigations involving respirometry.

Materials and methods

Collection of animals

Experimental specimens of both species were collected in January and February 1991 from sites near the Brazilian Antarctic station 'Commandante Ferraz' (62°05'S, 58°24'W) in Admiralty Bay, King George Island. In the bay water temperature ranges from −0.75°C in winter to 0.0°C in summer, salinity is always close to 34‰, and oxygen levels in the water column never fall below 75% saturation (Samp 1980; Lipski 1987). However, there may be local fresh-water input at some sites from melting glaciers during the summer. These conditions are typical of the high stability of some environmental parameters which is characteristic of the Antarctic marine environment (Clarke 1988). In Admiralty Bay *W. obesa* is common between depths of 15 and 150 m (Jazdzewski et al. 1992), and specimens were collected using baited traps connected to an acoustic release mechanism at depths between 60 and 100 m. Specimens of *B. gigantea* were collected between depths of 2 and 20 m using an Agassiz trawl.

Specimens were transported back to the UK in March 1991 by air in cool boxes at 0°C. On arrival they were held in a recirculating sea-water aquarium at 0.0 ± 0.5°C. Salinity was maintained at 34 ± 0.5‰, and a L:D 12:12 h photoperiod was adopted. Both species were fed twice weekly with thawed Antarctic krill.

Measurement of metabolic rates

Assessments of oxygen consumption were started in June 1991, 3 months after their transportation. All trials were conducted in small, cylindrical perspex chambers (Fig. 1) that had screw-threaded lids fitted with O-ring seals. Measurements were made using closed

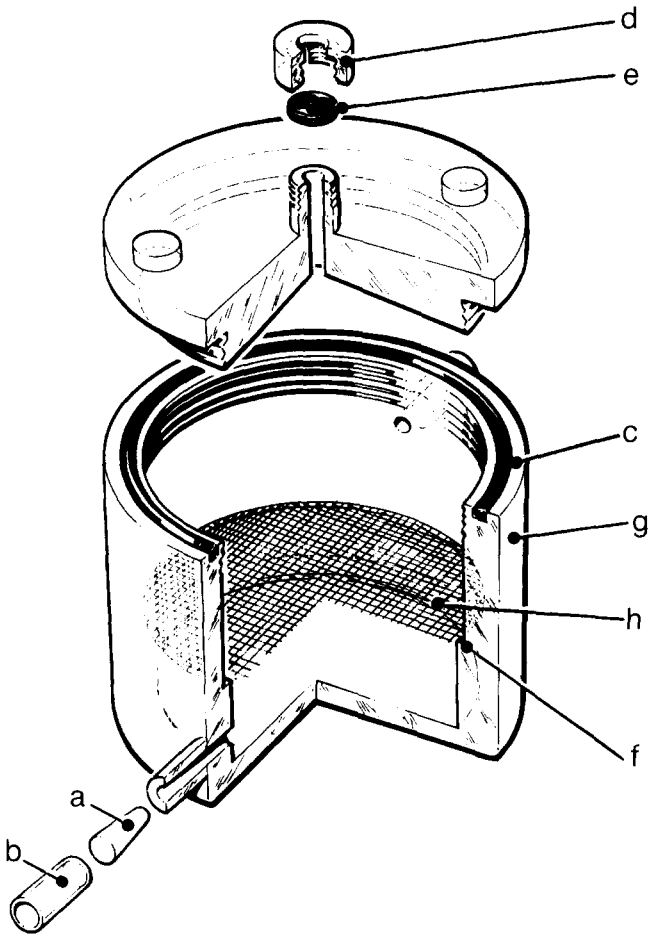

Fig. 1 The perspex respirometer used in this study (*a* rubber bung, *b* silicone tubing, *c* rubber O-ring, *d* cap, *e* rubber septum, *f* groove, *g* perspex, *h* piece of net to act as substratum)

bottle techniques because of the low respiration rates of the amphipods. Two different sized chambers were used. The trials testing for the effect of a substratum to attach to were conducted in 79 cm^3 chambers, whereas all other trials were carried out using chambers of 198 cm^3 volume. These chambers were placed in a water bath set in a jacketed container that was connected to a thermocirculator. The whole apparatus was sited in a constant temperature room with the air temperature set at 0.0°C. Water temperatures in the chambers were maintained at 0.0 ± 0.1°C in experiments using this method. Prior to setting up experiments the seawater for the trials was aerated for between 10 and 24 h. The initial seawater oxygen content was, therefore, always close to saturation. During the experiments a maximum depletion of 20–30% was allowed. Final levels were thus at, or above, the minimum values recorded for the water column in Admiralty Bay.

Oxygen consumption by the amphipods was assessed by measuring the seawater oxygen content in the chambers at regular intervals. Prior to taking samples for analysis, the chambers were gently inverted three times to mix the contents and ensure that the measurements made were representative. For each assessment a 25 µl sample was taken, using a gas-tight syringe, through the septum in the lid of a chamber (Fig. 1). The oxygen contents of samples were obtained by injecting them into a couloximeter similar to that of Peck and Uglow (1990), with the modifications described by Peck and Whitehouse (1992). The wet mass of the amphipods was measured after removing surface water. Dry mass was obtained after drying to constant weight at 60°C.

Experimental protocol

Two factors were manipulated in the investigation of the effects of stress on metabolic rate. These were the presence of a substratum to attach to, and the effect of an acclimation period after being placed in a respirometer. Initially behavioural observations were made over 2 days to assess the effects of the presence of material for the amphipods to attach to. On the 1st day half of the animals were held in the presence of the substratum, and the other half were kept in respirometers with nothing else inside. The second half of this trial, on day 2, was run with the positions of each half of the group reversed. From these behavioural trials 1 mm nylon mesh was chosen as a suitable substratum for *W. obesa*. Trials were then run simultaneously to test for the metabolic effects of the presence of the substratum by following the oxygen consumption of four animals. Measurements were made immediately after putting the amphipods in respirometers, and a further 8–10 assessments were made over the following 4 h. Similar behavioural observations on the effects of the presence of a substratum were made for *B. gigantea*. Pieces of corrugated plastic tubing (30 mm diameter, 40 mm length) were found to be good substrata. Metabolic measurements were made on six amphipods, and measurements were made every 1.5 h over the first 12 h after setting up trials.

The effect of an acclimation period on oxygen consumption was only studied in *W. obesa*. This was investigated by the use of a time course experiment in the presence of a net substratum. The oxygen content of water in the chambers was measured 12 h and 30 h after the start of each trial. A 12 h assessment was chosen because this period has frequently been used to acclimate animals to experimental systems in the past (Holeton 1973; Luxmoore 1984; Wells 1986; Saint-Paul et al. 1988; Klages, personal communication). After this, all other trials were conducted following a 12 h acclimation period. The protocol for these later experiments was to put the animals in chambers between 8 p.m. and 10 p.m. and to start the measurements the following morning, after 12 h had elapsed.

Standard metabolic rates were measured in *W. obesa* using the above protocol. Thirty-four animals were used in this experiment. They were taken from the routine holding tanks and held in the absence of food until their metabolic rates had declined to standard levels. During the starvation period oxygen consumption was measured every 3 days in a subgroup of eight of the experimental animals. When the metabolic rates of this subgroup had been reduced to standard levels, oxygen consumption was measured for all of the animals in the experiment.

Data manipulation and statistical treatments were conducted using the MINITAB statistical package.

Results

Substratum

In the absence of a substratum *Waldeckia obesa* could not stand upright, and repeatedly fell onto their sides. They spent much time righting themselves, followed by periods of walking on the smooth perspex surface or, in a few instances, they swam around the respirometer. In all cases when a piece of mesh was added to the respirometer the experimental animals rapidly settled on it. They spent most of their time motionless in one position, although occasionally there was some walking. No swimming activity was observed in trials with mesh.

Rates of oxygen consumption by individuals with mesh were always lower than when they were held without a substratum (Fig. 2). The mean respiration rate was 2.8 times lower when mesh was present than when it was absent (Table 1), and the difference in oxygen consumption between trials with and without mesh was significant ($t = 3.36$, $P < 0.05$).

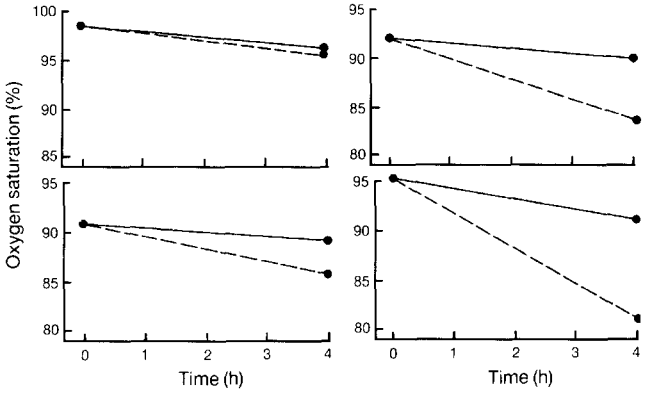

Fig. 2 Substratum effect on the metabolic rate of *Waldeckia obesa*. Oxygen consumption is shown in presence (*solid lines*) or absence (*dotted lines*) of a piece of 1 mm mesh nylon net, for four different animals. 100% oxygen saturation is defined here as water equilibrated with air at 1 atm pressure, or a PO_2 of about 160 mmHg

Table 1 Substratum effect: ratio of oxygen consumption rate without substratum to oxygen consumption rate with substratum, for *Waldeckia obesa* and *Bovallia gigantea*. The mean ratio for *W. obesa* was significantly greater than 1 ($t = 3.36$, $P < 0.05$), but the mean for *B. gigantea* was not ($t = 2.05$, $0.1 > P > 0.05$) (*STDev* standard deviation)

Species	N	Mean	STDev	Max	Min
Waldeckia obesa	4	2.78	1.06	3.62	1.24
Bovallia gigantea	6	1.86	1.03	3.86	1.14

The effects of the presence of pieces of corrugated tubing on the behaviour of *Bovallia gigantea* were similar to those of mesh on *W. obesa*. In all instances when tubing was present the *B. gigantea* went inside and settled in an alert, but inactive, posture. However, in the absence of the plastic tubing *B. gigantea* behaved very differently to *W. obesa*. Specimens settled quickly on the bottoms of chambers and adopted a similar resting position to that seen inside the tubing. It is not, therefore, surprising to find that the effects of the presence of the tubing on oxygen consumption in *B. gigantea* were less than the effects of the presence of mesh on oxygen consumption in *W. obesa*. However, oxygen consumption in the presence of tubing was still 1.86 times lower than when no substratum was present (Table 1). This ratio of oxygen consumption with and without tubing was not significantly different from 1 ($t = 2.05$, $0.1 > P > 0.05$). Despite this, oxygen consumption in *B. gigantea* was lowered by the presence of tubing in all cases. On this basis a non-parametric Wilcoxon sign-rank test was used and showed a significant difference between treatments ($W = 21.0$, medians = 192.0, 123.5, $P < 0.05$). Thus the presence of pieces of corrugated plastic tubing reduced the metabolic rate of the *B. gigantea* by a factor of 1.9 times.

Acclimation time

In these trials ten *W. obesa* were placed in respirometers with mesh and their respiration rates measured (Fig. 3). The mean oxygen consumption rate over the first 12 h of this experiment was 16.24 µl O_2 animal^{-1} h^{-1}. This was 3.6 times higher than the mean rate between 12 and 30 h (4.47 µl O_2 animal^{-1} h^{-1}), and the difference was highly significant ($t = 5.01$, $P < 0.001$).

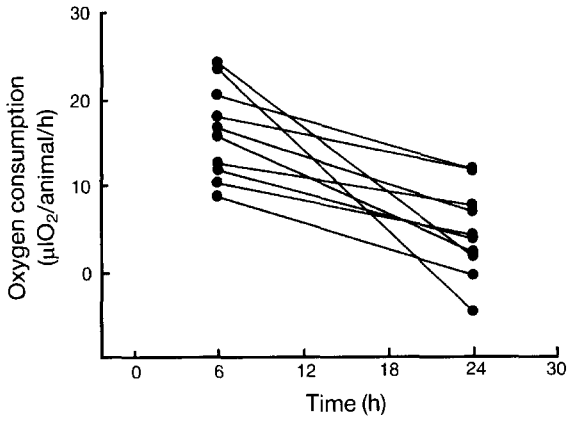

Fig. 3 Effect of acclimation on metabolic rate of *Waldeckia obesa*. Values shown are mean oxygen consumption rates per individual (µl O_2 animal^{-1} h^{-1}) over the periods 0–12 h and 12–30 h. Data points for each specimen are joined by *lines*

Table 2 Standard metabolism of *Waldeckia obesa*: *OxCons* oxygen consumption (µl O_2 animal^{-1} h^{-1}) per animal for a standard animal of 0.113 g DM, *DM* dry mass (g), *WM* wet mass (g) ($n = 34$)

	OxCons	DM	WM
Mean	2.54	0.113	0.295
STDev	2.63	0.049	0.148

Standard metabolism

Standard metabolism was estimated here by the measurement of oxygen consumption after a period of starvation of 60–64 days. Thirty-four animals were used in this experiment. The mean size of the group was 0.113 g dry mass, and the mean rate of oxygen consumption was 2.54 µl O_2 animal^{-1} h^{-1} (Table 2).

Discussion

Stress

It is impossible to make valid comparisons between species without evidence that the animals were at similar activity levels (Everson 1977). Stress then becomes a major factor in any estimation of metabolic rates, and its minimisation is of prime importance. In investigations similar to the present study, the first problem is to minimise the effects of stress caused by collection, transportation and holding specimens in laboratory aquaria. Physiological measurements should only be made after experimental animals have recovered from such stresses. A delay after collection also allows sensitive or damaged animals to be identified and excluded from trials. A minimum period of 24 h has been shown to be necessary for some temperate species (Edwards and Learner 1960; Ivleva 1973), and for polar species a longer delay between collection and measurement may be necessary (Wells et al. 1984). Periods of 12–24 h have routinely been used to allow Antarctic invertebrates to recover after collection (Klekowski et al. 1973; Opalinski 1974; White 1975; Ralph and Maxwell 1977b), while some studies have used longer periods (Rakusa-Suszczewski 1982; Luxmoore 1984). Delays similar to the 3 months in the present study are much rarer (Hiller-Adams and Childress 1983; Saint-Paul et al. 1988), because good maintenance facilities are needed as well as suitable species for holding in experimental systems. Information on recovery periods after collection, which is crucial to the understanding of physiological state, is, unfortunately, not quoted by all authors.

Antarctic seas are cold and thermally stable, although other factors such as phytoplankton levels and sea-ice cover vary greatly from season to season (Clarke et al. 1988). This is as likely to be true of benthic

habitats as for the water column, however, data from benthic sites are scarce (Gilbert 1991). Naturally stable physical environmental parameters were therefore, controlled as rigorously as possible in the present study. Temperature, salinity and oxygen concentration were held at $0.0 \pm 0.1°C$, $34 \pm 0.5‰$ and 70–100%, respectively. Oxygen consumption differences between day and night were not assessed, although in the amphipods *Hippomedon* (formerly *Tryphosella*) *kerguelensis* and *Cheirimedon femoratus* metabolic variations of this type have been found (Bregazzi 1973). In measuring standard metabolic rates here, an attempt was made to minimise errors due to variations in oxygen consumption between day and night by adopting a constant regime for making the measurements. Acclimation periods were always conducted during the night and oxygen consumption assessments made over the following day.

Substrata for experimental animals to attach to were used to reduce stress levels during trials. The effects of such substrata have received little attention in the past. Where they have been used, plastic grids were employed to separate animals from magnetic stirrers (e.g. Kangas and Lappalainen 1978). Other materials, such as gravel (Ivleva 1973), gauze (Bulnheim 1974) and cotton wool (Klekowski et al. 1973; Opalinski 1974; Rakusa-Suszczewski and McWinnie 1976) have also been utilised. Data on the behavioural and physiological effects of such substrata are scarce, and this is especially so for polar crustaceans. In the present study oxygen consumption and acclimation times were always reduced by the presence of a substratum. The rapidity with which both species utilised substrata emphasises the importance of an understanding of the biology and ecology of the species under investigation.

Another factor increasing stress levels in experimental specimens is the handling associated with putting animals in respirometry chambers. An acclimation period after placing animals in the chambers is usually used to reduce this effect, and a lack of knowledge of acclimation effects was a major argument used by Holeton (1974) against previous studies by Scholander et al. (1953) and Wohlschlag (1957, 1960, 1964), which had formed the basis for the concept of MCA. Since the mid-1970s the use of an acclimation period has become common, and 12–24 h has been routinely used. Oxygen consumption by *Waldeckia obesa* was 3.5 times lower after an acclimation period of 12 h than it was during that period. A similar effect was found in the fish *Pogonophryne scotti*, where a 12 h acclimation period reduced oxygen consumption by factors between 2 and 4 (Saint-Paul et al. 1988), and in the decapod *Chorismus antarcticus* the reduction was by a factor of approximately 2 (Maxwell and Ralph 1985).

Metabolic rates in Antarctic amphipods and MCA

In many previous studies of metabolic rates in Antarctic amphipods factors have been utilised, which, in the light of the above discussion on stress, would be expected to raise the measured rates of oxygen consumption. These factors include the lack of an acclimation period, no substratum and the use of very small volume respirometers (Table 3). Some authors have also placed more than one experimental animal in a chamber during trials, a factor which could affect oxygen consumption, either raising or lowering it, depending on the lifestyle of a given species (Edwards and Learner 1960). This emphasises the need for more knowledge of the biology and ecology of the species under physiological investigation.

Table 3 Oxygen consumption and the various handling parameters, when quoted, for Antarctic amphipods. *OxCons* oxygen consumption in $\mu l\ O_2\ mg^{-1}$ wwt h^{-1} *VolR* volume of respirometers in ml, *Accl* acclimation time in the respirometers in h, *Subs* substratum (*CW* cotton wool, *LB* lucite bottom, *PN* pieces of net), *Starv* time between beginning of starvation and measurement being made in h, *Res* respirometric technique (*VR* volumetric respirometry, *CE* Clark electrode, *WKL* Winkler respirometry; *GR* Gilson respirometry, *CX* couloximetry), *NM* not mentioned.

Species	Ox Cons	T°	VolR	Accl	Subs	Starv	Res	Reference
Parathemisto gaudichaudi	0.121	2.4	40	NM	NM	NM	VR	Opalinski and Jazdzewski (1978)
Paramoera walkeri	0.06	−1.8	10	18–24	CW	18–24	VR	Klekowski et al. (1973)
Paramoera walkeri	0.04–0.07	2	10	NM	CW	24	VR	Opalinski (1974)
Parandania boecki	0.07	1.8	40	NM	NM	NM	VR	Opalinski and Jazdzewski (1978)
Byblis securiger	0.172–0.3	2.4	40	NM	NM	NM	VR	Opalinski and Jazdzewski (1978)
Eusirus perdentatus	0.024	−1	40	NM	NM	NM	VR	Opalinski and Jazdzewski (1978)
Eusirus antarcticus[a]	0.028–0.07	0.5	NM	4	LB	NM	CE	Aarset and Torres (1989)
Cyphocaris richardi	0.039	−1.4	40	NM	NM	NM	VR	Opalinski and Jazdzewski (1978)
Orchomenella chilensis	0.17	0	60	NM	NM	24	WKL	Armitage (1962)
Abyssorchomene plebs	0.09	−1.8	NM	NM	CW	NM	GR	Rakusa-Suszczewski and McWinnie (1976)
Abyssorchomene plebs	0.056	0	8–15	NM	NM	NM	GR	Rakusa-Suszczewski (1990)
Waldeckia obesa	0.027	0	8–15	NM	NM	NM	GR	Rakusa-Suszczewski (1990)
Waldeckia obesa	0.009	0	200	12	PN	2 months	CX	This study

[a]For this species, 0.2 was used as the DW/WW ratio

Most studies have not measured standard metabolic rates, and where starvation periods have been employed they have been generally of short duration, and less than 24 h (Table 3). Feeding causes a rise in metabolism, called the specific dynamic action of feeding (SDA). In *W. obesa* this effect has been shown to raise oxygen consumption by a factor of 4, and the peak of the SDA occurred around 24 h after feeding (Chapelle et al. 1994). This effect could have been important in the investigation by Armitage (1962). He studied '*Orchomonella chilensis* (Heller)', which was probably *Abyssorchomene plebs* (Hurley), or *A. rossi* (Walker), or a mixture of both species (De Broyer 1983). These are scavenging species, and if they were fed immediately prior to experimentation, or collected by baited traps, the data on *W. obesa* would suggest that the rates measured by Armitage (1962) could have been at the peak of any SDA effect, as a 24 h acclimation period was employed. Chapelle et al. (1994) also showed that the post-prandial rise in metabolism took around 8–10 days to decay to pre-feeding levels, which suggests that many of the studies quoted in Table 3 may have measured elevated rates of oxygen consumption because of SDA effects.

Not knowing the feeding status of the animals investigated, combined with possible acclimation, substratum and small respirometer volume effects makes comparisons across species more difficult. However, the rate reported here for *W. obesa* is 2.7–33.3 times lower than rates for other Antarctic benthic amphipods (Table 3). The relatively high metabolic rates found in previous studies of cold-water amphipods have been cited as supporting the concept of MCA (Opalinski 1982) during a period where carefully controlled studies found low metabolic rates in a wide range of other benthic taxa (Maxwell 1976; Ralph and Maxwell 1977a, b; Clarke 1983; Luxmoore 1984; Peck et al. 1987; Peck 1989). The results produced here are clearly not consistent with the MCA hypothesis, and this is emphasised by comparisons with oxygen consumption in temperate amphipods (Fig. 4). Oxygen consumption in four species of temperate gammaridean amphipods declined with temperature. The data for *W. obesa* clearly fit this overall trend, whereas the results from previous investigations on *Orchomene plebs* are much higher (Fig. 4). Much of the difference between the data on *O. plebs* and *W. obesa* are probably due to some or all of the stress factors discussed above.

It is possible that the data for *W. obesa* in this study are slightly lower than is absolutely justified for the comparison in Fig. 4. Mitigating factors include the stringency with which conditions were controlled for *W. obesa*, and the fact that this species has a more substantial cuticle than many other amphipods. Results expressed in terms of ash-free dry mass might, therefore, be closer to values for other species. *W. obesa* is also a scavenging species, probably in the 'batch reactor digestion' group as defined by Sainte-Marie

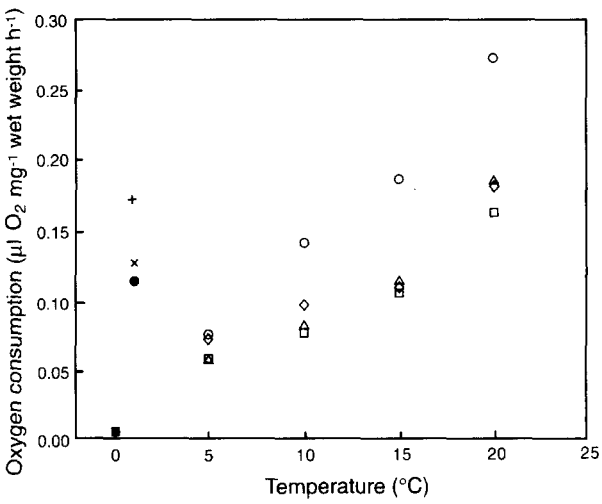

Fig. 4 A comparison of oxygen consumption by Antarctic and temperate amphipods. Data for the Antarctic species *Waldeckia obesa* (■) were taken from the present investigation and for *Orchomene plebs* (●, X, +) from Rakusa-Suszczeweki (1982). Data for the temperate species (*Gammarus locusta* (○), *G. zaddachi* (□), *G. salinus* (△) and *G. oceanicus* (◇) were taken from Bulnheim (1979). All the data in this figure were kindly supplied by M. Klages

(1992). It is likely that it is well adapted to a feeding regime with long periods of starvation. In the current study specimens survived without food for 2 months, and possible starvation periods of 18 months have been reported (Coleman 1991), although the exact holding conditions were not described, and factors such as cannibalism may have been sustaining animals. Because of this, care should be taken before extrapolating the low rates of oxygen consumption measured in this study directly to the approximately 400 other species of benthic Antarctic amphipods. Furthermore, there is now a need for investigations of the metabolism of Antarctic amphipods, and for comparisons with temperate and tropical species, under rigorously controlled conditions.

Despite these difficulties, the data on oxygen consumption in *W. obesa* presented here are clearly not consistent with the concept of MCA, and suggest that some, if not the majority of, earlier assessments showing high metabolic rates in Antarctic amphipods were probably produced, in part, by stress. Respiration should also be viewed as a cost to an organism, as it is energy lost and, therefore, not available for other parts of the energy balance such as growth or reproduction (Clarke 1991). From this point of view, a high standard metabolic rate, such as that postulated by the MCA theory, is a powerful selective disadvantage, especially in the resource-limited environments common in polar seas. High metabolic rates, such as those found recently for the Antarctic fish *Trematomus bernacchii* (Eastman 1993), can be expected in some species for ecological reasons. Where careful comparisons of metabolism have been made across polar, temperate and tropical

boundaries, low rates of oxygen consumption have been reported for polar species (Johnston et al. 1991). Several authors (Wells 1986; Saint-Paul et al. 1988; Johnston and Battram 1993) have also emphasised that for investigations of this type a good knowledge of the ecology and biology of the species studied is necessary before any experiments are conducted.

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