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# The effect of hydrostatic pressure on grazing in three calanoid copepods

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The vertical distribution of copepods, and the factors determining it, has been studied extensively. Some copepods appear to maintain their vertical position in the water column with high precision, even against downwelling and upwelling currents. The adaptive benefits of this behavior are unknown. We hypothesized that changes in hydrostatic pressure alter the buoyancy of copepods and, in turn, affect their grazing rate. We tested this hypothesis in laboratory grazing experiments with three calanoid species, *Calanus helgolandicus*, *Pleuromamma indica* and *Rhincalanus nasutus*, grazing on cultured phytoplankton. The study was based on a comparison of gut pigment content between individuals that fed in chambers at two different pressure levels: 1 bar and 4 bars. A significant effect of pressure was found for *C. helgolandicus* (lower gut content at 4 bars) but not for *P. indica* and *R. nasutus*. If the effect observed in *C. helgolandicus* is common, it would add a new dimension to our understanding of copepod ecology in the oceanic realm, where plankton is often exposed to vertical currents and internal waves.

**KEYWORDS:** zooplankton; vertical distribution; gut pigment content; grazing experiment; depth retention; *Calanus helgolandicus*; *Pleuromamma indica*; *Rhincalanus nasutus*

## INTRODUCTION

The vertical distribution of copepods is governed by a number of factors, including temperature (McLaren, 1963), turbulence (Haury *et al.*, 1990; Incze *et al.*, 2001),

light (Bollens and Frost, 1990) and hydrostatic pressure (Banse, 1964). At certain locations, such as oceanic fronts, copepods maintain their depth against downwelling and upwelling currents with a precision of

centimeters (Genin *et al.*, 2005). The ability of copepods to sense changes in pressure that take place during downward/upward flows, and to react to them, might explain this. Observations of the pressure response in calanoid copepods are inconsistent: Knight-Jones and Qasim (Knight-Jones and Qasim, 1955; *Calanus* sp.) and Hardy and Bainbridge (Hardy and Bainbridge, 1951; *Calanus finmarchicus*) reported no response to pressure changes, whereas Rice (Rice, 1962; *C. finmarchicus*) and Lincoln (Lincoln, 1971; *C. helgolandicus*) found that the copepods swam upward in response to increasing pressure and downward in response to decreasing pressure.

Many calanoid copepods generate feeding currents (Strickler, 1982, 1985), driving water over their mechano- and chemo-sensors in order to divert food particles toward their feeding appendages (Fields and Yen, 1993; Jiang and Strickler, 2007). If copepods were neutrally buoyant, these feeding currents would propel them through the water column. In reality, negatively (or rarely positively) buoyant copepods are “anchored”, allowing them to entrain fluid toward the mouth in a predictable way (Strickler, 1982; Andrews, 1983). The strength of the anchor determines the pattern and intensity of the feeding currents (Jiang and Strickler, 2005) and, possibly, the animal’s ability to effectively use chemical cues (Moore *et al.*, 1999).

Many calanoid copepods accumulate lipid as an energy store and sequester it in different parts of their body (Lee *et al.*, 2006). Since lipids are less dense than proteins, differences in the center of gravity and buoyancy of a copepod, caused by the relative distribution of protein and lipid in the body, result in the copepod maintaining a specific orientation in the water column (Strickler, 1982; Fields and Yen, 1997). The animal’s orientation determines the direction of the feeding current. In addition to being less dense, lipids are also more compressible and more thermally expandable than seawater (Yayanos *et al.*, 1978). As a result, changes in temperature and pressure can alter a copepod’s orientation and buoyancy in the water column (Visser and Jónasdóttir, 1999), which can also affect the magnitude and direction of its feeding currents. In theory, the shape and intensity of feeding currents are function of the aforementioned “anchor strength,” that is, the density difference between a copepod’s body and the surrounding water, a parameter that can vary strongly with depth (Jiang and Strickler, 2005). For instance, a copepod with a small density difference at the surface may generate stronger feeding currents at a greater depth at which the density difference with the ambient water is larger (Jiang and Strickler, 2005). As a consequence, grazing rates of individual copepods are expected to vary as a function of depth (given a similar food concentration). In order to maintain optimal buoyancy, a copepod could either

adjust its lipid content or actively select a suitable depth. The observation that individual copepods of the same species and same life stage differ in lipid content across depth differences as small as 12–15 m (Zarubin *et al.*, 2014) is consistent with the hypothesis that depth selection among individual copepods is based on buoyancy. Such active depth selection would explain how and why copepods maintain their depth in zones of vertical currents, such as oceanic fronts (Genin *et al.*, 2005).

The objective of this study was to test the hypothesis that small changes in hydrostatic pressure, corresponding to a depth difference of 30 m, affect grazing rates in three species of calanoid copepods.

## METHOD

### Study species

We tested the effect of hydrostatic pressure on grazing in three species of calanoid copepods, *Calanus helgolandicus*, *Rhincalanus nasutus* and *Pleuromamma indica* (adult females only). These species were selected mainly because they are herbivorous and relatively large, simplifying the measurements of gut pigment content in individual animals. We expected to find an effect of pressure on grazing in two of these species, *C. helgolandicus* and *R. nasutus*, since both accumulate relatively large amounts of lipid [*C. helgolandicus* up to 30% dry weight (Kattner and Hagen, 2009) and *R. nasutus* on average 30% dry weight (Sommer *et al.*, 2002; Cass *et al.*, 2014)]. The third species, *P. indica*, only sometimes exhibits a few small lipid droplets (personal observation). Thus, we expected the effect of pressure to be small or absent in *P. indica*, rendering this species an “internal control” that would allow us to resolve an effect of pressure (if found for this species) that is unrelated to lipid content.

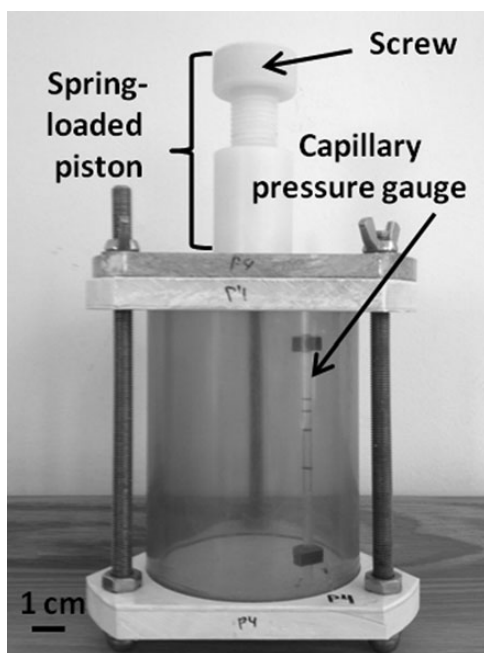
*Calanus helgolandicus*’ vertical migratory behavior varies regionally (Bonnet *et al.*, 2005). For instance, in the Mediterranean Sea and the North Sea, the diel vertical migration (DVM) of this species is negligible (Bonnet *et al.*, 2005). However, in the Celtic Sea DVM is seasonal and is part of a more complex pattern of behavior (Williams and Conway, 1984). The source of the *C. helgolandicus* in our experiments was a population that had been cultured for several generations in 2.35-m deep 2800-L silos at the Austevoll Research Station, Norway.

The Red-Sea *P. indica* is a daily vertical migrator, found at depths >250 m during the day (Farstey, 2001). *Rhincalanus nasutus* in the Gulf of Aqaba is a seasonal vertical migrator, found at depths ~500 m during most of the year, ascending to the surface layer for a brief period during cold winters when the vertical mixing is sufficiently deep (Farstey, 2001).

## Pressure chambers

Our experiments were based on a comparison of grazing under two levels of hydrostatic pressure: 1 bar (atmospheric pressure) and 4 bars. All of the incubations were carried out in custom-built chambers made of a transparent PVC cylinder, 10 cm diameter, 15.5 cm high, with a pressure-generating piston made of Delrin mounted on the end cap (Fig. 1). The volume of each chamber was  $\sim 1.1$  L and the number of copepods per chamber ranged between 21 and 25 (average 22 copepods). Although this density of animals is higher than that found *in situ*, it was chosen to ensure that the sample size for each experiment was sufficient. Importantly, Wong (Wong, 1988) reported that the gut pigment content of individual copepods (*Calanus*, *Metridia*, *Pseudocalanus* sp.) did not vary in experiments at animal densities similar to those that we used. In addition, the gut pigment content of animals feeding alone was never significantly different from that of animals feeding in the presence of conspecifics (Wong, 1988).

The pressure inside the chambers was set by turning the piston's screw, allowing it to be fine-tuned to  $\pm 0.1$  bar. A calibrated, transparent capillary tube was mounted inside the chamber to monitor the pressure from the outside. Repetitive readings during the trials indicated that



**Fig. 1.** The experimental pressure chamber. The diameter of the transparent PVC cylinder is 10 cm and its height 15.5 cm. The screw at the top of the spring-loaded pressure-generating piston is used to set the pressure inside the cylinder. The transparent capillary pressure gauge allows fine tuning of the pressure inside the chamber and verification of its constancy throughout trials.

the set pressure remained unchanged for  $>24$  h. The pressure level of the increased pressure treatment was chosen to be 4 bars because of the maximum pressure recommended for the PVC cylinder. Four to six incubation chambers were used in each trial (depending on the number of copepods caught), with half of them set to 4 bars and the other half set to 1 bar. All of the chambers were mounted on a rotating wheel (0.3 rpm) in order to keep the algae and the animals mixed.

## Experiment protocols

The experiments with *C. helgolandicus* were carried out in October 2012 at the Institute of Marine Research, Austevoll Research Station, Norway. On the evening prior to each experiment, adult *C. helgolandicus* females were gently sampled from the Station's mono-specific rearing tanks using a light trap. Captured animals were rapidly transferred to a temperature-controlled room ( $10^{\circ}\text{C}$ ), and sorted using a wide-mouth pipette. Care was taken to use only undamaged individuals. Sorted copepods were acclimated overnight in a 2-L vessel containing  $2 \times 10^4$  cells  $\text{mL}^{-1}$  of the algae *Isochrysis galbana* ( $4\text{--}5\ \mu\text{m}$  cell size) collected from a culture that was in exponential growth phase. After the acclimation period, active copepods were transferred to the incubation chambers filled with a fresh suspension of *I. galbana* at a concentration of  $2 \times 10^4$  cells  $\text{mL}^{-1}$ . The same algal suspension was used to fill the high- and low-pressure chambers. The concentration of algae was measured using a Beckman Coulter<sup>TM</sup> Z2 Coulter Particle Counter and Size Analyzer. Six such feeding trials were conducted in the dark at  $10^{\circ}\text{C}$  and lasted 24 h.

The experiments with *P. indica* and *R. nasutus* were conducted at the Interuniversity Institute for Marine Sciences of Eilat, Israel. The copepods were sampled in the Gulf of Aqaba, Northern Red Sea ( $29^{\circ} 30' \text{N}$ ,  $34^{\circ} 55' \text{E}$ ), in February and March 2014. *Pleuromamma indica* was sampled approximately 1 h after sunset (because it is a vertical migrator that is found in the surface waters of the Gulf at night) by slowly ( $0.8\text{--}1.5$  knots) towing a plankton net horizontally ( $300\text{-}\mu\text{m}$  mesh size,  $0.25\ \text{m}^2$  mouth opening) for 5–10 min at 5–10 m below surface. In 2014, the mixing depth was not sufficiently deep for *R. nasutus* to ascend to the Gulf's surface layer. Therefore, *R. nasutus* was sampled from the deep water ( $>400$  m) 2 days prior to the grazing experiment, using vertical tows from 600 m to the surface (bottom depth of  $\sim 650$  m), using a plankton net ( $300\text{-}\mu\text{m}$  mesh size,  $0.25\ \text{m}^2$  mouth opening). For both species, the contents of the cod-end were gently transferred to several 1-L jars and taken to the laboratory within  $\sim 30$  min. Individuals were then sorted and transferred to the acclimation

containers. Care was taken to select only undamaged individuals. Acclimation lasted 12–15 h for *P. indica*, as it was caught near the surface, and 48 h for *R. nasutus*, which was collected at greater depths. The acclimation was carried out in the dark at a constant temperature of 25°C in a suspension of the algae *Tetraselmis tetrahele* (~12–15 µm cell size) at an initial concentration of  $2.0 \times 10^4$  cells mL<sup>-1</sup>. *Tetraselmis tetrahele* in exponential growth phase was obtained from the Israel Oceanographic and Limnological Research Institute in Eilat on the same day or on the day before the experiment. Phytoplankton concentrations in the stock culture were determined microscopically using a hemocytometer, and then diluted with filtered seawater (FSW; filtered on 0.7-µm GF/F filters) to obtain the target concentration. After acclimation, the copepods were gently transferred to the incubation chambers. A total of four (*P. indica*) and three (*R. nasutus*) trials with one to three chambers of each type (1 bar and 4 bars) per trial (depending of the number of copepods collected) were conducted.

At the end of the incubation, the chambers were taken off the rotating wheel and each copepod was sampled, gently rinsed in FSW, and examined under a microscope to determine its condition. Only specimens that were alive and active at the end of the incubation were measured for gut fluorescence.

In the case of the *C. helgolandicus* experiments in Norway, after the incubations the sorted animals were transferred to cryovials, the excess water was removed using a thin Pasteur pipette, and the cryovials were frozen in liquid nitrogen. The samples were transported to Eilat in liquid nitrogen and then stored at -80°C. Prior to pigment measurements (described below), the frozen cryovials were thawed one by one, the thawed copepods placed in a Petri dish in FSW, cooled with ice from underneath and examined under the microscope for any damage. Only undamaged individuals were used for pigment extraction (see below). In the case of *P. indica* and *R. nasutus*, for which the incubations were carried out in Eilat, after the incubation each live individual was immediately transferred to the pigment extraction vial.

To determine the amount of pigments in the copepod gut we followed Mackas and Bohrer (Mackas and Bohrer, 1976) and used the sum of chlorophyll *a* and phaeopigments (in ng copepod<sup>-1</sup>) as a measure of gut pigment content. The pigments of each individual copepod were extracted for 24 h in 140 µL of 90% acetone at 4°C in the dark. Pigment concentration in the extracted solution was fluorometrically determined using “mini-cells” of a pre-calibrated Trilogy Fluorometer (Turner Designs) equipped with an “acidified module.” A quantity of 120 µL of supernatant acetone solution was carefully pipetted into a mini-cell for the “before”

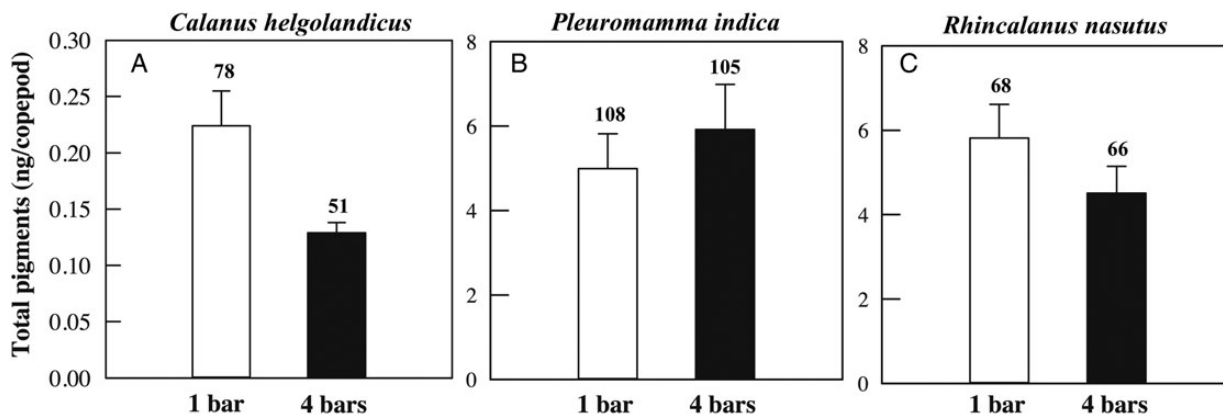
reading followed by acidification with 5-µL 10% HCl for the “after” reading. The instrument’s detection threshold was approximately 0.15 ng Chl *a* mL<sup>-1</sup>, corresponding to 0.021-ng pigments copepod<sup>-1</sup> in an extraction volume of 140 µL. Hereafter, we use the term “pigments” to indicate the sum of chlorophyll *a* and phaeopigments. Following Kleppel *et al.* (Kleppel *et al.*, 1988), the copepods were not macerated prior to extraction. To determine the fluorescence of starved animals, copepods were placed into FSW and allowed to empty their guts in the dark (*C. helgolandicus* for ~24 h, *P. indica* for ~26 h and *R. nasutus* for ~47 h). They were then processed for individual pigment extraction and analysis as described above. Copepods that did not appear to be feeding during the high- and low-pressure trials (i.e. their gut pigment content did not exceed the pigment content values recorded for starved individuals of the same species) were omitted from the analysis. The total number of copepods that actively grazed during the trials was 494 individuals: 129 *C. helgolandicus*, 213 *P. indica* and 134 *R. nasutus*. The values presented here are not corrected for background fluorescence (following Baars and Oosterhuis, 1984).

### Statistical analysis

For each species, permutation-based ANOVA was used to test the effect of pressure on gut fluorescence. The analysis was carried out using R version 3.0.1. (R Development Core Team, 2011), with the lmPerm package (Wheeler, 2010).

## RESULTS

Individual gut pigment content was measurable in all species examined. The gut pigment content of starved animals was on average 0.066, 0.057 and 0.064 ng copepod<sup>-1</sup> for *C. helgolandicus*, *P. indica* and *R. nasutus*, respectively. The percentage of animals in the experimental incubations with gut pigment content indicative of empty guts was, for *C. helgolandicus* 31% in the 1-bar treatment (36 of 115) and 36% at 4 bars (29 of 80), for *P. indica* 0.9% (1 of 109) and 3.7% (4 of 109) for the 1- and 4-bar treatment, respectively, and for *R. nasutus* all of the animals appeared to be feeding. Removing the non-feeding individuals from the analysis slightly raised the averages of each treatment but did not affect the overall trend. In *C. helgolandicus*, the gut pigment content (disregarding the values of non-feeding copepods) among individuals within an experimental trial and within a treatment differed by a mean factor of 14.6 (ranging 1–104). In one trial in the 1-bar treatment, a single animal had an exceptionally high gut pigment content (6.96 ng copepod<sup>-1</sup>). When



**Fig. 2.** Average gut pigment content of *Calanus helgolandicus* (A), *Pleuromamma indica* (B) and *Rhincalanus nasutus* (C) after grazing at low (1 bar) and at increased pressure (4 bars). For *C. helgolandicus* (A), the outlier from the 1-bar treatment was removed, for details see text. Had the outlier been included, the average gut pigment in the 1-bar treatment would be 0.309 ng copepod<sup>-1</sup>. Numbers above the bars indicate sample size. Error bars are standard error.

this value was omitted from the analysis, the mean factor became 6.7 (ranging from 1 to 26). For *P. indica*, this factor was on average 45 (range 6–77) and for *R. nasutus* 16 (range 9–23).

In *C. helgolandicus*, the gut pigment content of individuals that grazed at 4 bars was significantly lower than that at 1 bar (Fig. 2A; permutation-based ANOVA,  $P < 0.04$ ; statistical analysis was performed without the above mentioned exceptionally high value, rendering it more conservative). The average gut pigment content (disregarding the non-feeding copepods) of *C. helgolandicus* at 4 bars was 0.129 ng copepod<sup>-1</sup> (range 0.07–0.34) (Fig. 2A). The gut pigment content at 1 bar, if the above mentioned exceptionally high value (6.96 ng copepod<sup>-1</sup>) is omitted from the analysis, was 0.224 ng copepod<sup>-1</sup> (Fig. 2A). If the outlier was not removed, the average gut pigment content was 0.309 ng copepod<sup>-1</sup> (range 0.07–6.96).

Hydrostatic pressure had no significant effect on the gut pigment content of *P. indica* (Fig. 2B; permutation-based ANOVA,  $P = 0.569$ ) and *R. nasutus* (Fig. 2C; permutation-based ANOVA,  $P = 0.208$ ). The gut pigment content in *P. indica* at 4 bars was on average 5.92 ng copepod<sup>-1</sup> (range: 0.06–65.64) (Fig. 2B). The average gut pigment content in the 1-bar treatment was 4.99 ng copepod<sup>-1</sup> (range 0.06–50.2). In *R. nasutus*, the average gut pigment content was 4.51 and 5.81 ng copepod<sup>-1</sup> for the 4- and 1-bar treatments, respectively (Fig. 2C) (ranges 0.27–27.67 and 0.26–28.24, respectively) (Fig. 2C).

## DISCUSSION

Overall, the gut pigment contents of all three species examined were lower than those of copepods sampled *in situ*: 0.306–1.447 ng copepod<sup>-1</sup> for *C. helgolandicus*

(Baars and Oosterhuis, 1984) and  $\sim 1$ –9 ng copepod<sup>-1</sup>, depending on food in the ambient water (Bautista *et al.*, 1992); for *P. indica* 14–15 ng copepod<sup>-1</sup> (Farstey, 2001); for *R. nasutus* 0.2–66 ng copepod<sup>-1</sup>, depending on depth and time of year (Farstey, 2001). Obviously, experimental conditions in the laboratory do not perfectly simulate natural conditions. Nonetheless, the majority of the animals in our experiments were feeding at levels discernibly greater than the background levels for non-feeding animals.

A more common technique to determine copepod gut content is to extract the pigments from a pooled group of individuals, sometimes 10s of animals in a single extraction vial, thereby amplifying the fluorometric signal. A major shortcoming of such pooling is the loss of information on inter-individual variability. Our measurements indicate that this variability can be substantial: some of the individuals did not feed during the incubation and the gut pigments of those that fed differed by factors averaging 6.7–45, depending on the species. Similarly, a 10-fold variability in gut pigment content was reported between individuals of *Acartia tonsa* by Kleppel *et al.* (Kleppel *et al.*, 1988) and *Calanus pacificus* and *Metridia pacifica* by Mackas and Burns (Mackas and Burns, 1986). Using video recordings, Paffenhöfer *et al.* (Paffenhöfer *et al.*, 1995) observed that some *Paracalanus aculeatus* individuals ingested twice as much as others. These results suggest that ingestion rates calculated from group averages underestimate the maximum rates of individuals and their ability to exploit local food resources.

The gut contents of *P. indica*, the species with the smallest lipid content that we considered as a “control” species, was not affected by increased pressure (Fig. 2B). The gut content of the two more lipid-rich species, *C. helgolandicus* and *R. nasutus*, showed a trend of lower average gut content at 4 bars compared with 1 bar (Fig. 2A and C).

However, this effect was only statistically significant in *C. helgolandicus*; the average gut pigment content at 4 bars was 57% lower than at 1 bar (or 42% lower if the outlier individual was removed). We suggest that the reduced grazing rate under increased pressure was related to the high lipid content of these species. Due to the higher compressibility of lipids relative to water (Yayanos *et al.*, 1978), the buoyancy of the individuals (Visser and Jónasdóttir, 1999; Campbell and Dower, 2003) would have been affected in the 4-bar treatment which, in turn, modulated their feeding currents (Jiang and Strickler, 2005) and lowered their grazing efficiency. The absence of a statistically significant pressure effect in *R. nasutus* could be related to the fact that the individuals used in our experiments were collected at great depths (>400 m) at which they had been feeding on unidentified organic particles (Farstey, 2001). Since all of the *R. nasutus* actively fed on phytoplankton during our grazing experiments, they were clearly not dormant. However, being accustomed to graze at great depths (>40 bars), the two levels of pressure used in our experiment (1 and 4 bars) were perhaps not different enough to produce a statistically significant effect on grazing. In other words, their grazing history and adaptation to high-pressure conditions during capture might have led to grazing patterns that are not representative of their normal grazing activity during their natural ascent to near-surface waters. Following this reasoning, it is also possible that an effect of pressure was found in *C. helgolandicus* because the individuals used in our experiments were from a culture in which they had been grown in 2.35 m of water for several generations. A thorough examination of the pressure effect in many species and over a much wider range of hydrostatic pressures using individuals that are freshly caught *in situ* is required to clarify this.

While most studies have focused on the central role of lipids in buoyancy control of copepods, newer hypotheses emphasize the role of ion replacement and ammonium accumulation in the buoyancy control of Antarctic (Sartorius *et al.*, 2010; Schründer *et al.*, 2013) and Arctic (Freese *et al.*, 2015) copepods. Although *R. nasutus* accumulates large amounts of lipids, we did not find a significant effect of pressure on grazing in this species. A possible reason, in addition to those mentioned above, could be that this species uses a non-lipid-related buoyancy regulation mechanism, for example the accumulation of low-density ions, which might be independent of hydrostatic pressure.

Differences in the composition of lipids between temperate and subtropical copepods (Lee and Hirota, 1973; Kattner and Hagen, 2009), and the differences in compressibility and buoyancy related to this, lead to the expectation of a species-specific effect of hydrostatic pressure. It was recently suggested that copepod species adjust the amount, composition and anatomical location

of lipids according to their preferred habitat and life history traits in a manner that maximizes fitness (Pond, 2012). Different lipid mixtures will have different pressure–volume–temperature (*PVT*) properties and, thus, a distinct response to changes in pressure (Pond and Tarling, 2011). Unfortunately, the *PVT* properties of lipid mixtures have so far only been characterized in *Calanus plumchrus*, a species from temperate waters (Yayanos *et al.*, 1978). This is an area where more work is required.

The pressure that was used in our experiments (4 bars) was low compared with previous studies that investigated metabolic rates and responses of crustaceans to hydrostatic pressure: 40 atm ( $\approx$ 40 bars) (Torres and Childress, 1983), up to 100 atm (Teal, 1971) and several hundreds of atmospheres (George and Marum, 1974), corresponding to hundreds and even thousands of meters in depth. In this study, we found that a small pressure increase of several bars can affect grazing in copepods. If also observed in freshly collected wild individuals, a two-fold decline in grazing rates in response to a pressure change of 3 bars (as observed here in cultured *C. helgolandicus*) has significant ecological implications. For example, copepods “riding” strong internal waves can be displaced 10s of meters (Hauray *et al.*, 1979) with concomitant changes in feeding rates equivalent to those reported here. It also implies that copepods entrained in downwelling currents (not waves) would gain substantially (in terms of grazing rate) if they actively maintain a shallower depth. A more comprehensive test of the pressure effect, to determine whether or not it is widespread in copepods, is required before a thorough consideration of the ecological and evolutionary implications of pressure is possible.

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## REFERENCES

- Andrews, J. C. (1983) Deformation of the active space in the low Reynolds number feeding current of calanoid copepods. *Can. J. Fish Aquat. Sci.*, **40**, 1293–1302.
- Baars, M. A. and Oosterhuis, S. S. (1984) Diurnal feeding rhythms in North Sea copepods measured by gut fluorescence, digestive enzyme activity and grazing on labelled food. *Neth. J. Sea Res.*, **18**, 97–119.
- Banse, K. (1964) On the vertical distribution of zooplankton in the sea. *Prog. Oceanogr.*, **2**, 53–125.
- Bautista, B., Harris, R. P., Tranter, P. R. G. and Harbour, D. (1992) In situ copepod feeding and grazing rates during a spring bloom dominated by *Phaeocystis* sp. in the English Channel. *J. Plankton Res.*, **14**, 691–703.
- Bollens, S. M. and Frost, B. W. (1990) UV light and vertical distribution of the marine planktonic copepod *Acartia hudsonica* Pinhey. *J. Exp. Mar. Biol. Ecol.*, **137**, 89–93.
- Bonnet, D., Richardson, A., Harris, R., Hirst, A., Beaugrand, G., Edwards, M., Ceballos, S., Diekman, R. et al. (2005) An overview of *Calanus helgolandicus* ecology in European waters. *Prog. Oceanogr.*, **65**, 1–53.
- Campbell, R. W. and Dower, J. F. (2003) Role of lipids in the maintenance of neutral buoyancy by zooplankton. *Mar. Ecol. Prog. Ser.*, **263**, 93–99.
- Cass, C. J., Daly, K. L. and Wakeham, S. G. (2014) Assessment of storage lipid accumulation patterns in eucalanoid copepods from the eastern tropical Pacific Ocean. *Deep Sea Res. Part I*, **93**, 117–130.
- Farstey, V. (2001) Feeding and vertical distribution of the calanoid copepods *Rhincalanus nasutus* Giesbrecht and *Pleuromamma indica* Wolfenden in the seasonally mixed water column in the northern part of the Gulf of Aqaba. PhD Thesis. University of Jerusalem.
- Fields, D. M. and Yen, J. (1993) Outer limits and inner structure: the 3-dimensional flow field of *Pleuromamma xiphias* (Copepoda). *Bull. Mar. Sci.*, **53**, 84–95.
- Fields, D. M. and Yen, J. (1997) Implication of copepod feeding currents on the spatial orientation of their prey. *J. Plankton Res.*, **19**, 79–85.
- Freese, D., Niehoff, B., Soreide, J. E. and Sartoris, F. J. (2015) Seasonal patterns in extracellular ion concentrations and pH of the Arctic copepod *Calanus glacialis*. *Limnol. Oceanogr.*, **60**, 2121–2129.
- Hardy, A. C. and Bainbridge, R. (1951) Effect of pressure on the behaviour of decapod larvae (Crustacea). *Nature*, **167**, 354–355.
- Haurly, L. R., Briscoe, M. G. and Orr, M. H. (1979) Tidally generated internal wave packets in Massachusetts Bay. *Nature*, **278**, 312–317.
- Haurly, L. R., Yamazaki, H. and Itsweire, E. C. (1990) Effects of turbulent shear flow on zooplankton distribution. *Deep Sea Res. Part I*, **37**, 447–461.
- Genin, A., Jaffé, J. S., Reef, R., Richter, C. and Franks, P. J. (2005) Swimming against the flow: a mechanism of zooplankton aggregation. *Science*, **308**, 860–862.
- George, R. Y. and Marum, J. P. (1974) The effects of hydrostatic pressure on living aquatic organisms III. Behavior and tolerance of euplanktonic organisms to increased hydrostatic pressure. *Int. Rev. Hydrobiol.*, **59**, 175–186.
- Incze, L. S., Hebert, D., Wolff, N., Oakey, N. and Dye, D. (2001) Changes in copepod distributions associated with increased turbulence from wind stress. *Mar. Ecol. Prog. Ser.*, **213**, 229–240.
- Jiang, H. and Strickler, J. R. (2005) Mass density contrast in relation to the feeding currents in calanoid copepods. *J. Plankton Res.*, **27**, 1003–1012.
- Jiang, H. and Strickler, J. R. (2007) Copepod flow modes and modulation: a modelling study of the water currents produced by an unsteadily swimming copepod. *Phil. Trans. B*, **362**, 1959–1971. doi:10.1098/rstb.2007.2081
- Kattner, G. and Hagen, W. (2009) Lipids in marine copepods: latitudinal characteristics and perspective to global warming. In Arts, M. T., Brett, M. T. and Kainz, M. (eds.), *Lipids in Aquatic Ecosystems*. Springer, New York, pp. 257–280.
- Kleppel, G. S., Pieper, R. E. and Trager, G. (1988) Variability in the gut contents of individual *Acartia tonsa* from waters off Southern California. *Mar. Biol.*, **97**, 185–190.
- Knight-Jones, E. W. and Qasim, S. Z. (1955) Responses of some marine plankton animals to changes in hydrostatic pressure. *Nature*, **175**, 941–942.
- Lee, R. F. and Hirota, J. (1973) Wax esters in tropical zooplankton and nekton and the geographical distribution of wax esters in marine copepods. *Limnol. Oceanogr.*, **18**, 227–239.
- Lee, R. F., Hagen, W. and Kattner, G. (2006) Lipid storage in marine zooplankton. *Mar. Ecol. Prog. Ser.*, **307**, 273–306.
- Lincoln, R. J. (1971) Observations of the effects of changes in hydrostatic pressure and illumination on the behaviour of some planktonic crustaceans. *J. Exp. Biol.*, **54**, 677–688.
- Mackas, D. and Bohrer, R. (1976) Fluorescence analysis of zooplankton gut contents and an investigation of diel feeding patterns. *J. Exp. Mar. Biol. Ecol.*, **25**, 77–85.
- Mackas, D. L. and Burns, K. E. (1986) Poststarvation feeding and swimming activity in *Calanus pacificus* and *Metridia pacifica*. *Limnol. Oceanogr.*, **31**, 383–392.
- McLaren, I. A. (1963) Effects of temperature on growth of zooplankton, and the adaptive value of vertical migration. *J. Fish. Board Can.*, **20**, 685–727.
- Moore, P. A., Fields, D. M. and Yen, J. (1999) Physical constraints of chemoreception in foraging copepods. *Limnol. Oceanogr.*, **44**, 166–177.
- Paffenhöfer, G. A., Bundy, M. H., Lewis, K. D. and Metz, C. (1995) Rates of ingestion and their variability between individual calanoid copepods: direct observations. *J. Plankton Res.*, **17**, 1573–1585.
- Pond, D. W. (2012) The physical properties of lipids and their role in controlling the distribution of zooplankton in the oceans. *J. Plankton Res.*, **34**, 443–453.
- Pond, D. W. and Tarling, G. A. (2011) Phase transitions of wax esters adjust buoyancy in diapausing *Calanoides acutus*. *Limnol. Oceanogr.*, **56**, 1310–1318.
- R Development Core Team. (2011) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, <http://www.R-project.org/>.
- Rice, A. L. (1962) Responses of *Calanus finmarchicus* (Gunnerus) to changes of hydrostatic pressure. *Nature*, **194**, 1189–1190.
- Sartoris, F. J., Thomas, D. N., Cornils, A. and Schnack-Schiel, S. B. (2010) Buoyancy and diapause in Antarctic copepods: the role of ammonium accumulation. *Limnol. Oceanogr.*, **55**, 1860–1864.
- Schründer, S., Schnack-Schiel, S. B., Auel, H. and Sartoris, F. J. (2013) Control of diapause by acidic pH and ammonium accumulation in the haemolymph of Antarctic copepods. *PLoS One*, **8**, e77498.

- Sommer, U., Berninger, U. G., Böttger-Schnack, R., Cornils, A., Hagen, W., Hansen, T., Al-Najjar, T., Post, A. F. *et al.* (2002) Grazing during early spring in the Gulf of Aqaba and the northern Red Sea. *Mar. Ecol. Progr. Ser.*, **239**, 251–261.
- Strickler, J. R. (1982) Calanoid copepods, feeding currents, and the role of gravity. *Science*, **218**, 158–160.
- Strickler, J. R. (1985) Feeding currents in calanoid copepods: two new hypotheses. *In Symp. Soc. Exp. Biol.*, **89**, 459–485.
- Teal, J. M. (1971) Pressure effects on the respiration of vertically migrating decapod Crustacea. *Am. Zool.*, **11**, 571–576.
- Torres, J. J. and Childress, J. J. (1983) Relationship of oxygen consumption to swimming speed in *Euphausia pacifica*. *Mar. Biol.*, **74**, 79–86.
- Visser, A. W. and Jónasdóttir, S. H. (1999) Lipids, buoyancy and the seasonal vertical migration of *Calanus finmarchicus*. *Fish. Oceanogr.*, **8**, 100–106.
- Wheeler, B. (2010) lmPerm: Permutation tests for linear models. R package version 1.1–2. <http://CRAN.R-project.org/package=lmPerm>.
- Williams, R. and Conway, D. V. P. (1984) Vertical distribution, and seasonal and diurnal migration of *Calanus helgolandicus* in the Celtic Sea. *Mar. Biol.*, **79**, 63–73.
- Wong, C. K. (1988) Effects of competitors, predators, and prey on the grazing behavior of herbivorous calanoid copepods. *Bull. Mar. Sci.*, **43**, 573–582.
- Yayanos, A. A., Benson, A. A. and Nevenzel, J. C. (1978) The pressure-volume-temperature (PVT) properties of a lipid mixture from a marine copepod, *Calanus plumchrus*: implications for buoyancy and sound scattering. *Deep Sea Res.*, **25**, 257–268.
- Zarubin, M., Farstey, V., Wold, A., Falk-Petersen, S. and Genin, A. (2014) Intraspecific differences in lipid content of calanoid copepods across fine-scale depth ranges within the photic layer. *PLoS One*, **9**, e92935.