

## Observations of neutral buoyancy in diapausing copepods *Calanoides acutus* during Antarctic winter

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**Abstract** The herbivorous Antarctic copepod *Calanoides acutus* overwinters inactively in a resting stage (diapause) at depths below 500 m. It is assumed that during diapause *C. acutus* is neutrally buoyant in order to retain energy reserves otherwise depleted by swimming activities. However, so far, no experimental observations on its buoyancy have been reported and our knowledge of buoyancy regulation mechanisms is incomplete. In the present study, species-specific differences in buoyancy were assessed visually. Observations were made of specimens from the diapausing cohort of *C. acutus* and compared to another herbivorous copepod *Calanus propinquus*, which overwinters actively feeding in the upper water layers. Freshly caught copepods were anaesthetized in a 3-amino-benzoic acid ethyl ester (MS222) in seawater solution in order to exclude the influence of swimming movements on buoyancy control. It was shown that *C. propinquus* was negatively buoyant, whereas diapausing *C. acutus* remained neutrally buoyant. This is the first record that neutral buoyancy in diapausing copepods is maintained by the biochemical body composition without the additional need of swimming movements.

**Keywords** Zooplankton · Copepod · *Calanoides acutus* · Antarctic · Diapause · Buoyancy · Ammonium

### Introduction

The two dominant Antarctic copepod species, *Calanoides acutus* and *Calanus propinquus*, have developed opposing life-cycle strategies in order to survive periods of food scarcity in austral winter. They show distinct seasonal, inter-specific, and stage-dependent differences in behavior, physiological condition, and biochemical composition. *C. acutus* descends to depths below 500 m and survives the food-limited winter season by remaining inactive during a resting stage (diapause) for several months. In contrast, *C. propinquus* is a winter-active species, remaining in the upper 500 m throughout the entire year, and switching to a more opportunistic diet during winter (e.g., Donnelly et al. 1994; Schnack-Schiel and Hagen 1995; Atkinson 1998). Both species rely on large lipid deposits as energy reserves accumulated in the previous productive season, but they accumulate different lipid classes (Schnack-Schiel et al. 1991; Kattner et al. 1994; Hagen and Auel 2001).

In *C. acutus*, neutral buoyancy during diapause would reduce a depletion of energy reserves caused by swimming activities and, hence, ensure the successful restart of development and reproduction in the following spring. In contrast, actively overwintering species are presumably negatively buoyant and need to swim to counteract sinking (Haury and Weihs 1976). Until now, these species-specific differences in buoyancy have never been observed, neither under experimental conditions, nor in situ.

The present study aims to clarify whether diapausing copepods achieve and maintain neutral buoyancy without the necessity of active swimming.

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## Materials and Methods

Copepods were caught on board R/V *Polarstern* during expedition ANT XXIX/6 in austral winter at station 500 (68° 0.8' S, 6° 40.4' W; 2 July 2013; bottom depth 4,799 m) and 507 (66° 33.7' S, 27° 2.4' W; 17 July 2013; bottom depth 4,880 m) in the Weddell Sea. Hydrographical data were recorded prior to the zooplankton haul using a Conductivity-Temperature-Depth (CTD) profiler (SBE 911plus). Vertically stratified samples were collected with a multiple opening/closing net equipped with five nets of 100- $\mu$ m mesh size from 2,000–1,500, 1,500–1,000, 1,000–500, 500–200, and 200–0 m depth. Copepods were separated according to species, sex, and ontogenetic stage and kept in filtered seawater in a temperature-controlled refrigerator (0.4 °C) prior to the experiments. *C. propinquus* copepodite stages CV were taken from the upper water layer (200–0 m) and for diapausing *C. acutus* the main overwintering stages CIV were chosen from deeper layers (1,500–1,000 m). A graduated measuring cylinder (polymethylpentene, volume 2 l, external diameter 84 mm, and 531 mm height) was used as a settling chamber, filled with filtered seawater. Due to a pronounced thermocline in the upper 200 m where *C. propinquus* were caught, a mean experimental temperature representing conditions occurring in both water layers was chosen according to the CTD profile (0.4 °C, salinity 34.4, and calculated density 1.026 g cm<sup>-3</sup>). Previous studies may have suffered from bias by dissection, preservation, freezing, and rinsing. Therefore, we deliberately avoided any form of preparation of the copepods before the experiments to exclude effects on the osmotic or biochemical composition and, thus, on the overall density of an individual. Copepods were only anaesthetized in a MS222 in seawater solution to exclude the influence of swimming movements. The exposure time to the anesthetic varied depending on the species (see “Results”). A successful anesthetization was defined as the state in which external stimuli (gentle contact/touch of the large anterior antennae) did not result in any reaction or swimming/escape activities. Successfully anesthetized individuals were gently and separately transferred into the surface layer of the measuring cylinder using a small pipette. The behavior in the water column was monitored visually and sinking speeds were estimated with a stopwatch. The length of monitoring was adapted to the duration of the anesthesia. After a certain time in filtered seawater, the anesthetizing effects subsided and individuals were checked for their condition.

## Results and Conclusions

Copepods were successfully anesthetized at a concentration of 0.3 g MS222 per liter seawater at 0.4 °C. The

exposure times, until narcotic effects were ascertained, varied between 4 and 5 min for *C. acutus* and up to 40 min for *C. propinquus*. After approximately 30 min in fresh and filtrated seawater, the narcotic effects of the anesthetic agent subsided in both species.

Once introduced into the settling chamber, all individuals of anesthetized winter-active *C. propinquus* started sinking. The sinking velocities differed due to the orientation of the copepod in the water column. Whereas most of the individuals sank rapidly ( $\sim 1$  cm s<sup>-1</sup>) with their head first and legs folded alongside their bodies, some sank sidewise with one or both first antennae slightly spread, resulting in slower sinking velocities ( $\sim 0.5$  cm s<sup>-1</sup>). After a maximum of 1.5 min, all individuals were found on the bottom of the measuring cylinder. After the narcotic effects of the anesthetic agent had subsided, all copepods started swimming again and were found distributed over the entire water column.

In contrast, all individuals of the anesthetized diapausing copepod *C. acutus* reached a point of neutral to positive buoyancy just after having them transferred to the vessel and remained floating in the surface layer, where they were located irrespective of whether the anesthetic effects had worn off.

Species-specific density determinations for Antarctic copepods do not exist so far, whereas several studies have focused on the density of boreal or Arctic copepods, predominantly in relation to their lipid content and lipid class composition (e.g., Gross and Raymont 1942; Kögeler et al. 1987; Visser and Jónasdóttir 1999; Knutsen et al. 2001). Knutsen et al. (2001) critically reviewed density measurements of pelagic copepods and showed that all of these studies comprise systematic errors. Knutsen et al. (2001) used an experimental setup and procedure similar to the present study. Sinking speeds were determined in freshly caught and anesthetized individuals of diapausing *Calanus finmarchicus*, but with one major difference to our study. Prior to determination of sinking speeds, the large anterior antennae were removed to avoid an influence on sinking velocities. Stokes' law was applied to calculate their density, with distinctly lowest densities during winter. However, the removal of the antennae should be critically viewed, since leakage of hemolymph may have contributed substantially to the overall density of the copepod and may have biased the density determination.

The fact that large proportions of low-density lipids affect the overall density and, hence, buoyancy regulation in copepods has been recognized for a long time, and several authors consider lipids as the main constituent determining buoyancy and overwintering depth (e.g., Visser and Jónasdóttir 1999; Irigoien 2004; Pond and Tarling 2011; Pond et al. 2012). However, pure lipid-based neutral buoyancy is inherently unstable and difficult to regulate as lipids

are more compressible and have a larger thermal expansion than the surrounding seawater (Yayanos et al. 1978). Large compositional variability is evident for diapausing copepods and lipid levels may vary between 10 and 35 % of dry mass in overwintering copepodites CV in late winter (Hagen and Schnack-Schiel 1996). Small changes in lipid content and/or composition may have tremendous effects on the overall density of the organism and require additional buoyancy regulation mechanisms for fine-tuning (Campbell and Dower 2003; Campbell 2004).

Only recently were highly elevated levels of ammonium ( $\text{NH}_4^+$ ) measured in the hemolymph of diapausing *C. acutus*, while concentrations of other cations, particularly those with a higher molecular weight (i.e.,  $\text{Na}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ), were low. In contrast, none of the actively overwintering species showed elevated ammonium concentrations and their cation composition approximated that of the surrounding seawater (Sartoris et al. 2010; Schröder et al. 2013). Ammonium storage provides a potential mechanism for fine-scale buoyancy regulation. It is a waste product of protein and amino acid catabolism. Therefore, energetic costs for the accumulation of ammonium are low compared to active swimming or the accumulation of lipids. Ammonium-aided buoyancy is independent of ambient pressure, and therefore is well suited for the extensive vertical migration of copepods in autumn, as well as in assisting in the upward migration to the surface with reduced lipid levels in the following spring (Schnack-Schiel et al. 1991; Pasternak et al. 2009).

The present experimental approach suggests that diapausing *C. acutus* achieve neutral buoyancy through their biochemical body composition, without the need of swimming movements. This has a fundamental advantage as it reduces the depletion of energy reserves during diapause, when copepods do not feed. Moreover, it diminishes the risk of attracting predators, which otherwise would respond to swimming activities. To clarify how far neutral buoyancy can be maintained throughout the entire water column with varying pressure and densities represents a challenging task for future investigations.

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