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HORIZONS

The physical properties of lipids and their role in controlling the distribution of zooplankton in the oceans

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A new perspective on the role of lipids in zooplankton is proposed, with solid–liquid phase transitions of lipids being a factor regulating their buoyancy. These phase transitions are controlled by zooplankton in relation to their physical environment, through the selective accumulation of specific lipids with optimum levels of unsaturation. The necessity to control buoyancy and maintain an optimum depth is a fundamental evolutionary force, driving anatomical, biochemical and behavioural adaptations of all organisms within the aquatic realm. It is hypothesized that each species adjusts the amount, composition and anatomical location of lipids, to maximize fitness according to their preferred habitat and life history traits. Recent discoveries regarding the role of phase transitions of lipids in marine zooplankton and their role in regulating buoyancy will require re-interpretation of existing data and stimulate future scientific endeavours in zooplankton research.

KEYWORDS: zooplankton; lipid; phase transitions; buoyancy; omega 3 fatty acids

INTRODUCTION

Lipids are of fundamental importance to many zooplankton taxa (Lee *et al.*, 2006). Being the most chemically reduced organic molecules, lipids are utilized as metabolic energy reserves and are often accumulated in substantial amounts by many aquatic organisms (Bensen and Lee, 1972). An intrinsic physical property of lipids is a specific gravity less than seawater, and this has consequences for buoyancy (Lewis, 1970; Yayanos *et al.*, 1978; Sargent and Henderson, 1986). Furthermore, the compressibility and thermal expansivities of lipids are also greater than that of seawater, with profound consequences for the buoyancy of vertically migrating zooplankton that experience variation in pressure, temperature and salinity (Visser and Jónasdóttir, 1999; Campbell and Dower, 2003). Dominant storage lipids in zooplankton are triacylglycerols (TAG), wax esters (WE) and diacylglyceryl ethers (DAGE) (Lee *et al.*, 2006, Fig. 1). Each of these lipids has a different density and hence each will differ in their effectiveness as hydrostatic agents (Lewis, 1970). Although the role of lipids in regulating buoyancy in

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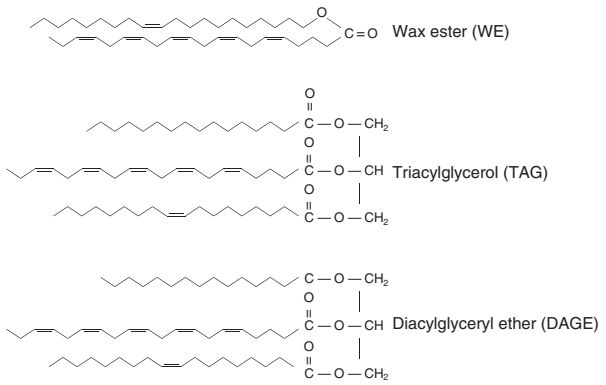

Fig. 1. Examples of the dominant lipids in marine zooplankton. WE containing 20:5(*n*-3) fatty acid and 20:1(*n*-9) alcohol. This is the dominant WE in *Calanoides acutus* and key to the pressure sensitive phase transitions observed by Pond and Tarling (Pond and Tarling, 2011). TAG with 16:0, 20:5(*n*-3) and 18:1(*n*-9) acids. DAGE with 15:0 glyceryl ether and 20:5(*n*-3) and 18:1(*n*-9) fatty acids.

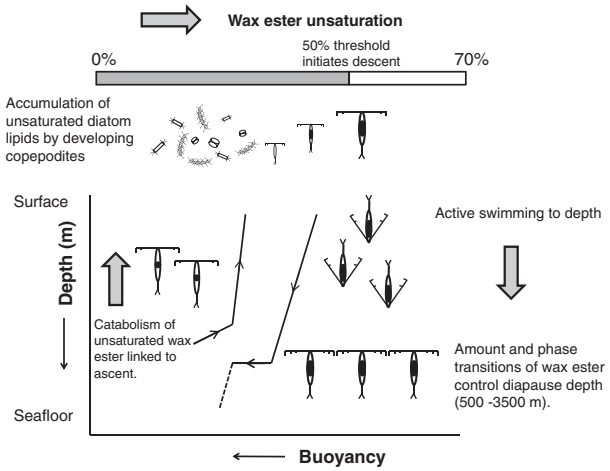

Fig. 2. Schematic of the role lipids play in the life cycle of those calanoid copepods that diapause. Diagram should be read in a clock-wise direction. Developing copepodites feed on and accumulate unsaturated lipids from diatoms. When WE reach an unsaturation threshold of approximately 50%, the positively buoyant copepodites initiate active swimming to depth. At depths >500 m, a combination of pressure-induced phase transitions of the WE and the total amount of WE determine the depth of neutral buoyancy. The dotted line indicates the potential for migrating below the point of neutral buoyancy, although clearly for the majority of the population this does not occur, since the copepodites would become increasingly negatively buoyant and lost from the population. An opposing buoyancy control mechanism such as ion exchange could potentially operate to counteract this (Sartoris *et al.*, 2010). During diapause, selective catabolism of the unsaturated WE, i.e. those most dense components of the lipid pool are proposed to be a timing and buoyancy mechanism linked to the ascent of the copepods back to surface waters. Note that the actual diapause depth will be a combination of the overall buoyancy of the copepod in relation to its physical environment, and that the figure focuses on the role that lipids play in this process.

zooplankton has been extensively studied, it has only recently been recognized that the composition of lipids can determine pressure-induced liquid–solid phase transitions (Pond and Tarling, 2011). These phase transitions are thought to be involved in buoyancy regulation and are a controlling factor of the life cycles of calanoid copepods that diapause (Clark *et al.*, 2012; Pond *et al.*, 2012). This article aims to (i) summarize recent findings on how the biophysical properties of lipids are exploited by zooplankton to maximize life-history fitness, (ii) collate current information on the physical properties of marine lipids, and (iii) propose future areas of research on how the physical properties of lipids are linked to the behaviour and distribution of zooplankton in the oceans.

A NEW PERSPECTIVE ON THE ROLE OF LIPIDS IN THE DIAPAUSE LIFE PHASE OF CALANOID COPEPODS

Initiation of diapause and buoyancy control

Many large calanoids, and particularly those at high latitudes overwinter in a dormant state, termed diapause. Understanding the diapause life-phase of calanoid copepods has long been an aim of biological oceanography, particularly since the development of predictive models coupling zooplankton behaviour with three-dimensional ocean flow fields (Miller *et al.*, 1998). Progress in addressing this issue has been hindered by both an inability to initiate diapause in captive calanoid populations and also the expense of obtaining deep water samples, particularly during winter. In a previous Horizons article, Irigoien (Irigoien, 2004) considered the role of lipids in the life cycle of *Calanus finmarchicus* and identified a number key issues relating to this topic. The current article extends the hypotheses of Irigoien (Irigoien, 2004) by highlighting recent findings on the significance of the physical properties of lipids in the life cycles of zooplankton.

Two recent scientific advances have indicated that the composition of WE is critical in controlling the diapause behaviour of calanoid copepods (Pond and Tarling, 2011; Clark *et al.*, 2012). In a study of *Calanoides acutus*, the dominant large calanoid species in the Southern Ocean, it was discovered that copepods only initiate descent to the deep ocean when their WE reached a threshold of 50% unsaturation (Pond and Tarling, 2011; note that unsaturation relates to the number of double bonds between carbon atoms in the WE molecule). Furthermore, this level of unsaturation was achieved primarily by accumulating eicosapentanoic acid, i.e. 20:5(*n*-3) originating from diatoms. Sufficiently provisioned with large reserves of highly

unsaturated WE, the positively buoyant copepods initiate descent and actively swim into the ocean depths (Fig. 2). Crucially, at depths >500 m, hydrostatic pressure and 50% unsaturation induces liquid–solid phase changes of WE (Pond and Tarling, 2011). These phase changes are accompanied by an increase in density of the lipid that reduces hydrostatic lift and enable copepods to achieve neutral buoyancy. Diapause depth will be a combination of the total amount of WE and the degree of unsaturation that controls the phase transition in relation to the physical environment (temperature and salinity). Achieving neutral buoyancy is essential for copepods that overwinter in diapause, since active swimming behaviour in the deep ocean will both attract predators, and deplete metabolic energy reserves. Jónasdóttir (Jónasdóttir, 1999) speculated that a major role for WE in diapausing copepods is the attainment of neutral buoyancy. The recent findings of Pond and Tarling (Pond and Tarling, 2011) have reaffirmed this theory and provided more evidence for the specific mechanism involved.

Termination of diapause

It can be seen that the composition of lipids in calanoid copepods and their physical properties offers a mechanism that explains both the initiation of diapause and the control of neutral buoyancy while at depth. The obvious remaining question in the life cycle of copepods is what controls the termination diapause and re-ascent to surface waters? Clark *et al.* (Clark *et al.*, 2012) conducted a seasonal sampling programme of a population of *Calanus finmarchcius* in Loch Etive on the west coast of Scotland, a habitat with limited advection and where populations can be sampled year round. Although Loch Etive is comparatively shallow, with a maximum depth of 140 m, copepods do undergo diapause, but to a lesser degree than in open ocean deep water environments (Clark *et al.*, 2012). It was discovered that during the period of diapause, *C. finmarchicus* selectively catabolize polyunsaturated WE. Given that polyunsaturated WE are pressure sensitive, changing from liquid to solid phase under pressure (Pond and Tarling, 2011), the CV *C. finmarchicus* in Loch Etive were catabolizing those lipids that in a deep water situation (>500 m) would reduce the overall buoyancy imparted by the large lipid pool, i.e. the copepods were catabolizing what would be the more dense components of the lipid pool during diapause. Copepods in Loch Etive would be unable to descend to depths and hydrostatic pressures where phase changes of WE occur and would remain positively buoyant. This would necessitate active swimming to maintain depth. Such active swimming behaviour during winter is consistent with the comparatively high rates of lipid utilization of *C. finmarchicus* in Loch Etive and other neritic waters (Campbell *et al.*, 2004; Clark *et al.*, 2012). It could be argued that Loch Etive populations of *C. finmarchicus* are not representative of those from deep water environments and that the selective utilization of polyunsaturated WE is specific to shallow environments (Mayor *et al.*, 2011). However, previous studies have indicated that polyunsaturated WE are also selectively catabolized in deep water environments (Pepin *et al.*, 2011; Pond *et al.*, 2012), suggesting that fundamental controls on diapause behaviour of copepods inhabiting open ocean and neritic environments are similar.

As well as controlling buoyancy, the composition of WE and specifically the process of selective catabolism of polyunsaturated WE could act as a timing device involved in the termination of diapause and re-ascent to surface waters. Selective catabolism of polyunsaturated WE is likely to be regulated by substrate specificity of hormone sensitive lipases. Such a mechanism has not been studied in zooplankton, but does operate in a species of Notothenioid fish where PUFA-rich TAG are selectively catabolized, resulting in the accumulation of TAG rich in saturated and monounsaturated fatty acids (Hazel and Sidell, 2004). Interestingly, it is also thought that the accumulation of TAG facilitates buoyancy control in these fish (Devries and Eastman, 1978; Eastman, 1988).

In summary, the recent findings on the composition and biophysics of WE offer a unifying explanation of the key controls of the life cycle of calanoid copepods that diapause (Fig. 2). It is now apparent that copepods carefully regulate the composition of the oil sac both during the feeding/accumulation and dormant/utilization life phases and this affects pressure-dependent phase transitions of these lipids. Turnover of waxes in the oil sac can be surprisingly high during the development of copepodites prior to diapause. Using ^{13}C labelled diatoms as a tracer of dietary fatty acids, Graeve *et al.* (Graeve *et al.*, 2005) found that the WE reserves of CV *Calanus hyperboreus* were exchanged after only 11 days of feeding, suggesting an extremely dynamic turnover of lipids across the oil sac membrane. Furthermore, in a now classic feeding study of Arctic calanoid copepods, Graeve *et al.* (Graeve *et al.*, 1994) established that wax reserves were heavily influenced by diet with 16:1(*n*-7) and 18:4(*n*-3), biomarkers for diatoms and flagellates, respectively, increasing in copepods fed these prey items. However, the proportions of 20:5(*n*-3), a diatom biomarker thought to be a key factor controlling the pressure sensitivities of WE (Pond and Tarling, 2011), remained relatively constant

in these feeding trials, irrespective of dietary input. Thus, it is proposed that composition of the WE in the oil sac of calanoid copepods does not simply reflect the diet of the copepods, but is also controlled during selective catabolism and retention of dietary and *de novo* synthesized lipids. The specific mechanism by which the composition of the oil sac is regulated and how this regulation could potentially serve as a timing mechanism in calanoid copepods remains to be revealed, but is likely to be mediated by hormone sensitive lipases under genetic control. A multi-faceted approach using traditional biochemistry in conjunction with transcriptomics will enable these issues to be addressed.

The next step towards developing more predictive models of the life cycles of calanoid copepods is to quantify changes in specific gravity associated with WE phase transitions and determine how this impacts on the overall buoyancy of copepods in relation to their physical environment. Such an approach has been successfully achieved for the sperm whale, which was demonstrated to be capable of using phase changes of WE to regulate buoyancy over its entire latitudinal range, i.e. from the tropics to the poles (Clarke *et al.*, 1978b). Furthermore, the necessity to optimize both the amount and composition of lipids accumulated by copepods that diapause is likely to involve trade-offs in terms of elevated predation risks associated with either a prolonged feeding phase in surface waters compared with early descent to depth. Such parameters should be considered in state-dependent life history models to evaluate population-level consequences of lipid phase transitions (Varpe *et al.*, 2009).

WHY ARE THE LIPID COMPOSITIONS OF ZOOPLANKTON TAXA SO VARIABLE?

What are the functional roles of fatty alcohols?

WE are long-chain molecules comprising a fatty acid esterified to a fatty alcohol and are unquestionably important sources of metabolic energy (Fig. 1, Lee *et al.*, 1970). The fatty acid component of the molecule is largely derived from dietary fatty acids, while the fatty alcohol is synthesized *de novo* from dietary lipid, carbohydrate and protein (Sargent and Henderson, 1986). The fatty acid components of WE have received the most attention and are widely used as trophic markers (Graeve *et al.*, 1994). However, the fatty alcohol composition of many marine zooplankton has also been well documented, suggesting major species-specific differences (Albers *et al.*, 1996; Scott *et al.*, 2002; Lee *et al.*, 2006, Cass *et al.*, 2011). It has been observed that herbivorous zooplankton from high latitude ecosystems with a highly seasonal diet accumulate long-chain 20:1(*n*-9) and 22:1(*n*-11) alcohols, while omnivorous and carnivorous taxa accumulate 14:0 and 16:0 alcohols (Lee *et al.*, 2006). Since long-chain fatty acids and alcohols are more chemically reduced than their shorter chain counterparts, this has been widely cited in the literature as an adaptation of high latitude zooplankton to maximizing energy accumulation in a highly seasonal environment (Albers *et al.*, 1996; Scott *et al.*, 2002). Albers *et al.* (Albers *et al.*, 1996) presents one of the few studies actually to calculate energy yield for short- and long-chain molecules, although these values were calculated on the basis of kjoule/mole rather than mass and this can be misleading. For instance, the mass of one mole of 16:0 fatty acid is 256 g and this will obviously contain less energy than one mole of 22:1(*n*-11) with a mass of 338 g. Consideration of energy yields of comparable masses of fatty acids and the adenosine triphosphate (ATP) they generate during catabolism is a more realistic analysis of the energy yields between different lipids. For example, the complete catabolism of one molecule of 16:0 generates 129 ATP, while 22:1(*n*-11) yields 176 ATP (note: these energy values are based on fatty acids since long-chain alcohols are converted to their acid analogues before catabolism: Sargent and Henderson, 1986). After standardizing for mass, it can be calculated that 1-unit mass of the long-chain 22:1(*n*-11) fatty acid contains only 3.2% more energy than the short-chain 16:0. A 3.2% energetic advantage appears insufficient to explain the high levels of long-chain fatty alcohols in some high latitude copepods.

An alternative explanation for the functional roles of long-chain fatty alcohols is provided by the insight that these molecules are only accumulated by copepods that over winter in diapause (Lee *et al.*, 2006). In studying the diapause behaviour of *C. acutus*, Pond *et al.* (Pond *et al.*, 2012) provided data suggesting that fatty alcohols may play a role in phase changes that are implicated in buoyancy regulation of these copepods. Detailed analyses of the fatty alcohol composition of WE from depth-stratified samples of *C. acutus* indicated that copepods at depth contain more unsaturated fatty alcohols than those from surface waters. This led Pond *et al.* (Pond *et al.*, 2012) to postulate that the fatty alcohols, and in particular 20:1(*n*-9), contribute to the pressure-dependent phase changes observed for the WE of

C. acutus. It is therefore probable that beyond being a simple metabolic storage reserve, fatty alcohols, through their biophysical properties, can also facilitate the various life-history strategies adopted by zooplankton, particularly those where long-term maintenance of neutral buoyancy is crucial. This theory can be addressed by determining the biophysical properties and biochemical composition of WE from a range of zooplankton in relation to their life cycle.

WE versus TAG

Some zooplankton store lipids as WE and others TAG; a good illustration of this difference is the comparison of the Southern Ocean copepods, *Calanoides acutus* and *Calanus propinquus*. Both species store their lipid reserves in a prominent oil sac running the entire length of the prosome (Fig. 3). However, *C. acutus* accumulates WE in its oil sac, while *C. propinquus* stores TAG (Schnack-Schiel *et al*., 1991). As detailed previously in this article, diapause is a key feature of the life cycle of *C. acutus* and the pressure sensitivity of its WE stores, at ecologically relevant hydrostatic pressures, are implicated in facilitating this behaviour. In contrast, *C. propinquus* does not diapause, rarely descends below 500 m and remains comparatively active throughout the winter (Schnack-Schiel *et al*., 1991; Atkinson, 1998). Why do such non-diapausing calanoids store TAG? A plausible explanation is that TAG does not exhibit the same pressure sensitivities as WE and that this is either beneficial to an organism largely restricted to occupying the upper 500 m of the water column, or that an absence of pressure sensitive WE precludes an ability to achieve neutral buoyancy and deep water diapause. There are many other examples of calanoid copepods storing predominantly TAG (Lee *et al*., 2006). Understanding why this is so will require a detailed analysis of the physical properties of TAG in relation to the evolutionary histories, life cycles and environmental preferences of different species.

It could be argued that the variation in lift provided by different lipids in zooplankton is very small (Lewis, 1970) and perhaps not significant in terms of overall buoyancy. However, the actual changes in specific gravity required to achieve neutral buoyancy in an organism or object can be surprisingly small. A good example is provided by the bathyscaphe Trieste, until recently was the only manned submersible to dive to the deepest ocean depth, i.e. the Challenger Deep of the Mariana Trench (Piccard and Dietz, 1961). During initial development dives, it proved impossible to maintain neutral buoyancy and effectively 'hover' the bathyscape a few metres above the sea floor to facilitate scientific observations. This problem was solved by attaching a 'tail' of rope that hung below the submersible. The submersible descended until it rested on the sea floor and ballast, in the form of iron pellets, was released causing the craft to slowly rise in the water column until sufficient mass of rope lifted off the sea floor to counteract the mass of the dropped ballast. Thus, the mass of a few metres of rope enabled a submersible with a 50 ton displacement to achieve neutral buoyancy in the deep sea.

Why do some zooplankton accumulate DAGE?

Phleger *et al*. (Phleger *et al*., 1997 and references therein) provided a comprehensive summary of current

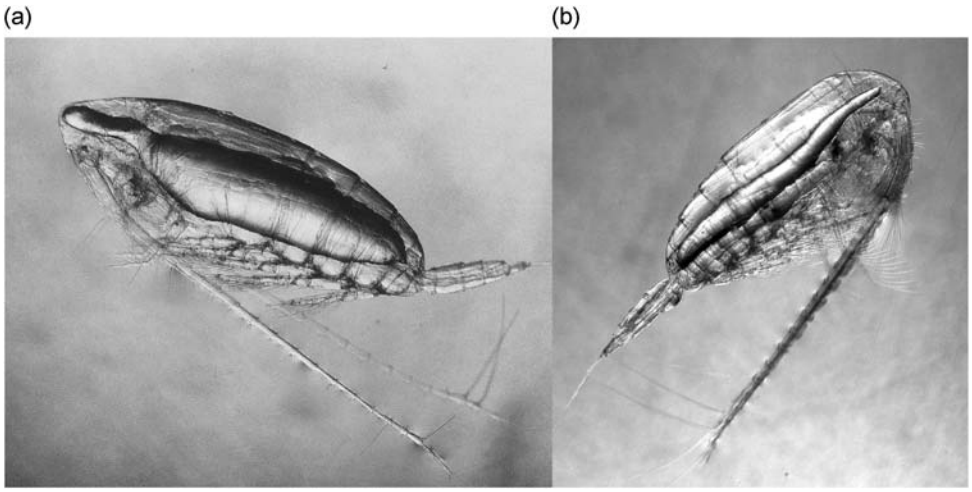

Fig. 3. (**a**) Copepodite stage CV *Calanoides acutus* with large oil sac containing WE and (**b**) female *Calanus propinquus* oil sac containing TAG. No image of a stage CV *C. propinquus* is available for this article, but note that these often contain amounts of lipid in their oil sac that are comparable to CV *C. acutus*.

knowledge of DAGE in a wide range of aquatic animals. From the perspective of zooplankton, the pteropod *Clione limacina* contains the highest amounts of this lipid of any taxon, and around 28% of their lipid comprises DAGE. Given that DAGE has a lower density than TAG (Lewis, 1970), Phleger *et al.* (Phleger *et al.*, 1997) suggested that this lipid would provide lift for *C. limacina* and enable this predatory pteropod to maintain a similar depth horizon as its prey. Why does *C. limacina* predominantly synthesize DAGE rather than TAG or WE? What are the consequences for the ether links and comparatively high levels of odd chain 15:0 and 17:0 glyceryl ethers for the phase transitions of this lipid? Given the importance of pteropods in the oceans (Hunt *et al.*, 2008), these questions require answers.

How universal are phase transitions of zooplankton lipids?

The emphasis of the Horizons article so far has been on calanoid copepods, but many other zooplankton store substantial amounts of lipid with important fitness implications. As with copepods, Euphausiidae also have tendencies to accumulate either TAG or WE, although the ecophysiological significance of these lipid storage modes has not been identified (Lee *et al.*, 2006). The importance of lipids for buoyancy in some euphausiid species has been considered by Falk-Petersen *et al.* (Falk-Petersen *et al.*, 2000) and has been shown to vary seasonally in relation to both lipid accumulation and catabolism. Antarctic krill (*Euphausia superba*) primarily accumulate TAG and are often perceived as containing comparatively low levels of lipid, perhaps because the lipids are not stored in large obvious vacuoles, but are located intracellularly throughout the body and often concentrated in the hepatopancreas (Fig. 4). However, levels of lipid in Antarctic krill can actually be extremely high, often reaching ~45% of dry weight at the onset of winter (Falk-Petersen *et al.*, 2000; Atkinson *et al.*, 2002). Preliminary analyses of the temperature-dependent phase transitions of lipids in *E. superba* from the Southern Ocean have indicated major differences between individuals. Lipids of three (A–C) immature krill (~45 mm total length) from the Scotia Sea were subjected to differential scanning calorimetry (DSC). Peak of melt, i.e. the temperature at which most lipid undergoes phase transition from solid to liquid varied from 1.2°C for krill A to 11.9°C for krill C (Fig. 5a). Total heat flow (mJ) indicated by the integrated area

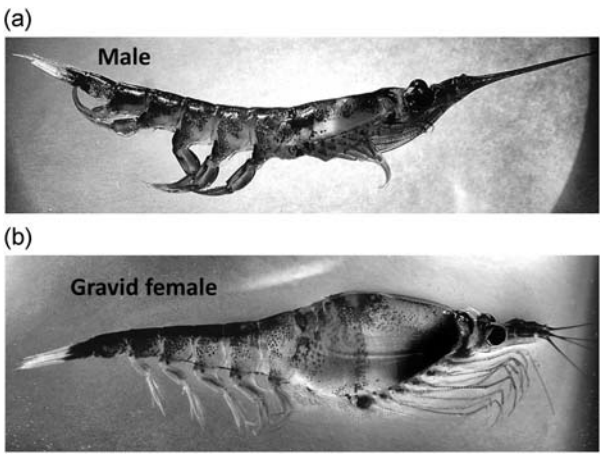

Fig. 4. The bentho-pelagic euphausid Antarctic krill, *Euphausia superba* which can accumulate substantial lipid stores (**a**) male and (**b**) gravid female. Both specimens collected from the upper 100 m of the water column of the Scotia Sea during the austral summer 2003.

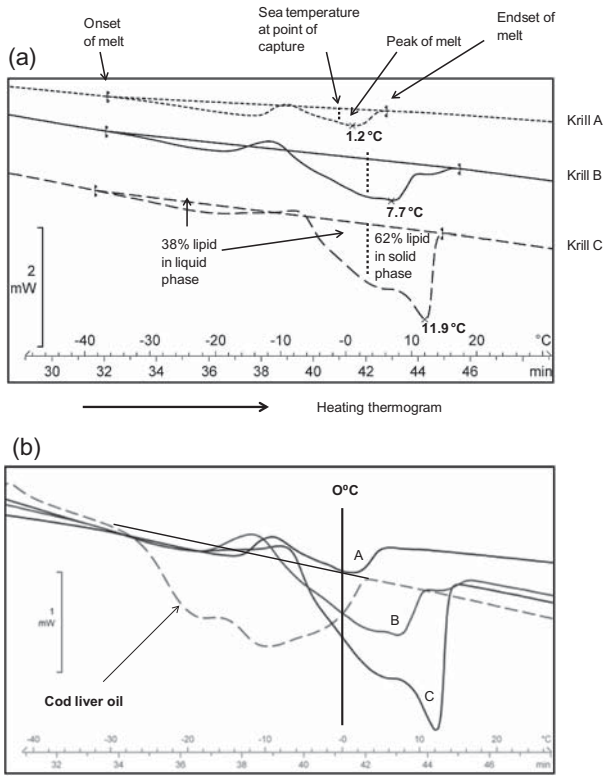

Fig. 5. (**a**) DSC heating thermograms of total lipid of individual Antarctic krill (*Euphausia superba*). Sample initially cooled to −60°C and heated to 40°C at 1°C min^{-1}. All specimens ~45 mm total length. Temperatures annotated on plots indicate 'peak of melts', i.e. the temperature at which most lipids changes from solid to liquid during the DSC analysis. Vertical line denotes sea temperature at the instant of capture. Thermogram peak area to the right of this line indicates lipid in solid phase and to the left, the lipid in liquid phase in the krill, i.e. for krill C, 62% of lipid would be in solid phase when caught. (**b**) Composite DCS thermograms of the three krill including data for cod liver oil. Note that phase transitions of cod liver oil are much lower than those of krill, suggesting that in contrast to krill, the majority of lipid in the liver of cod would be in liquid phase in its environment.

Table I: Sea temperature, lipid concentration and DSC-derived melting characteristics of three krill collected from the Scotia Sea during the austral summer 2007

Krill	Sea temperature at sampling location (°C)	Lipid content (mg/g dry weight)	Peak of melt (°C)	% of lipid in solid phase at capture
A	−1.06	157	1.2	27
B	3.32	293	7.7	44
C	3.32	333	11.9	62

under the baseline for the endothermic peaks provide an index of the amount of lipid in solid or liquid phase at any given temperature (Fig. 5a). Given that the sea temperature from where the krill were captured was determined, an estimate of the amount of lipid that would have been in solid and liquid phase for each krill in its environment can be derived (Fig. 5a, Table I). These are only preliminary data, but two important points are immediately apparent: (i) there are considerable differences in the phase-transition temperatures of the lipids in different krill and (ii) a substantial amount of that lipid would be in solid phase at ambient environmental temperatures. For one of these krill, a surprising 62% of its lipids would have been in solid state at the time of capture. These data raise a number of questions regarding the role of lipids in *E. superba*. Is it simply coincidence that the phase-transition temperatures of the lipids in krill span environmental temperatures, where small changes in temperature will have consequences for the proportions of lipid in solid and liquid phase? Is it also coincidence that those krill containing the most lipid have a greater proportion of their lipid in solid phase, which would potentially counteract the buoyant properties of large lipid reserves? The importance of lipid phase changes of WE for buoyancy control in calanoiod copepods has been discussed previously. The preliminary data for the TAG storage reserves of *E. superba* presented in Fig. 5a indicates that lipid phase transitions could also influence the buoyancy of euphausids.

Inclusion of DSC data for oil extracted from the liver of Atlantic cod, *Gadus morhua*, is also informative in this respect (Fig. 5b). Peak of melt for cod liver oil is −10°C, suggesting that the vast majority of the lipid pool would be in liquid phase in the environment. Cod are active, fast-swimming predators that employ a swim bladder to regulate buoyancy. Having highly unsaturated lipids in liquid phase in the liver would be advantageous to a mobile predator since these lipids are more readily metabolized compared with those in the solid phase (Ohtsu *et al.*, 1993). In contrast, the lipids of krill melt at much higher temperatures and a substantial proportion of their lipids would therefore be in the solid phase in their environment. Given that the microplanktonic food of krill comprises highly unsaturated lipids with low melting points, it is perhaps surprising that the phase transitions of lipids accumulated by these euphausids occur at such high temperatures. The penalty in terms of a less readily metabolized energy source must be offset by a positive benefit and I hypothesize that this is the control of buoyancy. It seems implausible that krill can achieve the level of buoyancy control demonstrated by diapausing copepods, but any energetic efficiencies, perhaps linked to feeding behaviour, could significantly reduce their overall metabolic budget.

Many species of krill including *E. superba* are well documented as undertaking substantial diurnal vertical migrations (Schmidt *et al.*, 2011) and in doing so will experience rapid changes in temperature that will adjust the proportions of solid- versus liquid-phase lipid. Recently, Antarctic krill and, in particular, gravid females have been observed mating and feeding at great depths (1000−3500 m, Clarke and Tyler, 2008; Kawaguchi *et al.*, 2011). How these euphausids adapt to such extremes of hydrostatic pressure, and the role of storage lipids in regulating buoyancy and their reproductive behaviour in the deep ocean also merits attention. Exploiting recent developments in thermomechanical analytical techniques and carefully designed laboratory experiments will enable these theories to be tested on euphausids and other taxa.

How significant are differences in the anatomical location of lipids?

How lipids are stored anatomically differs among the various zooplankton taxa, with single oil sacs, multiple smaller vacuoles and/or intracellular storage being employed by different species. Are these features of the lipid reserves of some zooplankton linked to locomotion or feeding guild? Previous studies have recognized that zooplankton inhabiting the deep sea often store lipid in multiple vesicles and that these lipids are comparatively saturated (Lee *et al.*, 1971; Bensen and Lee, 1972).

Eurythenes obesus is a predatory/scavenging deep sea amphipod and the considerable lipid reserves it contains are stored throughout its body in multiple vesicles (Fig. 6). Such a mode of lipid storage would enable the amphipod to float freely in any orientation and could be an advantage for an animal that is largely a scavenger (Stoddart and Lowry, 2004). Active, filter-feeding calanoid copepods have oil sacs that run the length of the body and utilizing extended antennae 'hang' head up in the water column (e.g. *Calanus simillimus*, Fig. 7).

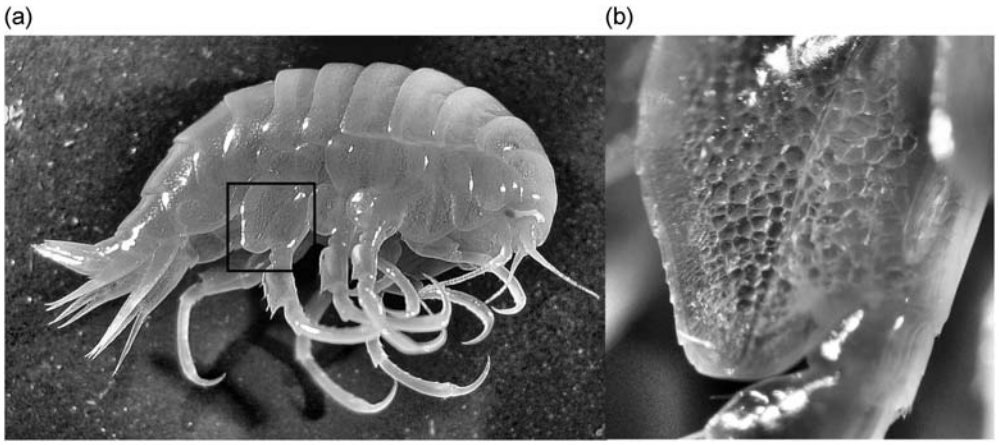

Fig. 6. (**a**) *Eurythenes obesus* with substantial lipid reserves stored in vesicles throughout the body and (**b**) an enlarged view of the coxal plate revealing the numerous lipid vesicles contained within this deep water amphipod. Specimen collected March 2004 in the vicinity of South Georgia, Southern Ocean (750–1000 m depth horizon).

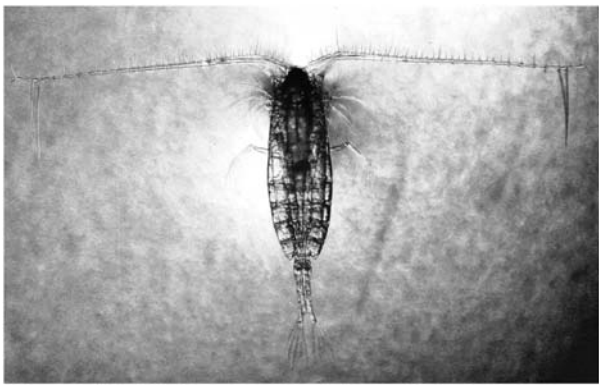

Fig. 7. The Southern Ocean copepod, *Calanus simillimus* indicating the typical head up, outstretched antennae orientation of filter-feeding calanoids.

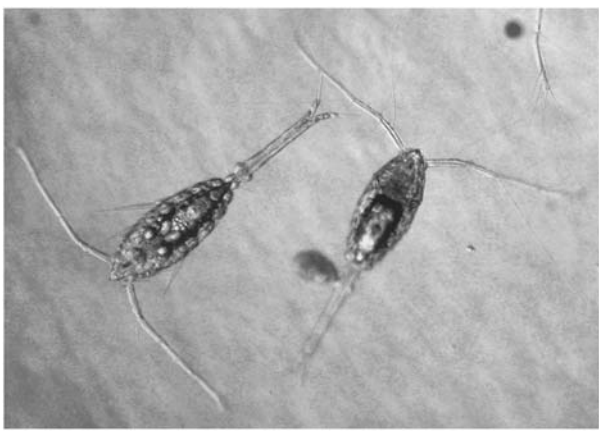

Fig. 8. *Oithona frigida* females with multiple oil vesicles throughout the body, although these are often concentrated in the lower prosome.

In contrast, *Oithona* sp. often contain numerous oil vesicles concentrated in the posterior of the prosome. *Oithona* are not active swimmers, have no particular orientation with respect to gravity, preferentially ingest motile prey and are sac spawners (Atkinson, 1995, Fig. 8). To what extent do the lipid stores in these species link with locomotory, feeding and reproductive behaviour?

In a study of deep water decapods, Herring (Herring, 1973) postulated that the large WE stores in the thorax of deepwater decapods shrimp would affect the 'trim', i.e. the orientation of the organism in the water column with respect to gravity. Herring noted that during reproduction, some female decapods produce lipid-rich eggs that are retained on the pleopods. Such a transfer of lipid from the hepatopancreas on the dorsal side of the thorax to the ventral side of the abdomen could have considerable implications for the animal orientation in its environment. However, Herring (Herring, 1973) also noted that the eggs of these decapods were rich in TAG, i.e. lipids that are less buoyant than WE (Lewis, 1970) and that any affect on trim would therefore be counteracted. This is another example of where a zooplankter modifies its lipid composition according to its life history traits.

Gelatinous zooplankton are generally considered to accumulate only low levels of lipid, but some species do contain oil vacuoles and particularly those inhabiting the deep sea (Terazaki, 1993; Lee *et al.*, 2006; personal observations). Chaetognaths (arrow worms) often contain a prominent oil vacuole located centrally in their elongated body (Fig. 9). Not all chaetognaths within a population contain these oil vacuoles, which also vary considerably in size, and it is not clear if the lipids are actually storage reserves, or simply indicate

Fig. 9. Chaetognaths (*Eukrohnia hamata*) with prominent, centrally located oil vacuoles. It is hypothesized that the lipid contributes to both buoyancy and trim of these ambush predators. Collected January 2012 from the Sofia Deep north of Svalbard (81°45′N).

recent feeding activity on lipid-rich copepods. The obvious question is whether these prominent centrally located lipid vacuoles impact on buoyancy? Chaetognaths are ambush predators which typically 'hover' in midwater in a horizontal position (J. Børge, personal communication). If the lipid vacuole, transient or otherwise, contained in chaetognaths impacts on their buoyancy, where would they be located to facilitate the horizontal orientation commonly adopted by these predators? Any position other than mid-body would have detrimental consequences for their trim and could hinder locomotion. The specific anatomical positioning of the oil vacuole in chaetognaths suggests that this is an adaptation to negate any detrimental impacts on trim that these lipids may have (Fig. 9).

Unusual lipid storage of hydrothermal vent shrimp

The lipids of the planktonic postlarvae of benthic shrimp that inhabit Mid-Atlantic ridge deep sea hydrothermal vents exhibit intriguing characteristics. These larvae typically have a saddle of lipid droplets which encircle the posterior cephalothorax and extend into the abdominal segments (Herring and Dixon, 1998). As with most other zooplankton inhabiting the bathypelagic zone, the lipids of postlarval vent shrimp are

Fig. 10. The deep water decapod *Pasiphaea scotiae,* with a large lipid vacuole clearly visible in the thorax. Specimen collected March 2004 in the vicinity of South Georgia, Southern Ocean (750–1000 m depth horizon). Such a large positively buoyant lipid vacuole in the thorax of the decapod must have significant implications for the 'trim' of the zooplankter within the water column.

dominated by WE, and these are thought to fuel the energy requirements for dispersal between geographically disparate vent sites (Pond *et al.*, 1997, 2000a). A striking feature of the WE in vent shrimp are high amounts of polyunsaturated fatty acids, particularly 22:6(*n*-3), which accounted for up to 50% of WE fatty acids (Pond *et al.*, 2000b). The accumulation of these unprecedented levels of 22:6(*n*-3) in WE is thought to be intimately linked to the life cycle of the shrimp. Dominant food sources for the adult shrimp at the vent sites are filamentous chemoauthotrophic bacteria that do not synthesize the essential long-chain PUFA, such as 22:6(*n*-3) (Pond *et al.*, 2000b). As a consequence, the shrimp utilize their bathypelagic, dispersal phase to accumulate high levels of lipid rich in PUFA to sustain growth and development after descent to an active vent habitat (Pond *et al.*, 2000b). However, Herring and Dixon (Herring and Dixon, 1998) also noted that the large lipid reserves contributed to buoyancy of postlarval shrimp, which either floated or were neutrally buoyant in chilled seawater on capture. In terms of buoyancy, it is noteworthy that when vent shrimp depart the plankton and adopt a benthic lifestyle at a suitable vent site, WE stores are converted into TAG (Pond *et al.*, 2000b). As discussed previously, the hydrostatic lift provided by WE exceeds that of TAG, and the transition in the dominant lipid storage mode between larvae and adults could be driven by the requirement to adjust buoyancy during the switch from planktonic to benthic habitats.

CONCLUSIONS

The image of deepwater decapod *Pasiphaea scotiae* sampled from the Southern Ocean provides a powerful example of how large amounts of lipid can be accumulated in specific anatomical locations within a zooplankter (Fig. 10). Such lipid storage modes must

have profound implications for buoyancy, trim and the behaviour of organisms inhabiting the planktonic realm. The interplay between locomotion and feeding behaviour and the necessity to maintain position within the water column is fundamental to the success of zooplankton (Genin *et al.*, 2005; Kiørboe, 2011). It is now clear that the composition of lipids in zooplankton is carefully regulated, and it is hypothesized that the principal factor driving this regulation is the control of buoyancy. The patterns we observe suggest that different species, through genetic control on dietary selection, assimilation and the synthesis and catabolism of specific lipids, have evolved defined lipid compositions. It is suggested that these lipid compositions are linked to temperature- and pressure-induced phase transitions that adjust specific gravity and hydrostatic lift. The concept of aquatic organisms using phase transitions of lipids to control buoyancy is not new: sperm whales (Clarke, 1970, 1978a) and orange roughy (Phleger and Grigor, 1990) have previously been shown to exploit this mechanism, which has until now been overlooked by those studying zooplankton.

The necessity to control buoyancy and maintain an optimum depth is a fundamental evolutionary force, driving anatomical, biochemical and behavioural adaptations of organisms within the aquatic realm. The requirement to overcome gravity in the oceans is as important for plankton through to whales, as it is for flying insects and birds in air. Recent discoveries regarding the role of phase transitions of lipids have given zooplankton research an entirely new perspective, which will require the re-interpretation of existing data and stimulate future scientific endeavours in this field.

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