The Ecology and Evolution of Deep-Sea Copepods: Niche Separation in a Three-Dimensional Habitat

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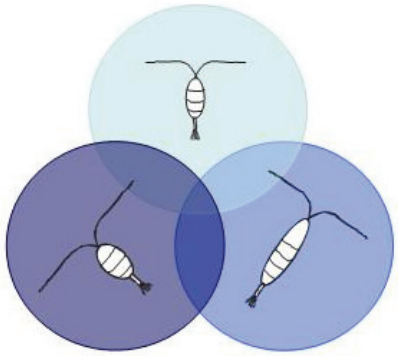

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Die Natur hat zehntausend Farben,
und wir haben es uns in den Kopf gesetzt,
die Skala auf zwanzig zu reduzieren.

Hermann Hesse, Klingsors letzter Sommer

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ABSTRACT

Specialisation and niche separation are key processes for minimising or avoiding competition between co-occurring species. This study aimed at elucidating these processes for co-occurring meso- and bathypelagic representatives of two calanoid copepod families (Euchaetidae and Aetideidae) by characterising their respective ecological niches based on ecological, biochemical and molecular phylogenetic aspects. Euchaetidae and Aetideidae were sampled by stratified hauls in the two high-latitude habitats of Arctic Fram Strait and the Atlantic sector of the Southern Ocean, in the vicinity of the Antarctic Polar Front.

Spatial niches were described by vertical distribution and abundance of the respective species. Lipid content and composition were specified in order to investigate energy storage. Fatty acids and stable isotopes were applied as trophic markers to elucidate general feeding patterns and trophic level within the pelagic food web. While fatty acid composition was determined as a general indicator of feeding habits by taking advantage of the specific origin of distinct fatty acids, stable isotope signatures provided information on food web interactions and predator-prey relationships.

In Fram Strait three euchaetid species endemic to the Arctic and boreal-Atlantic regions occurred, i.e. *Paraeuchaeta norvegica*, *P. glacialis* and *P. polaris.* In the Southern Ocean *P. antarctica*, *P. rasa* and *P. biloba* were the three endemic abundant species. The bathypelagic cosmopolitan *P. barbata* occurred regularly in both regions. Two aetideid genera occurred regularly, i.e. *Gaetanus* and *Aetideopsis*, while *Chiridius* was only found in Fram Strait.

Paraeuchaeta species stored high amounts of wax esters as dominant lipid class, presumably as long-term energy stores and as buoyancy aids, enabling these heavily built copepods to pursue their tactile predatory behaviour. Wax esters were mainly composed of the two fatty acids 18:1(n-9) and 16:1(n-7) as well as of the unsaturated fatty alcohols 14:0 and 16:0 in Antarctic species and 20:1 and 22:1 in Arctic species. Next to high levels of the fatty acid 18:1(n-9), carnivorous feeding of *Paraeuchaeta* species was proven by trophic biomarkers of calanid copepods. Conspicuously high levels of 16:1(n-7), a typical diatom fatty acid and in predatory copepods generally interpreted as trophic marker for herbivorous prey, leads to two explanations: 1) dietary derived 16:1(n-7) is selectively retained in the storage lipids or 2) *Paraeuchaeta* spp. are able to synthesise 16:1(n-7) *de novo*, a metabolic pathway that is rarely found in animals. Wax esters occurred in moderate amounts in *Gaetanus* spp. and were nearly absent in *Aetideopsis* spp. as well as in *Chiridius obtusifrons*, which possibly mainly stored triacylglycerols (TAG) considered to be a short-term energy depot. The more balanced fatty acid composition suggests omnivorous feeding habits for aetideid species.

Arctic species strongly preyed on the abundant calanid copepods, as indicated by high levels of C20 and C22 fatty acids and alcohols and by at least one trophic level between calanid species and predators based on stable isotope ratios of nitrogen. Even bathypelagic *P. barbata* seem to benefit from these seasonally vertically migrating copepods as food items, which provide a short-cut in the food web and accelerate the vertical transport of organic matter into the deep sea. A similar feeding behaviour in the Southern Ocean could only be proven for *P. barbata*.

Phylogenetically closely related species are generally characterised by similar trophic niches and minimise inter-specific competition by vertical partitioning, resulting in a stepwise arrangement within the water column. The restriction to different depth strata is supposed to play an important role in speciation processes. Species sharing the same main vertical distribution range (same spatial niche) occupy different trophic niches.

The molecular phylogenetic analysis on the basis of four sequence markers could not separate taxa on family level. The nuclear non-coding internal transcribed spacer 2 (ITS2) emerged as a valuable marker for resolving phylogenetic relationships on genera and species level. The highly variable mitochondrial cytochrome C oxidase subunit I (COI) was not suitable for analysing phylogenetic relationships on deeper nodes, since this fast evolving region only showed a resolution on species level. On the one hand, phylogenetic analysis of this marker suggested genetic homogeneity of Arctic and Antarctic individuals in the cosmopolitan bathypelagic species *P. barbata* and *Gaetanus brevispinus*. On the other hand, the mesopelagic cosmopolitan species *G. tenuispinus* and bipolar *Aetideopsis minor* showed a clear phylogeographic pattern, separating individuals from the Arctic and Antarctic in different clades. This might be attributed to a higher demand for adaptation potential and ecological tolerances by mesopelagic species compared to bathypelagic ones, which live in a more constant environment over a wide geographic range.

In conclusion, this interdisciplinary approach on two copepod families provided new knowledge on niche separation in the pelagic deep sea, which can be transferred to a variety of other deep-sea inhabitants. Furthermore, the congruence of phylogeny and biology demonstrates the power of combining these approaches to better understand evolutionary processes.

ZUSAMMENFASSUNG

Spezialisierung und Nischenaufteilung sind Schlüsselprozesse zur Vermeidung bzw. Minderung interspezifischer Konkurrenz sympatrischer Arten. Mithilfe von ökologischen, biochemischen und molekulargenetischen Methoden werden in dieser Arbeit die ökologischen Nischen von co-existierenden meso- und bathypelagischen Vertretern zweier calanoider Copepodenfamilien (Euchaetidae und Aetideidae) beschrieben. Euchaetiden und Aetideiden wurden sowohl in der arktischen Framstraße als auch im atlantischen Sektor des Südlichen Ozeans in der Nähe der Antarktischen Polarfront, Tiefenstufen aufgelöst beprobt.

Die räumlichen Nischen der Arten wurden anhand ihrer Abundanz und Vertikalverteilung beschrieben. Die Energiespeicherung der Arten wurde auf der Grundlage des Lipidgehalts und dessen Zusammensetzung analysiert. Generelle Ernährungsgewohnheiten sowie trophische Positionen innerhalb des pelagischen Nahrungsnetzes wurden mit trophischen Markern, d.h. Fettsäuren und stabilen Isotopen, untersucht. Die spezifische Herkunft bestimmter Fettsäuren kann genutzt werden, um Ernährungsgewohnheiten zu bestimmen, während stabile Isotopenwerte Aufschluss über Nahrungsnetzinteraktionen und Räuber-Beute-Beziehungen geben können.

In der Framstraße wurden drei in der Arktis und boreal-Atlantischen Gebieten endemische Euchaetiden beprobt: *Paraeuchaeta norvegic*a, *P. glacialis* und *P. polaris*. Im Südlichen Ozean waren die drei endemischen Arten *P. antarctica*, *P. rasa* und *P. biloba* abundant. Die bathypelagische kosmopolitische Art *P. barbata* kam in beiden Regionen vor. Von den Aetideiden traten Arten der beiden Gattungen *Gaetanus* und *Aetideopsis* in beiden Polarregionen, *Chiridius* sp. jedoch nur in der Framstraße auf.

Paraeuchaeta Arten zeigten hohe Wachsester (WE) Einlagerungen. Diese dienen vermutlich als Langzeitenergiespeicher und spielen weiterhin eine wichtige Rolle bei der Tarierung in der Tiefe, welche es diesen großen und schweren Copepoden erlaubt, ihrem taktilen Jagdverhalten nachzugehen. Die WE waren hauptsächlich aus den zwei Fettsäuren 18:1(n-9) und 16:1(n-7) aufgebaut sowie aus den Fettalkoholen 14:0 und 16:0 in den antarktischen und aus C20- und C22-Fettalkoholen in den arktischen Arten. Letztere sind trophische Fettsäuremarker für calanide Copepoden und spiegeln neben den hohen Mengen der Fettsäure 18:1(n-9) die karnivore Ernährungsweise dieser Arten wider. Auffallend hohe Mengen der Fettsäure 16:1(n-7), ein typischer Marker für Diatomeen und bei räuberischen Arten generell als Indikator für herbivore Beuteorganismen interpretiert, führt zu zwei Erklärungen: 1) die aus der Nahrung stammende Fettsäure 16:1(n-7) wird selektiv in den Speicherlipiden zurückgehalten oder 2) *Paraeuchaeta* Arten sind in der Lage, 16:1(n-7) *de novo* zu synthetisieren, ein Stoffwechselprozess der nur selten in Tieren vorkommt. Aetideiden speicherten mäßige (*Gaetanus* spp.) oder nur sehr geringe Mengen (*Aetideopsis* spp. und *Chiridius obtusifrons*) an WE. Vermutlich

stellt Triacylglycerin die wichtigste Lipidklasse in diesen Tieren dar, welches als Kurzzeitenergiespeicher interpretiert wird. Die eher ausgewogene Fettsäurezusammensetzung der Aetideiden weist auf eine omnivore Ernährungsweise hin.

Für die arktischen Arten stellten calanide Copepoden eine wichtige Beute dar. Dies konnte anhand hoher Mengen von C20- und C22-Fettsäuren und -alkoholen sowie durch die Ermittlung von mindestens einer trophischen Ebene zwischen den calaniden Arten und den Räubern anhand der stabilen Stickstoffisotopenverhältnisse nachgewiesen werden. Auch bathypelagische Arten wie *P. barbata* nutzten diese saisonal vertikal wandernden Organismen als Beute, die eine Abkürzung innerhalb des Nahrungsnetzes darstellen und somit den Vertikaltransport von organischem Material in die Tiefsee beschleunigen. Wie in der Framstraße, ernährte sich *P. barbata* auch im Südlichen Ozean von calaniden Copepoden.

Im Allgemeinen sind die trophischen Nischen phylogenetisch nah verwandter Arten sehr ähnlich und interspezifische Konkurrenz wird durch die vertikale Aufteilung innerhalb der Wassersäule gemindert. Die Beschränkung auf unterschiedliche Tiefenzonen scheint eine wichtige Rolle in Artentstehungsprozessen zu spielen. Arten, die miteinander innerhalb einer Tiefenzone vorkommen (die gleiche räumlichen Nische), besetzen unterschiedliche trophische Nischen.

Molekulargenetische Untersuchungen anhand von vier genetischen Markern konnten die Beziehung der beiden Familien zueinander nicht auflösen. Mit Hilfe des nicht-kodierenden Kernmarkers „Internal Transcribed Spacer 2“ (ITS2) konnten die phylogenetischen Beziehungen zwischen den Gattungen und Arten aufgelöst werden. Der hoch variable, mitochondriale Marker Cytochrom C Oxidase Untereinheit I (COI) konnte zwar die einzelnen Arten voneinander trennen, war aber nicht geeignet phylogenetische Beziehungen auf höherer taxonomischer Ebene aufzulösen. Auf der einen Seite konnte anhand dieses Markers eine genetische Homogenität arktischer und antarktischer Individuen der kosmopolitischen bathypelagischen Arten *P. barbata* and *Gaetanus brevispinus* gezeigt werden. Auf der anderen Seite wurde beim mesopelagischen Kosmopoliten *G. tenuispinus* und der bipolaren Art *Aetideopsis minor* eine klare phylogeographische Struktur nachgewiesen, die die einzelnen Individuen dieser Arten einer arktischen und einer antarktischen Klade zuordneten. Dieses Muster könnte das Ergebnis der Notwendigkeit eines höheren Anpassungspotentials und einer erhöhten ökologischen Toleranz der im Mesopelagial lebenden Arten sein. Im Gegensatz dazu herrschen im Bathypelagial konstantere Lebensbedingungen.

Insgesamt konnten mit Hilfe dieses interdisziplinären Ansatzes neue Kenntnisse über Nischeneinteilung pelagischer Tiefseecopepoden gewonnen werden, der auf andere Tiefseebewohner übertragen werden kann. Die Übereinstimmung phylogenetischer und biologischer Charakteristika verdeutlicht, dass die Verknüpfung dieser beiden Methoden zu aussagekräftigen Ergebnissen für unser Verständnis von Evolutionsprozessen führt.

1 SCIENTIFIC BACKGROUND AND OBJECTIVES

The deep-sea environment hosts the largest ecosystem on earth. Many new species were discovered in the last decades (e.g. Brandt et al. 2007a,b, Kaiser et al. 2007, Markhaseva and Schulz 2008) leading to the perception that deep-sea biodiversity has been underestimated so far. High biodiversity in the deep sea raises the question of mechanisms sustaining co-existence of many species in this almost homogeneous environment. Although numerous studies on deep-sea benthic communities have been conducted so far, our knowledge on the deep-sea pelagic environment is still scarce. The present approach, thus, focuses on deep-sea pelagic zooplankton communities and the co-existence of closely related species in order to achieve new insights into this rarely explored habitat.

Two well-represented meso- and bathypelagic calanoid copepod families (Euchaetidae and Aetideidae) were chosen as case studies for this topic of general ecological interest. Special focus is given to spatial niches, e.g. abundance and depth distribution, as well as to trophic niches, e.g. trophic level, feeding behaviour and dietary composition, and to phylogenetic relationships.

Theory of competition, competitive exclusion principle and ecological niches

Competition is an interrelationship between individuals, resulting from the same requirement on a limited resource and affects survival, growth and/or reproduction of at least some of the competing individuals (Begon et al. 1996). Hence, competition is an important mechanism in structuring communities and occurs where resources are shared or limited. Intra-specific competition (between individuals of one species) is mainly density-dependent. The higher the density of a population (abundance) the smaller is the proportion of the limited resource for each individual, resulting in higher mortality rates, decreasing birth rate and limitation of population growth. Regarding inter-specific competition (between species) the growth of a species is determined by their abundance but furthermore limited by a competing species. Thus inter-specific competition occurs when two or more species of a community rely on the same limited resource. It occurs in two interactions: the interference competition and the exploitative competition. The interference competition is the unequal access to resources whereby the access to resources is refused to competitors by dominant species or individuals (direct interaction) (Begon et al. 1996). The exploitation competition is the consumption of a resource by which its availability is reduced to a competing species or organism without direct interrelationship (indirect interaction) (Begon et al. 1996). Both types of competition may occur simultaneously.

The possible outcomes of competitions are: 1) a competitive exclusion, the local extinction of at least one species, 2) reduction in abundance of at least one species, 3) both species co-exist, resulting in niche differentiation, character displacements or generic change, and/or 4) neither

species is the clear winner. The first outcome is referred to the competition exclusion principle, developed by Lotka and Volterra in 1925/1926 and tested by Gause in 1934 (also named Gause's principle). According to this principle, two species with similar requirements cannot stably and spatially co-exist, because one species would have a benefit of the resource, resulting in better reproduction for the one species and the extinction of the inferior species (Begon et al. 1996). In other words, when two competing species inhabit the same ecological niche, the advantaged species will suppress and exclude the inferior species. An ecological niche is the range of n-dimensional factors, within an organism can live and reproduce or in other words the utilisation of all abiotic and biotic resources of a habitat by an organism. Closely related species cannot co-exist when their niches do not differ in at least one aspect.

However, often, competing species can co-exist by niche differentiation, which is the fundament of the high biodiversity on earth. Effects of competition can be reduced through specialisation and niche separation (e.g. Hayward and McGowan 1979). For the division of the oceanic habitat by organisms, Madin and Madin (1995) proposed three ways: 1) spatial separation mainly vertically resulting in faunal differences between different depth strata, 2) a temporal component based on movement, mainly as vertical migration of organisms resulting in avoidance and a partitioning of the environment, and 3) differences in physiology and behaviour of feeding and reproduction, resulting in specialisation of species and sometimes whole families.

The copepod families Euchaetidae and Aetideidae

Species of the clausocalanoid families Euchaetidae and Aetideidae mainly occur in meso- to bathypelagic depths (Park 1994a, Markhaseva 1996, Auel 1999, Braga et al. 1999) in all parts of the world's oceans (Bakke 1977, Båmstedt 1978, Yen 1985, 1991, Hopkins 1987, Shuert and Hopkins 1987, Ward and Wood 1988, Richter 1994, 1995, Øresland 1991, 1995, Mauchline 1995, Ikeda and Hirakawa 1996, Auel 1999, Weikert et al. 2001, Yamaguchi and Ikeda 2002, Skarra and Kaartvedt 2003, Auel and Hagen 2005, Tönnessen et al. 2006, Kosobokova et al. 2007, Schnack-Schiel et al. 2008). Both families comprise endemic, bipolar and cosmopolitan species (Park 1994a, Markhaseva 1996, Yamaguchi and Ikeda 2002, Park and Ferrari 2009).

The family Euchaetidae (Giesbrecht 1892) comprises the two genera *Euchaeta*, represented by three groups with 14 species, and *Paraeuchaeta* with six groups (and three independent species) and in total 61 species (Park 1994b). All species are pelagic and generally occur in oceanic waters with *Euchaeta* spp. being generally distributed in epipelagic horizons while *Paraeuchaeta* species mainly occur at bathypelagic depths (Park 1994b). The family Aetideidae (Giesbrecht 1892) is highly diverse, comprising 25 genera and 180 species (Markhaseva 1996). They are pelagic or benthopelagic and occur throughout the water column in

oceanic waters (Markhaseva 1996), but with a greater diversity in the deep sea (Bradford-Grieve 2004).

Especially *Paraeuchaeta* species can play important roles in the pelagic food webs (Båmstedt 1981, Yen 1987, Auel 1999, Skarra and Kaartvedt 2003) as major predators on other meso-zooplankton, especially on copepods (Øresland 1991, 1995, Conover and Huntley 1991, Fleddum et al. 2001) and on larvae of commercially important fish such as Atlantic cod and Pacific hake (Bailey and Yen 1983, Yen 1987). They also represent important prey items for deep-sea organisms such as deep-sea fish (Gartner and Musick 1989, Hopkins et al. 1996, Sutton 2005). Aetideidae are less abundant than Euchaetidae but are characteristic in deep waters of the Arctic and Antarctic (Båmstedt 1981, Richter 1995, Markhaseva 1996, Auel 1999). They may consume ≥40% of the vertical carbon flux in the Greenland Sea (Auel 1999). In Kosterfjorden, Sweden, *Aetideopsis armata* along with *Paraeuchaeta norvegica* are responsible for 29 to 77% of the total energy flow through the carnivorous trophic level (Båmstedt 1981).

The sympatric co-existence of members of these two families is well documented. 14 *Paraeuchaeta* species co-occur in the Southern Ocean around South Georgia (Ward and Wood 1988) and in the North East Atlantic Rockall Trough (Mauchline 1994a,b, 1995). In the Arctic Ocean, Greenland Sea and Fram Strait, four congeners co-exist (Kosobokova et al. 1998, Auel 1999, Auel 2004). In the Western Sub-Arctic Pacific Ocean, three *Paraeuchaeta* species live sympatrically in meso- and bathypelagic depths (Yamaguchi and Ikeda 2002, Yamaguchi et al. 2004). Among Aetideidae the co-existence of eight species was documented for the Arctic Ocean (Markhaseva 1984) and of five species in the Greenland Sea and Fram Strait (Richter 1994, 1995, Seiler and Brandt 1997).

Among co-occurring *Paraeuchaeta* species, the species with the shallowest vertical distribution is usually the most abundant one, e.g. *P. antarctica* in the Antarctic (Ward and Wood 1988, Boysen-Ennen et al. 1991, Schnack-Schiel et al. 1998, Razouls et al. 2000), *P. norvegica* in boreal-Atlantic regions (e.g. Mauchline 1995) and *P. glacialis* in the Arctic (e.g. Kosobokova 1982, Auel and Hagen 2002), while deeper distributed species mainly occur in lower numbers (Ward and Wood 1988, Mauchline 1995, Auel 1999). Often the abundant species have been studied in detail (e.g. Bakke 1977, Yen 1987, Ward and Robins 1987, Øresland 1991, 1995, Øresland and Ward 1993, Hagen et al. 1995, Alonzo et al. 2000a,b, Fleddum et al. 2001, Tönnessen et al. 2006), however, little information is available on deeper living and less abundant species.

Euchaetidae and Aetideidae thus provide suitable case studies for elucidating the patterns of co-occurrence in the deep-sea realm. These consumers differ in predation and feeding behav-

iour (e.g. Olsen et al. 2000). *Paraeuchaeta* are non-visual, tactile predators, detecting their prey by reception of mechanical stimuli. Thus they are able to detect motile prey only (e.g. Yen 1982, Olsen et al. 2000). Prey is attacked with a rapid swimming burst and trapped with the predator's large maxillipeds (Bailey 1984). Very long setae on the first antennules and at the caudal rami of Euchaetidae are mechanoreceptors and balancing structures, respectively, and associated with the predatory feeding mode (Yen and Nicoll 1990, Park 1994b). In contrast, copepods of the family Aetideidae are considered omnivorous, detritivorous or coprophageous (Hopkins 1985a, Greene 1985, 1988, Richter 1995, Falkenhaug et al. 1997). They can detect both, motile and non-motile food items (Olsen et al. 2000).

Deep-sea ecosystems

The deep sea differs from shallow-water and terrestrial habitats in the spatial separation of production and remineralisation processes with phytoplankton production restricted to the epipelagic zone (0-200 m, euphotic surface layer) while substantial parts of secondary production and remineralisation take place at greater depths and on the seafloor (Wassmann 1998). Photosynthetically fixed carbon from the euphotic zone is exported into mesopelagic layers (200-1000 m) by fast sinking large particles known as marine snow as well as dead or dying animals and plants, protists or faecal pellets as well as carcasses (Honjo 1980, Urrère and Knauer 1981, Karl et al. 1988). Organisms living at mesopelagic depths accelerate the export of carbon into the deep sea by diel or seasonal vertical migration (e.g. Steinberg et al. 2000) and by repackaging sinking particles by feeding and production of faecal pellets (e.g. Wilson et al. 2008). Thereby the vertical carbon transport is accelerated. It contributes to the biological pump (Longhurst and Harrison 1989) and pelago-benthic coupling processes. Carnivorous zooplankton plays an important role for the vertical carbon flux in oceanic environments (Froneman et al. 2002), e.g. contributing to a downward flux of faeces equivalent to 5% of the local mesozooplankton stock in the Atlantic sector of Southern Ocean (Pakhomov et al. 1999).

In general, organisms living in the mesopelagic environment obtain their nutrition by feeding at the surface (by vertical migration) or by carnivory and particle feeding within mesopelagic depth ranges (e.g. Yamaguchi and Ikeda 2000, Auel and Hagen 2002, Schnetzer and Steinberg 2002). In bathypelagic zones (1000-4000 m), environmental conditions are constant and characterised by cold water temperature, high hydrostatic pressure and darkness. Since organic matter strongly decreases with increasing depth (Honjo 1980, Karl et al. 1988, Tseitlin 2001) with the sharpest decrease in the twilight zone due to rapid biological consumption and remineralisation of carbon (Karl et al. 1988 and references therein, Buesseler et al. 2007), food resource limitation is expected as a main problem the organisms have to cope with in the deep-sea pelagic realm. Hence, competition for food sources may play an important role in deep-sea ecosystems (Madin and Madin 1995). In a general view, the deep-sea environment provides stable and almost homogeneous abiotic conditions on a global scale through recent geological

times. However, seasonal components like vertical fluxes can influence deep-sea communities, resulting in spatial variations of biomass and abundance as a function of surface production (Hayward 1986 in Ward and Shreeve 2001, Koppelmann and Weikert 1999).

Deep-sea biodiversity

Variability in environmental factors plays a key role in the dynamic evolution of life. Because this variability is low in the deep sea, evolutionary processes have been assumed to be slow in this region of the ocean, resulting in a low biodiversity. The lack of isolating barriers should favour the development of cosmopolitan species (Wilson and Hessler 1987 and references therein). However, recent discoveries of high numbers of new deep-sea species, mainly benthic ones (e.g. Brandt et al. 2007a,b, Kaiser et al. 2007, Markhaseva and Schulz 2008) underline that deep-sea biodiversity has been underestimated and re-fuel scientific interest in the issue of deep-sea biodiversity (e.g. Miya and Nishida 1997, Morin and Fox 2004). A lot of newly described species are rare and have only been sampled in low numbers (e.g. Brandt et al. 2007b). For example 12 *Paraeuchaeta* species occur worldwide in all great oceans, but with the exception of the two species *P. barbata* and *P. sarsi*, they are relatively rare throughout their ranges (Park 1994a). One key to understand the high biodiversity in the deep sea may be the proportions of different species. It is estimated that over 80% of all oceanic species are rare and this rarity may reduce competition because the species are too few and dispersed to interact very often (Madin and Madin 1995).

Biodiversity includes the variation of life on all levels of biological organisation including diversity within and among individuals, species and ecosystems. The mechanisms leading to a high biodiversity are speciation processes, beginning with microevolution on the level of populations to macroevolution on the level of new species. The variation of phenotype within a population leads to speciation, often by isolation mechanisms (Knoop and Müller 2006). Separation of populations over long periods of time results in a reduced gene flow and finally leads to speciation and can be caused by different isolation mechanisms. The allopatric speciation is a result of geographic isolation of a part of the population (Ridley 2004). As a version of this type of speciation the peripatric speciation occurs in a small population which is isolated at the edge of a larger population. Within not geographically isolated populations, parapatric and sympatric speciation can occur. Parapatric speciation arises when the population does not mate randomly but more likely with their geographic neighbours. Sympatric speciation can be a result of the exploitation of new niches which automatically reduce gene flow with the individuals exploiting other niches (selection for specialisation) (Ridley 2004). Speciation can be detectable on molecular genetic level, although morphological character differences may not be identifiable yet.

As a result, next to taxonomic characterisation and identification of new species, molecular analyses help in identifying and discovering sibling and cryptic species (species which are morphologically similar but reproductively isolated) (e.g. de Vargas et al. 1999, Lee 2000, Goetze 2003, Castro-Longoria et al. 2003, Peijnenburg et al. 2004). In general, recent molecular genetic studies suggest that pelagic species diversity in the open ocean is higher than interfered from many morphological taxonomies and cryptic species biodiversity is high and well structured in the open ocean (Norris 2000). Furthermore molecular phylogenetic analysis represents a valuable tool to detect ancestral states and close relationships of species, genera and families.

Objectives

The present study focuses on the regional and vertical distribution, ecological niches and phylogeny of co-existing species of the two clausocalanoid copepod families Euchaetidae and Aetideidae in order to elucidate mechanisms enabling co-occurrence. These results provide new insights into possible speciation processes within the vast and largely undiscovered deep-sea pelagic realm. The mechanisms and processes are investigated by applying a variety of methods including lipid analysis, trophic marker concepts of fatty acids and stable isotopes as well as molecular phylogenetics. In detail, this work addresses five objectives which are summarised below. In the first two objectives information for the following objectives are provided by focusing on occurrence and abundance of species in both polar regions as well as on lipid biochemistry and the use of fatty acids as trophic biomarkers. In objective 3 trophic structures and vertical transport in the polar deep sea are analysed. As a synthesis, mechanisms enabling co-occurrence are discussed by combining data on occurrence, vertical distribution and trophic characterisation of species in objective 4. The last objective deals with the phylogenetic classification of species and the integration of all results obtained in the present study.

Objective 1: Similarities in deep-water community of both polar regions

Epipelagic zooplankton organisms in polar oceans are well adapted to seasonality in primary production and are generally characterised by low numbers of species which are highly abundant and often endemic to this region (Knox 2007). For epipelagic species the Antarctic Circumpolar Current with the two major frontal systems, the Subtropical Front and the Antarctic Polar Front can act as major biogeographical boundaries for distribution and dispersal (Pakhomov and McQuaid 1996, Ward and Shreeve 2001, Ward et al. 2003). In consequence, these species are endemic to the Southern Ocean. In contrast, for mesopelagic species oceanic fronts like the Antarctic Polar Front do not represent a dispersal boundary (Atkinson and Sinclair 2000). Furthermore omnivorous and carnivorous zooplankton does not directly depend on primary production resulting in the higher potential for a wide distribution (Machida et al. 2006).

Hypothesis: In contrast to the high endemic epipelagic zooplankton community in polar seas the deep-water community bears many cosmopolitan species.

To prove this hypothesis, occurrence and abundance of species will be discussed in the light of habitat-specific adaptations, distribution boundaries as well as on phylogeographic aspects based on the collected and on published data.

Objective 2: Lipid storage strategies and validation of fatty acids as trophic biomarkers

The amount and composition of lipids provide information on life-cycle patterns and condition of organisms (e.g. Sargent and Falk-Petersen 1988). Adaptations to seasonally varying food supply are mirrored in lipid-storage strategies, since lipid depots act as important energy buffers for times of starvation (e.g. Lee and Hirota 1973, Lee 1975, Kattner et al. 1994). These adaptations are well studied in epipelagic herbivorous copepods, which are directly affected by strong seasonal fluctuations in food supply (e.g. Graeve and Kattner 1992, Kattner et al. 1994, Albers et al. 1996, Scott et al. 2000, Hagen and Auel 2001).
Since certain fatty acids can be used as trophic biomarkers, they provide a useful tool to determine feeding history and general food preferences of species (see Dalsgaard et al. 2003). Lipids are composed of polar and neutral lipids. While polar lipids are mainly compounds of biomembranes, fatty acid composition of neutral lipids mainly reflect recent feeding history. It is thus of major interest, in which lipid classes fatty acid trophic biomarkers are stored and how they mirror general feeding habits. In general, fatty acids of triacylglycerol reflect recent feeding, whereas wax ester fatty acids and alcohols reflect dietary influences and *de novo* synthesis (Lee et al. 2006).

Hypothesis: Deep-sea Euchaetidae and Aetideidae occupy different ecological niches, with a higher degree of omnivory in the latter representatives, leading to differences in the respective fatty acids composition as well as lipid storing strategies.

In this objective it will be elucidated how lipids of the deep-sea Euchaetidae and Aetideidae are composed, whether specific fatty acids are associated to specific lipid classes and how the fatty acid biomarker approach is applicable to omnivorous and carnivorous copepods.

Objective 3: Vertical migrations provide a trophic short-cut for deep-sea copepods

In general, it is assumed that the influence of seasonality decreases with increasing depth and deep-sea ecosystems provide rather stable environmental conditions (Mauchline 1995). In polar waters, copepods of the family Calanidae (mainly of the genera *Calanus*, *Calanoides* and *Neocalanus*) are dominant components of the epipelagic zooplankton community (e.g. Kosobokova 1986, Boysen-Ennen et al. 1991, Zmijewska and Yen 1993, Richter 1995, Norrbin

et al. 2008, Schnack-Schiel et al. 2008). These copepods are adapted to seasonality in primary production regime by high lipid deposits as energy reserves to overwinter at great depth during polar winter (Richter 1995, Scott et al. 2000, Hagen and Auel 2001).

Hypothesis: The seasonal vertical migrations of abundant herbivorous copepods provide a trophic short-cut for carnivorous and omnivorous deep-sea copepods. They represent important prey items throughout the water column and their seasonal vertical migration leads to a peak in food availability in the deep sea. As a consequence, the deep sea in polar regions is structured in spatial and temporal terms.

In order to trace the role of Calanidae for the nutrition of omnivorous and carnivorous deep-sea copepods, trophic markers, i.e. fatty acids and stable isotope signatures will be applied.

Objective 4: Co-existence in the pelagic deep sea: spatial and trophic niche separation

The vast, three-dimensional deep-sea habitat is not spatially distinctive in physical factors and bears many co-occurring closely related species. Referred to the theory of competition and the competition exclusion principle, two or more species with similar requirements cannot stably co-exist. The co-occurrence of closely related species is enabled solely by specialisation and niche separation, which reduce the effects of competition (e.g. Hayward and McGowan 1979). These mechanisms would enable and sustain the co-occurrence of many species and thus high biodiversity. For the deep-sea pelagic realm, three ways of dividing a habitat are described: 1) by spatial separation, 2) by temporal separation and 3) by differences in physiology and behaviour of feeding and reproduction (Madin and Madin 1995). These mechanisms will be tested on the deep-sea copepods by examining vertical distribution patterns and trophic preferences of the species.

Hypothesis: Closely related species occupy rather similar trophic niches and minimise interspecific competition by vertical separation (spatial niches). Species within one depth stratum usually differ in feeding behaviour and/or dietary preferences (trophic niches).

In order to test this hypothesis, overlaps as well as disparities of the respective ecological niches (trophic and spatial niches) will be synoptically compared.

Objective 5: Combining molecular phylogenetics and ecology to understand evolutionary processes

In the previous objectives, the occurrence, vertical distribution, lipid composition, and dietary preferences of deep-sea copepods of the families Euchaetidae and Aetideidae have been used to characterise and illustrate their ecological niches in order to understand their co-occurrence

with minimised or without inter-specific competition. This illustration demonstrates a present-day picture of these deep-sea copepods with genus- and species-specific characteristics.
Some of these specific characteristics can be related to morphological characters that support the status as sister families (Park 1994b). The main differences are four synapomorphic features in the Euchaetidae of which two are only found in the Euchaetidae among all calanoid copepods and the other two compared to the Aetideidae are associated with their predatory feeding behaviour (Yen and Nicoll 1990, Park 1994b).

Hypothesis: Phylogenetic clades exhibit high similarities in ecological and physiological traits.

The ecological and morphological characteristics of the species will be assigned to the phylogenetic tree in order to reveal evolutionary processes that govern speciation in the deep-sea pelagic realm.

2 MATERIALS AND METHODS

2.1 Study areas

Individuals of Euchaetidae and Aetideidae were sampled during two cruises to high latitude waters in Fram Strait between Greenland and Svalbard and in the Atlantic sector of the Southern Ocean (Fig. 1). These two polar sampling regions differ in oceanographic conditions due to the distinct prevailing current systems.

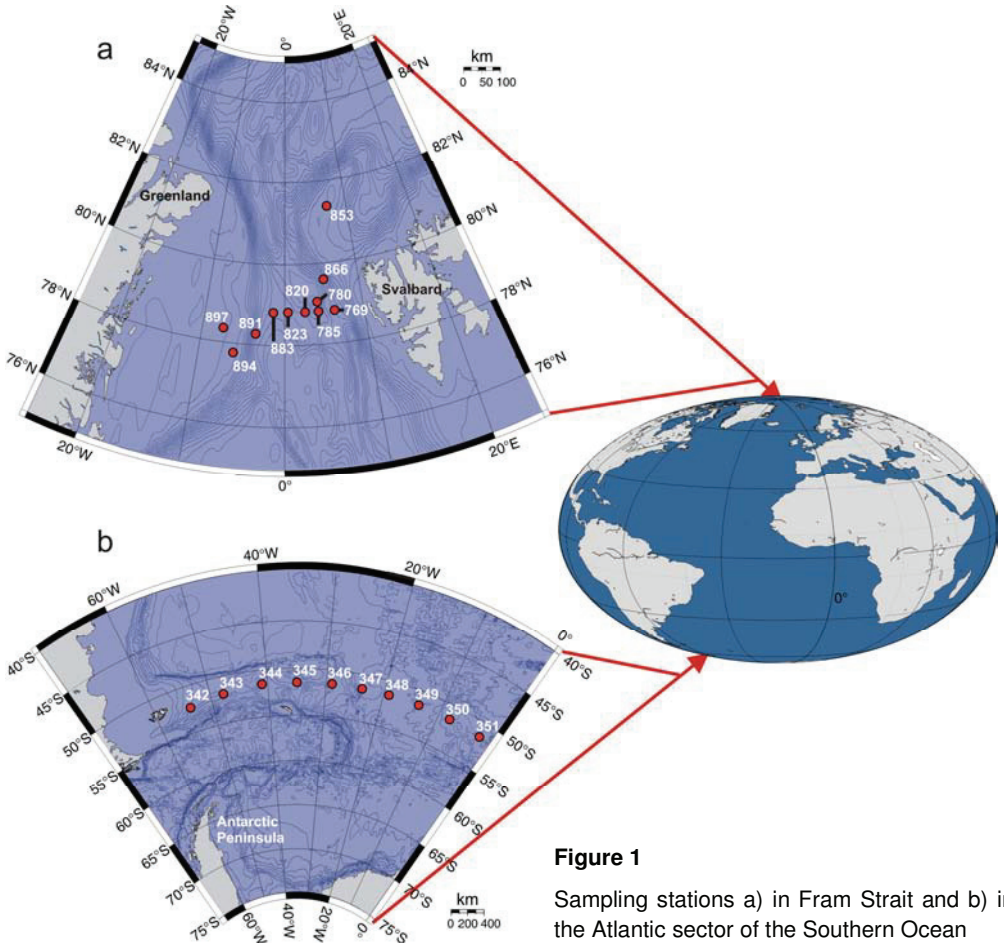


Figure 1

Sampling stations a) in Fram Strait and b) in the Atlantic sector of the Southern Ocean

In the northern hemisphere, samples were taken in Fram Strait, the only deep-water connection between the Arctic Ocean and other parts of the world ocean (Fig. 1a). Two opposing meridional ocean currents prevail in Fram Strait. Warm and saline water masses are transported northwards on the east side of Fram Strait by the West Spitsbergen Current (WSC), an extension of the Norwegian Atlantic Current. On the western side of Fram Strait, the East Greenland Current (EGC) transports cold polar surface water originating from the Transpolar Drift Current southward along the East Greenland Shelf (e.g. Hop et al. 2006). The close neighbourhood of

different water masses results in hydrographical fronts and separates different hydrographical, and associated with this, biological regimes. Fram Strait represents a sampling location, where high-Arctic, sub-Arctic and boreal-Atlantic species can be sampled in close proximity (Hop et al. 2006). In Fram Strait, sampling was conducted in autumn from September 20th, until October 16th in 2006. Ten stations were sampled, situated along a transect at approximately 79°N from 6°20'E to 7°29'W with a southward shift to 77°46'N and 78°21'N at westernmost stations due to the prevailing ice conditions and one station on Yermak plateau at 81°22'N, 6°52'E (Fig.1a, chapter I).

The Southern Ocean represents the water masses south of approximately 40°S (Deacon 1982) and is characterised by one of the largest current systems in the world's oceans: the Antarctic Circumpolar Current (ACC, West Wind Drift). The ACC flows eastwards around the Antarctic continent and isolates the Antarctic Ocean from adjacent ocean parts (Longhurst 2007). Due to the prevailing current system, most of the major taxonomic groups have a circumpolar distribution (Knox 2007).

Surface water masses of the ACC and their characteristics partition the Southern Ocean into zonal bands, which are separated by different frontal systems: Subtropical Front (STF), Sub-Antarctic Front (SAF), Antarctic Polar Front (APF) and the Antarctic Divergence. STF is the northernmost convergence front (41-45°S), with transition from subtropical to sub-Antarctic surface waters. Here, as well as at the further south located SAF, cold sub-Antarctic surface waters sink under warmer and more saline water with a sharp decrease in temperature of 4 to 5°C (Deacon 1982). Between the SAF and more southerly APF extends the Polar Frontal Zone. Intermediate water masses are formed within ACC by frontal jets of the SAF and APF via sinking and subduction, before it is distributed throughout the world's oceans. The APF represents one of the major frontal systems with more southerly regions belonging to Antarctic waters with large extend of sea ice. At the APF cold Antarctic surface water is subducted by warmer surface water and the temperature stays around 2°C all year round. Due to geographic and oceanographic conditions of the Drake Passage, Antarctic Peninsula and Scotian Arch, the APF is shifted northwards further than 50°S into the Atlantic Ocean sector of the Polar Sea.

Due to these features, the Antarctic Ocean is mostly separated from the world's oceans and sub-Antarctic and Antarctic picture two different biogeographical provinces. In many cases zooplankton taxa, especially epipelagic species are associated with distinct biogeographical regions and limited in distribution by ocean fronts as boundaries (i.e. surface isotherms). Both, STF and APF can thus act as dispersal barriers for many Antarctic species living at or near the surface (Deacon 1982 and references therein, Pakhomov et al. 2000). However, for mesopelagic organisms no evidence was found that the APF represents a major biogeographical boundary to their distribution (Atkinson and Sinclair 2000). In the Atlantic sector of the Southern

Ocean, samples were taken in austral autumn from April 16th until 25th in 2006 at 10 stations along a transect at approximately 51°30´S from 53°54´W to 2°05´W (Fig. 1b, chapters II and III), including sub-Antarctic and Antarctic waters. Due to the prevailing oceanographic conditions, mentioned above, similar species are sampled due to their circumpolar distribution.

2.2. Analytical work

2.2.1 Lipid storage

Lipids are major sources of metabolic energy and essential compounds for the formation of cell and tissue membranes. They comprise diverse functions in organisms, are included in physiological and reproductive processes whereby specific functions can be ascribed to specific lipid classes. Polar glycerophospholipids like phosphatidyl ethanolamine (PE) and phosphatidyl choline (PC) are involved in structural functions of biomembranes as they serve as components of the membrane lipid bilayer (Sargent and Whittle 1981), whereas the two neutral lipid classes wax ester (WE) and triacylglycerol (TAG) represent the major form of lipid storage in marine copepods (e.g. Lee 1975, Hagen et al. 1993). Increased lipid deposition is thus, often reflected by an increase in WE and TAG and serves as an important energy buffer against starvation especially in polar herbivorous copepods. High levels of these lipid classes enable species to survive long periods of food limitation resulting from the strong seasonality of the production regime in polar regions.

Due to a slower catabolism, WE are regarded as long-term energy storage (e.g. Lee and Hirota 1973, Lee et al. 1974, Sargent and Henderson 1986, Hagen et al. 1995). Because of the low density of WE, they are considered important for maintaining buoyancy in marine zooplankton (Nevenzel 1970, Visser and Jónasdóttir 1999). Neutral lipid TAG is mainly important as short-term energy reserve (e.g. Lee 1974, Sargent and Falk-Petersen 1988, Lee et al. 2006). Storage of TAG precedes the synthesis of WE (Miller et al. 1998) and WE can be reconverted into TAG, for example to provide energy for reproductive demands such as egg production. Furthermore, both WE and TAG can be converted into phospholipids during oogenesis (Lee et al. 2006). Analysis of lipids thus provides information on life-cycle patterns, reproduction and dormant stages and is a valuable tool to determine the condition of an organism.

Lipid extraction was performed after Folch et al. (1957), modified by Hagen (2000). Prior to extraction, tricosanoic acid was added as an internal standard. Extraction was carried out in dichloromethane/methanol (2:1 per volume) using both a potter (Sartorius Potter S) and ultrasonic for sample homogenisation. Aqueous KCl solution was used in the washing step prior to centrifugation. Lipid content was measured gravimetrically, accounting for the added amount of the internal standard.

2.2.2 Trophic markers

Trophic biomarkers give insight in energy flows through food webs. For use as a trophic biomarker, a compound has to fulfil specific requirements: it has to be of unique origin (taxon- or species-specific), it has to be inert, metabolically stable and unaltered in order to be transferred qualitatively and quantitatively from one trophic level to the next (e.g. Dalsgaard et al. 2003 and references therein).

Trophic biomarkers are particularly useful when working with organisms in remote areas. Sampling of organisms from polar regions and the deep sea is generally accompanied by certain logistic constraints, since sampling in polar regions is largely restricted to the less extreme summer season and thus high temporal resolution is not possible. Sampling of the deep sea demands high efforts with respect to time and cost. Furthermore, this approach is useful when working with animals that are hardly accessible for feeding experiments and when gut content analyses are highly time consuming regarding the high number of individuals. Feeding experiments are hardly practicable, when trying to keep deep-sea species, and foremost tactile predators, alive in captivity for a sufficiently long period of time. Moreover the long hauling time of individuals and stowage in the cod end of the net from deep waters to the surface compromise the fitness of the collected specimens for subsequent experiments. Stowage in the cod end results in high prey density for carnivorous organisms and a potential bias of gut contents and thus rather mirrors recent feeding instead of general feeding habits under natural conditions. Based on these considerations, trophic markers represent a valuable tool to investigate general feeding preferences of deep-sea organisms, integrated over a period of weeks to months.

Fatty acid and fatty alcohol composition

The analysis of lipid composition, i.e. fatty acids and fatty alcohols, is a useful tool to elucidate feeding preferences. Certain fatty acids are only synthesised *de novo* by specific groups of phyto- and zooplankton and their conservative transfer along the food chain enables the reconstruction of trophic pathways (Dalsgaard et al. 2003 and references therein). Food-specific fatty acids and alcohols display a good basis for interpretation, when they are involved in storage processes, such as in TAG or in the long-term storage WE, where they mirror feeding of an organism over the last weeks to months (Sargent and Henderson 1986).

Fatty acid biomarkers for marine primary producers are for example 16:1(n-7) and 20:5(n-3), which are typical for diatoms (e.g. Graeve et al. 1994a,b) and 18:4(n-3) as well as 22:6(n-3), generally produced by dinoflagellates (e.g. Graeve et al. 1994b). Oleic acid 18:1(n-9) is enriched in secondary consumers and is often used as a marker for carnivorous feeding (Falk-Petersen et al. 1990, Graeve et al. 1997). Characteristic for herbivorous calanid copepods are the long-chain monounsaturated 20:1 and 22:1 fatty acids as well as alcohols (Sargent and

Falk-Petersen 1988, Graeve et al. 1994a, Kattner et al. 1994, Kattner and Hagen 1995, Albers et al. 1996), and useful in detecting predation on these species. Further indications on feeding history of organisms are provided by fatty acid biomarker ratios (e.g. St. John and Lund 1996, Auel et al. 2002). For example, on the basis of the ratio of the fatty acid isomers 18:1(n-9) and 18:1(n-7) degree of carnivory can be derived (e.g. Graeve et al. 1997).

For the gas chromatographic determination of the fatty acid composition, subsamples of the lipid extracts were transesterified into methyl esters by heating in hexane and methanol containing 3% concentrated sulphuric acid for four hours at 80°C (Kattner and Fricke 1986). The fatty acid and alcohol composition was determined with a Hewlett-Packard gas chromatograph (HP 6890A), equipped with a DB-FFAP column of 30 m length and 0.25 mm diameter. Peaks were identified by comparing retention times with those of a fish oil standard and a copepod lipid sample of known composition.

Lipid class-specific fatty acid and fatty alcohol composition

To elucidate lipid-class specific fatty acid and alcohol composition, high performance thin layer chromatography was applied. Subsamples of the total lipid extracts were spotted on a self-made silica-coated glass plate (silica gel Merck H60, coat thickness 750 µm) using a CAMAG Linomat IV. Polar lipids were developed in methylacetate : isopropanol : chloroform : methanol : 0.25% KCl (25:25:25:10:9, V:V) for 5 min and neutral lipids in hexane : diethylether : acetic acid (80:20:2, V:V) for 17 min. Developing solvent was evaporated with nitrogen and lipid bands were visualised by iodine vapour. Bands containing the lipid classes of interest were scraped off the plates and extracted according to the total lipid extraction (Hagen 2000). Lipid-class specific composition was analysed according to Kattner and Fricke (1986) as described above.

Stable isotopes

Stable isotopes (SI) are suitable markers for detecting pathways of organic matter within biological systems, since isotopic fractionation processes lead to the accumulation of the heavier isotope in the consumer (e.g. Minagawa and Wada 1984, Michener and Schell 1994, Hobson 1999, Peters et al. 2005). This retention and progressive enrichment of the heavier isotope with increasing trophic level can be used for food web analysis (e.g. DeNiro and Epstein 1978, 1981).

In this work, SI ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) were determined and especially $\delta^{15}N$ applied as trophic marker in order to detect differences in trophic signals and predator-prey relationships since dietary input can be integrated over several weeks to months (e.g. Tieszen et al. 1983). With a low enrichment factor of 0-1‰ per trophic level (DeNiro and Epstein 1978, Rau et al. 1983, Wada et al. 1987, Hobson and Welch 1992), $\delta^{13}C$ is less sensitive as a marker of trophic level, but often applied for detecting sources of carbon and primary production within

a food web (Post 2002, McCutchan Jr. et al. 2003). In contrast, $\delta^{15}N$ accumulates by 3-5‰ per trophic level and can mirror predator-prey relationships (Rau et al. 1983, Minagawa and Wada 1984, Hobson and Welch 1992, Kurle and Worthy 2002). For comprehensive food web analysis, a baseline (in general particulate organic matter in marine food webs) has to be defined in order to calculate the trophic level of consumers in relation to this baseline (Vander Zanden and Rasmussen 1999, Søreide et al. 2006). In this thesis, the SI approach is used to compare species on a relative basis in connection with the information from fatty acid and fatty alcohol analyses.

On the basis of variations in SI signatures with latitude and longitude, they can also be applied in tracking foraging locations of organisms. Generally, organisms from high latitudes have lower $\delta^{13}C$ values compared to those in lower latitudes, related to the higher concentration of dissolved CO_2 in seawater at lower temperatures (e.g. Rau et al. 1989) and to differences in phytoplankton growth rate, cell size, and membrane permeability or to the degree of fractionation during carbon fixation (François et al. 1993). Thus, regional differences in SI can be used to trace movement of seasonal migrating organisms as demonstrated in southern right whale and a variety of seabirds (Best and Schell 1996, Hobson 1999, Cherel et al. 2007, Gladbach et al. 2007). In the Southern Ocean also $\delta^{15}N$ of suspended particles and phytoplankton decreases towards higher latitudes (Wada et al. 1987, Altabet and François 1994).

Stable isotope ratios of nitrogen and carbon of lyophilised samples were analysed at Agroisolab GmbH in Jülich, Germany. Determination was performed in a mass spectrometer (EA NA1500 Series 2, Carlo Erba Instruments), using helium as carrier gas. Ratios were provided by using standards IAEA-PDB (IAEA-C1, Vienna) for carbon and AIR, atmospheric air (IAEA-N1, Vienna) for nitrogen and expressed as $\delta^{13}C$ and $\delta^{15}N$ as parts per thousand (‰) according to the equation:

$$\delta X = \left[\left(R_{sample} - R_{standard}\right) - 1\right] \times 1000$$

where X is ^{13}C or ^{15}N of the sample and R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$

Molar C/N ratios were calculated from percentage data of carbon and nitrogen. Compared to other body compounds, lipids are depleted in ^{13}C (Tieszen et al. 1983) and thus lipid content can bias $\delta^{13}C$. Therefore several authors recommend the removal of lipids prior SI analysis (e.g. Hobson et al. 2002, Søreide et al. 2006). To obtain adequate biomass for SI analysis even for small species and stages, a lipid-corrected $\delta^{13}C$ value was determined on the basis of C/N ratios according to the equations proposed by McConnaughey and McRoy (1979):

$$L = \frac{93}{1 + (0.246\ [CN] - 0.775)^{-1}} \quad \text{(equation 1)}$$

$$\delta^{13}C' = \delta^{13}C + D\left(-0.207 + \frac{3.9}{1 + 287/L}\right) \quad \text{(equation 2)}$$

where L is the lipid factor, $\delta^{13}C'$ is the corrected value and D is the isotopic difference between protein and lipid (6‰, based on published data). This approach is applicable for C/N≥4.0 (McConnaughey and McRoy 1979). Equation 1 evaluated the lipid factor, using C/N ratio, whereas equation 2 calculates the corrected $\delta^{13}C$

2.3 Molecular phylogenetics

Besides morphological characters, molecular phylogenetic analysis is a valuable tool to analyse relationship of species and to detect ancestral states. It also allows the identification of sibling and cryptic species, which are morphologically similar but reproductively isolated (e.g. de Vargas et al. 1999, Lee 2000, Goetze 2003, Castro-Longoria et al. 2003). In the present work, a molecular phylogenetic approach is used in order to elucidate different aspects: 1) phylogenetic relationship of the two calanoid copepod families, since the two families share specific morphological characteristics and are regarded as sister families (Park 1994b), 2) to plot data on feeding traits, energy storage as well reproductive strategies on the phylogenetic tree and 3) to compare individuals of the same species, including bipolar and cosmopolitan species, occurring in the Arctic and Antarctic.

The different mutation rates of genes can be used to resolve phylogenetic relationships on different taxonomic levels. Genes with low mutation rates mirror early separation and resolve deep lineages. In general, nuclear genes are more conserved than mitochondrial ones. The higher mutation rate in mitochondrial genes is due to several reasons. It has a tenfold higher nucleotid substitution rate compared to nuclear DNA because of a higher error rate of the mitochondrial replication (in animals) (Storch et al. 2007). The generally maternal inheritance of mitochondria results in a smaller effective number of its genes in a population and thereby reduces the gene diversity relative to nuclear genes (Birky et al. 1989). Furthermore, mitochondrial DNA is not protected by histones like nuclear DNA, which makes it more vulnerable to mutagens.

In the present work, two nuclear coding (18S and 28S), one nuclear non-coding (internal transcribed spacer 2: ITS2) and one mitochondrial coding marker (cytochrome C oxidase subunit I: COI) are applied. Eukaryotic nuclear ribosomal genes are typically multicopy and are arranged as a series of tandem repeats, separated by non-transcribed external spacers (ETS). Each transcriptional unit codes for 18S, 5.8S and 28S, which are separated by internal transcribed spacer, ITS1 between 18S and 5.8S and ITS2 between 5.8S and 28S. In other studies on zooplankton species it was shown that the three nuclear markers applied in this

study can resolve relationships on higher taxonomic ranks (e.g. Braga et al. 1999, Goetze 2003, Bucklin et al. 2003, Thum 2004, Song et al. 2008).

Cytochrome C oxidase subunit I, coding for a subunit of an important enzyme in the respiratory chain, is one of the 37 genes of the circular mitochondrial genome which consists of a light and a heavy strand. Higher mutation rates compared to nuclear ones, lead to high variability in sequences which makes these genes suitable in analysis and identification of species. For instance, COI is used in population genetics, providing information on phylogeographic breaks between populations (e.g. Burton and Lee 1994, Bucklin et al. 2000, Peijnenburg et al. 2004, Goetze 2005). In addition, partial sequences of the comparably highly variable COI gene are used as DNA barcodes for species identification. DNA barcoding is a technique for characterising species using a short, standardised DNA sequence. These barcode sequences are very short (e.g. COI about 650 base pairs) and they can be obtained quickly and cheaply. COI as a standard DNA barcode is applied to a variety of animal taxa, using universal primers and a standard protocol for DNA amplification (Hebert et al. 2002, Bucklin et al. 2003, Valentini et al. 2008). Since species-specific sequence data in public databases (e.g. GenBank, Barcode of Life Data System (BOLD)) are available, specimens can be identified by sequences similarities. In GenBank sequences can be assigned by the Basic Local Alignment Search Tool (BLAST) and checked for orthology or in BOLD by the BOLD Identification System (IDS). These methods provide species identification on the basis of molecular sequence data, when taxonomic identification on the basis of morphological characters does not provide an unambiguous result.

Extraction of copepod genomic DNA was performed using three different methods, i.e. GeneReleaser™ (Bioventures, Murfreesboro) modified after Schizas et al. (1997), FTA® Elute cards (Whatman), and QIAGEN DNeasy tissue kit. The quantitatively and qualitatively best results were obtained using QIAGEN DNeasy tissue kit, following the manufacturer's protocol and were therefore used for the majority of samples. Polymerase chain reaction (PCR) was carried out for amplification of specific markers, using an Eppendorf Mastercycler gradient thermocycler with heated lid (Eppendorf, Hamburg) (chapter IV). Nuclear markers were amplified using specific primer pairs, known to be applicable for the investigated species (White et al. 1990, Hillis and Dixon 1991, Zardoya et al. 1995, Goetze 2003, Bucklin et al. 2003, chapter IV). In addition to universal primers (Folmer et al. 1994), three new primer pairs were designed for COI. After amplification, length of the amplified gene was determined by electrophoresis prior to purification of PCR products using peqGOLD Cycle-Pure Kit (peqLab) following the manufacturer's protocol. Cycle PCR using BigDyeTM terminator chemistry with subsequent purification by means of ethanol precipitation and sequencing (automatic sequencer 3730xl) was performed by Macrogen in Seoul, Korea.

Sequences were edited using the software Seqman (version 4.05 ©1989-2000 DNASTAR Inc.) and aligned using Clustal W (Thompson et al. 1997) as implemented in the software Bioedit (version 7.0.0.1, Hall 1999). After determination of best-fit evolutionary model using the software ModelTest (version 3.06, Posada and Crandall 1998), phylogenetic analyses were performed within the software PAUP* (version 4.0b10, Swofford 1998). Phylogenies were determined on the basis of Neighbour Joining (NJ), Maximum Parsimony (MP) and Maximum Likelihood (ML) algorithms followed by non-parametric bootstrap analysis, a statistical re-sampling procedure, in order to evaluate statistical confidences by 1000 bootstraps for NJ and MP and 100 for ML.

Phylogenetic analyses are based on different algorithms, comprising distance- (NJ) and character-based (MP and ML) methods. Neighbour Joining is a distance-based method, where characters are reduced to distances and the topology of the tree is defined by the genetic distances between taxa. The calculation of the genetic distances is based on a best-fit evolutionary model. This model is determined by the software Modeltest, which calculates the probability which model (of a variety of given models) fits the best on the data set (Posada and Crandall 1998). In NJ the numbers of pairwise differences in character states are simply counted and closest characters share branches (Saitou and Nei 1987). This analysis is a form of star decomposition, starting with a tree in which all taxa are combined in the centre. From this, sequences of lowest genetic distances are combined in one branch, genetic distances are again calculated and the next related taxon is added on this branch, until the structure of the star like tree is resolved.

Maximum Parsimony constructs a phylogenetic tree with the least character changes (nucleotides) and thus evolutionary changes/steps (Felsenstein 1983). When two taxa share the same character, it is assumed that they are genetically related, meaning that evolution is parsimonious and sharing of characters is not a result of parallel evolution. Within this analysis missing data, such as sequence gaps in the multiple alignments, can be treated in the same manner as the other bases in the DNA sequences. Thus, this method involves species-specific indels as evolutionary information. With a simple algorithm, the number of steps is evaluated, which are required to explain the distribution of each character. Thus, one step means one change from one character to another.

Maximum Likelihood analysis is an estimation of the probability that the phylogeny (the tree) with a given evolutionary process (evolutionary model) generate the distribution of character states (the sequence data set), which is observed in the terminal taxa (Wägele 2001). The outcome of ML analysis thus depends on the quality of the evolutionary model. The model with the highest likelihood score is employed within the analysis in order to resemble more realistic natural conditions of evolution (Felsenstein 1981). The sequences serve as starting points for the search for appropriate parameters (e.g. substitution rates, branch lengths of the appropriate tree). The values are optimised to obtain the maximum probability (likelihood) that the

sequences are the result of the estimated process (Wägele 2001). The total probability of the tree is the product of the probabilities for each column in the sequence alignment.
In all three analyses, branch lengths are meaningful (=phylogram) as they are proportional to the average probability of change of characters on the respective branch.

2.4 Statistics

Prior to statistical analysis percentage data were transformed by arc sine square root transformation. Significant differences between means of species- and stage-specific data were tested using one-way analysis of variance (One-way ANOVA) with Dunnett T3 Post-Hoc test. Principal component analysis (PCA) is used to identify patterns in species-specific lipid composition and to highlight their similarities and differences. This non-rotated factor analysis was carried out on a correlation matrix with eigenvalues >1. Both, One-way ANOVA as well as PCA were performed using the software SPSS (version 15.0 and 16.0). Parametric unpaired t-tests as well as non-parametric Mann Whitney tests were performed using the software Prism version 5.02 with prior Bartlett's-test for testing variances and Kolmogorov-Smirnov test for testing normal distribution.

For the identification of species-specific pattern in lipid composition, a hierarchical cluster analysis with group-linkage was performed on the basis of a similarity matrix (Bray-Curtis), calculated from the percentage fatty acid and fatty alcohol compositions with prior square root transformation. This analysis was performed using the software Primer 5 (version 5.2.2, Clarke and Gorley 2001).

3 RESULTS AND SYNOPTIC DISCUSSION

3.1 Similarities in deep-water community of both polar regions

Euchaetidae and Aetideidae were sampled in both polar regions, comprising *Paraeuchaeta* and nine aetideid genera (*Aetideopsis*, *Aetideus*, *Chiridius*, *Chiridiella*, *Chirundina*, *Gaetanus*, *Euchirella*, *Pseudochirella* and *Undeuchaeta*) (Table 1). The genus *Valdiviella* was not included in any of the two families because its classification is unclear. It is either considered an aetideid genus or a euchaetid genus or even suggested to form a separate family (Markhaseva 1996, Bradford-Grieve et al. 1999).

Table 1

Species of the families Euchaetidae and Aetideidae sampled in the Southern Ocean and in Fram Strait. Data on abundance are combined from own observations and from literature (Ward and Wood 1988, Park 1994a, Park and Ferrari 2009). Definitions of abundances resulted from Park (1994a) and earlier studies as well as from Park and Ferrari (2009). Rare: species represented in the study by 10-30 individuals and/or the number of specimens found per sample was usually less than 5; common: species represented in the study by more than 30 individuals and/or the number of specimens found per sample frequently exceeded 5; very common: species found to be the most abundant in some samples and often represented in a sample by more than 100 individuals

FRAM STRAIT			SOUTHERN OCEAN		
Aetideidae (Giesbrecht 1892)		Abundance	**Aetideidae (Giesbrecht 1892)**		Abundance
Aetideopsis minor	Wolfenden 1911	common	*Aetideopsis minor*	Wolfenden 1911	common
A. rostrata	Sars 1903	common	*A. rostrata*	Sars 1903	rare
Chiridius obtusifrons	Sars 1902	common	*Aetideus* sp.		rare
C. armatus	Boeck, 1872	rare	*Chirundina* cf. *streetsii*	Giesbrecht, 1895	rare
Chiridiella abyssalis	Brodsky 1950	rare	*Chiridius* cf. *gracilis*	Farran 1908	rare
Chiridiella sp.		rare	*Chiridius* sp.		rare
Gaetanus brevispinus	Sars 1900	common	*Euchirella* sp.		rare
G. tenuispinus	Sars 1900	common	*Gaetanus pileatus*	Farran 1903	rare
Pseudochirella cf.*spectabilis*	Sars 1900	rare	*G. brevispinus*	Sars 1900	common
			G. tenuispinus	Sars 1900	common
			Pseudochirella cf. *spectabilis*	Sars 1900	rare
			Pseudochirella sp.		rare
			Undeuchaeta cf. *incisa*	Esterly 1911	rare
			Undeuchaeta cf. *major*	Giesbrecht 1888	rare
			Undeuchaeta cf. *plumosa*	Lubbock 1856	rare
			Undeuchaeta sp.		rare
Euchaetidae (Giesbrecht 1892)			**Euchaetidae (Giesbrecht 1892)**		
Paraeucheata norvegica	Boeck 1872	very common	*P. antarctica*	Giesbrecht 1902	very common
P. glacialis	Hansen 1887	common	*P. rasa*	Farran 1929	common
P. barbata	Brady 1883	common	*P. biloba*	Farran 1929	common
P. polaris	Brodsky 1950	common	*P. barbata*	Brady 1883	common
			P. exigua	Wolfenden 1911	rare
			Valdiviella sp.		rare

In Fram Strait, four *Paraeuchaeta* species were identified with three endemics (species which are unique to a habitat/region), the boreal-Atlantic *P. norvegica*, Arctic *P. glacialis* and *P. polaris* and one cosmopolitan (species with a world wide distribution), *P. barbata* (Table 1, chapter I, Park 1994a, Auel 1999, Park and Ferrari 2009). In the Antarctic, five *Paraeuchaeta*

species were sampled with three species endemic to sub-Antarctic and Antarctic waters (*P. antarctica*, *P. rasa* and *P. biloba*), one sub-Antarctic species (*P. exigua*) and *P. barbata* with cosmopolitan distribution (Table 1, chapter II, Park 1994a, Park and Ferrari 2009). Except for *P. exigua*, all species were common or very common. The dominant *Paraeuchaeta* species were those with the shallowest distribution: *P. norvegica* in Fram Strait and *P. antarctica* in the Antarctic. These species are considered to occupy similar vertical and ecological niches in the respective polar regions. Both show an ontogenetic vertical partitioning of the water column (chapters I and II, Fleddum et al. 2001, Irigoien and Harris 2006).

The most abundant aetideid species were *Aetideopsis minor*, *A. rostrata*, *Chiridius obtusifrons*, *Gaetanus brevispinus* and *G. tenuispinus* in Fram Strait (chapter I) and *A. minor*, *G. brevispinus* and *G. tenuispinus* in the Antarctic (chapter II). Other aetideid species occurred in very low numbers only, but the species list indicates higher aetideid species richness in the Southern Ocean than in Fram Strait (Table 1). Among aetideids, *G. brevispinus* and *G. tenuispinus* have a cosmopolitan distribution (Markhaseva 1996). *A. minor* and *A. rostrata* are considered as bipolar (Park and Ferrari 2009). Both species are found south of the Antarctic Polar Front and in the Arctic basin but in contrast to *A. minor*, *A. rostrata* also occurred in adjacent boreal seas of the Arctic basin (Park and Ferrari 2009). The bipolar distribution may be a result from continuous extinction in middle and low latitudes with a shallow population in polar seas, i.e. polar emergence (Markhaseva 1996, Auel 1999, Kosobokova et al. 2007, Park and Ferrari 2009).

Hence, the deep-water assemblages of Euchaetidae and Aetideidae comprise endemic species as well as species with cosmopolitan or bipolar distribution. Endemism is mainly found in species with a distribution at epi- to mesopelagic depths, i.e. *P. norvegica*, *P. glacialis*, *P. antarctica*, *P. biloba*, *P. rasa* and *C. obtusifrons* and only in one bathypelagic species, i.e. *P. polaris* (chapters I and II). *P. polaris* has a bathypelagic distribution endemic to the Arctic Ocean (Park 1994a) and occurs in deep ranges, even deeper than *P. barbata* (Auel 1999). Topographic barriers like the connection between Arctic Ocean and Fram Strait or boarders of the deep Greenland Sea basin may act as dispersal barriers for this species. Endemic *P. norvegica*/*P. glacialis* and *P. antarctica* are considered as congener pair with similar niches in polar seas, while niches of *P. biloba* and *P. rasa* were not occupied by euchaetid species in the Fram Strait and the Arctic (chapter II compared to chapter I). In general *Paraeuchaeta* fauna of the Southern Ocean is more diverse than of northern polar regions with 44% of all *Paraeuchaeta* species occurring in the Southern Ocean being endemic and most of them are rare (Ward and Shreeve 2001). This pattern may be due to the fact that the Antarctic is an older polar habitat than the Arctic.

Like for the epi- to mesopelagic *Paraeuchaeta* spp., high degree of endemism is found in epipelagic Antarctic species, i.e. the dominant *Calanoides acutus*, *Calanus propinquus*, *Rhincalanus gigas*, *Clausocalanus laticeps* and *Metridia gerlachei* (e.g. Boysen-Ennen et al. 1991, Atkinson 1998, Schnack-Schiel et al. 1998, 2008, Park and Ferrari 2009). Species of the two genera *Metridia* and *Calanus* are found in Fram Strait with *Metridia longa*, *Calanus hyperboreus*, *C. glacialis* and *C. finmarchicus* (Richter 1995, Kosobokova et al. 1998, Arnkværn et al. 2005, Hop et al. 2006). They occupy similar trophic niches like their Antarctic congeners. For example, both *Metridia* species feed omnivorously and year-round and store WE as main energy reserve (Lee and Hirota 1973, Albers et al. 1996, Hagen and Auel 2001 and references therein). The epipelagic copepod fauna of polar seas thus includes endemics as well as congener pairs but is not represented by cosmopolitan or bipolar species.

The endemic distribution of the Antarctic epipelagic species can be explained by physical and biotic restrictions to the respective habitat. The convergences of water masses at the Subtropical and Antarctic Polar Front represent boundaries for distribution and dispersal for epipelagic organisms (Pakhomov and McQuaid 1996, Ward and Shreeve 2001, Ward et al. 2003) and these species are circumpolar distributed. However, even without physical boundaries, dispersal abilities would be low, since epipelagic species are coupled to the euphotic zone with low temperatures as well as seasonal rich food supply in polar regions, which both change towards lower latitudes. For mesopelagic species, the Subtropical Front represents to some extent a dispersal boundary (Ward and Shreeve 2001) in contrast to the Antarctic Polar Front (Atkinson and Sinclair 2000). However, for the carnivorous *Paraeuchaeta* species, coupling to environmental factors may not be as tight as for primarily herbivorous copepods. Park (1994a) demonstrated for *Paraeuchaeta* that endemic species are considered “eutrophic” species, adapted to high food supply in highly productive habitats whereas cosmopolitan species are “oligotrophic”, adapted to low food availability and able to survive on a large geographical scale.

For deep mesopelagic *G. tenuispinus* and *A. minor* as well as bathypelagic *G. brevispinus*, *A. rostrata* and *P. barbata* neither frontal systems nor habitat-specific productivity represent dispersal boundaries, leading to a bipolar or cosmopolitan distribution. These species exhibit adaptive strategies allowing them to survive over a wide geographical range. However, occurrence of species in both polar regions does not imply constant genetic exchange between populations, as indicated by the molecular phylogenetic analysis of mitochondrial COI (chapter IV). High COI diversity in Antarctic and Arctic individuals of the mesopelagic *G. tenuispinus* and *A. minor* suggests the existence of different geographic forms, while there were no indications of genetic difference in the bathypelagic *G. brevispinus* and *P. barbata* (chapter IV, Fig. 2).

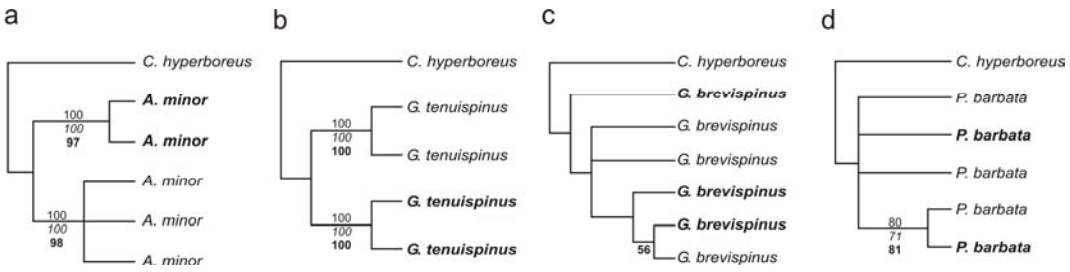


Figure 2

Maximum Likelihood tree on the basis of cytochrome C oxidase subunit I sequences of a) *Aetideopsis minor*, b) *Gaetanus tenuispinus*, c) *G. brevispinus* and d) *Paraeuchaeta barbata*. Regular: Arctic individuals, bold: Antarctic individuals. Numbers: Bootstrap values of Neighbour Joining (regular), Maximum Parsimony (italics) and Maximum Likelihood (bold)

In general polar deep-sea zooplankton can be carried and distributed by the global dispersal of Antarctic Bottom Water (Brandt et al. 2007a) and North Atlantic Deep Water, spreading from the Weddell and Greenland Sea, respectively, throughout the deep basins of the world's oceans (e.g. Mantyla and Reid 1983). Such deep-water links for transequatorial exchange of populations are possible for carnivorous or omnivorous zooplankton, which complete their life-cycles in meso- or bathypelagic depths and are not directly dependent on primary production (Machida et al. 2006). Since the variability of the oceanic habitat decreases with increasing depth, it can be assumed that mesopelagic species are subject to a higher selection pressure over a wide geographic range than bathypelagic species, resulting in higher adaptation potentials and ecological tolerances of mesopelagic species. Stronger impact of forcing factors on mesopelagic organisms demands on adaptation to varying conditions and the ability to maintain viable populations over a wide geographic range for population exchange. As a result, changes in the vertical structure and seasonality of water masses play an important role in the evolution of pelagic organisms (Norris 2000).

Molecular genetic studies conducted on wide-spread organisms revealed both, genetic differences and genetic similarities in species. Genetic differences were found in various epipelagic organisms like in foraminifera (de Vargas et al. 1999), copepods (Bucklin et al. 2000, Goetze 2003, Nuwer et al. 2008), euphausiids (Zane et al. 2000, Papetti et al. 2005), and chaetognaths (Peijnenburg et al. 2004). Strong genetic differences in surface water organisms are coupled to considerable environmental variation, playing an important role in species distribution and thus in ecological and genetic subdivisions of populations. For deep-living species however, these variations are less pronounced with increasing depths, but even in the circum-globally distributed deep-sea fish genus *Cyclothone* several cryptic allopatric lineages occur (Miya and Nishida 1997), although physical and geographic isolation mechanisms are almost absent in the deep-sea realm. High genetic similarities were found between deep-sea benthic Arctic and Antarctic meiofaunal iso- and amphipod and foraminifera populations (Brandt et al. 2007b, Pawlowski et al. 2007) with almost identical sequences of nuclear internal transcribed spacer

(ITS) in these geographically distant populations (Brandt et al. 2007b). However, in contrast to COI, ITS2 sequences of Arctic and Antarctic euchaetid and aetideid individuals were identical, suggesting that ITS2 does not have the power to resolve relation-ships on population level in these copepods (chapter IV).

Hypothesis: *In contrast to the high endemic epipelagic zooplankton community in polar seas the deep-water community bears many cosmopolitan species.*

Conclusions:

- Epipelagic calanoid copepods of the Southern Ocean are endemic and restricted by physical barriers like frontal systems (Subtropical and Antarctic Polar Front) as well as by biotic barriers, i.e. adaptations to high productivity. There are no epipelagic cosmopolitan or bipolar species, but rather species pairs of the same genera that occupy similar ecological niches in both polar systems.
- In contrast, deep mesopelagic and bathypelagic species of both families Euchaetidae and Aetideidae occur in both polar regions with a bipolar and cosmopolitan distribution.
- For deep-water species endemic to the Southern Ocean, the Subtropical Front may represent a faunal boundary and species are considered to be adapted to seasonal productivity.
- Cosmopolitan species are distributed by deep-water circulation systems and are not restricted by frontal systems. They have high survival capacities, coupled to their carnivorous or omnivorous feeding mode.
- Mesopelagic cosmopolitan and bipolar species are supposed to be more affected by the regional variability of their habitat than bathypelagic ones, resulting in different geographic forms in the Arctic and Antarctic, based on high diversity of mitochondrial cytochrome C oxidase subunit I.

3.2 Lipid storage strategies and validation of fatty acids as trophic biomarkers

Euchaetidae copepodite stages 5 (C5) and females had high lipid levels of 10-45% of dry mass (DM) and Aetideidae C5 and females had moderate ones of 12-32% DM (chapters I and II). The main difference between the two families and even between genera were the types of dominant lipid classes as main energy storage. *Paraeuchaeta* C5 and females were characterised by high wax ester (WE) amounts of 6-38% DM and 45-83% of total lipids (TL), while in aetideid *Gaetanus* C5 and females this lipid class occurred in moderate amounts only (2-12% DM, 13-38% TL). In the aetideid genera *Aetideopsis* and *Chiridius* WE were nearly absent with

0-4%DM and 2-14%TL and other lipid classes like triacylglycerol (TAG) may play an important role in energy storage (chapters I and II).

The ecological meaning of high WE levels is still controversially discussed. Generally, WE are considered long-term energy reserve to overcome extended periods of food scarcity and over-wintering, as exhibited by herbivorous copepods in polar regions with strongly pulsed primary productivity. This assumption is based on the observation of extremely high WE levels (up to 90% TL) in herbivorous calanid copepods from the Arctic and Antarctic (Lee et al. 1972, Lee and Hirota 1973, Lee 1974, Hagen et al. 1993, Kattner et al. 1994, Kattner and Hagen 1995, Albers et al. 1996). However, in the sub-Antarctic and Antarctic proper the herbivorous *Calanus propinquus* and *Calanus simillimus* as well as the omnivorous aetideid *Euchirella rostromagna* store high amounts of TAG instead of WE (Kattner et al. 1994, Hagen et al. 1995, Albers et al. 1996, Ward et al. 1996). These species feed continuously throughout the year, switching to other food sources than phytoplankton during polar winter (Kattner et al. 1994, Albers et al. 1996). These observations demonstrate that polar epipelagic species have adapted differently to the seasonal food availability. In *Paraeuchaeta* species, WE might not play an important role as long-term energy reserve. These predators might be less affected by seasonal food scarcity, since the four dominant Antarctic *Paraeuchaeta* species were shown to feed year-round with little differences in amounts taken with time of the year (Øresland and Ward 1993).

Wax esters also play an important role in maintaining neutral buoyancy (Nevenzel 1970, Visser and Jónasdóttir 1999). For deep-water zooplankton species in general it is assumed that neutral buoyancy provided by WE in cold deep water may be advantageous (Lee et al. 2006). This function is most likely for the heavily built *Paraeuchaeta* with their strongly skeletorised maxillipeds. Wax ester as buoyancy aid may balance these non-visual, tactile predators, which lurk in the water column and respond to vibrations caused by potential prey (Yen 1987). Furthermore, *Paraeuchaeta* are balanced by highly developed appendicular caudal setae (Park 1994b). These two features thus enable these species to follow this mechanotactic preying behaviour. In conclusion, it cannot be determined which primarily function WE adopt in the investigated species, but for the large carnivorous *Paraeuchaeta* species, the role as buoyancy aid seems to be the most conclusive one.

Lipids of *Paraeuchaeta* were dominated by the two monounsaturated fatty acids 16:1(n-7) and 18:1(n-9) (chapters I and II, Hagen et al. 1995). Only in young stages, important biomembrane components such as the polyunsaturated fatty acids 20:5(n-3) and 22:6(n-3) were present in higher proportions, reflecting the low lipid level of the young stages.

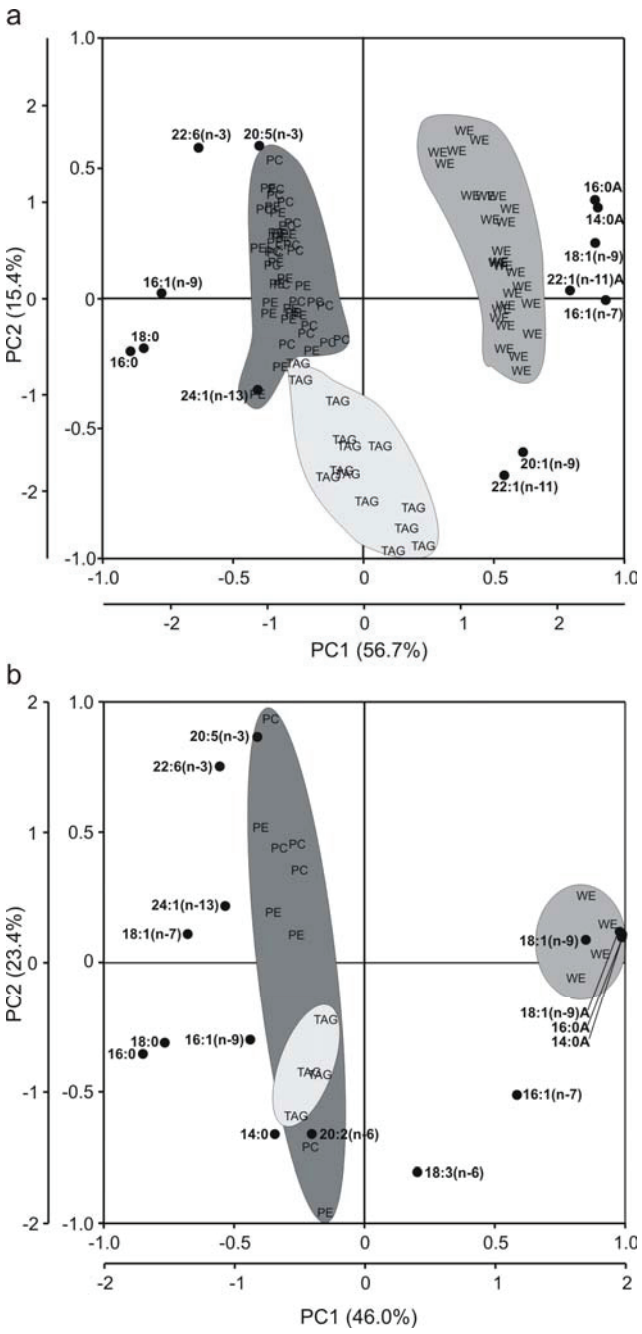


Figure 3

Principal component analysis of lipid class-specific fatty acids and alcohols (as percentages of lipids of respective lipid class) in a) *Paraeuchaeta* spp. and b) *Gaetanus brevispinus*. Fatty acids and alcohols <10% for *Paraeuchaeta* and <6% for *Gaetanus* are not shown. TAG: triacylglycerol, WE: wax ester, PC: phosphatidyl choline, PE: phosphatidyl ethanolamine. Sample plot for the principal components PC1 and PC2 (outer axes) and superimposed loading plot (inner axes) with the relevant fatty acids and alcohols. Loading differences >0.5 are significant

The separation of lipid classes for *G. brevispinus* and *Paraeuchaeta* species, including *P. barbata*, *P. norvegica* and *P. rasa* demonstrated that specific fatty acids were associated with certain lipid classes as illustrated by principal component analysis (Fig. 3). Along principal component 1 (PC1) with highest variance, phospholipids (phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE)) and partly TAG are separated from WE in both, *Paraeuchaeta* and *Gaetanus*. To some extent, phospholipids and TAG separated along PC2 (15.4% and 23.4% of variance for *Paraeuchaeta* and *G. brevispinus*, respectively). Biomembrane

compounds like the fatty acids 16:0, 20:5(n-3) and 22:6(n-3) (Lee 1975) were included in the phospholipids components PC and PE and high levels of these fatty acids revealed a lipid-poor status of organisms, prevalent in young stages.

The two dominant fatty acids in *Paraeuchaeta*, 16:1(n-7) and 18:1(n-9) were mainly stored in the WE, together with saturated (14:0A, 16:0A) and the long-chain monounsaturated 22:1(n-11) fatty alcohol that accumulated during ontogenesis (Fig. 3a). The fatty acids 20:1(n-9) and 22:1(n-11), which are generally considered as trophic biomarkers for calanid copepods (Sargent and Falk-Petersen 1988, Graeve et al. 1994a, Kattner et al. 1994, Kattner and Hagen 1995, Albers et al. 1996), were situated in both, TAG and WE fractions, reflecting their origin in the diet of these carnivorous species. In *G. brevispinus*, WE were mainly composed of the three fatty alcohols 14:0A, 16:0A and 18:1(n-9)A together with the biomarker of carnivorous feeding 18:1(n-9) fatty acid (Fig. 3b). In contrast to *Paraeuchaeta*, 16:1(n-7) was accumulated in both neutral lipid classes, WE and TAG.

Trophic biomarkers, associated with neutral lipids like WE and TAG, can be applied for detecting trophic interactions and for tracing the feeding history of consumers. In contrast, fatty acid trophic biomarkers which are also associated with biomembranes are not suitable for the interpretation of feeding history when solely considering the total lipid content. These fatty acids are 20:5(n-3), a marker for diatoms (e.g. Graeve et al. 1994a,b) and 22:6(n-3), a marker for dinoflagellates (e.g. Graeve et al. 1994b). High levels of the biomarker for carnivorous feeding 18:1(n-9) (Falk-Petersen et al. 1990, Graeve et al. 1997) in the WE fraction support the view of predatory feeding behaviour of *Paraeuchaeta*. The carnivorous feeding mode of *Paraeuchaeta* species was demonstrated in feeding experiments, guts content analyses as well as concluded from morphology of the mouth parts (Hopkins 1987, Yen 1991, Øresland and Ward 1993, Olsen et al. 2000, Michels and Schnack-Schiel 2005). In addition, the carnivorous feeding on calanid copepods was supported in many *Paraeuchaeta* species by high levels of 20:1 and 22:1 fatty acids and alcohols, mainly stored in the TAG and WE fractions (Fig. 3a, chapter I). However, WE also contain high levels of the biomarker for herbivorous feeding on diatoms 16:1(n-7) (e.g. Graeve et al. 1994a,b). These high levels are conspicuous for a true carnivorous species like *Paraeuchaeta*. Prey movement is an important stimulus triggering predatory responses in *Paraeuchaeta* (e.g. Bailey 1984, Yen 1987) and the morphology of mouthparts is designed for feeding on large prey items (Michels and Schnack-Schiel 2005). It is highly unlikely, that *Paraeuchaeta* feeds selectively on phytoplankton. High amounts of 16:1(n-7) rather resulted from a selective retention of this fatty acid which might attain high levels in the copepod prey or from *de novo* synthesis. Interestingly, both, 18:1(n-9) and 16:1(n-7) were suggested to result from *de novo* synthesis in this genus (Hagen et al. 1995).

In contrast to *Paraeuchaeta*, the fatty acid composition of Aetideidae was more balanced (chapters I and II), implying a more omnivorous feeding mode as well as a higher influence of biomembrane fatty acids related to the lower total lipid content. Nevertheless, carnivorous feeding played an important role in *G. brevispinus*, as indicated by high levels of 18:1(n-9) fatty acid and alcohol in the WE fraction (Fig. 3b).

Hypothesis: *Deep-sea Euchaetidae and Aetideidae occupy different ecological niches, with a higher degree of omnivory in the latter representatives, leading to differences in the respective fatty acids composition as well as lipid storing strategies.*

Conclusions:

- High wax ester levels in *Paraeuchaeta* species mainly contain saturated fatty alcohols; however enhanced predation on calanid copepods is reflected by elevated levels of 20:1 and 22:1 fatty alcohols. The carnivory marker 18:1(n-9) as well as 16:1(n-7) were the main fatty acids in the wax esters, but their origin (*de novo* synthesis vs. food derived) is still unclear.
- Deep-sea Aetideidae store moderate to low amounts of wax ester. Triacylglycerols are presumably the dominating storage lipid class.
- Aetideidae have a more balanced fatty acid composition than *Paraeuchaeta* species, in line with their omnivorous and opportunistic feeding behaviour.

3.3 Vertical migrations provide a trophic short-cut for deep-sea copepods

Feeding on *Calanus* species was determined on the basis of calanid-specific 20:1(n-9) and 22:1(n-11) fatty acids and alcohols (chapters I and II). For the Arctic, stable isotope signatures of nitrogen and carbon provided insights in the food web structure, since additional data were collected for potential prey organisms *Calanus hyperboreus*, *C. glacialis* and *C. finmarchicus* (chapter I). The combination of both approaches allowed conclusions on feeding habits of the carnivorous Euchaetidae and omnivorous Aetideidae.

Seasonally migrating calanid copepods are abundant in both polar regions and thus may represent potentially important food items for euchaetid and aetideid deep-water species. In the Arctic, the three *Calanus* species *C. hyperboreus*, *C. glacialis* and *C. finmarchicus* form the key link between primary producers and higher trophic levels (e.g. Falk-Petersen et al. 1990). Arctic *Calanus* species perform seasonal vertical migrations to overwinter at bathypelagic depths (Dale et al. 1999, Hirche et al. 2006, Blachowiak-Samolyk et al. 2007). Similarly, the abundant

Antarctic *Calanoides acutus* descends below 750 m during winter, while only a part of the population of the sub-Antarctic *Calanus simillimus* is found below 500 m during winter (Atkinson 1991).

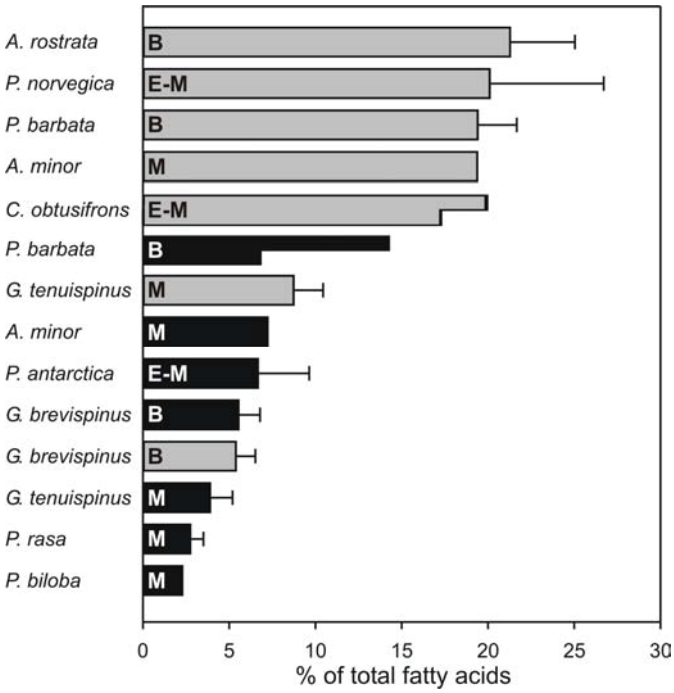


Figure 4

Proportions of calanid fatty acid trophic biomarkers of total fatty acids in Arctic (grey) and Antarctic (black) euchaetid and aetideid females. Calanid biomarkers 20:1(n-9) and 22:1(n-11) were summed up. E-M: epi- to meso-pelagic, M: meso-pelagic, B: bathy-pelagic distribution. In case of only two replicates, both values are shown as staged columns

In general, proportions of 20:1(n-9) and 22:1(n-11) fatty acids were higher in Arctic than in Antarctic species and specimens, with the exception of Arctic *G. brevispinus* individuals (Fig. 4). Elevated levels of calanid biomarkers in Arctic species from all depths demonstrated the availability and utilisation of these copepods as prey items at all depth horizons. They provide a rich food source for the omnivorous and carnivorous deep-sea zooplankton community (Fig. 4, chapter I).

Feeding on *Calanus* by aetideid and euchaetid species in Fram Strait was further supported by stable isotope signatures (Fig. 5, chapter I). Arctic *Paraeuchaeta* females were identical in $\delta^{15}N$ and $\delta^{13}C$, indicating predation on possibly the same prey items (Fig. 5, chapter I). Applying an enrichment factor of 3-5‰ per trophic level for $\delta^{15}N$ (Rau et al. 1983, Minagawa and Wada 1984, Hobson and Welch 1992, Kurle and Worthy 2002), *C. finmarchicus* C5 and females from 200 to 1000 m depth were the most likely prey for *Paraeuchaeta* females (Fig. 5, chapter I). This is in accordance with the dominance of *C. finmarchicus* in Fram Strait during autumn (Blachowiak-Samolyk et al. 2007). Although it is assumed that diapausing copepods are inactive, tactile feeding *P. barbata* seem to be able to detect these prey. The occurrence of herbivorous copepods and thus primary consumers at depth provides a rich food source for deep-sea organisms and represents a short-cut from epipelagic primary production to the

bathypelagic food web. *Gaetanus* females had the same $\delta^{15}N$ as *Paraeuchaeta* females, although for *G. brevispinus* calanid fatty acid and alcohol moieties were less abundant.

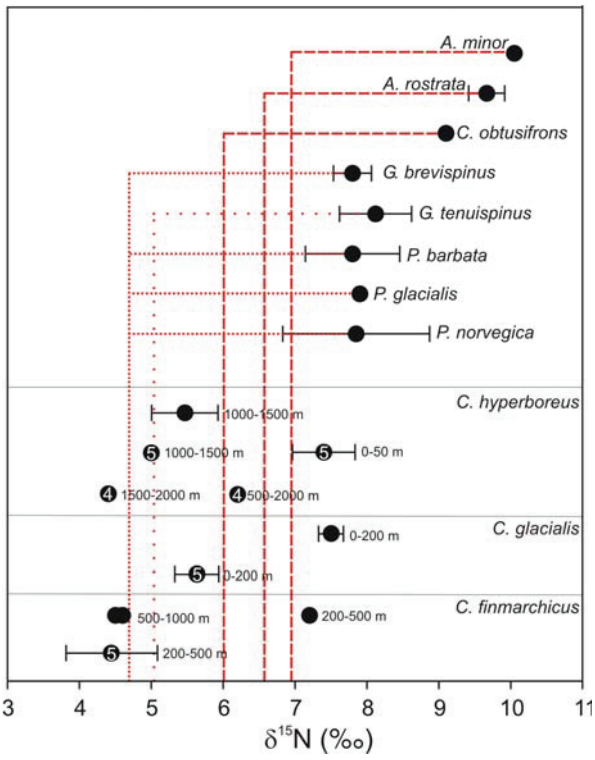


Figure 5

Stable nitrogen isotope ratio ($\delta^{15}N$) of Arctic euchaetid and aetideid females as well as of potential prey organisms *Calanus hyperboreus*, *C. glacialis* and *C. finmarchicus* with different ontogenetic stages and from different depth horizons. Red vertical lines represent the difference between predator and potential prey according to an enrichment of 3‰ per trophic level. Dots: females; 4: copepodite stage 4, 5: copepodite stage 5

Based on fatty acid calanid biomarkers, in the Southern Ocean, calanid copepods did not represent an important food item in epi- to mesopelagic realms during austral autumn (Fig. 4, chapter II). Only in bathypelagic *P. barbata* females, elevated levels of calanid biomarkers, especially alcohols could be detected (chapter III). However, it can not ruled out that mesopelagic *Paraeuchaeta* might have fed on *Calanus simillimus*, a common species in the sub-Antarctic (Atkinson 1991) with low levels of calanid-typical biomarkers (Ward et al. 1996). In general, calanid copepods like *Calanoides acutus* are an important prey for *P. antarctica* (Øresland and Ward 1993), but in polar winter this species descends below the vertical distribution of *P. antarctica* and is thus not available (Atkinson and Ward 1988). For *P. rasa* and *P. biloba C. acutus* does not seem to represent an important food item (Øresland and Ward 1993).

In conclusion, the importance of Calanidae as prey organisms for meso- and bathypelagic omnivorous and carnivorous copepods could be shown on the basis of trophic markers for Fram Strait but not for the Southern Ocean. *Calanus* species overwintering at depth provide a trophic short-cut for the deep-sea community. The vertical flux of fresh, organic material is accelerated by downward migrating primary consumers.

As a result, in polar regions the deep-sea environment may experience a seasonal peak in food supply in autumn and during winter, when calanid copepods overwinter at depth. Since this pattern is opposite to the primary production regime in the surface layer, it may be termed "inverse seasonality".

Hypothesis: *The seasonal vertical migrations of abundant herbivorous copepods provide a trophic short-cut for carnivorous and omnivorous deep-sea copepods. They represent important prey items throughout the water column and their seasonal vertical migration leads to a peak in food availability in the deep sea. As a consequence, the deep sea in polar regions is structured in spatial and temporal terms.*

Conclusions:

- *Calanus* represents an important prey item for Arctic Euchaetidae and Aetideidae in all depth horizons, proven by trophic markers.
- For the Southern Ocean, predation on calanid copepods was only detectable for bathypelagic *P. barbata*. However, this may be related to the lower content of typical calanid markers in sub-Antarctic calanid species.
- The seasonal vertical migration of *Calanus* and their overwintering at great depths
    a) provide a short-cut within the food web.
    b) accelerate the vertical flux of organic carbon.
    c) distinguish the deep sea in polar regions seasonally as well as spatially from other deep-sea regions
    d) lead to an inverse food supply, i.e. high food availability at depth when sea surface primary production is low.

3.4 Co-existence in the pelagic deep sea: spatial and trophic niche separation

Vertical partitioning of the water column by congeners seems to be the main mechanism for minimising inter-specific competition between closely related species resulting in a stepwise arrangement of species with similar trophic demands (chapters I and II).

In both high-latitude regions, species of the same genera (congeners) do generally not occur within the same depth range, thus avoiding competition (Fig. 6, chapters I and II).

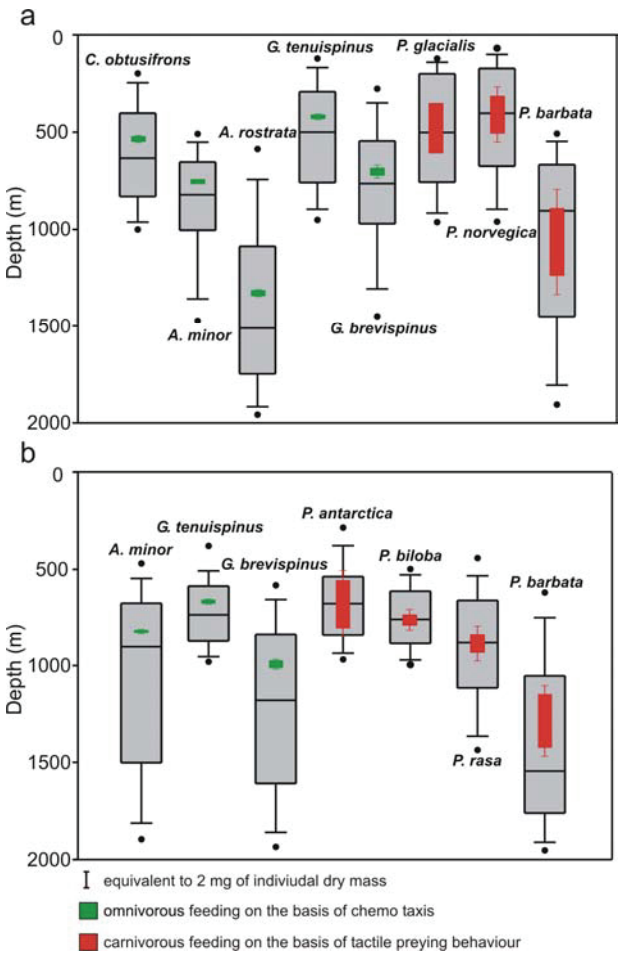


Figure 6

Vertical distribution of a) Arctic and b) Antarctic Euchaetidae and Aetideidae females (big grey box plots). Boxes include 50% of the population, while error bars encompass the 10th to the 90th percentile and dots 5th to the 95th. Smaller interior box plots: individual dry mass of females (mg); green colour: omnivorous feeding by means of chemotaxis; red colour: carnivorous feeding by means of tactile preying behaviour

In the Arctic, *Paraeuchaeta norvegica* and *P. glacialis* occurred in the epi- to mesopelagic zone, while *P. barbata* inhabited bathypelagic depths (Fig. 6a, chapter I). In the Antarctic, *P. antarctica* mainly occurred in epi- to mesopelagic depths and overlapped in distribution with *P. rasa* and *P. biloba*, concentrated in the mesopelagic realm (Fig. 6b, chapter II). *P. barbata* had the deepest distribution within the bathypelagic realm with a slightly deeper occurrence compared to Arctic individuals. In both polar regions, *Gaetanus* congeners occurred in different depth strata, with *G. tenuispinus* in mesopelagic and *G. brevispinus* in deep meso- to bathypelagic depths (Fig. 6, chapters I and II). The same was true for the closely related genera *Aetideopsis* and *Chiridius* in the Arctic. *C. obtusifrons* and *A. minor* inhabited mesopelagic and *A. rostrata* bathypelagic depths (Fig. 6a, chapter I). Partitioning of the water column thus allows closely related species to occur in the same region. Exceptions to this rule are the congener pairs *P. norvegica* and *P. glacialis* in the Arctic and *P. rasa* and *P. biloba* in the Antarctic, overlapping with *P. antarctica*. The rather similar *P. norvegica* and *P. glacialis* do not show any differences in the analysed characteristics of their ecological niches (Table 2a). These species are associated with two opposing current systems in the Fram Strait with boreal-Atlantic

P. norvegica linked to the warm West Spitsbergen Current (chapter I, Mumm et al. 1998, Auel 1999) and Arctic *P. glacialis* to the cold East Greenland Current (chapter I, Auel 1999). In consequence, the overlapping distribution in the Fram Strait and in the Greenland Sea despite the high similarity in the ecological niche can be explained by the continuous advection of specimens from the different areas of origin by the opposing currents (Auel 1999).

The lipid and fatty acid composition revealed similarities in trophic demands of closely related species (chapters I, II and IV). Occurrence of closely related species with similar trophic niches in the same depth strata would lead to competition and according to the competition exclusion principle two species with similar requirements cannot stably and spatially co-exist. This inter-specific competition is minimised by vertical separation (chapters I and II). Such structuring of the water column was also described for several congener species e.g. within the families Euchaetidae, Aetideidae, Eucalanidae, Metridiidae as well as Augaptilidae in the Pacific and in other ocean basins (e.g. Raymont 1983, Mauchline 1995, Richter 1995, Saltzman and Wishner 1997, Yamaguchi and Ikeda 2002, 2003). This strategy is generally considered to be a main mechanism of sustaining co-existence of zooplankton organisms in the same habitat (Hayward and McGowan 1979, Ambler and Miller 1987, Williams 1988).

Co-existence of species within the same depth range requires other mechanisms or differentiation in order to minimise competition. The diversity of communities is sustained by selectivity in feeding behaviour but each trophic level comprises several species, implying that competitive interactions have somehow been resolved (Ambler and Miller 1987). Co-occurring species have to partition resources and competition is buffered by inhabiting different trophic niches (Ambler and Miller 1987). As the deep-sea pelagic realm is assumed to be a resource-limited, almost homogeneous environment, lacking physico-chemical barriers, the ecological niches of their inhabitants are of main interest to understand co-occurrence.

Euchaetidae and Aetideidae, occurring within the same depth range, mainly differed in size, implying different prey size spectra (Fig. 6, Table 2). Furthermore, the different feeding behaviour of Euchaetidae and Aetideidae drives the selectivity of food. While Aetideidae feed on the basis of chemotaxis, enabling these species to detect both, motile and non-motile food items, Euchaetidae are tactile, rheotactic predators, implying localisation of motile prey only (Yen 1987, 1991, Greene 1988, Olsen et al. 2000). The predatory feeding behaviour of *Paraeuchaeta* species is further demonstrated by their higher index of carnivory (13-58) compared to the Aetideidae (1-10) ($p<0.001$) in both polar regions, based on the fatty acid isomer ratio 18:1(n-9)/18:1(n-7) (Fig. 7). The higher degree in carnivory of *Paraeuchaeta* thus differentiated species of these two families, resulting in a lower competition for same food items. In conclusion, the family-specific differences in marker ratios are in accordance with their generally assumed feeding modes. However since physiological pathways of fatty acid

synthesis, e.g. the *de novo* synthesis of 16:1(n-7), are not fully understood for these deep-sea species, a final interpretation as to be made with care.

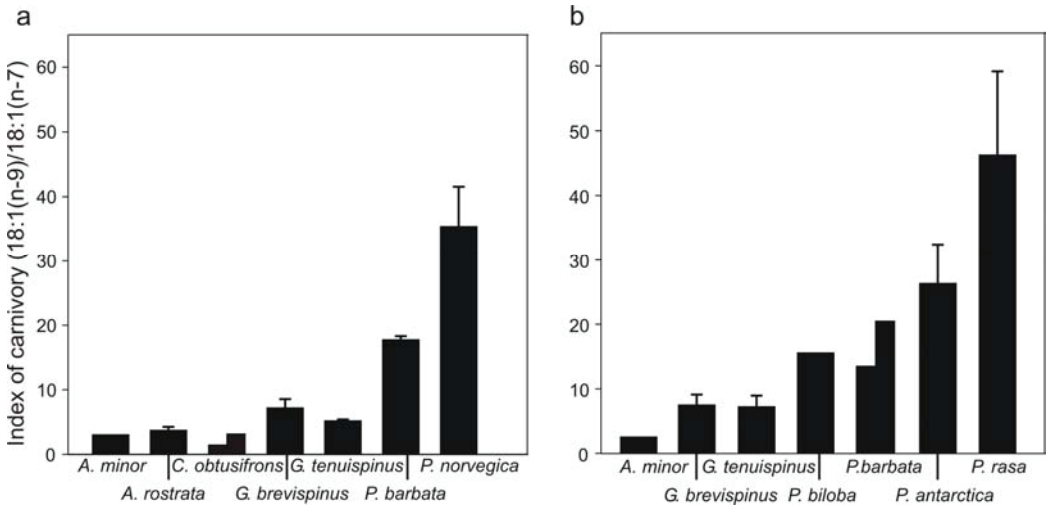


Figure 7

Index of carnivory on the basis of the fatty acid ratio 18:1(n-9)/18:1(n-7) for a) Arctic and b) Antarctic females of Euchaetidae and Aetideidae. In case of only two replicates, both values are shown as staged columns

In the mesopelagic zone of the Antarctic, competition between *Paraeuchaeta* species was minimised by the larger size of *P. antarctica* compared to *P. biloba* and *P. rasa* (Table 2b, Fig. 6b), and hence by different prey size spectra, as it was demonstrated by Øresland and Ward (1993). Compared to *P. biloba*, *P. rasa* fed more carnivorously as indicated by higher degree of carnivory (18:1(n-9)/18:1(n-7)) (Fig. 7b). Additionally, *P. rasa* fed on prey of higher trophic levels based on higher $\delta^{15}N$ signatures than *P. biloba* (chapter III). The epi- to mesopelagic *P. antarctica* responds to spatial and temporal variations in food availability, indicated by high predation on *Calanoides acutus* in summer (Øresland and Ward 1993) but not in autumn and winter, when the preferred prey is not abundant within their main distribution depth (chapter II).

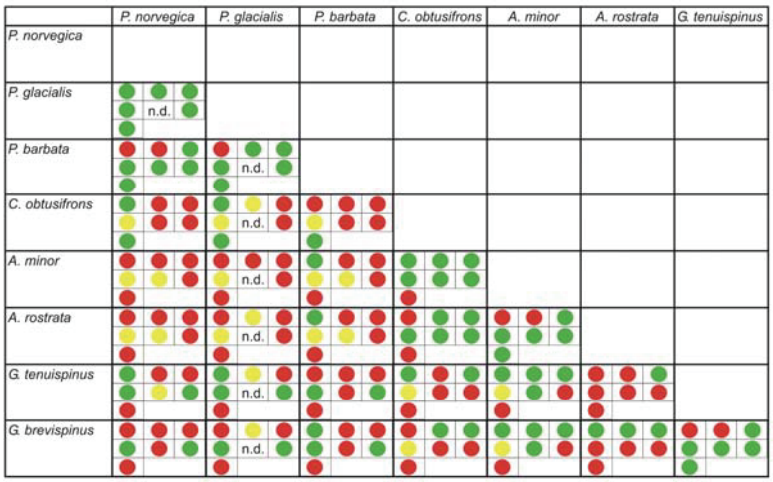
According to Williams (1988), next to vertical partitioning and different trophic levels, additional mechanisms are involved for minimising inter-specific competition of co-occurring species. Diverse reproductive strategies minimise competition, with *Paraeuchaeta* species and aetideid *C. obtusifrons* carrying egg sacs with a robust membrane, while *Gaetanus* spp. are broadcast spawners releasing floating eggs (Kosobokova et al. 2007). *Aetideopsis* spp. release a mass of eggs (*A. rostrata*) or carry egg sacs with a fragile membrane (*A. minor*) (Kosobokova et al. 2007). Eggs of *Aetideopsis* and *Gaetanus* species are coated with an adhesive substance (e.g. Kosobokova et al. 2007). Furthermore, a seasonal offset of the main reproductive period is likely for some species. For example, *P. norvegica* bears two generations per year in Korsfjorden, western Norway (Båmstedt and Matthews 1975, Båmstedt 1979), while *P. barbata*

breeds throughout the year with periods of greater intensity in the Rockall Trough (Mauchline 1992). As an example for aetideids, the timing of spawning of *Gaetanus variabilis* matched with the phytoplankton bloom (Yamaguchi and Ikeda 2000). In addition, different migratory behaviour throughout the year leads to avoidance but was not recorded for Arctic Aetideidae (Richter 1995). Diel vertical migration is recorded for *P. norvegica* (Hays et al. 1994, Kaartvedt et al. 2002, Skarra and Kaartvedt 2003), *P. antarctica* (Hopkins 1985b) as well as for the *Gaetanus* species *G. variabilis* in the western North Pacific and *G. minor* in the North East Atlantic (Roe 1984, Yamaguchi and Ikeda 2000).

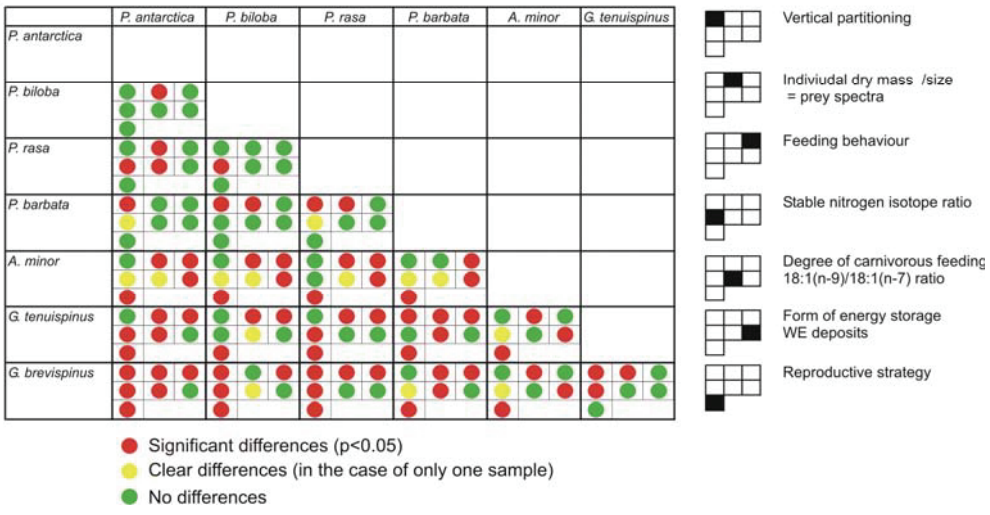
Table 2

Summary of species-specific differences in a) Arctic and b) Antarctic females of Euchaetidae and Aetideidae. Differences in dry mass, stable nitrogen isotope ratio and degree of carnivory are evaluated by parametric unpaired t-test and non-parametric Mann-Whitney-test ($p<0.05$) (red) and in the case of only one sample by clear differences (yellow). No difference (green). Published data were used for reproductive strategies (Kosobokova et al. 2007) and for *P. glacialis* (Auel 1999)

a



b



For the omnivorous Aetideidae, co-existence may be possible, even in a food-limited habitat, by shifting grazing pressure to other food size classes as this opportunistic behaviour was shown for a variety of omnivorous copepod species (Poulet 1978). Quantitative feeding, related to specific ingestion rates of particles, was a major difference in five co-occurring omnivorous copepod species in Nova Scotia (Poulet 1978).

In contrast, similar niches rather than specialisation were demonstrated for closely related copepod species in food-limited environments with all copepods being omnivores and food generalists and similar in terms of spatial and temporal feeding patterns (Hayward 1980). Furthermore, species were not distinct by temporal differences in population size and constant in relative abundance among seasons and years (McGowan and Walker 1979). Convergence in niche separation rather than specialisation was demonstrated for closely related copepod species on the basis of diet (Hayward 1980).

Regarding the differences between the species, co-occurrence in the same region as well as co-existence within one depth stratum can generally be explained by niche differentiation, mechanisms that control competitive displacement, and by chance. Furthermore, low abundance of deep-sea species lead to low frequencies of interactions, which further reduces the risk of inter-specific competition in oceanic species (Madin and Madin 1995). Euchaetidae and Aetideidae from both polar regions showed differences in trophic requirements, suggesting the occupation of different ecological niches. The very similar closely related species minimised competition by vertical partitioning of the water column (spatial niches). Vertical partitioning is not only an important component in sustaining a highly diverse community, but also seems to play an important role in speciation processes in the deep-sea realm (chapter IV).

In conclusion, two of the three processes for dividing an oceanic habitat by organisms, proposed by Madin and Madin (1995) could be demonstrated in the deep-sea copepods: spatial separation and differences in behaviour of feeding and reproduction. The third process, a temporal component based on movement (vertical migration) was not demonstrated on the present results.

Hypothesis: *Closely related species occupy rather similar trophic niches and minimise inter-specific competition by vertical separation (spatial niches). Species within one depth stratum usually differ in feeding behaviour and/or dietary preferences (trophic niches).*

Conclusions:

- Congeners with similar trophic niches partition the water column and avoid competition by vertical separation. This spatial partitioning plays an important role in allowing and sustaining co-occurrence and may also be involved in speciation processes in the deep sea.
- Competition of species inhabiting the same depth range is minimised by differences in
    a) body size (=prey size spectra).
    b) feeding behaviour (food selectivity on the basis of chemotaxis vs. rheotactic predation).
    c) degree of carnivorous feeding.
    d) mode of energy storage (adaptations to temporal and spatial food availability).
    e) trophic level.
    f) reproductive strategy.

3.5 Combining molecular phylogenetics and ecology to understand evolutionary processes

Phylogenetic relationships of Euchaetidae and Aetideidae were analysed on the basis of four different genetic markers. The variable mitochondrial DNA cytochrome C oxidase subunit I (COI), a subunit of an important enzyme in the respiratory chain, was chosen as genetic marker. COI sequences of species were highly variable, and even resolved genetic differences among individuals of one species (chapter IV). In consequence, this marker is suitable for identification of species as well as for differentiation on population level. However, this marker did not provide information on phylogenetic relationships on the level of genera or families (chapter IV). Supplemented COI sequences of euchaetid and aetideid species from GenBank illustrate the classification of many individuals to one species by high bootstrap values (in general 100%) and show polytomy of all species on the main branch, thus no resolution of phylogenetic relationship of species (Fig. 8).

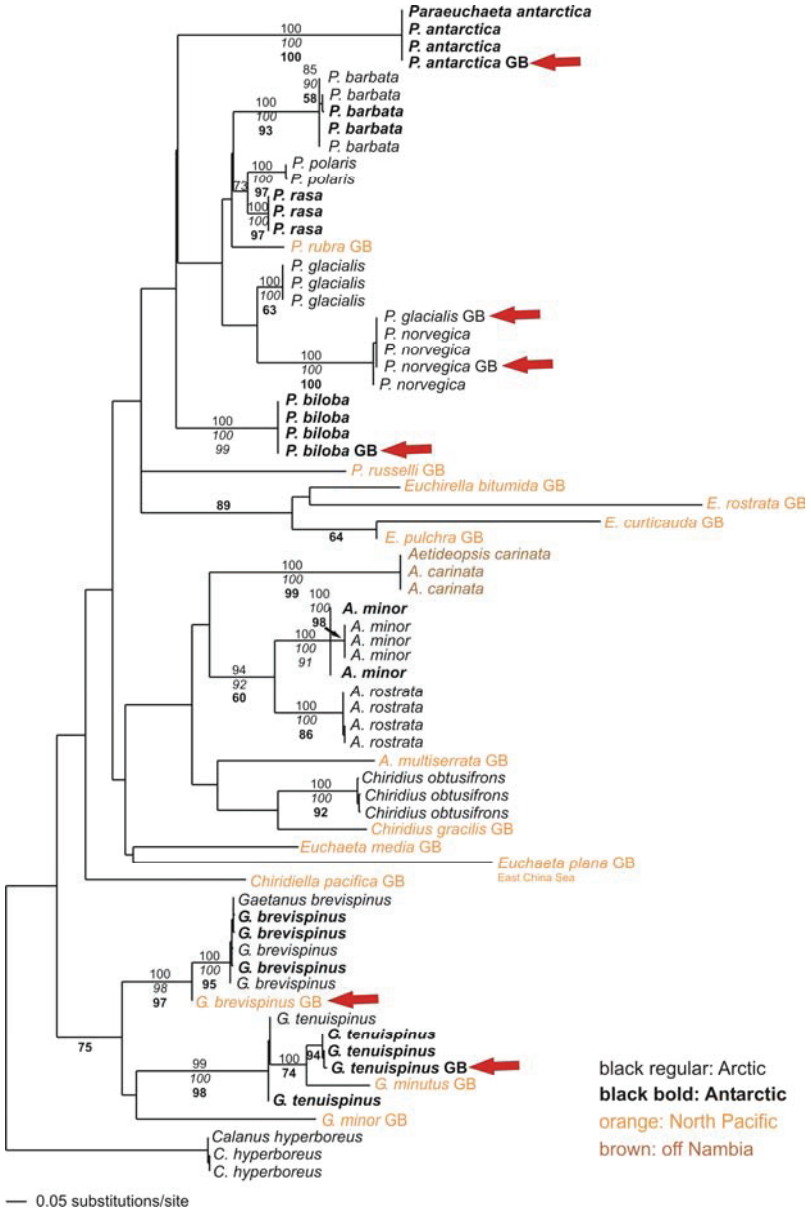


Figure 8

Maximum Likelihood phylogram of euchaetid and aetideid species on the basis of COI sequences including individuals from the Arctic (black regular), Antarctic (black bold), North Pacific (orange) and off Namibia (brown). Analysis is based on a fragment of 487 base pairs and Tamura Nei with proportion of invariable sites (0.51) and a gamma distribution shape parameter (0.44) as best-fit model of evolution (TrN, Tamura and Nei 1993). Bootstrap values: Neighbour Joining (regular), Maximum Parsimony (italics), Maximum Likelihood (bold); GB: sequences from GenBank with accession numbers: B380003, AB380014, AB380021, AB380010, AB380011, AB380006, AB379978, AB380007, AB380002, AB379980, AF531742, AB379979, EF104261, AF531748, AF531749, EF015491, AY660600, AB379996, AB380008; red arrows: investigated species from GenBank

Sequences of the investigated species from GenBank clustered together with those determined in the present study, except for one *Paraeuchaeta glacialis* sequence, which is placed in a clade with *P. norvegica* and *Gaetanus minutus* clustering together with *G. tenuispinus* (Fig. 8).

The example of *P. glacialis* and *P. norvegica* demonstrates the power of DNA barcoding for species identification, since young stages of these two species are difficult to distinguish based on morphological characters. *P. glacialis* and *P. norvegica* co-occur in Fram Strait and Greenland Sea with *P. norvegica* being transported northwards by the warm West Spitsbergen Current and *P. glacialis* southwards by the cold East Greenland Current. During the sampling period in Fram Strait in 2006 boreal-Atlantic *P. norvegica* was more abundant and had a broader distribution than Arctic *P. glacialis*, as compared to previous years (1997, see Auel 1999, chapter I). This result was in line with a stronger inflow of Atlantic water masses, accompanied by an increased water temperature of more than 1°C from 1996 to 2006 (Schauer et al. 2008). This example represents a reliable approach of DNA barcoding, since distribution and abundance of these two indicator species provide evidence of variances in the Arctic realm and therefore these species are a bioindicator for progressive global warming.

Fast evolving COI sequences have been proven to be applicable as barcodes for organisms. DNA barcoding, based on sequence diversity of COI, is thus used for identification and discrimination of species (Hebert et al. 2002, Bucklin et al. 2003, Valentini et al. 2008). Initiatives such as Census of Marine Life (COML), Census of Marine Zooplankton (CMarZ) and Marine Barcode of Life (MarBOL) are comprehensive attempts to understand biodiversity of marine life and apply a combination of taxonomic identification and DNA barcoding. Collection of species barcodes thus enables scientists to rapidly identify species and furthermore to detect prey items in guts and faecal pellets, as shown for carnivorous *Paraeuchaeta* species (Vestheim et al. 2005).

Nuclear ribosomal markers 18S and 28S did not provide information on family, genus or even species level (chapter IV). Both, 18S and 28S are conserved sequences with low genetic distances between genera and none between species. Between the conservative nuclear coding (18S and 28S) and the fast evolving mitochondrial coding markers the nuclear non-coding region internal transcribed spacer 2 (ITS) emerged to be valuable since it highly supported the different genera and species as well as to a lesser degree the classification of the aetideid family (chapter IV). The present work thus proved the suitability of ITS2 in phylogenetic analysis, already demonstrated in previous studies (Rocha-Olivares et al. 2001, Goetze 2003, Young and Coleman 2004). ITS2 sequence phylogeny together with genus- and species-specific characteristics in feeding behaviour (e.g. Yen 1987, Olsen et al. 2000), lipid class-specific energy storage (chapters I and II) and reproductive strategy (Mauchline 1988, Auel 1999, Kosobokova et al. 2007), demonstrate uniformity within genera (Fig. 9, chapter IV) except for reproductive strategy within the *Aetideopsis*/*Chiridius* clade.

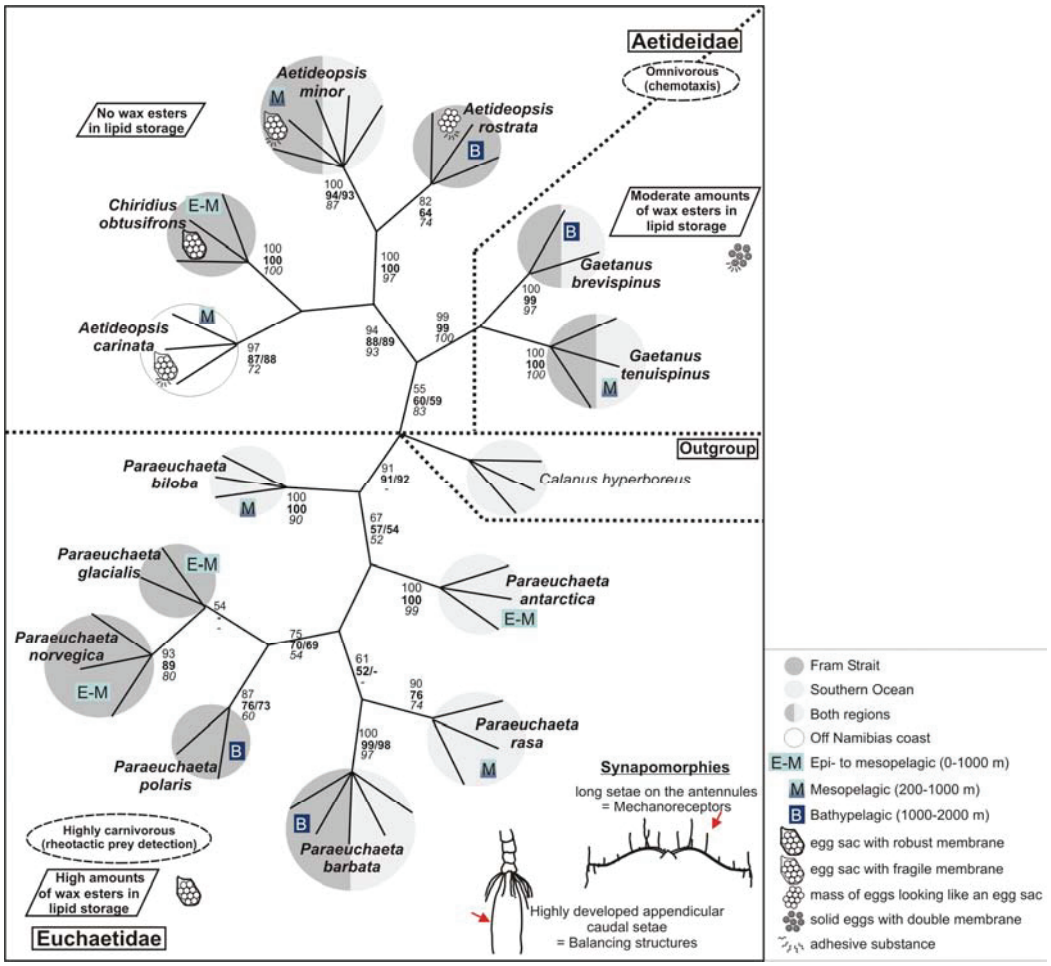


Figure 9

Maximum Likelihood tree on the basis of nuclear internal transcribed spacer 2 (ITS2) sequences of Euchaetidae and Aetideidae. ITS2 re-construction of a fragment of 510 base pairs is based on Tamura Nei model with equal frequencies (TrNef, Tamura and Nei 1993) with proportion of invariable sites (0.40) and a gamma distribution shape parameter (0.55). Bootstrap values: regular figures (Neighbour Joining), bold figures (Maximum Parsimony/MP with gaps handled as 5th base) and italic figures (Maximum Likelihood). Biological data, i.e. synapomorphies (Park 1994b), feeding behaviour (Yen 1987, Olsen et al. 2000), lipid class-specific energy storage (chapters I and II) and reproductive mode (Mauchline 1988, Auel 1999, Kosobokova et al. 2007, Auel own observation) is plotted on phylogenetic relationships.

All *Paraeuchaeta* species feed carnivorously on the basis of rheotactic prey detection, have high levels of wax esters (chapters I and II) and carry egg sacs with a robust membrane (Mauchline 1988, Auel 1999, Kosobokova et al. 2007) (Fig. 9). Carrying egg sacs or attached egg masses is generally only found in few calanoids, including families of freshwater Diaptomidae, marine Euchaetidae and in the genera *Pseudocalanus* and *Eurytemora* (Huys and Boxshall 1991). True egg sacs are mainly found in other copepod orders like in Harpacticoida, Mormonilloida, Cyclopoida, Siphonostomatoida and Poecilostomatoida (Huys and Boxshall 1991). Species of both euchaetid genera *Euchaeta* and *Paraeuchaeta* are uniform in terms of general high WE contents (e.g. Lee and Hirota 1973, Lee et al. 1974, Hagen et

al. 1995, Albers et al. 1996). Together with the synapomorphies of long setae at the antennules and the appendage as mechanoreceptors and balancing structures (Yen and Nicoll 1991, Park 1994b), the high WE levels are considered as an adaptation to their preying behaviour or even allow this predation behaviour in terms of maintaining buoyancy at depth. In addition, *Euchaeta* and *Paraeuchaeta* have the same gonad type (Niehoff 2007) and carry egg-sacs (e.g. Mauchline 1988, Auel 1999, Kosobokova et al. 2007). The Aetideidae are mixed feeders, detecting food items on the basis of chemotaxis and are more diverse, with *Gaetanus* spp. storing moderate amounts of WE (chapters I and II, Lee and Hirota 1973), being broadcast spawners producing adhesive eggs with a double membrane (Kosobokova et al. 2007). *Aetideopsis* spp. have fragile egg sacs as well as a broadcast spawning mode, *C. obtusifrons* has a robust egg sac and both, *Aetideopsis* spp. and *C. obtusifrons* do not store WE (chapters I and II, Auel 1999, Kosobokova et al. 2007) (Fig. 9). For *Aetideopsis* spp. and *C. obtusifrons*, TAG may play a more important role like in the other aetideid species of the genera *Chirundina*, *Euchirella* and *Undeuchaeta* (Lee and Hirota 1973, Hagen et al. 1995, Albers et al. 1996). Furthermore, several gonad types (Niehoff 2007) and reproductive strategies are found within Aetideidae, comprising egg-sacs with robust and fragile membranes, egg strings with membranous sacs as well as free spawning (Ohman and Townsend 1998, Auel 1999, Kosobokova et al. 2007). These results demonstrate the high diversity of characteristics in Aetideidae, while Euchaetidae are more uniform in the respective characteristics which can be partly found in the Aetideidae.

Euchaetidae and Aetideidae are currently presumed to be sister families, basically similar in morphology with the exception of four characters regarded as synapomorphies in Euchaetidae (Park 1994b). The combination of phylogenetic analysis and genus- as well as species-specific characteristics demonstrated that also WE storage and reproductive strategy could represent uniformly derived states within the Euchaetidae.

The resolution of phylogenetic relationships on the level of families and genera was not well resolved by using the conserved markers 18S and 28S (chapter IV). In contrast, 18S proved as appropriate proxy to resolve phylogenetic relationships among genera within the two calanoid families Calanidae and Clausocalanidae (Bucklin et al. 2003). To evaluate the present results, species of other calanoid copepod families were included as ingroup to the Euchaetidae and Aetideidae and phylogenetic relationship was analysed on the basis of 18S sequences (Fig. 10).

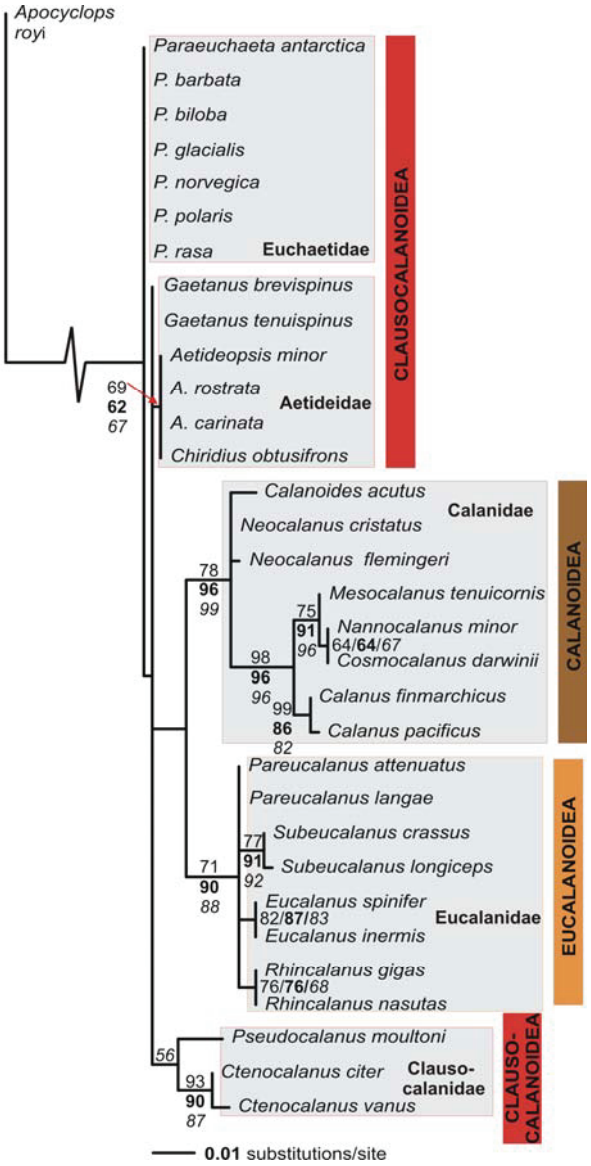


Figure 10

Phlyogram of Maximum Likelihood phylogram on the basis of 18S sequences comprising different calanoid superfamilies and families as ingroups and a cyclopoid copepod as outgroup. Analysis is based on an alignment of 541 base pairs and a best-fit evolutionary model (Tamura Nei with equal frequencies and a gamma distribution of 0.13, TrNef, Tamura and Nei 1993). Bootstrap values: regular figures (Neighbour Joining), bold figures (Maximum Parsimony) and italic figures (Maximum Likelihood). Sequences from the copepods from GenBank (all species except of Euchaetidae and Aetideidae) with the accession numbers: AY118071, AF514344, AF514339, AF367716, AF367715, AF462319, AB297704, AF367719, AY335852, AY335858, AY335862, AY335859, AY335855, AY335860, AF462320, AY118078, AF367717 and AY626997

The two calanoid copepod families Calanidae and Eucalanidae were supported by high bootstrap values (Fig. 10). Within the family Calanidae, the genus *Calanus* was highly supported; within the family Eucalanidae this was the case for the genera *Subeucalanus*, *Eucalanus*, and *Rhincalanus*. In contrast, the three families of the superfamily Clausocalanoidea were not

supported. Only the genus *Ctenocalanus* was supported by high bootstrap values and to a lower degree the clade comprising the Aetideidae *Aetideopsis* spp. and *C. obtusifrons* (Fig. 10).

These results demonstrate, that 18S is not applicable to resolve phylogenetic relationships on higher taxanomic levels in all copepod families or even superfamilies. The lack of resolution in the Clausocalanoidea might be referred to the comparably young age of this superfamily (Park 1986, Bradford-Grieve 2002).

Hypothesis: *Phylogenetic clades exhibit high similarities in ecological and physiological traits.*

Conclusions:

- Evaluation of species-specific characteristics (feeding, energy storage and reproductive strategy) on phylogenetic analysis revealed uniformity within Euchaetidae and diversity in Aetideidae.
- Mitochondrial cytochrome C oxidase subunit I (COI) does not provide information on phylogenetic relationships in deeper nodes below species level. However, their high variability makes this gene suitable for species identification.
- In comparison to other calanoid copepod families, Aetideidae and Euchaetidae could not be separated on the basis of the conservative 18S and 28S markers.

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4 PERSPECTIVES

This study aimed at improving our understanding of niche partitioning of closely related organisms inhabiting the same habitat, using two deep-sea copepod families as examples. Potential mechanisms for minimising inter-specific competition were identified by characterising their trophic as well as spatial niches and by comparing ecophysiology and life history traits of the species. A phylogenetic tree was used to discuss these characteristics and potential speciation processes on an evolutionary background. A lot of new questions both specific as well as derived emerged from this study and the arising research perspectives will be shortly outlined within this chapter.

A major methodological constraint in the work with deep-sea species consists in limitations of sampling which is generally restricted to a low spatially and temporarily resolution as well as to descriptive rather than to experimental work. These restrictions increase with increasing epipelagic water temperatures. Numerous studies, including this one, have thus applied trophic markers such as fatty acids and stable isotopes in order to elucidate feeding and trophic flow within the system. However, the concept of fatty acid markers was originally established for herbivorous epipelagic copepods and a ground truthing for deep-sea species, which are primarily omnivorous or carnivorous is lacking. The storage and *de novo* synthesis of specific fatty acid varies between copepod groups making a direct application of the markers problematic, as discussed in this thesis for the extremely high typical diatom markers found in a strictly carnivorous species. Molecular methods, such as species-specific DNA barcodes for identifying ingested food in faecal pellets (Vestheim et al. 2005) or quantitative PCR (Neijstgaard et al 2008) combined with more classical trophic markers (fatty acids and stable isotopes) seem to be promising approaches to better describe the trophic niches of deep sea species and maybe even used to quantify trophic flows within the system.

The composition of lipid classes and in particular the physiological potential to build up large amounts of wax esters is generally interpreted as the need of a species to store energy for longer times of food scarcity. As reverse conclusion the absence of wax esters is often used as indication for a less seasonal and more omnivorous feeding behaviour. While generally agreeing with the former interpretation of wax esters as long-term energy storage, the last inference is questioned in part by this thesis. A strong dependence of wax ester formation on phylogenetic affiliation was shown, implying that the potential for wax ester synthesis has to be interpreted rather on the evolutionary scale than on the actual feeding ecology of a species. Meta-analyses on the occurrence of not only wax esters but further ecophysiology traits combined with phylogenetic classification may help with ecological interpretations of biochemical and physiological proxies. In a second step this can be used to underline phylogenetic affiliation of species. The identification of evolutionary relationships will provide a useful hypothesis e.g. for

comparative morphological analyses and new hypotheses about systematic relationships (e.g. Bucklin et al. 2003). Hence, the combination of phylogenetics, morphology and ecology is an important and promising approach to elucidate patterns or processes in evolution, adaptations and distribution range of species.

Phylogenetic relationships on the level of families, genera and species were partly resolved in the present work. The application of different markers with different mutation rates demonstrated that information on separation on different taxonomic levels can be resolved. However, the present work demonstrated that specific markers which resolve phylogenetic relationship of other copepods (e.g.18S for Calanidae, Bucklin et al. 2003) are not applicable for resolving relationships within copepods in general (e.g. not for the Euchaetidae and Aetideidae). So far no ubiquitous taxon-spanning markers have been identified. However, an implementation of phylogenetic approaches into ecological research will depend on the success to develop and provide markers resolving on different taxonomic levels within specific taxa, such as copepods. This can only be achieved by comprehensive studies covering a wide range of different species, thus improving our knowledge on mutation rates of specific markers.

An interesting outcome of this thesis is that there are genetic differences in Arctic and Antarctic populations of cosmopolitan and bipolar species from the mesopelagic zone. However, no difference was detected in bathypelagic cosmopolitans. These results ask for further and more detailed studies on population genetics of circum-globally distributed species (e.g. by applying microsatellites, tandemly repeated short sequence motives of two to six bases that are spread over the eukaryote genome) which will expand our knowledge on formation of populations as well as speciation processes in the open ocean and thus identify temporal abiotic and biotic boundaries in the deep-sea realm. In general two hypotheses might be deduced from the high genetic uniformity of the bathypelagic species: 1) Mutation rates within bathypelagic species are slower since environmental conditions are less variable on spatial as well as evolutionary time scales and 2) the adaptive potential of these species to environmental change is lower than in many meso- or epipelagic species. A fundamental understanding of the interrelation between evolutionary history and genetic flexibility will be essential to forecast a species-specific potential to adapt to climate change. Comprehensive approaches comparing distribution, habitat-specific physiological, behavioural and life history aspects as well as tolerances to varying abiotic and biotic factors of different populations and congeneric species will be a first step to predict potential changes in ecosystem structures.

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OVERVIEW OF PUBLICATIONS

The following overview of the four publications included in this PhD thesis demonstrates the aims and sophisticated separation of themes. The general concept of this study was developed by Holger Auel and Marc Kochzius and funded by the Deutsche Forschungsgemeinschaft (DFG project grant AU 175/3). Realisation of the applied analysis and methods was done by Silke Laakmann with provision of laboratories and equipment by the department of Marine Zoology (Prof. Dr. W. Hagen) and the department of Biotechnology and Molecular Genetics (Prof. Dr. D. Blohm) at the University of Bremen.

Chapter I

Laakmann S, Kochzius M, Auel H (2009) Ecological niches of Arctic deep-sea copepods: Vertical partitioning, dietary preferences and different trophic levels minimize inter-specific competition. Deep-Sea Research 56: 741-756

Sampling was conducted by S. Laakmann, H. Auel and M. Kochzius. Analysis of samples, including extraction of lipids, determination of fatty acid composition and vertical distribution of the species as well as evaluation of the data was performed by S. Laakmann. Stable isotopes were measured at Agroisolab GmBH in Jülich, Germany. The manuscript was written by S. Laakmann, with input by H. Auel and M. Kochzius.

Chapter II

Laakmann S, Stumpp M, Auel H (2009) Vertical distribution and dietary preferences of deep-sea copepods (Euchaetidae and Aetideidae; Calanoida) in the vicinity of the Antarctic Polar Front. Polar Biology 32: 679-689

Sampling was conducted by S. Laakmann, H. Auel and M. Stumpp. Lipid analysis of the species *Paraeuchaeta antarctica* was implemented in the project thesis of M. Stumpp. Analysis of lipid and fatty acid samples of all other species as well as evaluation of the data was done by S. Laakmann. The manuscript was written by S. Laakmann with assistance of H. Auel.

Chapter III

Laakmann S, Auel H (in revision) Longitudinal and vertical trends in stable isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) of omnivorous and carnivorous copepods across the South Atlantic Ocean. Marine Biology

Sampling was conducted by S. Laakmann, H. Auel and M. Stumpp. Stable isotope and C/N data were analysed by Agroisolab GmBH in Jülich, Germany and S. Laakmann evaluated the data set. S. Laakmann and H. Auel developed and wrote the manuscript.

Chapter IV

Laakmann S, Auel H, Kochzius M (in preparation) Evolution in the deep sea: Biological traits, ecology and phylogenetics of pelagic copepods

Sampling was conducted by S. Laakmann, H. Auel, M. Kochzius and M. Stumpp. All laboratory work was performed by S. Laakmann, except for sequencing which was performed at Macrogen in Seoul, Korea. Sequences, alignments, and phylogenetic analysis were evaluated by S. Laakmann. For an expanded phylogenetic approach S. Laakmann traced and selected additional marker genes and developed new primers with assistance of M. Kochzius. The concept of the manuscript, combining phylogenetic analyses with biological and biochemical data was developed by S. Laakmann and M. Kochzius. The manuscript was written by S. Laakmann with assistance of M. Kochzius and H. Auel. This manuscript was presented on MarBOL (Marine Barcoding of Life) Symposia (April 16th and 17th 2009) in Bremerhaven, Germany and will be submitted to the open-access journal PLoS ONE within a collection of marine barcoding papers from the series of the symposium.

CHAPTER I

Ecological niches of Arctic deep-sea copepods:
Vertical partitioning, dietary preferences and different trophic levels minimize inter-specific competition

Laakmann S, Kochzius M and Auel H

published in Deep-Sea Research I 56: 741-756 (2009)

ECOLOGICAL NICHES OF ARCTIC DEEP-SEA COPEPODS: VERTICAL PARTITIONING, DIETARY PREFERENCES AND DIFFERENT TROPHIC LEVELS MINIMIZE INTER-SPECIFIC COMPETITION

Silke Laakmann, Marc Kochzius and Holger Auel

ABSTRACT

The biodiversity of pelagic deep-sea ecosystems has received growing scientific interest in the last decade, especially in the framework of international marine biodiversity initiatives, such as Census of Marine Life (CoML). While a growing number of deep-sea zooplankton species has been identified and genetically characterized, little information is available on the mechanisms minimizing inter-specific competition and thus allowing closely related species to co-occur in the deep-sea pelagic realm. Focussing on the two dominant calanoid copepod families Euchaetidae and Aetideidae in Fram Strait, Arctic Ocean, the present study strives to characterize ecological niches of co-occurring species, with regard to vertical distribution, dietary composition as derived from lipid biomarkers, and trophic level on the basis of stable isotope signatures. Closely related species were usually restricted to different depth layers, resulting in a multi-layered vertical distribution pattern. Thus, vertical partitioning was an important mechanism to avoid inter-specific competition. Species occurring in the same depth strata usually belonged to different genera. They differed in fatty acid composition and trophic level, indicating different food preferences. Herbivorous *Calanus* represent major prey items for many omnivorous and carnivorous species throughout the water column. The seasonal and ontogenetic vertical migration of *Calanus* acts as a short-cut in food supply for pelagic deep-sea ecosystems in the Arctic.

KEYWORDS

Euchaetidae; Aetideidae; *Paraeuchaeta*; *Pareuchaeta; Euchaeta; Gaetanus*; *Aetideopsis*; *Chiridius*; Fram Strait; Arctic Ocean; vertical distribution; dietary composition; trophic biomarker; fatty acid; stable isotope; biodiversity

INTRODUCTION

Biodiversity of deep-sea communities has received increasing scientific interest during the last decade (e.g. Miya and Nishida, 1997; Morin and Fox, 2004; Brandt et al., 2007). International initiatives especially in the framework of the Census of Marine Life and Census of Marine Zooplankton discovered many new species and proved that biodiversity in the deep ocean has been underestimated so far. However, studies on the functional role of biodiversity in the ocean remained scarce and the mechanisms sustaining high biodiversities in the almost homogeneous environment of the pelagic deep sea are still far from understood (Miya and Nishida, 1997).

Deep-sea ecosystems differ from shallow-water and terrestrial habitats in the spatial separation of production and remineralization processes (Wassmann, 1998). Phytoplankton production is restricted to a thin euphotic surface layer (max. 100-200 m), whereas substantial parts of secondary production and remineralization take place at greater depths and on the seafloor. The majority of the vertical flux of particulate organic matter (POM) to the deep sea is achieved by fast sinking large particles, such as faecal pellets of copepods (Honjo, 1980; Urrère and Knauer, 1981). Meso- and bathypelagic zooplankton strongly contributes to and accelerates the export of photosynthetically fixed carbon from the euphotic zone to deeper layers via several mechanisms, including feeding and faecal pellet production as well as vertical migrations. This biologically enhanced carbon transport contributes to the “biological pump” (Longhurst and Harrison, 1989). It can be expected that resource limitation represents an important driving force in the evolution of meso- and bathypelagic species since the availability of organic matter strongly decreases with increasing depth (Honjo, 1980; Tseitlin, 2001). Planktonic organisms are - by definition - limited in their mobility in relation to the surrounding water body. Therefore, they may be especially dependent on a sufficient food supply within close range. Based on these considerations, one can expect that zooplankton is potentially vulnerable to food limitations and that competition for food sources may play an important role in deep-sea ecosystems (Madin and Madin, 1995). In polar regions, ice cover and strong seasonality of the light regime further limit primary production and food supplies for pelagic organisms, especially during winter.

The present study examines the distribution and mechanisms of niche separation of deep-sea copepods of the calanoid families Euchaetidae and Aetideidae in the Arctic Fram Strait. Species of these two families are important components of pelagic communities throughout the world ocean, especially in deep oceanic waters and polar regions (Båmstedt, 1975, 1978; Bakke, 1977; Bakke and Valderhaug, 1978; Båmstedt and Holt, 1978; Yen 1985, 1991; Øresland, 1995; Ikeda and Hirakawa, 1996). Most species inhabit meso- and bathypelagic depths; some are epi- or benthopelagic (Park, 1994a; Markhaseva, 1996; Auel, 1999). In the Greenland Sea *Paraeuchaeta* spp. belong to the ten most important taxa with respect to

biomass (Richter, 1994). Less abundant, but represented by a species-rich, very diverse assemblage, Aetideidae are characteristic inhabitants of the Arctic and Antarctic deep sea (Båmstedt, 1981; Richter, 1995; Markhaseva, 1996; Auel, 1999). Aetideidae are generally referred to as omnivorous (Hopkins, 1985 and references therein; Richter, 1995; Auel, 1999). However, they comprise a very diverse group, so that significant variability in feeding behaviour and dietary composition is likely to occur. Carnivorous *Paraeuchaeta* are major predators (e.g. Yen, 1985, 1987, 1991; Øresland, 1991, 1995; Fleddum et al., 2001; Tönnesson et al., 2006) on other mesozooplankton and fish larvae. They can contribute to a top-down control of prey populations in the Arctic (Auel, 1999) and may even influence the recruitment of commercially important fish stocks (Yen, 1987). In Kosterfjorden, Sweden, *Aetideopsis armata* and *Paraeuchaeta norvegica* were responsible for 29 to 77% of the total energy flow through the carnivorous trophic level (Båmstedt, 1981), while in the Greenland Sea Aetideidae may consume ≥40% of the vertical carbon flux (Auel, 1999). Thus, omnivorous and carnivorous deep-sea copepods substantially affect pelago-benthic coupling processes and the structure of pelagic communities. Because of their ecological importance they provide an excellent example for mechanistic studies on deep-sea biodiversity.

The present study aims at characterizing the ecological niches of co-occurring deep-sea copepods in order to identify potential mechanisms sustaining a high biodiversity in the deep-sea pelagic realm. Major focus is given to vertical distribution patterns and dietary preferences. Since food availability, feeding behaviour and dietary preferences are crucial factors affecting the ecological niches of deep-sea animals, analyses of fatty acid trophic biomarkers (e.g. Graeve et al., 1994a) and stable isotope ratios of nitrogen $^{15}N/^{14}N$ and carbon $^{13}C/^{12}C$ (e.g. Rau et al., 1983; Minagawa and Wada, 1984; Hobson and Welch, 1992) were carried out to elucidate differences in dietary composition and trophic level.

MARERIALS AND METHODS

Study area

Fram Strait is situated between Greenland and the Svalbard Archipelago and represents the only deep-water connection between the Arctic Ocean and other parts of the world ocean. It is the major pathway for water exchange with the North Atlantic and of prime importance for the global thermohaline circulation/meridional overturning circulation. The hydrographic regime is governed by two opposing meridional currents (Fig. 1). Along the East Greenland Shelf, Polar Surface Water originating from the Transpolar Drift Current is transported southward by the East Greenland Current (EGC) (Quadfasel et al., 1987). In contrast, the West Spitsbergen Current (WSC), an extension of the Norwegian Atlantic Current, transports warm and saline Atlantic water masses northward on the eastern side of Fram Strait. One branch of the WSC, known as the Return Atlantic Current, is deflected to the West and entrained into the EGC in

sub-surface layers. Another branch flows northward along the western slope of Yermak Plateau transporting Atlantic water masses directly to high latitudes, while the major inflow turns eastward and follows the Eurasian continental shelf into the Arctic Ocean. Hydrographic fronts, resulting from the close neighbourhood of different water masses, separate different hydrographic domains. The complex hydrographic regime and opposing currents allow sampling of high-Arctic, sub-Arctic and boreal-Atlantic species in close proximity (Hop et al., 2006).

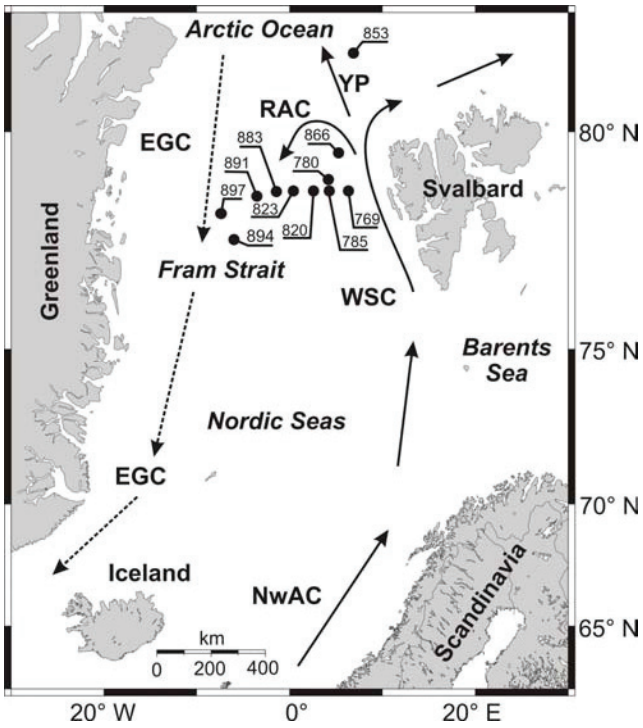

Figure 1

Map of the study area with major currents (EGC = East Greenland Current; WSC = West Spitsbergen Current; NwAC = Norwegian Atlantic Current; RAC = Return Atlantic Current; YP = Yermak Plateau) and stations

Field work and sampling

Deep-sea copepods of the two calanoid families Euchaetidae and Aetideidae were collected from August 20th to September 16th 2006 in Arctic Fram Strait between East Greenland and the Svalbard Archipelago during the expedition MSM 02/4 on board of RV Maria S. Merian. Sampling concentrated on ten stations along a transect at approx. 79°N from 6°20'E to 7°29'W (Table 1, Fig. 1). Because of the prevailing ice conditions, the westernmost stations had to be shifted southward to latitudes between 77°46'N and 78°21'N. One additional station was sampled at 81°22'N, 6°52'E on the Yermak Plateau at the entrance to the central Arctic Ocean. The expedition still took place during the period of midnight sun. Under these conditions, diel vertical migrations (DVM) are negligible in Arctic zooplankton (Blachowiak-Samolyk et al., 2006). With the exception of station 897, all samples were collected between 21:34 and 09:21, i.e. when the sun was low above the horizon, further reducing the potential risk of bias in vertical distribution pattern by DVM.

Mesozooplankton was collected by stratified multiple opening/closing net hauls (Hydro-Bios Multinet Midi, mouth opening: 0.25 m^2, mesh size: 300 µm). Standard depth intervals of 2000-1500-1000-500-200-100-50-0 m were sampled in order to study differences in the vertical and regional distributions of copepod species in relation to hydrographic regimes. Since only five discrete depth strata could be sampled in one haul of the Multinet, two successive hauls (one to 2000 m depth and another one to 200 m) were conducted at each station in order to combine deep sampling with a higher vertical resolution of the upper water layers.

Table 1

Multinet stations in Fram Strait during the expedition MSM 02/4

Station	Date in 2006	Start time at max. depth [UTC]	Position latitude	Position longitude	Bottom depth [m]	max. Sampling depth [m]	Number of stratified samples	Ice cover [%]	Sea surface temperature [°C]	Salinity
769	21.08.	21:34	78°50´N	6°20´E	2146	2000	7	0	7.5	-
780	24.08.	07:16	79°04´N	4°11´E	2413	2000	7	0	2.2	-
785	25.08.	02:40	78°50´N	4°17´E	2379	2000	7	0	6.4	-
820	30.08.	06:34	78°50´N	2°35´E	2475	2000	7	0	5.8	34.4
823	31.08.	00:09	78°50´N	0°24´E	2530	2000	7	0	5.2	34.4
853	03.09.	08:14	81°22´N	6°52´E	928	915	5	20-90	0.1	-
866	04.09.	00:45	79°36`N	5°16´E	2653	2000	7	0	6.5	34.7
883	08.09.	09:21	78°49´N	1°27´W	2636	2000	7	70-80	-1.0	30.5
891	10.09.	05:17	78°16´N	3°31´W	2488	2000	7	0	1.0	32.8
894	11.09.	02:11	77°46`N	6°00´W	381	375	4	<10	-0.5	29.6
897	11.09.	15:22	78°21´N	7°29´W	216	106	2	50	-0.9	29.8

Zooplankton samples were analyzed alive in a cold-room at 2°C immediately after the catch. Copepods of the families Euchaetidae and Aetideidae (as well as their potential prey *Calanus*) were sorted from the samples and identified using taxonomic guides of Park (1994b) and Markhaseva (1996). Species were staged and counted for vertical and regional patterns of abundance, and deep-frozen at -80°C for lipid and stable isotope analysis. The remains of the zooplankton samples were preserved in 4% formaldehyde and analysed again in the home laboratory to ensure that no individuals of Euchaetidae and Aetideidae had been missed.

Dry mass determination

Deep-frozen samples were lyophilized for 48 hours (Leybold-Heraeus, LYOVAC GT2). In case of small species or stages, individuals from neighbouring stations or adjacent depth layers were pooled to ensure adequate biomasses for lipid and stable isotope analyses. Dry mass was determined on a microbalance (Sartorius MC21 S, reproducibility <2 µg).

Lipid analysis

Lipid was extracted from dried samples with dichloromethane/methanol (2:1 per volume), essentially according to Folch et al. (1957) modified by Hagen (2000). Total lipid content was measured gravimetrically (Hagen, 2000). Fatty acids were converted to methyl esters and

analyzed together with the fatty alcohols by gas-liquid chromatography, according to Kattner and Fricke (1986). Methyl esters were prepared by transesterification with methanol containing 3% concentrated sulphuric acid at 80°C for four hours. Fatty acids and alcohols were separated and quantified using a Hewlett-Packard gas chromatograph (HP 6890A), equipped with a DB-FFAP column of 30 m length and 0.25 mm diameter. Peaks were identified according to retention times in comparison to a fish oil and a copepod lipid standard of known compositions. Hence, it was not necessary to use gas chromatography-mass spectrometry for the identification of each compound.

In addition, absolute amounts of fatty acids and alcohols were quantified by adding tricosanic acid (23:0) as an internal standard (Peters et al., 2006). The proportions of wax esters relative to total lipid and dry mass, respectively, were calculated from the fatty alcohol content of the samples, assuming equal masses for the fatty alcohol and fatty acid chains of each wax ester molecule.

Statistical analysis

Species-specific differences in biochemical composition were analyzed using One-way ANOVA and proximate Post-Hoc test (Dunnett T3) within the SPSS software package (version 15.0). Prior to statistical analysis percentage data were transformed by arc sine square root transformation. To investigate species-specific differences in fatty acid and fatty alcohol composition (percentages), a principal component analysis was conducted using the same software package with previous arc sine square root transformation.

Stable isotope analysis

Dried samples were transferred in tin capsules and stable isotope analyses were performed at Agroisolab GmbH in Jülich, Germany, using a mass spectrometer (EA NA1500 Series 2, Carlo Erba Instruments) and helium as carrier gas. For determination of carbon and nitrogen stable isotope ratios, IAEA-PDB (IAEA, Vienna) and AIR, atmospheric air, were used as standards for $\delta^{13}C$ and $\delta^{15}N$, respectively. Ratios were expressed as $\delta^{13}C$ and $\delta^{15}N$ in ppt (‰) between the measured values and standards, according to equations given in Iken et al. (2005) and Søreide et al. (2006). In addition, stable isotope signatures of dominant potential prey species, i.e. copepodite stages C4 to C6 of *Calanus finmarchicus*, *C. glacialis* and *C. hyperboreus*, were included in the analysis.

Because of isotopic fractionation, consumers become enriched in $\delta^{15}N$ by 3-5‰ per trophic level (Rau et al., 1983; Minagawa and Wada, 1984; Hobson and Welch, 1992). For Arctic copepods in Fram Strait feeding on particulate organic matter, Sasaki et al. (2001) determined a $\delta^{15}N$ enrichment of 3.2‰ per trophic level. Thus, differences in $\delta^{15}N$ can reveal trophic levels and predator-prey relationships for food-web analyses (DeNiro and Epstein, 1981).

We did not extract lipids prior to stable isotope analysis, as suggested by some authors (e.g. Søreide et al., 2006; Tamelander et al., 2006), since biomass values were generally low and other studies show a bias of $\delta^{15}N$ by lipid extraction (Jacob et al., 2005; Mintenbeck et al., 2008). The present study concentrates on trophic levels, for which $\delta^{15}N$ is the prime marker. Therefore, we wanted to exclude any risk of bias in $\delta^{15}N$ ratios by lipid extraction. Values of $\delta^{13}C$ were measured together with $\delta^{15}N$ and are given in the results section. However, the interpretation and discussion of trophic relationships is mainly based on $\delta^{15}N$ data.

RESULTS

Vertical distribution

During the present study, a total of four euchaetid and eight aetideid species were identified, of which *Paraeuchaeta polaris* and the three aetideids *Chiridiella abyssalis*, *Chiridius armatus* and *Pseudochirella* c.f. *spectabilis* were rather rare. Only the more abundant species allowed detailed analyses of vertical distribution patterns and trophic biomarkers.

Distribution patterns were determined for the copepodite stages of the three euchaetid species, *Paraeuchaeta barbata* (C1 to C6), *P. glacialis* and *P. norvegica* (C3 to C6) as well as for five aetideid copepods (C3 to C6), i.e. *Gaetanus brevispinus*, *G. tenuispinus*, *Aetideopsis minor*, *A. rostrata* and *Chiridius obtusifrons* (Fig. 2). The fourth Arctic euchaetid species *Paraeuchaeta polaris* was mainly found below 1000 m. All species were restricted to distinct depth strata, resulting in a multi-layered vertical distribution pattern. Congeners and representatives of the very similar genera *Aetideopsis* and *Chiridius* partitioned the water column (Fig. 2). In contrast, copepods of different genera often co-occurred in the same depth zones.

Among the Euchaetidae, *Paraeuchaeta norvegica* inhabited the epipelagic to upper mesopelagic layer (0-500 m) in high abundances of 80 to 720 individuals 1000^{-1} m^{-3} (Fig. 2a). This species was more abundant in the eastern part of Fram Strait, which is strongly influenced by the warm WSC, than on the East Greenland Shelf. It did not occur in the surface layer over the high-Arctic Yermak Plateau. Usually, young stages were distributed at shallower depth than older individuals (data not shown). Copepodids C1 to C4 were concentrated in the upper 200 m, whereas C5 occurred down to 500 m and females between 200 and 500 m. The polar congener *P. glacialis* was rather rare during the present study. It mainly occurred in the surface layer (0-50 m) on the East Greenland Shelf and below 100 m depth over the Yermak Plateau (Fig. 2a). Maximum abundances of 320 ind. 1000^{-1} m^{-3} were substantially lower than those of the boreal-Atlantic *P. norvegica*. In contrast, the third euchaetid species *P. barbata* showed a deeper distribution in the lower mesopelagic to bathypelagic layer (500-2000 m) throughout the deep-sea basin with abundances of generally 24 to 168 ind. 1000^{-1} m^{-3} (Fig. 2a). In contrast to

P. norvegica, the youngest copepodids C1 to C2 of *P. barbata* were distributed deeper (1000-2000 m) than older ones. In conclusion, there was a vertical partitioning of the water column between the epi- to mesopelagic *P. norvegica* and *P. glacialis* and the lower meso- to bathypelagic *P. barbata* and *P. polaris* as well as a regional segregation between the boreal-Atlantic *P. norvegica* in the Atlantic-influenced WSC and the polar *P. glacialis* in the EGC.

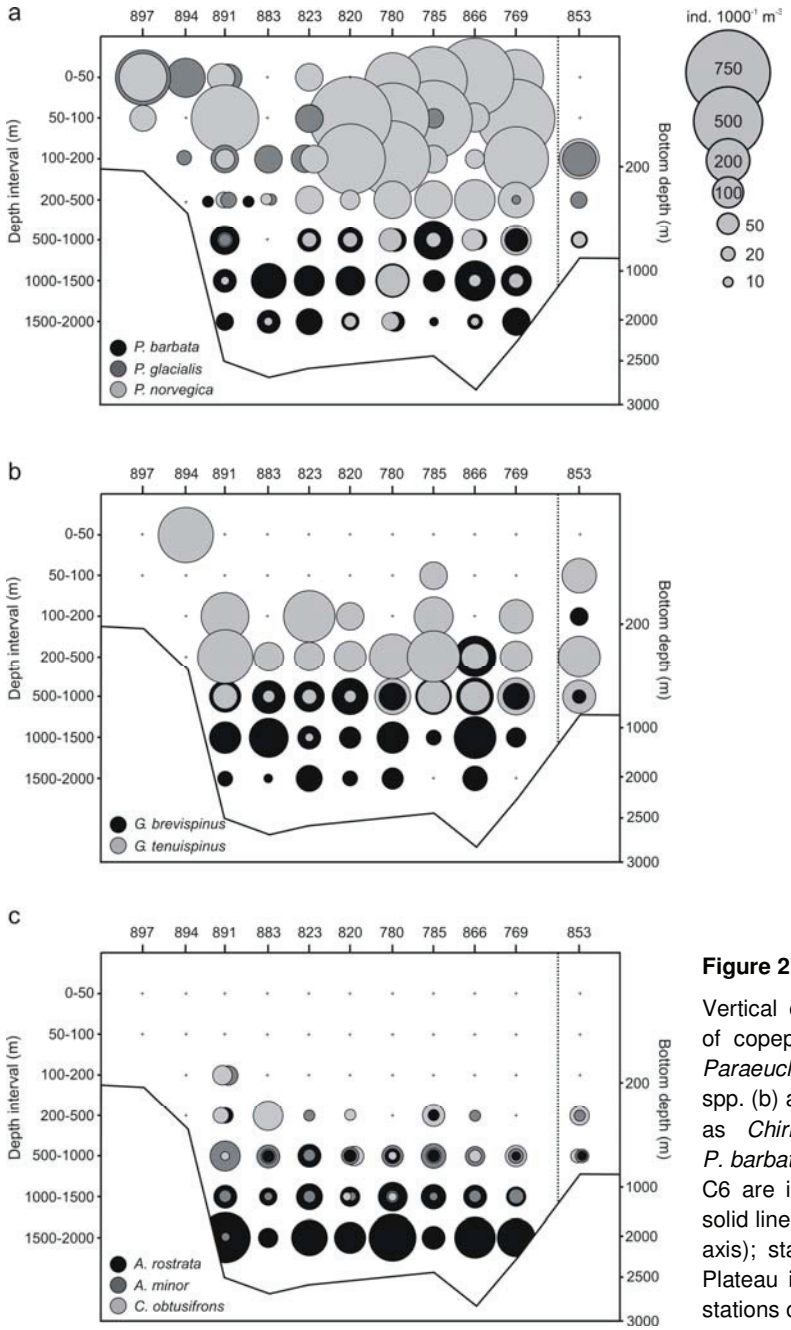

Figure 2

Vertical distribution and abundance of copepodite stages C3 to C6 of *Paraeuchaeta* spp. (a), *Gaetanus* spp. (b) and *Aetideopsis* spp. as well as *Chiridius obtusifrons* (c). For *P. barbata* copepodite stages C1 to C6 are included. +: no occurrence; solid line depicts bottom profile (right axis); station 853 over the Yermak Plateau is separated from the other stations on the transect

Accordingly, the two species of the aetideid genus *Gaetanus* occurred in different vertical zones (Fig. 2b). *Gaetanus tenuispinus* dominated between 100 and 500 m depth with abundances of 80 to 320 ind. 1000^{-1} m^{-3}. Exceptionally, high concentrations of 320 and 125 ind. 1000^{-1} m^{-3} were detected in the surface layer at station 894 over the East Greenland Shelf and in 50-100 m over the Yermak Plateau, respectively. The congener *G. brevispinus* was mainly distributed below 500 m, reaching abundances of 24 to 187 ind. 1000^{-1} m^{-3} and, thus, should be considered a lower meso- to bathypelagic species similar to *P. barbata*. There was some ontogenetic structure in the vertical distribution of both *Gaetanus* species with a deeper distribution of copepodite stages C5 as compared to other ontogenetic stages; albeit this trend was not pronounced. For the other aetideid species group, consisting of *Aetideopsis minor* and *A. rostrata* as well as the rather similar *Chiridius obtusifrons*, vertical partitioning of the water column was also evident, but with broadly overlapping depth ranges (Fig. 2c). *Chiridius obtusifrons* was mainly present between 200 and 1000 m with abundances of 8 to 93 ind. 1000^{-1} m^{-3}. In the area of the EGC (station 891) its distribution range extended into shallower depths of 100-200 m. *Aetideopsis minor* occurred between 200 and 1500 m, but was most abundant in 500-1000 m with 16 to 96 ind. 1000^{-1} m^{-3}. In contrast, the congener *A. rostrata* was generally found below 500 m with increasing abundance towards deeper layers (Fig. 2c). It reached considerably higher concentrations of 40 to 264 ind. 1000^{-1} m^{-3} in 1500-2000 m depth than its relatives in the mesopelagic zone.

In conclusion, in all three species groups, congeners were restricted to distinct depth ranges and partitioned the water column. Thus, inter-specific competition between closely related species is apparently reduced by vertical segregation. In contrast, members of different species groups did co-occur in the same depth zones, e.g. *P. norvegica*, *G. tenuispinus*, *A. minor* as well as *C. obtusifrons* in 100-500 m and *P. barbata*, *G. brevispinus* as well as *A. rostrata* in 500-2000 m, indicating that additional mechanisms besides vertical partitioning must act to minimize interspecific competition.

Inter-specific differences in dry mass, total lipid and wax ester content

Body dry mass deviated substantially between the different species of Arctic deep-sea copepods (Table 2). For two species, *Paraeuchaeta glacialis* and *P. polaris*, small sample size precluded further analysis of dry mass and lipid composition. *Aetideopsis minor* and *Gaetanus tenuispinus* were the smallest species with adult female dry masses of 0.56 to 0.57 mg, followed by *A. rostrata* and *Chiridius obtusifrons* with about 0.7 mg in adult females. The largest aetideids in this study were *G. brevispinus* females reaching 1.2 mg. By contrast, adult females of *P. norvegica* and *P. barbata* were considerably heavier with 4.7 mg and 8.7 mg, respectively. Data on dry mass of other ontogenetic stages is given in Table 2. Interestingly, the males of *P. norvegica*, which have reduced mouth parts and do not feed at all, showed dry mass values of 1.7 mg, very similar to those of copepodids C5 (1.5 mg).

The inter-specific comparison of total lipid and wax ester (WE) contents in copepodids C5 and females revealed moderate to medium lipid levels of 12 to 32% of dry mass (DM) in C5 and 14 to 45% DM in females (Table 2, Fig. 3). Lowest lipid levels occurred in stage C5 of *C. obtusifrons* (12% DM) and both *Gaetanus* species (16-17% DM) as well as in females of *G. tenuispinus* (14% DM). Among copepodite stages C5, lipid levels of *Paraeuchaeta* were significantly higher (27-32% DM) than those of *Gaetanus* ($p<0.05$) and similarly high to those of *Aetideopsis* (26-28% DM). In contrast, *Paraeuchaeta* females had significantly higher lipid contents of 40-45% DM than all aetideid females ($p<0.05$).

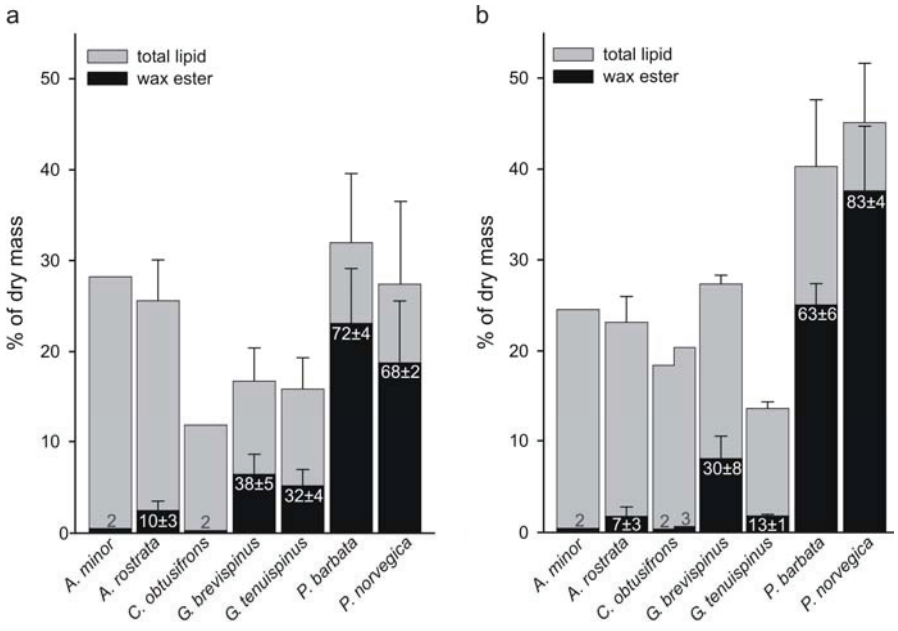

Figure 3

Total lipid (grey) and wax ester (black) content of copepodite stages C5 (a) and adult females (b) of Arctic deep-sea copepods as percentage of dry mass. Error bars depict standard deviations. In case of only two replicates, both values are shown as staged columns. White numbers give wax ester contents as percentage of total lipid

Wax esters were the dominant lipid component in copepodids C5 and females of *Paraeuchaeta* species, reaching 19-23% DM in C5 and 25-38% DM in females (Table 2, Fig. 3). In relation to total lipid (TL), WE made up between 63 and 83% TL in copepodids C5 and females of *Paraeuchaeta* species. Significantly ($p<0.001$) lower WE levels of 2-8% DM (13-38% TL) occurred in copepodids C5 and females of *Gaetanus*. In the other aetideid species, only traces of fatty alcohols and, hence, WE could be detected.

The ontogenetic development of *P. barbata* and *P. norvegica* was characterized by a pronounced accumulation of total lipid from ≤18% DM in copepodids C3 to ≥40% DM in adult females (Fig. 4a, Table 2). In *P. norvegica*, this increase in lipid levels was accompanied by a strong accumulation of WE from 5% DM in C3 to 38% DM in adult females (Fig. 4a, Table 2). In

P. barbata WE content increased from 8% DM in C3 to 23% DM in C5 and remained stable in adult females. Similarly to *Paraeuchaeta*, *G. brevispinus* showed a strong accumulation of total lipid from 9% DM in copepodid C3 to 27% DM in females. However, the increase in WE was less pronounced. In contrast, adult females of *G. tenuispinus* had lower WE contents (% TL) than copepodids C4 and C5 (Fig. 4b), while in *A. rostrata* there was no indication of ontogenetic changes in total lipid or WE content at all (Fig. 4c, Table 2). Already C3 and C4 contained relatively high lipid amounts of 18-27% DM, similarly to those of older copepodites C5 and adult females.

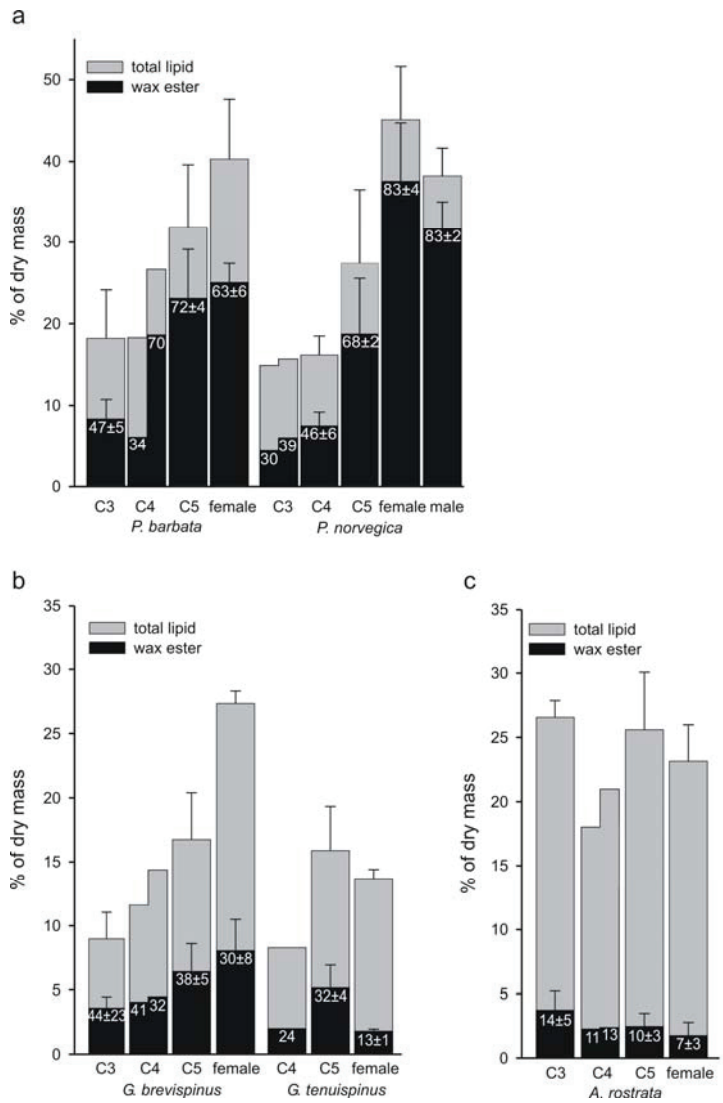

Figure 4

Ontogenetic trends in total lipid (grey) and wax ester (black) content of *Paraeuchaeta barbata* and *P. norvegica* (a), *Gaetanus brevispinus* and *G. tenuispinus* (b) as well as *Aetideopsis rostrata* (c) as percentage of dry mass. Error bars depict standard deviations. In case of only two replicates, both values are shown as staged columns. White numbers give wax ester contents as percentage of total lipid. C3-C5: copepodite stage C3 to C5

Fatty acid and fatty alcohol composition

The fatty acid composition of most species was characterized by a dominance of monounsaturated fatty acids (MUFAs), except for *Gaetanus tenuispinus*, where the proportion of polyunsaturated fatty acids (PUFAs) exceeded that of MUFAs (Table 2). Saturated fatty acids (SFAs) contributed minor amounts. Eight fatty acids, i.e. 16:0, 16:1(n-7), 18:1(n-9), 18:1(n-7), 20:1(n-9), 22:1(n-11), 20:5(n-3), and 22:6(n-3) generally dominated in all species, however, with quantitative differences between the different species or stages.

Paraeuchaeta was characterized by high amounts of the fatty acids 16:1(n-7) and 18:1(n-9), which in case of *P. norvegica* increased significantly during ontogenetic development ($p<0.05$, Table 2). In contrast, typical components of biomembranes, such as 16:0, 20:5(n-3) and 22:6(n-6), decreased during growth and lipid accumulation. In *P. norvegica*, the proportions of the long-chain MUFAs 20:1(n-9) and 22:1(n-11) increased towards adult females.

In aetideids, membrane fatty acids were more dominant than in *Paraeuchaeta* (Table 2). In *A. minor*, *C. obtusifrons* and *G. brevispinus*, they decreased during growth and lipid accumulation. In contrast, very high proportions of 20:5(n-3) and 22:6(n-3) were measured in *G. tenuispinus* females, coinciding with the lowest total lipid content of all females (Fig. 3b). Among aetideids, highest amounts of 18:1(n-9) were detected in *G. brevispinus* increasing with ontogenetic stages to 31% of total fatty acids (TFA) in females. In contrast to *Paraeuchaeta*, 16:1(n-7) never exceeded proportions of 10% TFA in aetideids, whereas levels of 18:1(n-7) were significantly higher in aetideids than in *Paraeuchaeta* ($p<0.001$). *Aetideopsis rostrata* contained relatively high levels of 20:1(n-9) and 22:1(n-11) throughout the ontogenetic stages C3 to adults.

In general, biomarkers of carnivorous feeding, i.e. 18:1(n-9), 20:1(n-9) and 22:1(n-11), were highest in *Paraeuchaeta* females with nearly half of TFA, followed by *G. brevispinus*, *Aetideopsis* spp. and *C. obtusifrons*. In *G. tenuispinus* these biomarkers contributed about one quarter of TFA.

The fatty alcohol composition of *Paraeuchaeta* and *Gaetanus* was characterized by five moieties, i.e. 14:0A, 16:0A, 18:1(n-9)A, 20:1A and 22:1A (Table 2). *Gaetanus* and *P. norvegica* differed from *P. barbata* in higher amounts of saturated alcohols, while *P. barbata* had more monounsaturated ones.

Table 2

Dry mass, total lipid and wax ester content as well as fatty acid and fatty alcohol composition of Arctic deep-sea copepods

	A. minor		*A. rostrata*					*C.obtusifrons*			*G. brevispinus*					*G. tenuispinus*		
	C5	♀	C3	C4	C4	C5	♀	C5	♀	♀	C3	C4	C4	C5	♀	C4	C5	♀
Individual dry mass [mg]	0.28	0.56	0.07 ± 0.01	0.15	0.14	0.57 ± 0.03	0.66 ± 0.09	0.30	0.71	0.67	0.09 ± 0.01	0.18	0.18	0.49 ± 0.06	1.20 ± 0.19	0.14	0.27 ± 0.02	0.57 ± 0.03
Lipid [%DM]	28	25	27 ± 1	21	18	26 ± 4	23 ± 3	12	20	18	9 ± 2	10	14	17 ± 4	27 ± 1	8	16 ± 3	14 ± 1
Wax ester [%DM]	1	0	4 ± 1	2	2	2 ± 1	2 ± 1	0	1	0	4 ± 1	4	5	6 ± 2	8 ± 2	2	5 ± 2	2 ± 0
Wax ester [%TL]	2	2	14 ± 5	11	13	9 ± 3	7 ± 3	2	3	2	44 ± 23	41	31	38 ± 5	29 ± 8	24	32 ± 4	13 ± 1
Fatty acids [%TFA]																		
16:0	18	16	11 ± 1	11	11	13 ± 1	13 ± 1	12	14	11	12 ± 1	8	9	7 ± 0	8 ± 0	12	8 ± 2	10 ± 1
16:1(n-9)	0	0	0 ± 0	0	1	1 ± 1	0 ± 0	0	0	0	0 ± 0	0	0	1 ± 0	1 ± 0	0	1 ± 0	0 ± 0
16:1(n-7)	5	4	10 ± 1	8	8	6 ± 0	5 ± 0	8	3	10	8 ± 2	6	9	8 ± 0	6 ± 3	7	9 ± 2	5 ± 0
18:1(n-9)	14	14	24 ± 1	20	21	14 ± 1	14 ± 1	10	13	8	21 ± 2	22	24	26 ± 2	31 ± 3	13	22 ± 3	15 ± 1
18:1(n-7)	4	5	4 ± 0	3	4	4 ± 0	4 ± 0	6	4	6	3 ± 0	3	3	3 ± 1	4 ± 0	2	2 ± 0	3 ± 0
20:1(n-9)	5	9	8 ± 2	10	8	10 ± 1	10 ± 1	5	11	9	3 ± 1	3	3	2 ± 0	3 ± 0	4	3 ± 1	5 ± 1
20:5(n-3)	11	9	8 ± 2	9	8	9 ± 0	9 ± 1	15	10	14	11 ± 1	14	13	13 ± 2	11 ± 0	15	14 ± 0	15 ± 1
22:1(n-11)	4	11	8 ± 2	10	7	10 ± 2	11 ± 2	3	9	8	2 ± 1	2	2	2 ± 0	2 ± 1	3	2 ± 1	4 ± 1
22:6(n-3)	14	11	12 ± 4	13	14	12 ± 1	12 ± 1	20	11	11	19 ± 2	22	20	18 ± 1	14 ± 1	24	21 ± 1	23 ± 1
FA<5%	23	21	17 ± 1	16	17	21 ± 1	22 ± 1	22	24	22	20 ± 1	21	19	20 ± 1	19 ± 1	20	20 ± 1	20 ± 0
SFA	23	21	14 ± 0	14	15	17 ± 1	16 ± 1	16	19	15	16 ± 1	11	12	10 ± 1	12 ± 1	17	11 ± 2	13 ± 1
MUFA	44	53	60 ± 6	59	56	54 ± 2	56 ± 3	41	52	52	44 ± 3	42	46	48 ± 1	53 ± 1	36	44 ± 2	38 ± 1
PUFA	33	26	26 ± 6	28	29	29 ± 1	28 ± 2	43	29	32	39 ± 4	46	42	42 ± 1	35 ± 1	47	45 ± 1	48 ± 1
Fatty alcohols [%TFAlc]																		
14:0A	30	34	8 ± 1	8	9	8 ± 2	8 ± 2	35	14	15	9 ± 2	10	14	19 ± 8	23 ± 1	11	10 ± 2	9 ± 1
16:0A	31	19	24 ± 4	19	21	13 ± 4	13 ± 6	16	15	26	35 ± 5	37	41	35 ± 4	32 ± 2	36	47 ± 5	39 ± 3
16:1A	0	0	1 ± 0	0	1	0 ± 0	0 ± 0	0	0	0	1 ± 1	0	1	0 ± 1	0 ± 0	1	1 ± 1	1 ± 1
18:0A	0	10	2 ± 1	2	2	0 ± 0	0 ± 0	15	6	10	2 ± 0	1	1	1 ± 0	2 ± 0	2	2 ± 1	3 ± 1
18:1(n-9)A	0	0	8 ± 4	0	6	1 ± 2	1 ± 1	0	0	0	10 ± 2	11	10	14 ± 1	8 ± 7	6	13 ± 3	3 ± 5
20:1A	18	9	17 ± 1	21	16	24 ± 4	22 ± 1	20	27	18	17 ± 1	17	14	14 ± 2	15 ± 3	20	13 ± 4	17 ± 1
22:1A	21	29	40 ± 9	50	44	55 ± 5	57 ± 8	14	38	30	25 ± 7	24	20	17 ± 3	21 ± 4	23	14 ± 6	28 ± 6
Number of samples	1	1	3	1	1	3	3	1	1	1	3	1	1	3	3	1	3	3
Number of individuals	10	9	32	16	9	31	32	7	10	8	19	5	7	15	13	10	31	27

	P. barbata					*P. norvegica*					
	C3	C4	C4	C5	♀	C3	C3	C4	C5	♀	♂
Individual dry mass [mg]	0.29 ± 0.05	1.10	0.70	2.62 ± 0.48	8.67 ± 2.76	0.16	0.12	0.55 ± 0.08	1.53 ± 0.28	4.72 ± 0.88	1.73 ± 0.11
Lipid [%DM]	18 ± 6	27	18	32 ± 8	40 ± 7	16	15	16 ± 2	27 ± 9	45 ± 7	38 ± 3
Wax ester [%DM]	8 ± 2	19	6	23 ± 6	25 ± 2	6	4	8 ± 2	19 ± 7	38 ± 7	32 ± 3
Wax ester [%TL]	47 ± 5	70	34	72 ± 4	63 ± 6	39	29	46 ± 6	68 ± 2	83 ± 4	83 ± 1
Fatty acids [%TFA]											
16:0	6 ± 1	2	5	2 ± 1	3 ± 1	8	9	6 ± 1	3 ± 1	2 ± 0	2 ± 0
16:1(n-9)	0 ± 0	1	1	1 ± 0	1 ± 0	2	13	1 ± 0	1 ± 1	2 ± 1	1 ± 0
16:1(n-7)	11 ± 1	15	13	19 ± 1	20 ± 4	10	5	7 ± 1	14 ± 2	18 ± 2	18 ± 2
18:1(n-9)	23 ± 3	28	23	32 ± 2	27 ± 3	29	19	23 ± 3	31 ± 3	30 ± 7	46 ± 4
18:1(n-7)	2 ± 0	1	2	1 ± 0	2 ± 0	2	1	1 ± 0	1 ± 0	1 ± 0	1 ± 0
20:1(n-9)	9 ± 0	9	7	7 ± 1	11 ± 1	3	2	7 ± 2	5 ± 1	8 ± 2	5 ± 1
20:5(n-3)	8 ± 1	9	8	7 ± 1	4 ± 2	11	10	13 ± 0	11 ± 2	6 ± 1	5 ± 1
22:1(n-11)	8 ± 1	6	7	4 ± 1	9 ± 1	1	0	1 ± 0	4 ± 1	12 ± 4	6 ± 2
22:6(n-3)	15 ± 1	12	16	8 ± 1	6 ± 0	18	22	23 ± 1	13 ± 2	6 ± 0	6 ± 1
FA<5%	18 ± 0	18	20	19 ± 1	18 ± 3	17	18	17 ± 0	16 ± 2	16 ± 2	11 ± 2
SFA	9 ± 1	4	7	4 ± 1	5 ± 1	12	13	9 ± 1	6 ± 2	3 ± 1	3 ± 0
MUFA	59 ± 1	63	60	69 ± 2	74 ± 4	51	45	44 ± 2	58 ± 4	73 ± 3	77 ± 4
PUFA	32 ± 0	32	33	27 ± 2	21 ± 3	38	42	47 ± 1	37 ± 3	23 ± 3	20 ± 4
Fatty alcohols [%TFAlc]											
14:0A	9 ± 1	12	9	17 ± 5	19 ± 3	18	21	26 ± 4	35 ± 3	36 ± 4	47 ± 2
16:0A	26 ± 2	24	19	20 ± 3	15 ± 2	40	55	41 ± 3	36 ± 2	26 ± 6	32 ± 2
16:1A	3 ± 0	4	3	4 ± 1	2 ± 1	6	0	1 ± 0	3 ± 1	2 ± 0	2 ± 0
18:0A	1 ± 0	1	1	0 ± 0	0 ± 0	2	4	2 ± 0	2 ± 0	1 ± 0	1 ± 0
18:1(n-9)A	5 ± 0	7	7	7 ± 1	4 ± 0	5	2	2 ± 0	3 ± 0	2 ± 0	2 ± 0
20:1A	17 ± 0	17	19	17 ± 1	19 ± 2	13	12	25 ± 6	16 ± 5	15 ± 2	8 ± 1
22:1A	39 ± 3	37	43	35 ± 4	40 ± 4	16	5	3 ± 1	6 ± 2	17 ± 7	9 ± 2
Number of samples	3	1	1	3	3	1	1	3	3	3	3
Number of individuals	16	5	4	9	6	6	5	22	16	9	6

In case of >2 replicates, data are given as mean ± standard deviation. DM: dry mass; TL: total lipid; TFA: total fatty acids; FA: fatty acid, SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; TFAlc: total fatty. C3-C5: copepodite stages C3 to C5

Principal component analysis of fatty acids and alcohols

The principal component analysis based on fatty acid and alcohol composition extracted three components with eigenvalues >1% of variance. Results were presented for the major two components, together accounting for 71.8% of variance (Fig. 5a).

Principal component 1 (PC1: 49.4% of variance) was characterized by positive loadings of 16:1(n-7) and 18:1(n-9) as well as all fatty alcohols and negative loadings of 18:1(n-7), 16:0 as well as PUFAs 20:5(n-3) and 22:6(n-3). Component 2 (PC2: 22.4% of variance) separated samples according to their contents of 20:5(n-3), 22:6(n-3) and 18:0A (positive loadings) and 16:1(n-7), long-chain MUFAs 20:1(n-9) and 22:1(n-11) as well as the alcohol 22:1A (negative loadings). Generally, samples of the same species were located close to one another (Fig. 5a). In *P. norvegica*, however, there was a wide spread from juveniles to adults along PC2 related to an ontogenetic shift from high levels in 20:5(n-3) and 22:6(n-3) towards 16:1(n-7). Species from the same genus were more similar to one another than those from different genera. The two *Aetideopsis* species and *C. obtusifrons* were characterized by negative values of PC1. In contrast, *G. tenuispinus* and *G. brevispinus* were neutral with regard to PC1, but had positive values of PC2. Compared to aetideids, both *Paraeuchaeta* species showed positive values of PC1 and in case of *P. barbata* negative values of PC2.

Stable isotopes

In euchaetid and aetideid species, $\delta^{13}C$ ranged from -22.4 to -27.2‰ and $\delta^{15}N$ from 4.9 to 11.7‰, while those of their potential prey *Calanus* ranged from -26.8 to -23.0‰ for $\delta^{13}C$ and lower $\delta^{15}N$ values of 4.4 to 7.5‰ (Fig. 5b). Females of *Aetideopsis minor*, *A. rostrata* and *Chiridius obtusifrons* had significantly higher $\delta^{15}N$ values of 9-10‰ than those of *Paraeuchaeta* and *Gaetanus* with 7.8-8.1‰ ($p<0.001$). Interestingly, juveniles of both, *P. barbata* and *P. glacialis* showed higher $\delta^{15}N$ values than adult females, whereas in *G. tenuispinus* and *Calanus glacialis* copepodids C5 had lower ratios than adult females. In the other aetideid species, ontogenetic differences in $\delta^{15}N$ were less pronounced.

In addition to ontogenetic trends, there were depth-related differences in $\delta^{15}N$ among copepodids C3 and C4 of *P. norvegica*. Individuals from 0-200 m had $\delta^{15}N$ values of 6.3-6.9‰, while specimens collected down to 2000 m were more enriched in $\delta^{15}N$ with 8.9-9.9‰ (Fig. 5b). In contrast, individuals of *Calanus hyperboreus* collected below 1000 m had lower $\delta^{15}N$ values and, in addition, higher $\delta^{13}C$ than specimens from the surface layer (0-50 m).

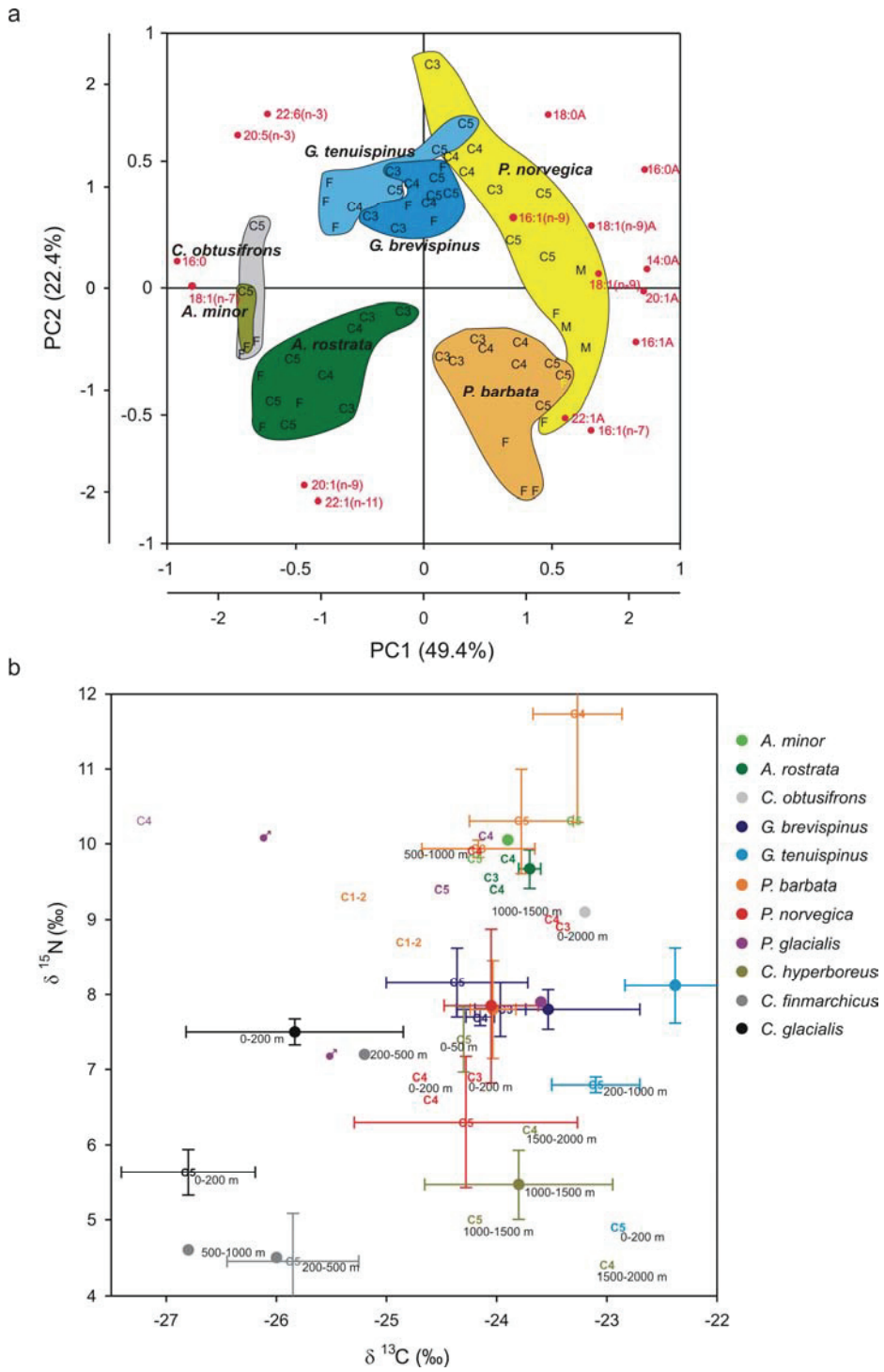

Figure 5

a) Principal component analysis of Arctic deep-sea copepods according to their fatty acid and alcohol compositions. Sample plot for the principal component PC1 and PC2 (outer axes) and superimposed loading plot (inner axes) with the relevant fatty acids and alcohols. Fatty acids and alcohols <5% are not shown. Loading differences >0.5% are significant. C3-C5: copepodite stages C3 to C5; F: female; M: male; A: fatty alcohol. b) Stable isotope signatures of $\delta^{15}N$ and $\delta^{13}C$ for Arctic deep-sea copepods and their potential prey *Calanus* spp.. Error bars represent standard deviations. C1-C5: copepodite stages C1 to C5; circles: females

Based on stable isotope data, *Calanus finmarchicus* C5 from 200-500 m and adult females from 500-1000 m depth ($\delta^{15}N$ = 4.5‰, $\delta^{13}C$ = -26.8 to -25.9‰) were the most likely prey of mesopelagic *Paraeuchaeta* females ($\delta^{15}N$ = 7.9‰, $\delta^{13}C$ = -24.1 to -23.6‰). However, there was a strong variability in the stable isotope signature of *C. finmarchicus* females with some individuals from 200-500 m reaching high $\delta^{15}N$ values of 7.2‰.

DISCUSSION

Deep-sea biodiversity has received increasing scientific interest over the last decade, but the majority of studies have focussed on benthic communities (e.g. Wilson and Hessler, 1987; Brandt et al., 2007). In contrast, the present study addresses mechanisms sustaining a species-rich fauna in the deep-sea pelagic realm despite the absence of physical barriers. In order to study these general aspects of deep-sea biodiversity in detail, we concentrate on two dominant families of deep-sea copepods, the Aetideidae and Euchaetidae. Representatives of both taxa are distributed throughout the world ocean and play important roles in marine food webs and carbon flux. For instance, in the Swedish Kosterfjorden they were responsible for 29 to 77% of the total energy flow through the carnivorous trophic level (Båmstedt, 1981), while Aetideidae may consume ≥40% of the vertical carbon flux in the Greenland Sea (Auel, 1999).

Many other zooplankton species coexist with these copepods and compete for the same resources. Carnivorous copepods of the families Heterorhabdidae and Augaptilidae, chaetognaths and amphipods may be potential competitors of predatory Euchaetidae, whereas *Scaphocalanus magnus*, other copepods, ostracods and the decapod *Hymenodora glacialis* can be potential competitors of omnivorous Aetideidae. We selected Euchaetidae and Aetideidae as case examples because of their ecological relevance and the fact that both families contain particularly high numbers of species, making them an ideal subject for studies on deep-sea biodiversity. Species-specific differences in vertical distribution, dietary composition and trophic level were examined in order to elucidate the mechanisms minimising inter-specific competition allowing a relatively large number of closely related species to co-exist in an almost homogeneous environment.

Vertical partitioning of the water column

Sympatric species might find individual niches in the three-dimensional environment of the deep-sea pelagic realm by vertical segregation (e.g. Ambler and Miller, 1987). In the present study, closely related species and congeners were usually confined to discrete depth ranges resulting in a multi-layered vertical distribution pattern and partitioning of the water column. This was true for the genus *Paraeuchaeta*, where mesopelagic *P. norvegica* and *P. glacialis* separate vertically from bathypelagic *P. barbata* and *P. polaris*, as well as for aetideids, such as

the two *Gaetanus* species and the two *Aetideopsis* species together with *Chiridius obtusifrons*. Our data on the species' vertical distribution ranges are in accordance with previous studies in the same region (Auel, 1999), in the Greenland Sea (Richter, 1995; Seiler and Brandt, 1997) and in the Norwegian Sea (Fleddum et al., 2001). An ontogenetic partitioning of the water column with older stages inhabiting deeper layers was also observed by Fleddum et al. (2001) for *P. norvegica* in the Norwegian Sea and by Irigoien and Harris (2006) in the North Atlantic.

There was some indication of a polar emergence, i.e. the occurrence at shallower depth in Arctic waters as compared to more southerly regions, in *G. tenuispinus*, *A. minor* and *C. obtusifrons* in accordance with Markhaseva (1996) and Kosobokova et al. (2007). Moreover, opposing current regimes in eastern (West Spitsbergen Current, WSC) and western Fram Strait (East Greenland Current, EGC) affected zooplankton species composition. Polar *P. glacialis* were only encountered in the EGC and at the northernmost station over the Yermak Plateau, whereas the boreal-Atlantic congener *P. norvegica* and *G. tenuispinus* dominated in the eastern region influenced by the WSC (Mumm et al., 1998; present study). In contrast to previous studies conducted in 1997 (Auel, 1999), boreal-Atlantic species were more abundant and widespread in Fram Strait during 2006, supporting the oceanographers' observation of a stronger inflow of Atlantic Water in the Arctic Ocean and an increase in temperature of the northward flowing Atlantic Water by more than 1°C in Fram Strait from 1996 to 2006 (Schauer et al., 2008).

Thus, vertical partitioning of the water column is an important mechanism minimizing inter-specific competition among congeners. However, aetideid species of different genera do co-exist in the same depth strata, as could be shown for *G. tenuispinus* and *A. minor* as well as *G. brevispinus* and *A. rostrata.* In these cases other mechanisms must be involved to reduce inter-specific competition.

Characterization of trophic niches

The present study revealed substantial differences in total lipid content, fatty acid composition and stable isotope signature among aetideid and euchaetid deep-sea copepods. In line with previous studies from tropical to polar regions (Lee et al., 1974; Lee and Hirota, 1973; Sargent et al., 1974; Båmstedt and Matthews, 1975; Hagen et al., 1995; Auel and Hagen, 2005; Laakmann et al., 2009), *Paraeuchaeta* spp. had very high lipid and wax ester (WE) contents reaching 40 to 45% DM and 63 to 83% TL, respectively, in adult females. WE are considered efficient long-term energy reserves (Lee et al., 1972; Sargent and Falk-Petersen, 1988) and, due to their low density, serve as buoyancy aids, which may be especially important for such large and heavily built copepods as predatory *Paraeuchaeta* (Sargent and Henderson, 1986).

Wax esters of *P. norvegica* are predominantly composed of SFAs and long-chain MUFAs (Sargent and McIntosh, 1974; present study). The significant proportions of long-chain MUFAs and alcohols 20:1 and 22:1, especially in body lipids of *P. barbata,* indicate extensive feeding on herbivorous calanid copepods. The three dominant Arctic *Calanus* spp., *C. hyperboreus*, *C. glacialis*, and *C. finmarchicus* are able to synthesize these components *de novo* (Graeve et al., 1994a; Kattner et al., 1994).

Surprisingly, the bathypelagic congener *P. barbata* had higher levels of 20:1 and 22:1 moieties than the epi- to mesopelagic *P. norvegica*. This may be explained by high abundances of *Calanus* overwintering in diapause at great depth in Fram Strait, as has been shown for *C. hyperboreus* (Auel et al., 2003; Hirche et al., 2006). Thus, seasonal and ontogenetic vertical migrations of *Calanus* spp. are apparently an important export process for organic carbon from the euphotic zone to deeper layers. This trophic "short-cut" provides deep-sea inhabitants with a stable food source for many months during Arctic winter (Auel and Hagen, 2005).

Lower levels of long-chain monounsaturated moieties in *P. norvegica* may either be explained by generally less predation on *Calanus* spp., stronger selectivity for *C. finmarchicus*, which contains lower amounts of 22:1 as compared to *C. hyperboreus* (Scott et al., 2002), or more active catabolism of MUFAs. Based on our stable isotope data (Fig. 5b) and applying a $\delta^{15}N$ enrichment factor of 3.2‰ per trophic level (Sasaki et al., 2001 for copepods feeding on particulate organic matter in Fram Strait), *C. finmarchicus* C5 and females are the most likely prey of *P. norvegica* females in the mesopelagic zone. While adult females of *P. barbata* and *P. norvegica* had essentially the same $\delta^{15}N$ values, young stages C3 to C5 of *P. barbata* had higher $\delta^{15}N$ values than corresponding copepodite stages of *P. norvegica* indicating a higher trophic level of the bathypelagic congener. Higher $\delta^{15}N$ values in juvenile *P. barbata* and *P. glacialis* as compared to adult females may suggest an ontogenetic shift in dietary composition from higher trophic level prey in juveniles to mainly herbivorous prey, i.e. *Calanus* spp., in adults. By contrast, the opposite trend in *G. tenuispinus* and *Calanus glacialis* with lower $\delta^{15}N$ values in copepodids C5 than in adult females could indicate a higher proportion of phytoplankton or phytodetritus in the diet of juveniles, whereas adult *Calanus* also take microzooplankton and adult *G. tenuispinus* are omnivorous. In general, *Calanus* spp. represent important prey items for several *Paraeuchaeta* species in different regions of the world ocean (Sargent and McIntosh, 1974; Båmstedt and Holt, 1978; Øresland, 1991; Øresland and Ward, 1993; Fleddum et al., 2001).

In line with its predatory feeding mode and previous studies (Lee et al., 1974; Sargent and McIntosh, 1974; Hagen et al., 1995; Lee et al., 2006), *Paraeuchaeta* had rather high levels of the fatty acid 18:1(n-9), which is generally considered an indicator of carnivorous feeding (Falk-Petersen et al., 1990; Lee et al., 2006). Surprisingly, *Paraeuchaeta* also contained high levels

of 16:1(n-7), which is usually considered a trophic biomarker for diatoms (e.g. Graeve et al., 1994a, b; Dalsgaard et al., 2003 and references therein). However, a direct ingestion of algae by *Paraeuchaeta* is highly unlikely. The species is a tactile predator (Greene and Landry, 1985; Yen, 1987), exclusively feeding on motile prey (Olsen et al., 2000). Moreover, both the morphology of mouth parts (Michels and Schnack-Schiel, 2005) and stable isotope signatures (Hop et al., 2006 for *P. norvegica* in Fram Strait) demonstrate carnivory of *Paraeuchaeta*. Thus, *Paraeuchaeta* may incorporate 16:1(n-7) indirectly by feeding on herbivorous prey such as *Calanus*, which is rich in diatom markers (e.g. Albers et al., 1996). However, it remains enigmatic why 16:1(n-7) should be selectively retained during catabolic processes and accumulated by *Paraeuchaeta*. Maybe Arctic *Paraeuchaeta* spp. are also able to synthesize this fatty acid *de novo*, as proposed for *Paraeuchaeta antarctica* by Hagen et al. (1995).

Aetideids differ from *Paraeuchaeta* species in lower lipid and WE contents (Hagen et al., 1995; Auel, 1999; Laakmann et al. 2009; present study). In some aetideid genera including the Antarctic *Euchirella rostromagna*, triacylglycerols serve as major storage lipid instead of WE (Hagen et al., 1995). This may also be true for the Arctic genera *Aetideopsis* and *Chiridius* in the present study. According to Lee et al. (2006) triacylglycerol storage serves rather more as a short-term energy reserve, suggesting continuous feeding throughout the year (Hagen et al., 1993). This is in line with the general perception of aetideids as opportunistic omnivores (Hopkins, 1985 and references therein; Richter, 1995; Auel, 1999). The very low WE content of *G. tenuispinus* females compared to copepodids C5 may indicate recent reproduction, as WE are often converted into phospholipids to assemble lipovitellin for the oocytes (Lee et al., 2006).

In contrast to *Paraeuchaeta* spp., aetideids contained more fatty acids in moderate amounts leading to a more uniform fatty acid composition. This observation may support the opportunistic and generally omnivorous feeding behaviour of aetideids. However, higher proportions of the fatty acids 16:0, 20:5(n-3) and 22:6(n-3), which are major components of phospholipids in biomembranes, can be explained by generally lower levels of storage lipids in aetideids resulting in a more prominent role of biomembrane components.

Relatively high levels of 18:1(n-9) especially in *G. brevispinus* (Lee et al., 1972; present study) indicate a substantial contribution of carnivorous feeding to total ingestion. Accordingly, levels of about 10% TFA each for 20:1(n-9) and 22:1(n-11) in *A. rostrata* and females of *A. minor* are similar to the amounts in predatory *Paraeuchaeta* and suggest substantial predation on *Calanus* spp. by aetideids. Thus, our data demonstrate that differences in dietary composition do exist among deep-sea copepods that are generally considered omnivorous. This interpretation is also supported by stable isotope signatures of nitrogen that are higher in *Aetideopsis* than *Gaetanus* indicating differences in trophic level.

Conclusions

The present study revealed different mechanisms reducing inter-specific competition among deep-sea copepods in a generally food-limited environment.

Aetideid species from different genera, i.e. *Aetideopsis* and *Chiridius* vs. *Gaetanus*, which co-occur in the same depth layers, differ in trophic biomarker patterns indicating differences in dietary composition and trophic level. Even minute differences in feeding behaviour may allow specialization among co-existing omnivores as has been shown for filter-feeding amphipods (Caine, 1977).

In contrast, congeners (within the genera *Aetideopsis*, *Gaetanus*, or *Paraeuchaeta*) with presumably similar trophic demands inhabit discrete depth layers, thus partitioning the water column. Thus, the risk of inter-specific competition is compensated by a stepwise arrangement of species with depth (Raymont, 1983; Mauchline, 1995), allowing for a generic radiation within the Aetideidae (Razouls, 1993).

In addition, generally low abundances of the different deep-sea species lead to low frequencies of interaction and may, thus, further reduce the risk of inter-specific competition, as proposed by Madin and Madin (1995) for oceanic species in general.

Finally, the vast and stable environment of the deep-sea pelagic realm provided a very long time frame for evolutionary processes and speciation, leading to a high biodiversity in the pelagic deep sea.

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CHAPTER II

Vertical distribution and dietary preferences of deep-sea copepods (Euchaetidae and Aetideidae; Calanoida) in the vicinity of the Antarctic Polar Front

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VERTICAL DISTRIBUTION AND DIETARY PREFERENCES OF DEEP-SEA COPEPODS (EUCHAETIDAE AND AETDEIDAE; CALANOIDA) IN THE VICINITY OF THE ANTARCTIC POLAR FRONT

Silke Laakmann, Meike Stumpp and Holger Auel

ABSTRACT

Four *Paraeuchaeta* species and three aetideids were frequently encountered along 51°30'S in the Atlantic sector of the Southern Ocean. *Paraeuchaeta antarctica* was most abundant close to the Antarctic Polar Front (APF). Within the genera *Paraeuchaeta* and *Gaetanus*, congeners usually partitioned the water column. Euchaetidae had high lipid (≤37% dry mass, DM in adult females) and wax ester contents (≤22% DM). Fatty acid composition of *Paraeuchaeta* spp. was dominated by monounsaturated moieties, especially 16:1(n-7) and 18:1(n-9), while fatty alcohols were mainly saturated. Surprisingly, only the bathypelagic *P. barbata* contained moderate amounts of 20:1(n-9) and 22:1(n-11) fatty acids (≤14%) and high levels of the respective fatty alcohols (≤50%), generally considered trophic biomarkers for calanid copepods as prey. Thus, herbivorous calanid copepods seem to be a readily available prey source at bathypelagic depths, indicating that their seasonal vertical migration provides a "trophic shortcut" from primary production at the surface to the interior of the ocean. Aetideidae also contained substantial levels of total lipid (14 to 36% DM), but wax esters contributed only up to 12% DM in copepodite stages C5 of *Gaetanus* spp., whereas other stages of *Gaetanus* and *Aetideopsis minor* only contained ≤6% DM of wax esters. The fatty acid compositions of Aetideidae were more balanced with 16:0, 18:1(n-9), 20:5(n-3), and 22:6(n-3) as important components, indicating a generally omnivorous feeding behaviour.

KEYWORDS

Euchaetidae; Aetideidae; *Paraeuchaeta*; *Pareuchaeta; Euchaeta; Gaetanus*; *Aetideopsis*; Southern Ocean; South Atlantic; Antarctic; lipid composition; fatty acid; trophic biomarker; vertical distribution; abundance; partitioning

INTRODUCTION

Copepods of the calanoid families Euchaetidae and Aetideidae are widespread throughout the World Ocean, inhabiting epi- to bathypelagic depths in tropical, temperate and polar waters (e.g. Park 1994a, b; Markhaseva 1996). Around South Georgia in the Southern Ocean, 14 species of the genus *Paraeuchaeta* co-occur sympatrically (Ward and Wood 1988). This raises the question how interspecific competition between closely related species is minimized. The dominant euchaetid in the Southern Ocean is *P. antarctica* (Ward and Wood 1988; Razouls et al. 2000), which contributes between 11 and 18% to total mesozooplankton biomass in the Weddell Sea (Schnack-Schiel et al. 1998). Detailed studies have focussed on its distribution and abundance (Ward 1989; Boysen-Ennen et al. 1991; Ward and Shreeve 1999), reproduction (Alonzo et al. 2000a, b), dietary composition and feeding (Hopkins 1985a; Hopkins 1987; Øresland 1991; Øresland and Ward 1993) as well as overwintering behaviour (Øresland 1995) in different parts of the Southern Ocean. Around the Kerguelen archipelago, *P. antarctica* has a one-year life-cycle (Alonzo et al. 2000a, b) and the species represents a dominant predator in the pelagic ecosystem of the Southern Ocean (Øresland 1991). In other ecosystems, predation by *Paraeuchaeta* species on fish larvae may even affect the recruitment of commercially important fish stocks (Yen 1987).

Data on lipid content and composition of zooplankton species have received growing interest over the last few decades (Dalsgaard et al. 2003; Lee et al. 2006), since they provide information on overwintering strategies, dietary composition, trophic level, and nutritional value for predators. Trophic biomarkers integrate dietary information over longer time spans of several weeks to months than gut content analyses making them a particularly valuable tool for studies on deep-sea and/or polar zooplankton which accumulates large amounts of storage lipids. Thus, lipid trophic biomarkers represent a very useful alternative approach for deep-sea organisms from remote regions that can only be infrequently sampled. Moreover, they allow dietary studies on animals which cannot be kept in captivity over longer periods for feeding experiments.

However, data on lipid content and composition of Antarctic species of Euchaetidae and Aetideidae are scarce. Detailed information is only available for *P. antarctica* and *Euchirella rostromagna* (Hagen et al. 1995; Mayzaud et al. 1998; Alonzo 2000a).

The present study focuses on the regional and vertical distribution of dominant copepod species of the families Euchaetidae and Aetideidae across the Atlantic sector of the Southern Ocean. Field data are combined with biochemical analyses to determine species-specific differences in total lipid content as well as fatty acid and alcohol composition in order to elucidate different feeding strategies and dietary preferences. Thus, the study contributes to a better understanding how closely related deep-sea species find individual ecological niches in

the food-limited environment of the deep-sea pelagic realm, where physical gradients are generally weak and isolation mechanisms almost absent.

MATERIALS AND METHODS

Field work and sampling

Deep-sea copepods of the two calanoid families Euchaetidae and Aetideidae were collected from April, 16 till 25 2006 in the Atlantic sector of the Southern Ocean during the expedition ANT XXIII-5 on board of R/V Polarstern. Sampling concentrated on ten stations along a transect at approximately 51°30´S from 53°54´W to 2°05´W (Fig. 1). Supplementary data on sea surface temperature and salinity were recorded by a ship-mounted, continuously measuring thermo-salinograph (Table 1).

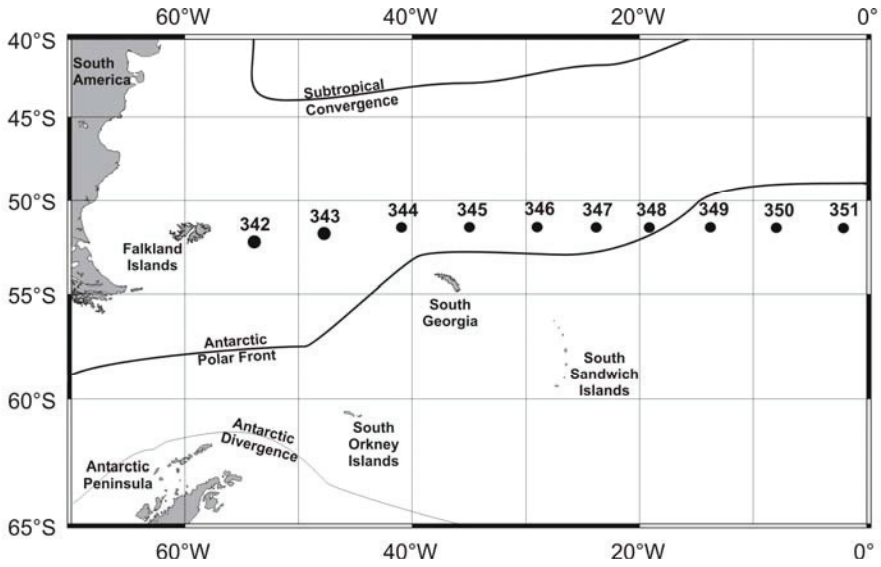

Figure 1

Map of stations along the transect across the Atlantic sector of the Southern Ocean

Samples were collected by stratified multiple opening/closing net hauls (Hydro-Bios Multinet Midi, mouth opening 0.25 m^2, mesh size 200 µm, vertical hauling speed 0.5 ms^{-1}). In order to study the vertical distribution of copepods, standard depth intervals of 2000-1500-1000-500-200-100-50-0 m were sampled. Since only five discrete depth strata could be sampled in one haul of the Multinet, two successive hauls (one to 2000 m depth and another one to 200 m) were conducted immediately one after the other at each station in order to combine deep sampling with a higher vertical resolution of the upper water layers.

Samples were analyzed in a temperature-controlled laboratory container at 2°C immediately after capture. Individuals of the families Euchaetidae and Aetideidae were sorted alive from the

samples and identified using taxonomic guides by Park (1978, 1994a), Razouls (1994) and Markhaseva (1996). For vertical and regional distribution patterns of abundance, species were staged, counted and deep-frozen at -80°C for later lipid analyses. All lipid analyses were carried out within 4 months after the end of the cruise. The remaining samples were preserved in 4% formaldehyde and analyzed again in the home laboratory to ensure that no individuals of Euchaetidae and Aetideidae had been missed.

Table 1

Multinet stations in the Atlantic sector of the Southern Ocean during the expedition ANT XXIII-5

Station	Date in 2006	Start time at 2000 m [UTC]	Position latitude	Position longitude	Bottom depth (m)	Sea surface temperature [°C]	Salinity
342	16.04.	14:45	52°18´S	53°54´W	2097	7.0	33.9
343	17.04.	13:16	51°51´S	47°45´W	2526	5.2	33.8
344	18.04.	13:04	51°30´S	40°57´W	3561	4.4	33.7
345	19.04.	12:09	51°30´S	34°59´W	4841	5.5	33.8
346	20.04.	12:10	51°29´S	29°00´W	3988	4.2	33.8
347	21.04.	12:10	51°30´S	23°50´W	4456	5.6	33.8
348	22.04.	13:41	51°30´S	19°08´W	4448	5.5	33.8
349	23.04.	11:06	51°30´S	13°47´W	4094	3.9	33.8
350	24.04.	10:10	51°32´S	7°58´W	2818	2.5	33.6
351	25.04.	10:08	51°33´S	2°05´W	2884	2.3	33.7

Dry mass determination and lipid analysis

A total of 49 deep-frozen samples including 285 individuals were lyophilized for 48 h (Leybold-Heraeus, LYOVAC GT2). In order to achieve adequate sample sizes for lipid analysis of small species and/or stages, individuals from neighbouring stations and/or adjacent depth layers were pooled. Dry mass was determined on a microbalance (Sartorius MC21 S, reproducibility <2 µg).

Lipid extraction was performed according to Folch et al. (1957) as modified by Hagen (2000). Lipid was extracted from lyophilized samples with dichloromethane/methanol (2:1 per volume) and total lipid content measured gravimetrically (Hagen 2000). Fatty acids were converted to methyl esters and analysed together with the fatty alcohols by gas-liquid chromatography (Kattner and Fricke 1986). For this purpose, fatty acids were modified to methyl esters by transesterification with methanol containing 3% concentrated sulphuric acid at 80°C for 4 h. Fatty acids and alcohols were separated and quantified using a Hewlett-Packard gas chromatograph (HP 6890A), equipped with a DB-FFAP column of 30 m length and 0.25 mm diameter. Peaks were identified according to retention times in comparison to a fish oil and a copepod lipid standard of known compositions.

In addition, by adding tricosanic acid (23:0) as an internal standard, the absolute amounts of fatty acids and alcohols were quantified (Peters et al. 2006). Proportions of wax esters relative to total lipid and dry mass, respectively, were calculated from the fatty alcohol content of the samples, assuming equal masses for the fatty alcohol and fatty acid chains of each wax ester molecule.

Statistical analysis

Vertical distribution data are presented as box plots, for which we assumed a random distribution of individuals within each of the sampled depth strata. In order to test for differences in dry mass, lipid, wax ester, fatty acid and alcohol proportions, one-way ANOVA and proximate Post-Hoc test (Dunnett T3) within the SPSS software package (version 15.0) were applied. Species- and stage-specific differences in lipid composition (percentages of fatty acids and fatty alcohols) were investigated on the basis of a principal component analysis within the same software package. Percentage data were transformed by arc sine square root transformation prior to both statistical analyses.

RESULTS

Hydrography

Sea surface temperature decreased along the transect from west to east from 7°C to 2.3°C (Table 1). The strongest decrease in temperature down to 2.5°C was recorded to the northeast of South Georgia between 19°08´W (stn. 348) and 7°58´W (stn. 350). Thus, the Antarctic Polar Front (APF), which is generally characterized by a sharp temperature decrease down to 2°C, apparently extended further to the north in the eastern section of the transect.

Vertical distribution and abundance

Across the Atlantic sector of the Southern Ocean, a total of seven euchaetid and 14 aetideid species were identified, of which four euchaetid species, i.e. *Paraeuchaeta antarctica*, *P. biloba*, *P. rasa* and *P. barbata* and three aetideid species, i.e. *Gaetanus brevispinus*, *G. tenuispinus* and *Aetideopsis minor* occurred more frequently and were, thus, chosen for the detailed analyses of the present study. Distribution patterns were determined for copepodite stages C3 to C6 for all dominant species. In case of *P. biloba*, only stages C5 and C6 were present and considered for the analysis (Fig. 2).

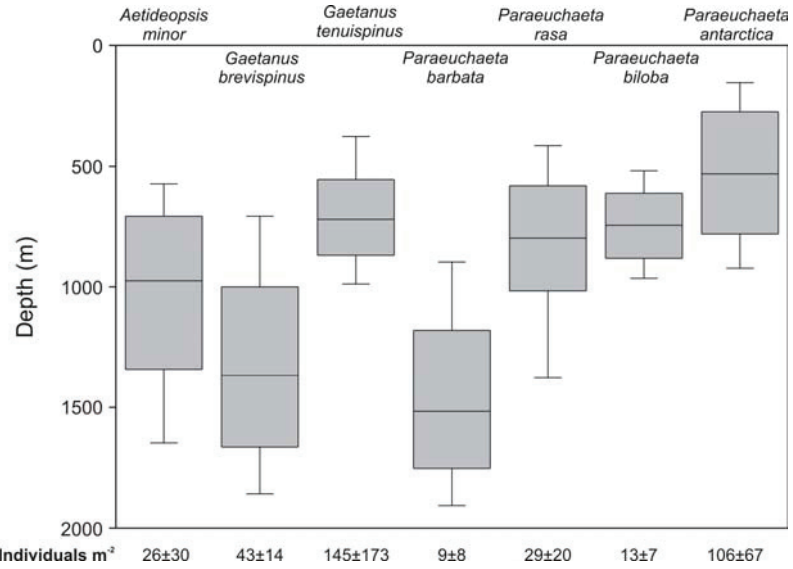

Figure 2

Vertical distribution and total abundance of copepodite stages C3 to C6 of *Aetideopsis minor*, *Gaetanus brevispinus*, *G. tenuispinus*, *Paraeuchaeta barbata*, *P. rasa*, *P. biloba* (only C5 to C6, see text), and *P. antarctica*. Boxes include 50% of the population, while error bars encompass the 5th to the 95th percentile. Abundance data are given as mean ± standard deviation

All species were restricted to distinct depth strata, resulting in a multi-layered vertical distribution pattern. *Aetideopsis minor* was mainly distributed between 715 and 1350 m (Fig. 2) with a low total abundance of 26±30 ind. m^{-2}. Copepodids C3 and C4 showed a slightly shallower distribution than copepodids C5 and females (data not shown). This species overlapped in distribution with both *Gaetanus* species. *Gaetanus brevispinus* occurred between 1000 and 1700 m, while its congener *G. tenuispinus* mainly inhabited depths between 550 and 880 m. Thus, both congeners partitioned the water column. Abundances of *G. brevispinus* were lower with 43±14 ind. m^{-2}, compared to those of *G. tenuispinus* with 145±173 ind. m^{-2}. An exceptionally high concentration of *G. tenuispinus* of >1 ind. m^{-3} in 500 to 1000 m depth, mainly composed of copepodite stage C5 and females, was observed at station 347 at 23°50'W.

Among Euchaetidae, *Paraeuchaeta antarctica* and *P. barbata* partitioned the water column, while *P. rasa* and *P. biloba* co-occurred within the same depth range and overlapped in their ranges with *P. antarctica* (Fig. 2). *P. barbata* inhabited the deepest layer between 1170 and 1770 m and showed a low total abundance of 9±8 ind. m^{-2}. *P. rasa* was mainly present between 600 and 1000 m with 29±20 ind. m^{-2} in total. Here, copepodids C3 were distributed deeper than older stages, while males occurred at shallower depth. Copepodite stage C5 was the dominant ontogenetic stage. *P. biloba* C5 to C6 (females and males) were concentrated between 600 and 880 m and reached 13±7 ind. m^{-2}. *P. antarctica* had the shallowest distribution range between 270 and 740 m (Fig. 2) and the highest abundance of 106±67 ind. m^{-2} in total.

Ontogenetic and regional trends in P. antarctica in relation to hydrography

Vertical profiles of abundance and stage composition for the dominant species *P. antarctica* are given in Fig. 3 separately for the two stations 348 and 349 at the APF (Fig. 3b) and the other stations along the transect (Fig. 3a). An ontogenetic partitioning of the water column was evident. Young copepodite stages C1 to C3 mainly occurred in the upper 200 m, while C4 and C5 dominated between 200 and 500 m and C5 as well as adult females and males were most abundant from 500 to 1000 m.

Extraordinary high concentrations of 800 ind. 1000^{-1} m^{-3} of *P. antarctica* occurred in 100 to 200 m close to the APF at stations 348 and 349 (Fig. 3b). Both stations differed markedly from other regions in the dominance of copepodids C4 and to a lesser extent C3, which were mainly responsible for the maximum abundances at the two stations. The dominance of young ontogenetic stages close to the APF may indicate recent reproductive activity in this region.

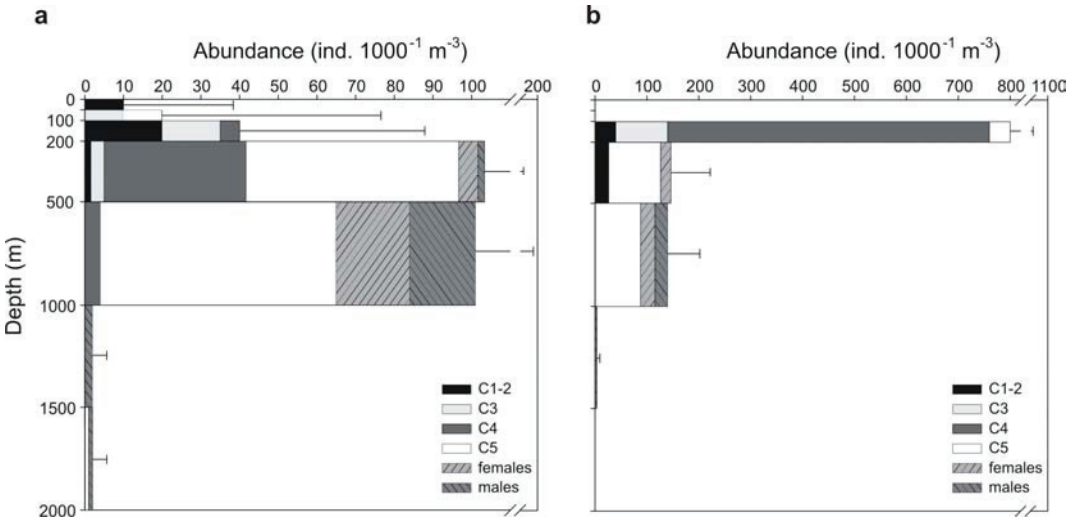

Figure 3

Paraeuchaeta antarctica: vertical distribution, abundance and stage composition (C1 to C6) at a) all stations along the transect with the exception of stns. 348 and 349 and b) at stns. 348 and 349 close to the Antarctic Polar Front

Inter-specific and ontogenetic differences in dry mass, total lipid and wax ester content

Deep-sea copepods strongly differed in body dry mass (DM), ranging from 0.17 to 2.59 mg in copepodids C5 and from 0.35 to 7.20 mg in adult females, with euchaetids generally heavier than aetideids. Among aetideids, *Gaetanus brevispinus* was the largest species with females weighing 1 mg, whereas *A. minor* was the smallest (female DM of 0.35 mg). *P. barbata* and *P. antarctica* were considerably larger (adult female DM of 7.20 and 4.93 mg, respectively) than *P. rasa* and *P. biloba* (1.73 and 0.58 mg DM, respectively) (Table 2).

Table 2

Dry mass, total lipid and wax ester content as well as fatty acid and fatty alcohol composition of Antarctic deep-sea copepods

	A. minor			*G. brevispinus*				*G. tenuispinus*			*P. barbata*			*P. rasa*			*P. antarctica*					*P. biloba*
	C5	C5	♀	C4	C4	C5	♀	C4	C5	♀	C5	♀	♀	C4	C5	♀	C3	C4	C5	♀	♂	♀
Individual dry mass [mg]	0.17	0.19	0.35	0.20	0.18	0.40 ± 0.10	1.01 ± 0.12	0.12	0.33 ± 0.05	0.50 ± 0.01	2.32	6.39	7.20	0.55	1.07 ± 0.03	1.73 ± 0.57	0.11	0.36 ± 0.19	2.59 ± 0.71	4.93 ± 1.24	2.83 ± 0.30	0.58
Lipid [%DM]	28	21	18	36	30	32 ± 2	21 ± 3	14	28 ± 7	15 ± 4	10	25	30	45	44 ± 6	37 ± 10	n.a.	28 ± 15	38 ± 7	31 ± 8	35 ± 14	7
Wax ester [%DM]	3	1	1	2	2	12 ± 6	6 ± 1	1	11 ± 6	3 ± 1	6	14	18	27	20 ± 3	22 ± 9	6	10 ± 4	20 ± 11	22 ± 9	24 ± 6	3
Wax ester [%TL]	11	5	3	5	6	36 ± 16	28 ± 1	8	38 ± 13	18 ± 8	60	56	62	60	45 ± 3	58 ± 10	n.a.	37 ± 10	50 ± 24	69 ± 18	70 ± 16	40
Fatty acids [%TFA]																						
14:0	3	4	2	1	1	1 ± 0	1 ± 1	2	1 ± 0	1 ± 0	0	0	1	0	0 ± 0	1 ± 0	1	1 ± 0	0 ± 0	0 ± 0	0 ± 0	0
16:0	16	16	16	9	8	5 ± 1	7 ± 2	12	5 ± 2	9 ± 1	6	2	4	2	3 ± 1	2 ± 0	8	7 ± 2	3 ± 3	2 ± 1	2 ± 1	10
16:1(n-7)	7	7	3	7	5	8 ± 1	8 ± 2	6	9 ± 1	7 ± 1	16	22	24	28	18 ± 4	30 ± 3	7	9 ± 2	19 ± 5	28 ± 3	28 ± 4	11
18:1(n-9)	17	16	18	25	21	31 ± 5	32 ± 1	12	28 ± 4	21 ± 2	28	41	33	39	39 ± 2	42 ± 2	23	27 ± 9	24 ± 5	30 ± 7	32 ± 4	27
18:1(n-7)	6	7	7	4	3	4 ± 2	4 ± 1	2	2 ± 0	3 ± 0	3	2	2	2	1 ± 0	1 ± 0	1	1 ± 0	1 ± 0	1 ± 0	1 ± 0	2
18:4(n-3)	1	1	1	1	1	1 ± 0	1 ± 0	1	1 ± 0	1 ± 0	1	1	0	1	2 ± 0	1 ± 1	3	2 ± 0	4 ± 1	2 ± 1	3 ± 0	1
18:4(n-1)	0	0	0	1	1	1 ± 1	1 ± 0	0	1 ± 0	1 ± 0	2	3	2	2	1 ± 1	4 ± 1	0	0 ± 0	1 ± 0	1 ± 0	1 ± 0	0
20:1(n-9)	4	4	6	3	2	4 ± 3	4 ± 1	1	2 ± 1	3 ± 1	6	5	8	4	3 ± 1	2 ± 1	0	0 ± 0	1 ± 0	3 ± 2	2 ± 1	1
20:5(n-3)	11	10	10	10	10	12 ± 2	10 ± 1	11	13 ± 1	12 ± 1	9	7	3	5	11 ± 3	4 ± 2	15	14 ± 4	14 ± 2	9 ± 3	10 ± 3	11
22:1(n-11)	1	1	2	1	1	1 ± 1	1 ± 0	0	0 ± 0	1 ± 0	1	2	6	1	0 ± 0	1 ± 0	0	0 ± 0	3 ± 2	3 ± 1	1 ± 0	1
22:6(n-3)	17	16	18	26	35	22 ± 6	17 ± 2	38	25 ± 2	26 ± 1	16	6	6	6	11 ± 1	5 ± 2	27	25 ± 3	20 ± 5	10 ± 5	11 ± 2	23
FA<4%	18	17	16	12	13	12 ± 1	13 ± 1	15	12 ± 1	15 ± 1	11	9	11	11	11 ± 2	8 ± 1	14	13 ± 1	11 ± 1	10 ± 2	9 ± 1	13
SFA	21	22	22	12	11	7 ± 2	10 ± 3	16	7 ± 3	12 ± 1	11	6	5	3	4 ± 1	3 ± 0	12	10 ± 3	5 ± 3	4 ± 1	3 ± 1	13
MUFA	44	45	42	44	36	51 ± 4	54 ± 3	27	44 ± 4	41 ± 2	57	73	79	75	64 ± 5	78 ± 5	35	41 ± 9	49 ± 8	68 ± 9	66 ± 7	47
PUFA	34	32	36	44	52	42 ± 5	35 ± 3	57	49 ± 2	47 ± 2	32	21	16	21	32 ± 4	19 ± 4	53	49 ± 6	46 ± 5	28 ± 8	31 ± 6	40
Fatty alcohols [%TFAlc]																						
14:0A	27	28	38	17	9	17 ± 6	15 ± 3	20	10 ± 1	11 ± 1	18	19	13	28	42 ± 7	29 ± 6	43	45 ± 12	36 ± 4	35 ± 5	39 ± 4	31
16:0A	39	35	50	43	41	42 ± 20	35 ± 7	49	48 ± 1	44 ± 5	32	28	22	38	43 ± 2	39 ± 4	50	49 ± 10	43 ± 3	36 ± 2	39 ± 3	48
16:1A	0	0	0	0	0	0 ± 0	0 ± 0	0	0 ± 0	0 ± 0	3	4	3	6	4 ± 3	12 ± 4	2	2 ± 1	4 ± 1	5 ± 1	4 ± 1	4
18:0A	5	0	0	2	3	2 ± 2	1 ± 1	4	4 ± 0	4 ± 0	1	1	1	2	1 ± 0	1 ± 0	2	1 ± 0	1 ± 0	1 ± 0	2 ± 0	2
18:1(n-9)A	10	10	0	19	19	16 ± 4	17 ± 6	11	19 ± 2	15 ± 2	16	15	11	10	5 ± 2	8 ± 1	3	2 ± 1	2 ± 0	3 ± 1	4 ± 1	6
20:1A	8	21	12	13	20	16 ± 10	17 ± 6	10	11 ± 1	13 ± 4	19	19	17	12	5 ± 1	5 ± 2	1	0 ± 0	1 ± 1	7 ± 4	7 ± 2	6
22:1A	11	6	0	7	7	7 ± 4	15 ± 2	7	8 ± 2	13 ± 2	11	14	33	5	1 ± 1	6 ± 2	0	0 ± 0	11 ± 8	13 ± 3	6 ± 1	2
Number of samples	1	1	1	1	1	3	3	1	5	4	1	1	1	1	3	3	1	3	4	4	5	1
Number of individuals	10	10	8	8	8	19	15	12	53	41	2	2	2	3	9	9	6	23	14	12	16	3

In case of >2 replicates, data are given as mean ± standard deviation. DM: dry mass; TL: total lipid; TFA: total fatty acids; FA: fatty acid, SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; TFAlc: total fatty alcohols; A: alcohol; C3-C5: copepodite stages C3 to C5

Total lipid content ranged from 10 to 44% DM in copepodite stage C5 and from 7 to 37% DM in females (Table 2). Among Euchaetidae, *P. antarctica* and *P. rasa* held high lipid levels of 38-44% DM in C5 and 31-37% DM in females. Total lipid in *P. barbata* was low in C5 with 10% DM (only one sample available), but increased to 25-30% DM in adult females. There was only one sample available for *P. biloba*, which showed an extremely low lipid proportion of 7% DM. In *Gaetanus* spp., lipid proportions were generally higher in copepodids C5 than in females ($p<0.05$), reaching 28 to 32% DM in C5 and 15 to 21% DM in females.

Wax esters (WE) ranged from 1 to 20% DM in copepodite stage C5 and from 1 to 22% DM in females (Table 2). Highest proportions of WE were determined for *P. rasa* and *P. antarctica* with 20 to 24% DM in copepodids C5 and adults, equivalent to 45 to 70% of total lipids. Lower, but still substantial amounts of WE of 11 to 18% DM occurred in adult females of *P. barbata* and copepodids C5 of both *Gaetanus* species, whereas adult females of *Gaetanus* spp. and copepodids C5 of *P. barbata* had WE levels accounting for 3 to 6% DM. *A. minor* only contained traces of WE (1 to 3% DM).

In *P. antarctica*, lipid levels increased from 28% DM in C4 to 38% DM in C5 stage (Table 2), while adult females and males had again slightly lower levels of 31 and 35% DM, respectively. In contrast, WE content increased from 10% DM in C4 via 20% DM in C5 to maximum values of 22 to 24% DM in adults.

Fatty acid and fatty alcohol composition

Fatty acid composition of *Paraeuchaeta* species was strongly dominated by the two monounsaturated fatty acids 16:1(n-7) and 18:1(n-9), which contributed up to 30 and 42% of total fatty acids (% TFA), respectively in adult females (Table 2). Only in *P. barbata*, 20:1(n-9) and 22:1(n-11), which are generally considered biomarkers for calanid copepods, reached 6 to 8% TFA in adult females. The only other fatty acids that occurred in amounts higher than 6% TFA were the polyunsaturated fatty acids (PUFA) 20:5(n-3) and 22:6(n-3), which represent major components of biomembranes. Highest levels of these PUFAs with 15 and 25-27% TFA, respectively, were found in young stages C3 to C4 of *P. antarctica*. Similarly, high levels of 20:5(n-3) and 22:6(n-3) were measured in one sample of adult females of *P. biloba*, which were severely depleted in total lipid (7% DM).

Aetideidae showed a rather more balanced fatty acid composition with five moieties contributing ≥9% TFA each, i.e. 16:0, 16:1(n-7), 18:1(n-9), 20:5(n-3), and 22:6(n-3) (Table 2). They also contained significantly higher amounts of 14:0 ($p<0.05$), 16:0, 18:1(n-7) (both $p<0.001$) and 22:6(n-3) ($p<0.05$) than *Paraeuchaeta* species. *A. minor* mainly differed from *Gaetanus* spp. in higher proportions of fatty acids 16:0, 18:1(n-7) (both $p<0.001$) and 22:1(n-11) ($p<0.05$), while *Gaetanus* spp. generally had higher levels of 18:1(n-9) ($p<0.001$)

and 22:6(n-3) ($p<0.001$). Thus, *A. minor* showed the most distinct fatty acid composition, whereas *Gaetanus* spp. fell in between *A. minor* and *Paraeuchaeta* spp.. Among the two *Gaetanus* species, *G. brevispinus* contained significantly higher amounts of 18:1(n-9) than *G. tenuispinus* ($p<0.05$).

In general, monounsaturated fatty acids were more dominant in both families of deep-sea copepods than saturated or PUFAs (Table 2). However, PUFAs prevailed in lipid-poor individuals such as young stages of *P. antarctica* and all ontogenetic stages of *G. tenuispinus*.

The fatty alcohol composition of *P. antarctica*, *P. biloba* and *P. rasa* was strongly dominated by 14:0A and 16:0A generally contributing 30 to 50% of total fatty alcohols (% TFAlc) each (Table 2). In addition, copepodite stages C4 of *P. rasa* contained substantial amounts of 18:1(n-9)A and 20:1A of 10 to 12% TFAlc each. Adult females of *P. antarctica* had only slightly elevated levels of 20:1A and 22:1A with 7 and 13% TFAlc, respectively. *P. barbata* differed from this general pattern with higher amounts of the carnivory biomarkers 18:1(n-9)A, 20:1A, and 22:1A with 11 to 33% TFAlc each. In both *Gaetanus* species, 16:0A was the dominant fatty alcohol. However, 14:0A, 18:1(n-9)A, 20:1A, and 22:1A also occurred in substantial amounts of 7 to 20% TFAlc. In contrast, *A. minor* only contained traces of wax esters and fatty alcohols.

Principal component analysis of fatty acids and alcohols

Principal component analysis based on fatty acid and alcohol composition data extracted three components with eigenvalues >1% of variance (Fig. 4). Results are presented for the major two components, together comprising 67.6% of variance (43.6 and 24.0% each). The third component was only responsible for a variance of 14.6% and therefore is not presented.

Principal component PC1 (43.6% of variance) was characterized by positive loadings of fatty acids 16:1(n-7), 18:1(n-9) and 22:1(n-11) as well as fatty alcohols 14:0A, 16:0A, 16:1A, 20:1A and 22:1A and negative loadings of fatty acids 16:0, 18:1(n-7), 20:1(n-9) as well as PUFAs 20:5(n-3) and 22:6(n-3) (Fig. 4). Principal component PC2 (24.0% of variance) included positive loadings of fatty acids 16:1(n-7), 18:1(n-7), 18:1(n-9), 20:1(n-9), 22:1(n-11) as well as fatty alcohols 18:1(n-9)A, 20:1A and 22:1A and negative loadings of PUFAs 20:5(n-3) and 22:6(n-3) as well as alcohols 14:0A, 16:0A and 16:1A.

Deep-sea copepod species were separated according to characteristic differences in their fatty acid and fatty alcohol compositions (Fig. 4). Generally, congeners were more similar to one another and clustered together, as was evident for *P. antarctica* and *P. rasa* as well as for *Gaetanus* species. Basically, differences in lipid composition of euchaetid and aetideid species were mainly pronounced along PC1, with generally lower values for aetideids. *P. antarctica* clustered along PC2 with negative loadings in young stages (due to high amounts of

biomembrane PUFAs) to positive ones in adult copepods. *P. rasa* clustered together with C5 and adult stages of *P. antarctica*, while *P. biloba* was rather similar to young stages of *P. antarctica* with negative loadings on PC2 and low levels of 16:1(n-7). *P. barbata* was isolated from other euchaetids by markedly positive loadings on PC2, namely 20:1 and 22:1 moieties and 18:1(n-9)A alcohol. Among aetideids, both *Gaetanus* species clustered together along PC1. Copepodids C5 and adult females of *G. brevispinus* were characterised by positive loadings of PC2, whereas *G. tenuispinus* was rather neutral regarding PC2. *A. minor* was also neutral along PC2 but separated from *Gaetanus* by more negative values on PC1 and, thus, deviated most strongly from euchaetid species. However, it must be taken into account that representatives of both families strongly differed in total lipid and WE content. Thus, some of the differences in fatty acid composition, such as high levels of 20:5(n-3) and 22:6(n-3) in *A. minor* and *G. tenuispinus*, can be explained by the dominance of biomembrane components in these lipid-poor species.

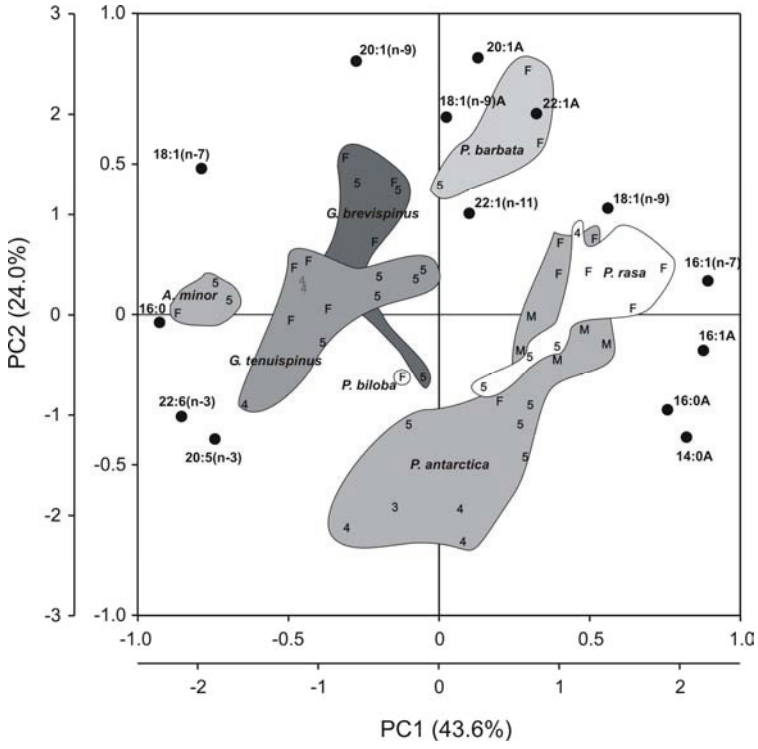

Figure 4

Principal component analysis of Antarctic deep-sea copepods according to their fatty acid and alcohol compositions. Sample plot for the principal components PC1 and PC2 (outer axes) and superimposed loading plot (inner axes) with the relevant fatty acids and alcohols. Fatty acids and alcohols <4% are not shown. Loading differences >0.5% are significant. 3 to 5: copepodite stages C3 to C5; F: female; M: male; A: fatty alcohol

DISCUSSION

Predatory copepods of the genus *Paraeuchaeta* and omnivorous Aetideidae comprised important components of mesozooplankton communities in the vicinity of the Antarctic Polar Front (APF). *P. antarctica*, *P. rasa*, *P. biloba* and *P. barbata*, are the most abundant euchaetid species in the Southern Ocean (present study; Park 1994b). The first three species are endemic to the sub-Antarctic and Antarctic (Park 1978, 1994b), while *P. barbata* is a cosmopolitan species, of which some representatives have been originally described as *P. farrani* (Mauchline 1992; Park 1994b). Among the Aetideidae, both the bathypelagic *Gaetanus brevispinus* and the mesopelagic *G. tenuispinus* have a cosmopolitan distribution, whereas *Aetideopsis minor* is considered a bipolar, mesopelagic species (Markhaseva 1996).

The vertical distribution of the dominant euchaetid and aetideid species was comparable to previous studies in more restricted sampling areas such as around the Antarctic Peninsula (Hopkins 1985b), off South Georgia (Ward and Wood 1988), and in the western Weddell Sea (Hopkins and Torres 1988). Close to the APF, *P. biloba*, *A. minor* and *G. tenuispinus* inhabited deeper layers than further south (Hopkins and Torres 1988; Ward and Wood 1988; Schnack-Schiel et al. 2008; present study). This phenomenon of a polar emergence is well known from Arctic Euchaetidae and Aetideidae (Markhaseva 1996; Auel 1999; Laakmann et al. submitted). The abundances of *P. antarctica* and *G. tenuispinus* were generally lower in the present study than at the Antarctic Peninsula (Hopkins 1985b) or in the Weddell Sea (Schnack-Schiel et al. 2008). Locally higher densities of both species at the easternmost stations and higher proportions of juvenile stages C3 and C4 in *P. antarctica* may either indicate a concentration of zooplankton (both predators and prey) related to downwelling or an elevated secondary production associated with the APF. In addition, downwelling can entrain organic matter from the euphotic zone into deeper layers and, thus, provide an enhanced food supply for omnivores at mesopelagic depths.

The ontogenetic vertical partitioning of *P. antarctica* can be explained by different dietary preferences. Young stages find smaller prey in higher concentrations at shallower depths. Mandibular gnathobases do not differ morphologically between different copepodite stages of *P. antarctica* (Michels and Schnack-Schiel 2005) indicating that even young stages are already predatory, feeding on small copepods such as *Microcalanus pygmaeus* (Yen 1991; Øresland and Ward 1993). Cannibalism, which is often assumed to be a major reason for ontogenetic partitioning, does not seem to be an important issue for *P. antarctica* (Øresland and Ward 1993).

High lipid and wax ester (WE) contents of up to 45% DM and 70% TL, respectively, in many deep-sea copepods especially *Paraeuchaeta* spp. and also *Gaetanus* spp. are usually considered an adaptive strategy to cope with a seasonally limited food supply (Lee et al. 1971;

Lee and Hirota 1973; Lee et al. 1974; Hagen et al. 1995). However, carnivorous and omnivorous copepods may be less affected by a seasonal production regime, since they can switch easier to other food sources than herbivores. Moreover, there are indications that the feeding conditions for bathypelagic carnivores such as *P. barbata* are even better during winter, when many herbivorous copepods descend to greater depth for overwintering (Auel and Hagen 2005; Laakmann et al. submitted). This situation, where feeding conditions are most favourable at times when primary productivity is at its minimum, can be termed “inverse seasonality”.

Thus, the long-standing hypothesis that high lipid and WE levels evolved primarily in polar herbivorous copepods, i.e. *Calanus* and *Calanoides*, as an energy reserve for overwintering and egg production independent of primary production, should not be simply adopted for omnivorous and carnivorous deep-sea copepods. Predatory *Paraeuchaeta* also contain high lipid and WE contents, which may act rather more likely as buoyancy aids (Sargent and Henderson 1986) than as internal energy reserves. Buoyancy is of prime importance for such large and heavily skeletorized copepods like *Paraeuchaeta*. Their “drift and wait” strategy and rheotactic prey detection require them to remain motionlessly for extended periods of time without sinking.

Aetideidae generally contained lower lipid and WE levels than *Paraeuchaeta*, implying a less important role of long-term energy storage and/or lipid-supported buoyancy (present study; Hagen et al. 1995; Auel 1999; Auel and Hagen 2005). Other lipid classes, potentially triacylglycerols (TAG) and/or phospholipids, may act as major energy stores in aetideids. For instance, the high-Antarctic epipelagic aetideid *Euchirella rostromagna* also stored TAG instead of WE (Hagen et al. 1995). TAG can be quickly hydrolyzed and, thus, provides an efficient short-term energy reserve (Lee et al. 2006). TAG accumulation appears to be an advantageous strategy for continuously feeding, omnivorous species such as aetideids (Hopkins et al. 1993; Hagen and Auel 2001). The higher lipid and WE levels in C5 stages of both *Gaetanus* species as compared to adult females may either be explained by better feeding conditions for smaller stages at the time of sampling or by lipid consumption for recent egg production in adult females. The assembly of lipovitellin for the oocytes requires considerable amounts of lipids (Lee et al. 2006).

Only a limited number of replicates for fatty acid and alcohol analysis was available particularly for small species and stages (Table 2). Thus, the interpretation of the results should be cautious. In *Paraeuchaeta*, WE were the major lipid class (up to 70% of total lipid). Therefore, the majority of fatty acids and alcohols of *Paraeuchaeta* were part of WE. Since fatty acid and alcohol patterns were analysed in total lipid extracts, information on lipid class-specific compositions is not available. The predominance of 16:1(n-7) and 18:1(n-9) as major fatty acids in *Paraeuchaeta* has already been observed in previous studies (Hagen et al. 1995;

Albers et al. 1996; Laakmann et al. submitted). Data on fatty acid and fatty alcohol trophic biomarkers support the general omnivorous and opportunistic feeding behaviour of Aetideidae and the carnivorous raptorial feeding of *Paraeuchaeta*, also confirmed by gut-content analysis and feeding experiments on selected species (Hopkins 1985a, 1987; Yen 1991; Hopkins et al. 1993; Olsen et al. 2000). Especially *Paraeuchaeta* species contained high amounts of 18:1(n-9) generally considered a trophic biomarker for carnivory (Falk-Petersen et al. 1990).

An interesting result of the present study is the difference in 20:1 and 22:1 moieties between *P. barbata* and the other congeners. The long-chain monounsaturated fatty acids 20:1(n-9) and 22:1(n-11) and the respective fatty alcohols are considered trophic biomarkers for calanid copepods as prey items (Graeve et al. 1994a; Kattner et al. 1994; Kattner and Hagen 1998). High levels of these compounds in bathypelagic *P. barbata* support the hypothesis of a "trophic shortcut" in polar oceans from primary production in the euphotic zone to the deep-sea, mediated by seasonally vertically migrating herbivorous copepods, as has also been shown for the Arctic (Laakmann et al. submitted).

In contrast, comparatively low levels of 20:1 and 22:1 moieties in epi- and mesopelagic *Paraeuchaeta* species may either be explained by feeding on *Calanus simillimus*, which is a common sub-Antarctic species (Atkinson 1991) but contains rather low levels of these compounds (Ward et al. 1996). Alternatively, non-calanid copepods and/or other zooplankton may be the dominant prey items during this time of the year. For instance off South Georgia, *Calanoides acutus* only represented an important food item for *P. antarctica* in summer (Øresland and Ward 1993), while during winter *C. acutus* descended below the vertical range of *P. antarctica* (Atkinson and Ward 1988). In winter, *P. antarctica* mainly preyed on *Metridia* spp. and *Drepanopus forcipatus* (Øresland and Ward 1993). The same two prey species also comprised the major food source for *P. biloba* during both summer and winter (Øresland and Ward 1993), while there is no indication of feeding on Calanidae in *P. biloba* or *P. rasa*, neither from trophic biomarker data (present study) nor from gut content analysis (Øresland and Ward 1993). Another, rather speculative interpretation would be that *P. antarctica*, whose centre of distribution lies south of the APF, does not find enough of its favourite lipid- and WE-rich prey *C. acutus* at the northernmost limit of its distribution range. Lower lipid and WE contents in females of *P. antarctica* during the present study as compared to specimens collected in high-Antarctic regions (Littlepage 1964; Hagen et al. 1995; Auel and Hagen 2005) support this hypothesis of a poorer condition together with the low levels of 20:1 and 22:1 trophic biomarkers.

High levels of the fatty acid 16:1(n-7), which is generally considered an indicator of diatoms (e.g. Graeve et al. 1994a, b; Dalsgaard et al. 2003), in predatory *Paraeuchaeta* especially from polar regions (present study; Laakmann et al. submitted) raise the question whether this

component is accumulated by feeding on herbivorous prey and selectively retained or if *Paraeuchaeta* is able to synthesize this fatty acid *de novo* as has been suggested by Hagen et al. (1995).

In Aetideidae, the more diverse fatty acid and fatty alcohol composition reflects the generally omnivorous, opportunistic feeding behaviour, which is also supported by gut-content analyses (Hopkins 1985a; Hopkins and Torres 1989; Hopkins et al. 1993). Higher levels of the carnivory marker 18:1(n-9) in *Gaetanus brevispinus* than in *G. tenuispinus* suggest a higher contribution of carnivorous feeding to the nutrition of the larger, bathypelagic species. In the smaller, mesopelagic *G. tenuispinus* phytoplankton was a frequent diet component besides protozoans (mostly ciliates) and metazoans (Hopkins and Torres 1989; Hopkins et al. 1993). Slightly elevated levels of the herbivory marker 18:1(n-7) in *A. minor* may reflect a higher contribution of phytoplankton and/or phytodetritus to its nutrition than to that of *Gaetanus* spp.. However, the proportions of 18:1(n-7) of all three aetideid species in the present study were substantially lower than in the epipelagic *Euchirella rostromagna* (Hagen et al. 1995), for which phytoplankton is easier available. Elevated levels of 20:5(n-3) and 22:6(n-3) in *A. minor* and *G. tenuispinus* – as compared to *Paraeuchaeta* – can be explained by the dominance of biomembrane components in these lipid- and WE-poor species. Thus, species- and stage-specific differences in total lipid content and lipid-class storage (i.e. WE vs. TAG accumulation) must be taken into account for the interpretation of fatty acid composition data.

In conclusion, the present study demonstrates that vertical partitioning of the water column and differences in feeding behaviour and dietary preferences allow closely related and co-occurring deep-sea copepods to find species-specific ecological niches in the deep-sea pelagic realm, where physical gradients are generally weak and isolation mechanisms almost absent. Members of the same genus usually partition the water column, while representatives of different genera, which co-occur at the same depth, generally differ in dietary preferences, as evident from trophic biomarkers. Thus, both mechanisms help minimizing inter-specific competition and sustain a relatively high biodiversity in polar deep-sea ecosystems. Nevertheless, biotic environmental factors, such as food availability, vary even in the bathypelagic zone on seasonal time scales. As shown in the present study, seasonal vertical migrations of herbivorous copepods can provide a “trophic shortcut” from the productive euphotic zone to the deep-sea and determine the seasonality of the food supply in the bathypelagic zone (“inverse seasonality”). These pelagic-benthic coupling processes and their relevance for the carbon flux are still far from understood and should be the focus of future studies on the biodiversity and functioning of pelagic deep-sea ecosystems.

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CHAPTER III

Longitudinal and vertical trends in stable isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) of omnivorous and carnivorous copepods across the South Atlantic Ocean

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LONGITUDINAL AND VERTICAL TRENDS IN STABLE ISOTOPE SIGNATURES ($\delta^{13}C$ AND $\delta^{15}N$) OF OMNIVOROUS AND CARNIVOROUS COPEPODS ACROSS THE SOUTH ATLANTIC OCEAN

Silke Laakmann and Holger Auel

ABSTRACT

Stable isotope (SI) ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) were measured in omnivorous and carnivorous deep-sea copepods of the families Euchaetidae and Aetideidae across the Atlantic sector of the Southern Ocean to establish their trophic positions. Due to high and variable C/N ratios related to differences in lipid content, $\delta^{13}C$ was corrected using a lipid-normalisation model. In general, $\delta^{15}N$ signals ranged from 3.0-6.9‰ in mesopelagic species to 7.0-9.5‰ in bathypelagic congeners. Among the carnivorous *Paraeuchaeta* species, the epi- to mesopelagic species *Paraeuchaeta antarctica* had lower $\delta^{15}N$ values than the mesopelagic *P. rasa* and bathypelagic *P. barbata*. Among the omnivorous Aetideidae no significant trend was observed. In the most abundant species *P. antarctica*, individuals from the western Atlantic had higher $\delta^{13}C$ and $\delta^{15}N$ values than specimens at the eastern stations. These regional differences were not related to differences in lipid content, since lipid normalisation of the data set did not weaken this trend. These longitudinal changes in $\delta^{13}C$ and $\delta^{15}N$ values were attributed to regional differences in hydrography and sea surface temperature (SST), in particular related to a northward extension of the Antarctic Polar Front (APF) at the easternmost stations. The results indicate that even in a mesopelagic carnivorous species the changes in surface stable isotope signatures are pronounced.

KEYWORDS

Pareuchaeta; Euchaeta; Gaetanus; *Aetideopsis*; deep-sea zooplankton

INTRODUCTION

Stable isotope (SI) ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) are widely used as trophic markers in order to trace pathways of organic matter in food webs (Hobson 1999; Peters et al. 2005). Isotopic fractionation by physiological processes leads to a retention and accumulation of the heavier isotope. Hence, $\delta^{15}N$ generally increases by 3.4±1.1‰ from one trophic level to

the next (Minagawa and Wada 1984). Therefore, nitrogen SI signals can be used to trace predator-prey relationships and to calculate the trophic level of an animal if the $\delta^{15}N$ baseline of the food web is known (e.g. Vander Zanden and Rasmussen 1999; Søreide et al. 2006). In contrast, the enrichment factor of $\delta^{13}C$ per trophic level is rather low (1‰, DeNiro and Epstein 1978), but $\delta^{13}C$ may provide valuable information on the fundamental carbon source of the food web and the primary producers at the base of the food chain (Post 2002; McCutchan et al. 2003).

Several protocols have been applied for sample processing prior to SI analysis. One of the still controversial issues is whether lipids should be extracted from the samples prior to SI analysis or not. Lipids are depleted in ^{13}C compared to other body compounds such as protein and muscle tissue (Tieszen et al. 1983). Therefore, high lipid content may bias $\delta^{13}C$ measurements towards lower values. In consequence, several authors recommend the removal of lipids prior to SI analysis (e.g. Hobson et al. 2002; Søreide et al. 2006). However, extraction of lipids can also affect the results of $\delta^{15}N$ determination (Jacob et al. 2005; Mintenbeck et al. 2008). Due to the lack of standard protocols for sample treatment and SI analysis, the respective results of different studies are hardly comparable. McConnaughey and McRoy (1979) proposed a lipid-normalisation model on the basis of C/N ratios, which are a regular by-product of SI analyses. This approach allows calculating standardised lipid-corrected $\delta^{13}C$ values for samples with varying lipid content and has already been tested for a variety of marine invertebrates (McConnaughey and McRoy 1979) and vertebrates (Rau et al. 1992a). Recently, Smyntek et al. (2007) validated the lipid-normalisation method again for zooplankton.

Baseline $\delta^{13}C$ values can be affected by the concentration of dissolved CO_2 in seawater, which in turn is determined by sea surface temperature (Rau et al. 1989, 1991b for the Southern Ocean). Thus, $\delta^{13}C$ of phytoplankton or seston generally decreases towards higher latitudes. In other studies, however, the latitudinal $\delta^{13}C$ depletion was independent of changes in dissolved CO_2 concentration (Rau et al. 1992b), but rather more related to differences in phytoplankton growth rate, cell size, membrane permeability or to the degree of fractionation during carbon fixation (François et al. 1993). In addition, $\delta^{15}N$ in Antarctic phytoplankton can also be depleted compared to lower latitudes (Wada et al. 1987) with an increase in surface NO_3^- concentration from north to south within a few degrees latitude at the Sub-Antarctic Front, accompanied by a decrease in suspended particles $\delta^{15}N$ by 11‰ (Altabet and François 1994b). The latitudinal changes of SI signatures more likely occur stepwise in frontal regions as it has been demonstrated for the Southern Subtropical Front (François et al. 1993; Altabet and François 1994a) as well as for the Sub-Antarctic Front and the Antarctic Polar Front (APF) (Trull and Armand 2001). Hence, variations in $\delta^{15}N$ along lines of latitude in the Southern Ocean are expected to result from changes in the position and extent of frontal systems (Altabet and François 1994b). In general, frontal zones can be identified by temperature and salinity

differences, for example the transition from the Polar Frontal Zone to the Antarctic Zone, which are separated by the APF is characterised by a summer subsurface temperature decrease to 2°C or less and low surface salinities (Pollard et al. 2002). The regional differences in $\delta^{13}C$ have been used to trace foraging locations of right whales, albatrosses, storm-petrels, and penguins in the Southern Ocean (Best and Schell 1996; Cherel et al. 2000; Gladbach et al. 2007; Cherel and Hobson 2007).

Since SI signatures integrate dietary input over several weeks to months (e.g. Tieszen et al. 1983) their application as trophic markers provides a valuable tool for studies on animals from remote areas, such as polar deep-sea zooplankton, that can neither be sampled in high temporal resolution, nor kept alive in captivity over longer periods for feeding experiments. The present study focuses on deep-sea copepods of the families Aetideidae and Euchaetidae from the Atlantic sector of the Southern Ocean in the vicinity of the APF. Species of both families are abundant in epi- to bathypelagic layers of the Southern Ocean (Hopkins 1985; Hopkins and Torres 1988; Ward and Wood 1988; Schnack-Schiel et al. 2008; Laakmann et al. 2009) with many co-occurring closely related species (e.g. Ward and Wood 1988). The dominant species of Euchaetidae is the shallowest distributed *Paraeuchaeta antarctica* (e.g. Ward and Wood 1988; Laakmann et al. 2009), which contributes between 11 and 18% to total mesozooplankton biomass in the high-Antarctic Weddell Sea (Schnack-Schiel et al. 1998). The two families display different feeding modes (Olsen et al. 2000). Euchaetidae are rheotactic predators feeding on a wide range of mesozooplankton (Øresland and Ward 1993; Øresland 1995) and even larvae of commercially important fish species (Yen 1987), while Aetideidae are generally considered to be omnivores (Hopkins et al. 1993; Auel 1999) and are in contrast to the Euchaetidae able to detect both, motile and non-motile food items (Olsen et al. 2000).

The aim of the present study is to determine the SI signatures of euchaetid and aetideid copepods from different depth horizons in the Atlantic sector of the Southern Ocean in order to get insights into the trophic characteristics of these pelagic deep-sea inhabitants. We further focus on large-scale regional trends in SI signatures of these omnivores and carnivores related to changes in the hydrographic regime, coupled to the longitudinal transect, located in the vicinity of the APF (close to 50°S between 40°W and 0° (Moore et al. 1999)).

MATERIALS AND METHODS

Sampling

Deep-sea copepods were collected from April 16th to 25th 2006 across the Atlantic sector of the Southern Ocean at approximately 51°30´S from 53°54´W to 2°05´W during the expedition ANT XXIII-5 on board of R/V Polarstern (Table 1, Fig. 1a). Sea surface temperature and salinity data were recorded by a ship-mounted, continuously measuring thermo-salinograph (Fig. 1b).

A multiple opening/closing net (Hydro-Bios Multinet Midi, mouth opening: 0.25 m^2, mesh size: 200 µm) was used for stratified vertical hauls with standard depth intervals (2000-1500-1000-500-200-100-50-0 m). Immediately after the catch, individuals of the calanoid copepod families Euchaetidae and Aetideidae were sorted alive from the samples in a temperature-controlled laboratory container at 2°C, staged and deep frozen at -80°C.

Table 1

Stations across the Atlantic sector of the Southern Ocean. Maximum sampling depth at all stations was 2000 m

Station	Date in 2006	Time [UTC]	Position latitude South	Position longitude West	Bottom depth [m]
342	16.04.	14:45	52°18´	53°54´	2097
343	17.04.	13:16	51°51´	47°45´	2526
344	18.04.	13:04	51°30´	40°57´	3561
345	19.04.	12:09	51°30´	34°59´	4841
346	20.04.	12:10	51°29´	29°00´	3988
347	21.04.	12:10	51°30´	23°50´	4456
348	22.04.	13:41	51°30´	19°08´	4448
349	23.04.	11:06	51°30´	13°47´	4094
350	24.04.	10:10	51°32´	7°58´	2818
351	25.04.	10:08	51°33´	2°05´	2884

Dry mass determination and stable isotope analysis

Deep-frozen samples were lyophilised for 48 hours (Leybold-Heraeus, LYOVAC GT2). For small species and/or stages, samples from neighbouring stations and/or adjacent depth layers were pooled to obtain sufficient biomass for SI analysis. Dry mass was determined on a microbalance (Sartorius MC21 S, reproducibility <2 µg). SI analyses were performed by Agroisolab GmbH in Jülich, Germany, using a mass spectrometer (EA NA1500 Series 2, Carlo Erba Instruments) and helium as carrier gas. Determination of carbon and nitrogen SI ratios $\delta^{13}C$ and $\delta^{15}N$, respectively, was conducted using the standards IAEA-PDB (IAEA-C1, Vienna) and AIR, atmospheric air (IAEA-N1, Vienna). Ratios are expressed as $\delta^{13}C$ and $\delta^{15}N$ in ppt (‰) between the measured samples and standards, according to equations given in Iken et al. (2005) and Søreide et al. (2006). Additionally, molar C/N ratios were determined as part of the standard analytical procedure. Since SI analysis were implemented in a comprehensive biochemical and phylogenetic study, biomass of samples for SI was generally low and lipids were not extracted prior to SI analysis. However, the polar copepods had moderate to high lipid deposits (see Laakmann et al. 2009) and thus, lipid content was calculated on the basis of molar C/N ratio and $\delta^{13}C$ values were corrected accordingly, applying a lipid-normalisation model, published by McConnaughey and McRoy (1979) and again tested for zooplankton by Smyntek et al. (2007).

For two samples of *Paraeuchaeta antarctica*, for which empirical C/N ratios were not available, average C/N ratios of the respective ontogenetic stages were used instead, since supplementary studies did not reveal substantial differences in lipid content of *P. antarctica* along the transect (Laakmann et al. 2009).

Statistical analysis

Differences between $\delta^{13}C$ and $\delta^{15}N$ of the different species were analysed on square root arc sin transformed data by One-way Analysis of Variance (ANOVA) with a prior Kolmogorov Smirnov test (normal distribution) and Tukeys Post Hoc test as well as by Kruskall Wallis test with Dunns Post Hoc test using the software GraphPad Prism (version 5.02).

RESULTS

The sea surface temperature (SST) decreased from west to east with values of ≥6°C until 52°W and temperatures on average of 5°C until 20°W, where the temperature decreased down to ≤2.5°C (stations 348 and 349) (Fig. 1b). These rather low temperatures at the eastern end of the transect indicate that those stations were located close to or within the APF, characterised by a temperature decrease down to 2°C or less (Pollard et al. 2002). The salinity along the transect showed a similar trend as SST, but changes were rather small (max. 3) (Fig. 1b).

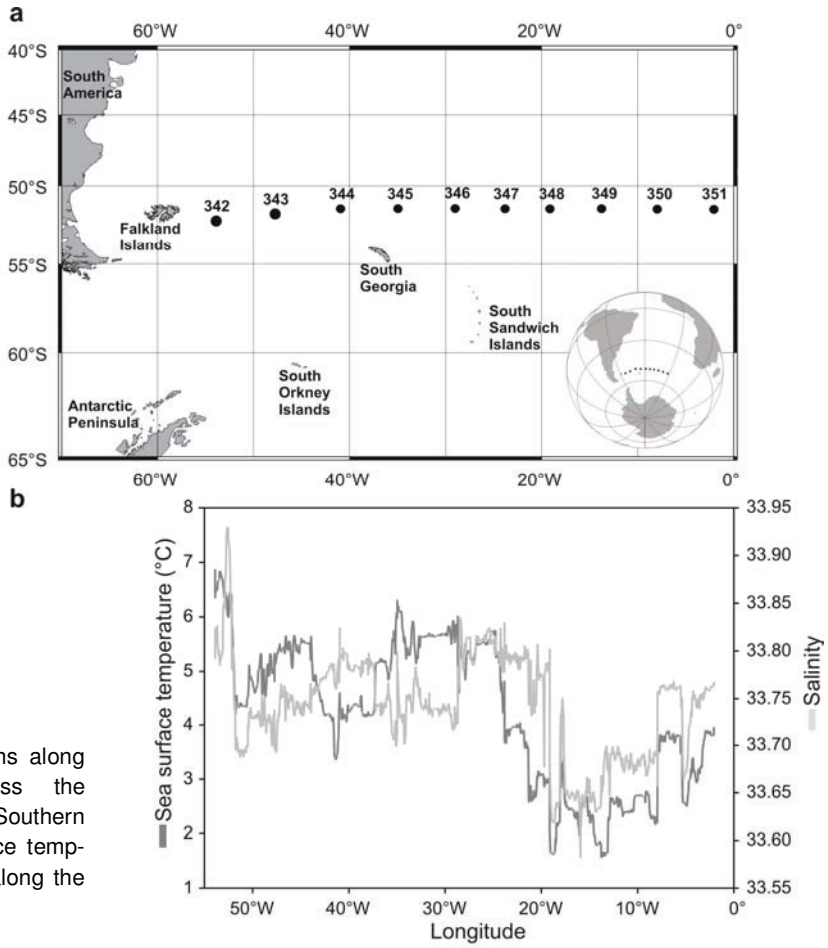

Figure 1

a) Location of stations along the transect across the Atlantic sector of the Southern Ocean, b) sea surface temperature and salinity along the transect

Table 2

Stable isotope ratios for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) as well as molar C/N ratio for euchaetid and aetideid copepods; $\delta^{13}C'$: lipid-corrected $\delta^{13}C$; A. *Aetideopsis*, G: *Gaetanus*, P: *Paraeuchaeta*

Species and stage	Station	Depth [m]	$\delta^{13}C$ [‰]	$\delta^{13}C'$ [‰]	$\delta^{15}N$ [‰]	C/N ratio	Individuals
A. minor							
female	344, 345	1000-2000	-23.4	-22.6	8.8	4.9	4
G. brevispinus							
C5	342, 348, 349	100-1500	-23.4	-22.9	7.0	4.4	3
female	343	1500-2000	-25.5	-25.6	7.6	3.9	2
	346	1000-1500	-23.5	-	8.1	-	1
	349	1000-1500	-22.0	-	7.8	-	1
G. tenuispinus							
C5	346	500-1000	-23.6	-22.4	6.9	5.4	3
	346	500-1000	-25.1	-23.9	6.6	5.5	4
	347	1000-1500	-23.6	-	7.5	-	4
	347	1000-1500	-24.8	-	6.6	-	3
	347	200-500	-23.9	-23.2	5.7	4.7	8
	351	200-500	-22.8	-23.4	5.7	3.5	7
female	342	500-1000	-25.9	-	5.4	-	3
	344	200-500	-24.8	-	7.2	-	2
	344	200-500	-25.3	-	6.6	-	2
	344	200-500	-25.1	-	6.3	-	2
	347	500-1000	-22.0	-21.8	6.2	4.2	10
P. antarctica							
C4	346	200-500	-23.5	-23.5	5.4	4.0	3
	349	100-200	-25.5	-24.1	4.7	5.8	5
	349	100-200	-26.0	-24.5	3.6	6.0	5
	349	100-200	-25.8	-23.9	4.0	7.0	5
	349	100-200	-25.6	-24.2	3.7	5.7	5
C5	344	200-500	-23.2	-21.3	6.4	6.8	2
	346	500-1000	-22.7	-20.9	5.9	6.6	1
	350	200-500	-25.3	-23.9	4.2	5.7	3
	350	500-1000	-26.9	-24.4	4.0	8.9	2
	351	200-500	-26.5	-25.7	3.0	4.8	1
	351	200-500	-26.2	-24.4	4.1	6.7	1
female	345	500-1000	-21.3	-19.2	6.1	7.4	1
	346	500-1000	-24.4	-22.3	5.5	7.6	1
	346	500-1000	-22.2	-19.8	5.2	8.4	1
	347	200-500	-24.9	-22.7	4.1	7.9	1
	351	500-1000	-27.0	-24.8	4.8	7.8	1
male	344	200-500	-26.2	-	6.3	-	1
	344	500-1000	-28.4	-26.9	4.9	6.0	1
	348	500-1000	-24.9	-22.7	4.6	7.8	1
	351	500-1000	-26.1	-24.2	4.8	7.0	1
P. biloba							
female	348	500-1000	-25.3	-24.3	5.7	5.1	3
	349	500-1000	-25.9	-	6.8	-	1
	350, 351	500-1000	-25.9	-23.6	6.6	8.2	3
male	350	500-1000	-27.6	-25.2	9.9	8.6	1
P. barbata							
C4	349	1500-2000	-22.9	-22.9	9.5	4.0	1
C5	343	1500-2000	-24.5	-24.1	8.0	4.4	1
C5	344	1500-2000	-24.2	-23.7	9.1	4.5	1
female	351	500-1000	-24.8	-22.5	6.7	8.2	1
male	348	1500-2000	-24.8	-22.5	8.5	8.1	1
P. rasa							
C5	344	500-1000	-28.0	-26.7	8.5	5.5	2
	346	500-1000	-26.7	-24.4	9.4	8.1	2
female	346	500-1000	-24.4	-21.7	8.5	9.9	1
	346	200-500	-24.3	-	9.8	-	1
	351	500-1000	-25.6	-23.5	9.9	7.6	1
	351	500-1000	-25.1	-22.6	9.3	9.1	1

Species-specific differences were discovered in SI signatures $\delta^{13}C$ and $\delta^{15}N$ as well as in C/N ratios (Table 2). Uncorrected $\delta^{13}C$ values ranged from -28.4 to -21.3‰ with both minimum and maximum of $\delta^{13}C$ were measured in adults of *Paraeuchaeta antarctica*. $\delta^{13}C$ values of two other euchaetid species, i.e. *P. biloba* and *P. rasa*, fell between -28.0 and -24.3‰, whereas *P. barbata* had higher ratios of -24.8 to -22.9‰. The two aetideid species *Gaetanus tenuispinus* and *G. brevispinus* showed rather similar $\delta^{13}C$ values of -25.9 to -22.0‰, while there was only one sample of *Aetideopsis minor*, which fell into the range of the *Gaetanus* species. In *P. antarctica*, for which the highest number of replicates was available, no stage-specific differences in $\delta^{13}C$ were identified.

Lipid-normalised $\delta^{13}C'$ values were generally higher than uncorrected $\delta^{13}C$ data and ranged from -26.9 to -19.2‰ (Table 2). Only for two *Gaetanus* samples, which had C/N ratios <4, lipid-normalisation lead to lower $\delta^{13}C'$ values as compared to $\delta^{13}C$. The effects of the lipid-normalisation were more pronounced in *Paraeuchaeta* than in Aetideidae, since *Paraeuchaeta* species often had higher C/N ratios of 7.0 to 9.9 than those of aetideid copepods with 3.5 to 5.5. These differences in C/N ratio were linked to generally higher lipid levels especially in older *Paraeuchaeta* stages (C5 and C6; for details see Laakmann et al. 2009). In *P. antarctica* and *P. barbata*, the ontogenetic increase in C/N ratio (related to an ontogenetic accumulation of lipid) further reduced ontogenetic differences in lipid-normalised $\delta^{13}C'$ as compared to $\delta^{13}C$ values, since adults with high C/N ratios were stronger affected by the normalisation than lipid-poor younger stages. However, lipid-normalised $\delta^{13}C'$ data generally showed equal similarities and differences between the species, indicating that the observed trends were independent of potential differences in lipid content and, hence, C/N ratio.

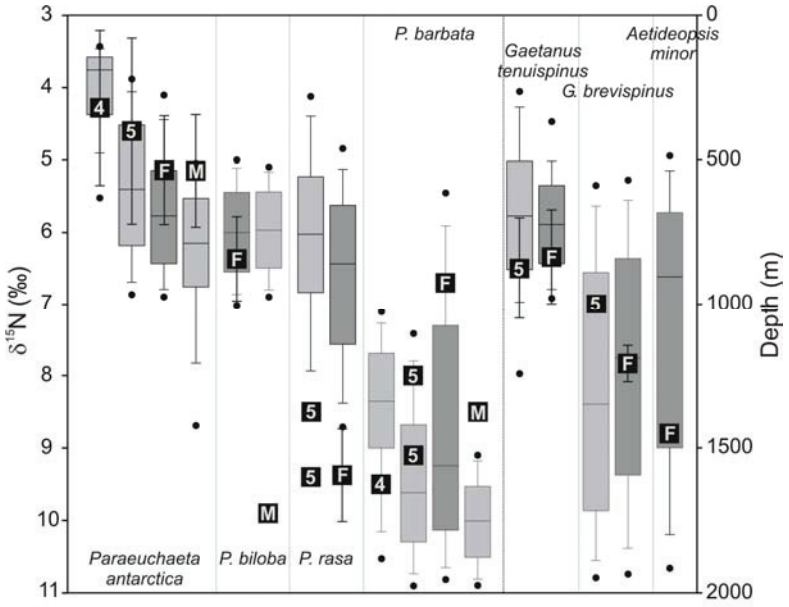

Figure 2

Stage- and species-specific $\delta^{15}N$ (black squares, left y-axis) and main vertical distribution (grey box plots, right axis) of *Paraeuchaeta* spp. and aetideid *Gaetanus* spp. as well as *Aetideopsis minor*; 4: copepodite stage 4 (C4), 5: C5, F: females, M: males

SI ratios of nitrogen ($\delta^{15}N$) ranged from 3.0 to 9.9‰ (Table 2, Fig. 2). As compared to $\delta^{13}C$, species-specific differences were more pronounced. Among Aetideidae, the bathypelagic *G. brevispinus* (main vertical distribution range at 840-1720 m) and *A. minor* (680-1500 m) had some higher $\delta^{15}N$ values of 7.6±0.5‰ and 8.8‰, respectively, than the mesopelagic (510-880 m) *G. tenuispinus* with 6.4±0.7‰ (Table 2, Fig. 2). *P. rasa* (560-1140 m) and *P. barbata* (except one female) (1170-1880 m) showed maximum $\delta^{15}N$ values of 8.0 to 9.9‰, while *P. biloba* females (610-890 m) had intermediate $\delta^{15}N$ ratios of 6.4±0.6‰, similar to the one sample of *P. barbata* females (1080-1780 m). In contrast, the epi- to mesopelagic *P. antarctica* (150-940 m) was characterised by the lowest $\delta^{15}N$ values of 3.0 to 6.4‰. Stage-specific differences in $\delta^{15}N$ of *P. antarctica* were very low and not significant (One way ANOVA with Tukey Post Hoc test). Copepodite stages C4 had a mean $\delta^{15}N$ value of 4.3±1.1‰, while copepodids C5 and especially adult females were more enriched in $\delta^{15}N$ with 4.6±1.3‰ and 5.1±0.8‰, respectively. Adult males showed a mean $\delta^{15}N$ of 5.2±0.8‰, rather similar to females. It should be considered that *P. antarctica* showed an ontogenetic partitioning of the water column. Copepodids C4 mainly occurred from 150-340 m, while C5 inhabited 380-800 m and adults were mainly sampled between 540 and 940 m (Fig. 2).

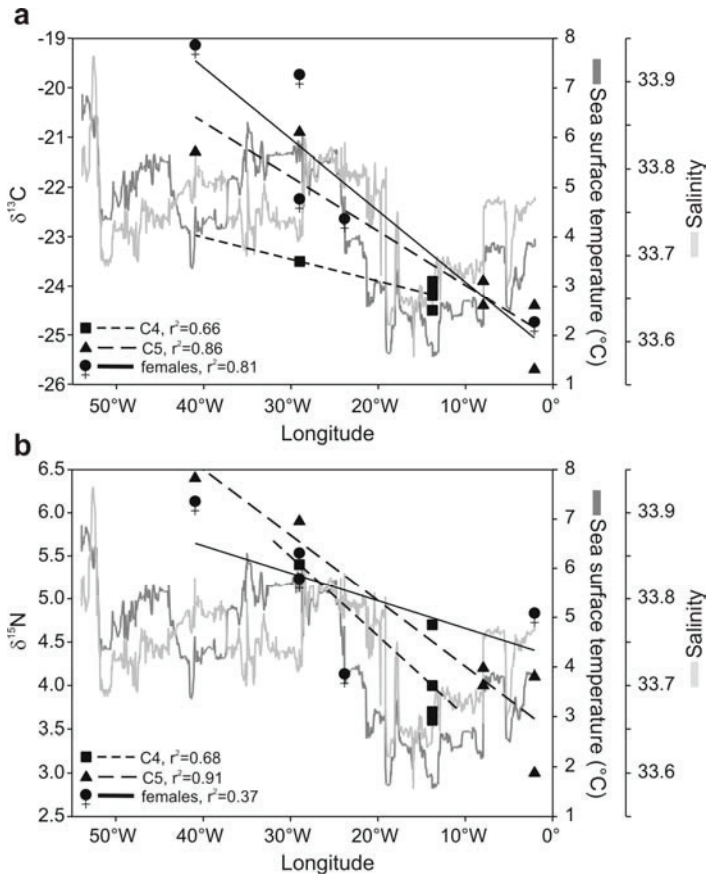

Figure 3

Paraeuchaeta antarctica: stable isotope ratios of a) $\delta^{13}C$ versus longitude (left axis) and b) $\delta^{15}N$ (left axis) versus longitude for copepodite stage C4 (squares), C5 (triangles) and adult females (♀) together with sea surface temperature (right axis) and salinity (superimposed right axis)

Comparing $\delta^{15}N$ of the different species, including only C5 and females the large epi- to mesopelagic distributed *P. antarctica* had a lower $\delta^{15}N$ than the smaller mesopelagic *P. rasa* and the large bathypelagic *P. barbata* ($p<0.001$) as well as the meso- and bathypelagic *Gaetanus* species ($p<0.001$). The mesopelagic *P. rasa* had the highest $\delta^{15}N$ and significantly differ from *P. antarctica* ($p<0.001$), *P. biloba* ($p<0.05$) and *G. tenuispinus* ($p<0.001$) (One way ANOVA with Tukey Post Hoc test).

In copepodite stages C4 and C5 as well as in adult females of *P. antarctica* longitudinal differences in both SI signatures $\delta^{13}C$ and $\delta^{15}N$ were detected and supported by generally high correlation coefficients, although the data set for C4 was bimodal (Fig. 3). $\delta^{13}C$ and $\delta^{15}N$ decreased with longitude from west to east across the Atlantic sector of the Southern Ocean from 41° to 2°W (Fig. 3a, b). This trend was evident in the uncorrected $\delta^{13}C$ and the lipid-normalised $\delta^{13}C$' values. Comparing molar C/N ratios with SST (as an indicator of frontal regimes), higher C/N ratios at low SST were only found for C4 stages of *P. antarctica*, while there was no relationship between the two parameters in C5 and females (Fig. 4). These results indicate that the longitudinal trend in $\delta^{13}C$ was not caused by differences in lipid content of the specimens but was rather linked to substantial changes in the SST along the transect (Fig. 3).

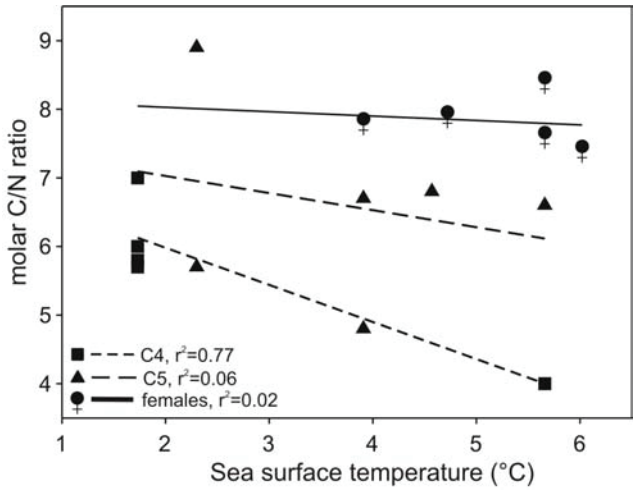

Figure 4

Paraeuchaeta antarctica: C/N ratio versus sea surface temperature for copepodite stages C4 (squares), C5 (triangles) and adult females (♀)

DISCUSSION

Depth gradient

SI can provide insight into trophic relationships and food-web interactions especially for deep-sea species, where alternative approaches such as feeding experiments are hardly practicable. The present study showed a depth-dependent increase in $\delta^{15}N$ from epi-/mesopelagic to bathypelagic carnivorous *Paraeuchaeta* species. An exception is the mesopelagic *P. rasa* oc-

curring in the same depth range like *P. biloba*, exhibiting higher $\delta^{15}N$ values. This result suggest that *P. rasa* feeds on prey items which are in a higher trophic level than those of *P. biloba* or that *P. rasa* feeds more carnivorously than *P. biloba* which is in line with higher values of the fatty acid trophic biomarker for carnivorous feeding 18:1(n-9) in this species (Laakmann et al. 2009). The difference in $\delta^{15}N$ is regarded as a difference in the ecological niche allowing co-existence in the same depth strata with minimised inter-specific competition of these congeners similar in body size. An increase in $\delta^{15}N$ of large mesozooplankton and in calanoid copepods with increasing depth was also demonstrated in the Mediterranean (Polunin et al. 2001; Koppelmann et al. 2009). These results can be explained by a general increase in trophic level with increasing depth (Sugisaki and Tsuda 1995) and a higher degree of carnivory in deep-sea species, related to the reduced availability of phytoplankton, phyto-detritus and herbivorously feeding prey with increasing depth (Auel and Hagen 2002 for Arctic zooplankton). Higher $\delta^{15}N$ in zooplankton with increasing depth can on the one hand be a result of on average more trophic steps between the consumers and basal materials at depth and on the other hand on a ^{15}N enrichment of the source material of the food web at depth (Polunin et al. 2001).

For omnivorous Aetideidae, feeding on sinking particles, the higher $\delta^{15}N$ may be explained by $\delta^{15}N$ of particulate organic matter (POM) values increasing with greater depth due to biodegradation (microbial consumption) in the water column (Mintenbeck et al. 2007), an enrichment of ^{15}N in sinking particles (Altabet 1988) and a higher δ^{15} of POM in the deep sea (Koppelmann et al. 2009). In contrast, other studies show a fairly constant $\delta^{15}N$ value of POM below the euphotic zone (Holmes et al. 1999 in Bergmann et al. 2009).

Meso- and bathypelagic omnivores and carnivores play an important role in the vertical flux from the surface to the deep sea. They feed on particles as well as on other organisms and produce faecal pellets. For herbivorous copepods it was shown that faecal pellets were highly depleted in ^{13}C and to a lower degree in ^{15}N relative to food ingested, resulting in a variability of $\delta^{13}C$ in marine POM (Tamelander et al 2006). Also in krill, $\delta^{15}N$ of faecal pellets were lower than those for the ingested copepods (Schmidt et al. 2003). In contrast, other studies showed that faecal material of zooplankton in the upper ocean is slightly enriched in $\delta^{15}N$ relative to food consumed (Checkley and Entzeroth 1985; Altabet and Small 1990) and depleted compared to body tissue $\delta^{15}N$ (Altabet and Small 1990; Montoya et al. 2002). In addition to the faecal pellet production, selection by macrozooplankton feeding on components with distinct $\delta^{15}N$ would alter $\delta^{15}N$ values between primary produced organic matter and sinking particles (Altabet and François 1994b). Thus, animals play an important role in cycling and transport of organic matter in the ocean whereby their feeding behaviour and physiology represent critical components of secondary production and the movement of elements through the food web, as proposed for marine animals in general (Montoya et al. 2002).

Longitudinal gradient

The longitudinal trend in $\delta^{13}C$ and $\delta^{15}N$ of *P. antarctica* was most likely related to changes in the SST associated with a northward extension of the APF along the eastern part of the transect. Changes in $\delta^{13}C$ and $\delta^{15}N$ in the copepod may be a result of changes in POM SI signatures by crossing the APF, as it was demonstrated at frontal zones in the Southern Ocean including the APF (François et al. 1993; Altabet and François 1994a; Trull and Armand 2001). The variations in $\delta^{15}N$ of POM along the lines of latitude are thus expected as a result from changes in the position and extent of frontal systems in the Southern Ocean (Altabet and François 1994b), with changes in phytoplankton composition, size and growth rate across the APF may have contributed to SI depletion (François et al. 1993). In general, the APF is characterised by elevated phytoplankton biomass and productivity as well as seasonal changes in $\delta^{13}C$ of POM during and after the phytoplankton bloom (Dehairs et al. 1997).

Generally, changes in $\delta^{15}N$ in zooplankton reflect shifts in $\delta^{15}N$ of particulate nitrogen at the base of the food chain (e.g. Goering et al. 1990) with seasonal changes during and after the spring bloom (Montoya et al. 1992 in Montoya 1994). However, seasonal changes in zooplankton are more slowly than those in phytoplankton and depend on the amount of trophic transfers between the consumer and the food web base as well as on the size (and turnover rate) and life span of the organisms (Montoya 1994). For the carnivorous, epi- to mesopelagic *P. antarctica,* which is large in size (body length, 8.4-9.9 mm, Park 1994), we assume that the longitudinal decrease in SI is a result of general changes in the food chain basis within the Antarctic Polar Frontal Zone rather than seasonal changes.

The detection of regional differences in SI signatures in the predatory epi- to mesopelagic *P. antarctica* thus indicates that fractionation processes by primary producers in the surface layer propagate into deeper layers and may affect even mesopelagic species directly. Active downwelling of surface water at the APF and, hence, a strong pelago-benthic coupling may accelerate the transmission of SI signals into the deep sea. $\delta^{15}N$ of POM in the Southern Ocean ranged from -6 to 3.5‰ (Rau et al. 1991b; Frazer 1996; Nyssen et al. 2002; Schmidt et al. 2003), with 0.8‰ at the APF and -6‰ in the Weddell Sea (Schmidt et al. 2003), thus showing a high variability which might also be reflected in epi- to mesopelagic carnivores, like *P. antarctica*. The differences in SI signatures across oceanic frontal systems and different regions in the Southern Ocean were already used for the determination of foraging locations and migration routes of right whales, albatrosses, storm-petrels, and penguins (Best and Schell 1996; Cherel et al. 2000; Gladbach et al. 2007; Cherel and Hobson 2007).

Published data on SI signatures of deep-sea copepods are generally scarce. Our lipid-corrected $\delta^{13}C$' for *P. antarctica* from the APF were similar to those from the Antarctic Peninsula (Schmidt et al. 2003), while individuals from the Weddell Sea (Rau et al. 1991a) and

from the Ross Sea (Wada et al. 1987) had generally lower $\delta^{13}C$ than our samples. Since data for Weddell and Ross Sea samples were not lipid-corrected and had higher C/N ratios (Weddell Sea, Rau et al. 1991a), higher lipid levels may have contributed to lower $\delta^{13}C$ values. In general, the available data on SI signatures of *P. antarctica* from high-Antarctic regions and our samples of the APF are in line with a latitudinal depletion in $\delta^{13}C$. Similar latitudinal trends have been recorded for Antarctic zooplankton in general (Schmidt et al. 2003). The $\delta^{15}N$ values of *P. antarctica* in the present study were generally lower than those of individuals sampled at the Antarctic Peninsula (Schmidt et al. 2003), in the Weddell Sea (Rau et al. 1991a) or in the Ross Sea (Wada et al. 1987).

In conclusion, the present study demonstrates vertical differences in SI signatures of omnivores and carnivores in the polar deep sea. Furthermore variations in SI across the APF in an epi- to mesopelagic carnivore possibly reflect sharp changes in SI signatures on the food web baseline across oceanic frontal systems.

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CHAPTER IV

Evolution in the deep sea:
Biological traits, ecology and phylogenetics of pelagic copepods

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EVOLUTION IN THE DEEP SEA: BIOLOGICAL TRAITS, ECOLOGY AND PHYLOGENETICS OF PELAGIC COPEPODS

Silke Laakmann, Holger Auel and Marc Kochzius

ABSTRACT

Biodiversity of the deep-sea fauna received a lot of interest in the last decades, mainly focussing on benthic organisms. In contrast, studies on zooplankton organisms of the deep-ocean pelagic zone are relatively scarce. For that reason, the present study focused on copepods of the two clausocalanoid families Euchaetidae and Aetideidae, which are well represented in the deep-sea pelagic realm. Phylogeny of the two sister families, mainly from Arctic and Antarctic regions, was examined together with species-specific biological data on feeding habits and preferences as well as energy storage and reproductive strategy. Relationships were analysed based on fragments of nuclear coding rDNA 18S and 28S genes, the non-coding internal transcribed spacer 2 (ITS2), and the mitochondrial cytochrome C oxidase subunit I gene (COI). Both 18S and 28S did not provide phylogenetic information with low genetic differences between genera of 0.30% in 18S and 0.55% in 28S. ITS2 resolved relationships on genera and species level with genetic differences ranging from ≤3.7% to 10.2%. Highest distances and thus, separation on species and individual level were obtained by COI sequences with maximum genetic difference of ≤5.0% on intra-specific and 19.4% on inter-specific level. Relationship could be resolved between aetideid species but not within the euchaetid genus *Paraeuchaeta*. *Paraeuchaeta* spp. (as well as the second genus *Euchaeta*) were similar in lipid-specific energy storage, reproductive strategy, as well as feeding habit and preference. In contrast, Aetideidae were highly diverse, comprising a variety of characteristics including those of *Paraeuchaeta*, and were generally similar within genus. These results, together with the indication of a fast radiation within Euchaetidae, support the close relationship of the two families. Closely related species (congeners), similar in biological and ecological characteristics, generally occurred in different depth horizons, suggesting that vertical partitioning of the water column represents an important mechanism in speciation processes for these deep-sea copepods. High COI diversity among Arctic and Antarctic individuals of mesopelagic cosmopolitan *Gaetanus tenuispinus* and bipolar *Aetideopsis minor* suggest different geographic forms. In contrast, Arctic and Antarctic individuals of deeper distributed bathypelagic cosmopolitans *G. brevispinus* and *P. barbata* emerged as very similar, indicating that driving forces for shaping populations leading to speciation were less pronounced in bathypelagic than in mesopelagic depths.

KEYWORDS
Pareuchaeta; *Chiridius*; wax ester

INTRODUCTION

Reduced environmental gradients in the deep sea compared to other ocean habitats provide no absolute barriers, which can act as barriers for genetic isolation. Speciation may thus be linked to environmental tolerance and dispersal abilitiy of species (Wilson and Hessler 1987). Ecological and physical factors are suggested to be important isolation mechanisms in the deep-sea realm, leading to speciation, as suggested for benthic deep-sea amphipods (France and Kocher 1996). However, studies on pelagic deep-sea organisms are relatively rare (Miya and Nishida 1997).

Recent results based on molecular phylogenetic analyses have revealed an impressive cryptic biodiversity, which is well structured in the open ocean and thus question the long-standing paradigm that oceanic species generally occupy broad distribution ranges (ocean basins to entire oceans), resulting in an overall low biodiversity (Wilson and Hessler 1987). These genetic studies suggest that species diversity is considerably higher in the pelagic realm than inferred from many morphological taxonomies and cryptic species (i.e. species that can not be distinguished on classical morphological characters) biodiversity is high and well structured in the open ocean (Norris 2000). Cryptic species are common in the oceanic realm, for example in epipelagic organisms like foraminifers (de Vargas et al. 1999), copepods (Bucklin et al. 2000, Goetze 2003, Nuwer et al. 2008), euphausiids (Zane et al. 2000, Papetti et al. 2005), and chaetognaths (Peijnenburg et al. 2004). Large genetic differences also exist within circum-globally distributed deep-sea fish, like in the very abundant meso- and bathypelagic genus *Cyclothone* (family Gonostomatidae) with several cryptic allopatric lineages which have split in the absence of discernible barriers (Miya and Nishida 1997). Such results imply that the oceanic and even deep-sea biodiversity may have been seriously underestimated and that the physically unstructured and homogeneous environment bears isolated, genetically structured populations. Recent expeditions exploring the deep-sea benthic fauna in the Southern Ocean revealed high biodiversity, describing hundreds of new species (e.g. Brandt et al. 2007a, b, Kaiser et al. 2007, Tchesunov 2008, Markhaseva and Schulz 2008). However, mechanisms that have structured genetic diversity and driven speciation processes remain obscure.

To elucidate mechanisms involved in speciation processes in the pelagic deep-sea realm as well as the phylogeny of closely related taxa, the two clausocalanoid copepod families Euchaetidae and Aetideidae were chosen as case studies. These two families share several morphological characters (Park 1994a) and are suggested to have a deep-living copepod as

common recent ancestor (Braga et al. 1999). The deep-living mode is evident in most of the species inhabiting meso- to bathypelagic depths as well as some are epi- or benthopelagic (Park 1994a, Markhaseva 1996, Auel 1999). Representatives of both families are found throughout the world's oceans, especially co-occurring in deep oceanic waters and polar regions, and are important components of pelagic communities (e.g. Båmstedt 1978, Hopkins 1987, Yen 1991, Øresland 1995, Auel 1999, Yamaguchi and Ikeda 2001, Laakmann et al. 2009a, b). For example, up to 14 *Paraeuchaeta* species co-exist in the Southern Ocean around South Georgia (Ward and Wood 1988) and in North Atlantic Rockall Trough (Mauchline 1995), respectively. In the Arctic Ocean, Greenland Sea and Fram Strait four species can be found (Auel 1999, Laakmann et al. 2009a) and three species co-occurred in the Western Subarctic Pacific Ocean (Yamaguchi and Ikeda 2001, 2002). Co-existence of Aetideidae was described for the Arctic Ocean with eight species (Markhaseva 1984) and five species for Greenland Sea and Fram Strait (Richter 1994, 1995, Seiler and Brandt 1997). Competition between species may occur when resources are limited, as expected for the deep-sea realm, since availability of organic matter strongly decreases with increasing depth (e.g. Karl et al. 1988). Competition in aetideid and euchaetid congeners is first of all minimised by vertical partitioning of the water column (Richter 1995, Yamaguchi and Ikeda 2001, Laakmann et al. 2009a, b), while species within the same depth stratum have different feeding strategies and preferences (Laakmann et al. 2009a, b). However, these mechanisms indeed allow co-occurrence of species but speciation processes within the vast deep-sea environment are still enigmatic.

This study aims to compare the phylogenetic relationships with the biology (e.g. vertical and geographic distribution, lipid composition, feeding behaviour and habits as well as reproductive strategies) of species from the two families Euchaetidae and Aetideidae. As a result from this comparison, this study aims to reveal evolutionary mechanisms of speciation in these pelagic deep-sea copepods, which are used as model-species in order to get new insights into evolutionary processes in the deep-sea pelagic realm.

MATERIALS AND METHODS

Sample collection

Deep-sea copepods of the two calanoid families Euchaetidae and Aetideidae were collected from the Atlantic sector of Southern Ocean, the Arctic Fram Strait and the Greenland Sea as well as off Namibia (Fig. 1, Table 1). Sampling in Fram Strait allowed to collect both Arctic and boreal-Atlantic organisms in close proximity because of the two opposing water currents of the warm West Spitsbergen Current flowing northward in the eastern part and the cold East Greenland Current flowing southward in the western part (Hop et al. 2006). In the Southern Ocean samples were collected in the Antarctic and the sub-Antarctic proper, divided by the Antarctic Polar Front, which represents a dispersal barrier for many organisms living near the

surface (Deacon 1982 and references therein, Pakhomov et al. 2000). The copepods were collected by stratified multiple opening/closing net hauls (Hydro-Bios Multinet Midi, mouth opening 0.25 m^2; mesh size 180-310 µm) and taxonomic identification was carried out on the basis of taxonomic guides by Park (1994a) and Markhaseva (1996). Species were deep-frozen at -80°C or preserved in ≥99.8% ethanol.

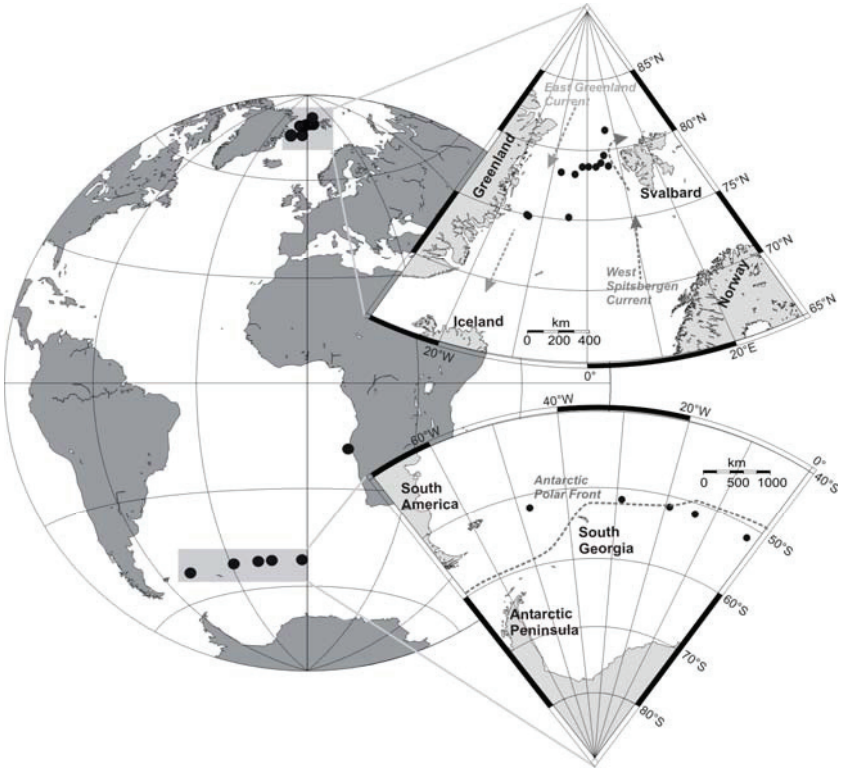

Figure 1

Sampling locations in Arctic Fram Strait and Greenland Sea, Southern Ocean and at Namibian coast

DNA extraction, PCR and sequencing

Genomic DNA of specimens was extracted using the QIAGEN DNeasy tissue kit following the manufacture's protocol. Nuclear markers were amplified using specific primer pairs, namely the primers 18S-E and 18S-639R for a fragment of the 18S gene (18S; Hillis and Dixon 1991, Bucklin et al. 2003), D9/10 Forward and D9/10 Reverse for a fragment of the 28S gene (28S; Zardoya et al. 1995), as well as ITS3F and ITS10R for the internal transcribed spacer 2 (ITS2; White et al. 1990 in Goetze 2003) (Table 2). A fragment of the mitochondrial cytochrome C oxidase subunit I gene (COI) could be amplified from the majority of samples with the universal primers LCO1490 and HCO2198 (Folmer et al. 1994, Table 2). Because universal primers did not provide PCR products for all investigated species, new primers for the amplification of COI were designed (Table 2). For *Paraeuchaeta barbata* and *P. polaris* the primers PantFor and PantRev were designed, and additionally for the latter species as well as for *P. rasa* the

primers EantFor and EantRev. The primers AetidFor and AetidRev were designed for the amplification of COI in the aetideids *Chiridius obtusifrons* and *Aetideopsis rostrata*.

Table 1

List of species, sampling location and sampling depth; P: *Paraeuchaeta*, A: *Aetideopsis*, C: *Chiridius*, G: *Gaetanus*

Species	Sampling location			Sampling depth	Described by
	region	latitude	longitude		
Euchaetidae					
P. antarctica	Southern Ocean	51°29´S	29°00´W	200-500 m	Giesbrecht (1902)
P. biloba	Southern Ocean	51°51´S	47°45´W	500-1000 m	Farran (1929)
	Southern Ocean	51°29´S	29°00´W	500-1000 m	
	Southern Ocean	51°33´S	02°05´W	200-500 m	
P. rasa	Southern Ocean	51°29´S	29°00´W	500-1000 m	Farran (1929)
	Southern Ocean	51°30´S	19°08´W	1000-1500 m	
P. norvegica	Fram Strait	78°50´N	00°24´E	200-500 m	Boeck (1872)
	Fram Strait	81°22´N	06°52´E	82-200 m	
	Fram Strait	81°22´N	06°52´E	500-915 m	
P. polaris	Fram Strait	78°50´N	06°20´E	1000-1500 m	Brodsky (1950)
	Fram Strait	79°36´N	05°16´E	500-1000 m	
	Fram Strait	79°36´N	05°16´E	1000-1500 m	
P. glacialis	Greenland Sea	74°58´N	13°36´W	0-190 m	Hansen (1886)
	Greenland Sea	74°55´N	13°07´W	0-400 m	
P. barbata	Southern Ocean	51°33´S	02°05´W	1500-2000 m	Brady (1883)
	Greenland Sea	75°10´N	04°09´W	0-1000 m	
Aetideidae					
A. carinata	off Namibia	18°26´S	11°22´E	400-500 m	Bradford (1969)
A. rostrata	Fram Strait	78°49´N	01°27´W	1500-2000 m	Sars (1903)
	Fram Strait	78°16´N	03°31´W	1000-1500 m	
A. minor	Southern Ocean	51°33´S	02°05´W	1500-2000 m	Wolfenden (1911)
	Southern Ocean	51°30´S	19°08´W	1000-1500 m	
	Fram Strait	81°22´N	06°52´E	200-500 m	
	Fram Strait	79°36`N	05°16´E	500-1000 m	
	Fram Strait	78°49´N	01°27´W	500-1000 m	
C. obtusifrons	Fram Strait	79°04´N	04°11´E	500-1000 m	Sars (1902)
	Fram Strait	81°22´N	06°52´E	500-915 m	
	Fram Strait	78°21´N	07°29´W	50-106 m	
G. brevispinus	Fram Strait	78°50´N	02°35´E	1000-1500 m	Sars (1900)
	Fram Strait	79°36`N	05°16´E	500-1000 m	
	Southern Ocean	51°30´S	13°47´W	50-100 m	
	Southern Ocean	51°51´S	47°45´W		
	Southern Ocean	51°30´S	19°08´W		
G. tenuispinus	Fram Strait	79°36`N	05°16´E	200-500 m	Sars (1900)
	Fram Strait	78°50´N	2°35´E	200-500 m	
	Southern Ocean	51°30´S	19°08´W	500-1000 m	
	Southern Ocean	51°30´S	13°47´W	500-1000 m	
	Southern Ocean	51°30´S	13°47´W	500-1000 m	
Outgroup: Calanidae					
Calanus hyperboreus	Fram Strait	78°49´N	01°27´W	0-200 m	

Composition of the PCR solution was identical for all markers and primer pairs, except for the primer pair LCO1490/HCO2198. The PCR reactions of a 50 µl volume contained 5 µl 10x PCR buffer (Molzym 10x PCR Buffer basic without $MgCl_2$), 0.1 µmol $MgCl_2$ (Molzym), 0.01 µmol

dNTPs (dNTPmix-OLS®), 5 pmol of each primer, 1 U Taq polymerase (Moltaq) and on average 80 ng of DNA template. The PCR solution for the primer pair LCO1490/HCO2198 was composed of: 5 µl 10x PCR buffer, 0.1-0.125 µmol $MgCl_2$, 0.01 µmol dNTPs, 5-15 pmol of each primer, 1-1.25 U Taq polymerase and on average 40-200 ng of DNA template. PCR amplification was carried out in an Eppendorf Masterthermocycler gradient with heated lid (Eppendorf, Hamburg). For the 18S and all COI primer pairs, the initial step was at 94°C (120 s), followed by 38 cycles for 18S and 40 cycles for COI of denaturation at 94°C (60 s), specific annealing temperature (see Table 2) and elongation at 72°C (180 s). The final step was at 72°C (300 s). ITS2 was amplified using 95°C (120 s) in the initial step, followed by 35 cycles of 95°C (30 s) for denaturation, 55°C (30 s) for annealing, and 72°C (60 s) for elongation. The final step was at 72°C (300 s). The initial step for 28S was 90°C (120 s). Denaturation at 90°C (120 s), annealing at 49°C (60 s), and elongation at (72°C (60 s) was carried out in 35 cycles, followed by a final step at 72°C (300 s).

Table 2

Different genetic markers and specific primer pairs with respective annealing conditions

Marker	Primer name	Sequence in 5´- 3´ direction	Reference	Annealing temperature [°C]	Annealing time [s]
18S	18S-E	CTG GTT GAT CCT GCC AGT	Hillis and Dixon (1991)	55	120
	18S-639R	AAA CCT CTG GCA AAA CTA CG	Bucklin et al. (2003)		
28S	D9/10 Forward	CGG CGG GAG TAA CTA TGA CTC TCT TAA GGT	Zardoya et al. (1995)	49	60
	D9/10 Reverse	CCG CCC CAG CCA AAC TCC CCA	Zardoya et al. (1995)		
ITS2	ITS3F	GCA TCG ATG AAG AAC GCA GC	White et al. (1990)	55	30
	ITS10R	TAC GGG CCT ATC ACC CTC TAC G	Goetze (2003)		
COI	LCO1490	GGT CAA CAA ATC ATA AAG ATA TTG G	Folmer et al. (1994)	42-45	120
	HCO2198	TAA ACT TCA GGG TGA CCA AAA AAT CA	Folmer et al. (1994)		
	PantFor	ATT CGG TTA GAG TTR GGY CAA GCA GG	This study	52	120
	PantRev	TGC TGG TAY AAA ATA GGR TCY CCC CC	This study		
	EantFor	GGG TGA CCA AAA AAT CAA AAT ARR TGC TGG	This study	52	120
	EantRev	ATA GCA GGW GCW TGA TCA GGT ATA G	This study		
	AetidFor	GTT AAA AGT ATA GTR ATA GCY CCM GC	This study	45-48	120
	AetidRev	TTC GAT TAG AAT TAG GTC AAG CAG G	This study		

An electrophorese with 5 µl of each PCR product was conducted in a 1% agarose gel, mixed with 5 µl 3x loading dye (Fermentas). DNA fragments were visualised by ethidium bromide staining. PCR products were purified using peqGOLD Cycle-Pure Kit (peqLab). Both strands were sequenced using the BigDye™ terminator chemistry and an ABI Prism 3730xl automated sequencer (Applied Biosystems). No sequence ambiguities were found on both strands of ITS2, indicating that ITS2 did not exist as multiple copies within the genome.

Editing, alignment and phylogenetic analysis

Sequences were edited using the software SeqMan (ver. 4.05; DNASTAR Inc. 1989-2000) and checked for orthology with BLAST (Altschul et al. 1990) against GenBank. A multiple alignment

of sequences was performed using the software CLUSTAL W (Thompson et al. 1997) as implemented in the software BioEdit (Hall 1999). The best-fit evolutionary model for tree construction was determined by using the software Modeltest (ver. 3.06; Posada and Crandall 1998). Maximum Parsimony (MP), Maximum Likelihood (ML), and Neighbour Joining (NJ) analyses were preformed with the software PAUP* (ver. 4.0b10; Swofford 1998), considering the calculated model (using corrected Akaike Information) for the two latter algorithms. Gaps in ITS2 sequences were treated as both, missing data and 5th character state in MP analysis. Statistical testing of the confidence in the nodes of the trees was done by bootstrap analysis with 1000 replicates for NJ and MP and 100 replicates for ML analyses.

Cluster analysis of lipid composition

Data on lipid composition were taken from Laakmann et al. (2009a, b) together with additional data from copepods sampled in Greenland Sea. Cluster analysis was performed using the software Primer (ver. 5, Clarke and Gorley 2001) on lipid composition, composed of fatty acids and fatty alcohols >4%. Prior to cluster analysis, a Bray-Curtis similarity matrix was carried out with samples transformed by square root. On this basis cluster analysis with group-average linking of species-specific lipid composition was performed.

RESULTS

Phylogenetic analysis

Phylogenetic analyses were performed with seven species of the euchaetid genus *Paraeuchaeta* and three, two and one species from the aetideid genera *Aetideopsis*, *Gaetanus* and *Chiridius*, respectively (Table 1). The analyses were performed with alignments of 668, 365, 510 and 487 base pairs for 18S, 28S, ITS2 and COI, respectively. For 18S and 28S each reconstruction was based on the Kimura model (Kimura 1980) and for ITS2 on the basis of the Tamura Nei model with equal frequencies (TrNef, Tamura and Nei 1993), a proportion of invariable sites (I) of 0.40 and a gamma distribution shape parameter (G) of 0.55. Phylogenetic analysis of COI was based on the Hasegawa-Kishino-Yano model (HKY, Hasegawa et. al. 1985) with I = 0.58 and G = 0.89. Markers were not combined for analysis due to significant differences in tree topology, based on the Shimodaira-Hasegawa (Shimodaira and Hasegawa 1999, Goldman et al. 2000) and Kishino-Hasegawa tests (Kishino and Hasegawa 1989).

The phylogenetic analyses of 18S and 28S did not support separation of families or even genera and species for all three used algorithms (NJ, MP and ML), due to low phylogenetic resolution. 18S analysis generated three clades that received only weak support by bootstrap analysis: (1) the genus *Paraeuchaeta* (NJ: 64, MP: 63 and ML: 63), (2) aetideid genera *Aetideopsis* and *Chiridius* (68, 62, 57) and aetideid genus *Gaetanus* showing polytomy on the

main branch. The phylogenetic tree based on 28S only slightly supported the clade of the family Aetideidae with low bootstrap values (64, 60, 66). Species of the same genus did not show genetic differences both in 18S and 28S sequences. Genetic differences in 18S were higher between species of the genera *Paraeuchaeta* and *Aetideopsis*/*Chiridius* (0.30%) than to the genus *Gaetanus*, which differed from all other genera by 0.15% (Table 3a). Genetic difference in 28S sequences was 0.27% between the genera *Paraeuchaeta*, *Gaetanus,* and *Aetideopsis*. *Gaetanus* and *Aetideopsis* showed the same distance to *Chiridius* (Table 3a). Greatest distance between genera was found between *Paraeuchaeta* and *Chiridius* with 0.55%.

Table 3

Inter-specific genetic pairwise maximum likelihood distances (%) in a) 28S (above diagonal) and 18S (below diagonal; italics) sequences, and b) ITS2 (above diagonal) and COI sequences (below diagonal: italics). Intra-specific differences were marked as bold figures. Abbreviations of horizontal species name according to vertical species list

a)

	Pa	*Pb*	*Pbil*	*Pg*	*Pn*	*Pp*	*Pr*	*Ac*	*Am*	*Ar*	*Co*	*Gb*	*Gt*
P. antarctica (Pa)	-	0	0	0	0	0	0	0.27	0.27	0.27	0.55	0.27	0.27
P. barbata (Pb)	*0*	-	0	0	0	0	0	0.27	0.27	0.27	0.55	0.27	0.27
P. biloba (Pbil)	*0*	*0*	-	0	0	0	0	0.27	0.27	0.27	0.55	0.27	0.27
P. glacialis (Pg)	*0*	*0*	*0*	-	0	0	0	0.27	0.27	0.27	0.55	0.27	0.27
P. norvegica (Pn)	*0*	*0*	*0*	*0*	-	0	0	0.27	0.27	0.27	0.55	0.27	0.27
P. polaris (Pp)	*0*	*0*	*0*	*0*	*0*	-	0	0.27	0.27	0.27	0.55	0.27	0.27
P. rasa (Pr)	*0*	*0*	*0*	*0*	*0*	*0*	-	0.27	0.27	0.27	0.55	0.27	0.27
A. carinata (Ac)	*0.30*	*0.30*	*0.30*	*0.30*	*0.30*	*0.30*	*0.30*	-	0	0	0.27	0	0
A. minor (Am)	*0.30*	*0.30*	*0.30*	*0.30*	*0.30*	*0.30*	*0.30*	*0*	-	0	0.27	0	0
A. rostrata (Ar)	*0.30*	*0.30*	*0.30*	*0.30*	*0.30*	*0.30*	*0.30*	*0*	*0*	-	0.27	0	0
C. obtusifrons (Co)	*0.30*	*0.30*	*0.30*	*0.30*	*0.30*	*0.30*	*0.30*	*0*	*0*	*0*	-	0.27	0.27
G. brevispinus (Gb)	*0.15*	*0.15*	*0.15*	*0.15*	*0.15*	*0.15*	*0.15*	*0.15*	*0.15*	*0.15*	*0.15*	-	0
G. tenuispinus (Gt)	*0.15*	*0.15*	*0.15*	*0.15*	*0.15*	*0.15*	*0.15*	*0.15*	*0.15*	*0.15*	*0.15*	*0*	-

b)

	Pa	*Pb*	*Pbil*	*Pg*	*Pn*	*Pp*	*Pr*	*Ac*	*Am*	*Ar*	*Co*	*Gb*	*Gt*
P. antarctica (Pa)	**0/*0***	3.3	3.5	2.0	2.6	2.2	2.2	8.3	10.2	9.7	9.5	8.1	8.4
P. barbata (Pb)	*16.9*	**0/*0.5***	3.5	2.0	2.7	2.2	1.7	7.7	9.8	9.4	9.4	7.9	8.9
P. biloba (Pbil)	*16.6*	*13.2*	**0/*0.1***	3.1	3.7	3.3	3.3	7.8	9.1	9.1	10.1	8.7	8.0
P. glacialis (Pg)	*15.6*	*12.8*	13,0	**0/*0.1***	0.6	0.6	1.0	7.3	9.5	9.1	8.9	7.3	7.7
P. norvegica (Pn)	*16.2*	*14.1*	*15.4*	*11.3*	**0/*0.6***	0.8	1.6	7.5	9.3	8.9	9.1	7.5	8.0
P. polaris (Pp)	*14.0*	*11.1*	*11.5*	*9.1*	*12.9*	**0/*0.2***	1.2	7.7	9.5	9.1	9.3	7.7	8.1
P. rasa (Pr)	*14.8*	*10.1*	*11.7*	*8.5*	*12.9*	*5.4*	**0/*0***	7.3	9.5	9.1	8.9	7.4	8.2
A. carinata (Ac)	*19.1*	*18.2*	*14.8*	*16.1*	*19.7*	*15.5*	*16.9*	**0.1/*0***	4.0	3.8	5.3	7.0	8.6
A. minor (Am)	*17.8*	*18.1*	*14.6*	*15.1*	*17.9*	*14.4*	*14.4*	*14.3*	**0/*1.7***	0.8	7.5	8.3	10.0
A. rostrata (Ar)	*19.3*	*17.3*	*16.6*	*15.6*	*19.4*	*15.7*	*13.4*	*14.3*	*10.4*	**0/*0.3***	7.3	8.0	9.7
C. obtusifrons (Co)	*18.3*	*17.4*	*14.0*	*14.3*	*17.3*	*15.0*	*13.8*	*13.4*	*13.6*	*14.6*	**0/*0.8***	9.5	10.1
G. brevispinus (Gb)	*17.8*	*18.2*	*16.2*	*16.0*	*18.1*	*15.9*	*14.7*	*15.6*	*16.0*	*17.4*	*15.1*	**0/*0.9***	4.0
G. tenuispinus (Gt)	*15.8*	*18.2*	*14.8*	*15.5*	*17.3*	*13.7*	*14.7*	*15.0*	*16.6*	*16.6*	*16.1*	*13.8*	**0.3/*5.0***

The phylogenetic analysis of ITS2 and COI separated taxa on different levels. While ITS2 resolved relationships of taxa on genus and species level, COI showed a good resolution on the level of species and individuals (Fig. 2a, b). ITS2 analysis supported monophyletic clades of

the genera and to a lesser degree a clade of the family Aetideidae (Fig. 2b). Aetideidae clade split into the genus *Gaetanus* with significant support of the two species *G. brevispinus* and *G. tenuispinus,* as well as into a clade comprising *Aetideopsis* species and *Chiridius obtusifrons*. This indicates the close relationship of these two genera and a close relationship between *A. minor* and *A. rostrata*, the latter also supported by phylogeny based on COI sequences (Fig. 2a, b). Additionally, COI data showed a highly significant separation of mesopelagic distributed *A. minor* specimens sampled in the Fram Strait and in the South Atlantic Ocean with a genetic difference of 2.6% (Fig. 2a). This separation of northern and southern Atlantic specimens was also observed in COI sequences of mesopelagic cosmopolitan *Gaetanus tenuispinus* (7.4% genetic distance, Fig. 2a). On the contrary, such a separation could not be found in the bathypelagic congener *G. brevispinus* (Fig. 2a).

Compared to the Aetideidae, bootstrap values for the resolution of relationships within the genus *Paraeuchaeta* were low, based on ITS2 (Fig. 2b). However, *P. biloba* separated from other *Paraeuchaeta* species by forming an individual group. Species from northern hemisphere, including *P. glacialis*, *P. norvegica* and *P. polaris,* clustered together in the ITS2 analysis (Fig. 2b). In the bathypelagic *P. barbata* individuals from the northern and southern hemisphere clustered together in the ITS2 and COI analysis, supporting its cosmopolitan state (Fig. 2a, b).

Intra- and inter-specific genetic distances were higher in COI than in ITS2 sequences (Table 3b). On the basis of ITS2 sequences, *Paraeuchaeta* species differed from one another by ≤3.7% and *Aetideopsis* as well as *Gaetanus* species by ≤4.0%, respectively. *Paraeuchaeta* species differed from *Gaetanus* by 7.3-8.9%, from *Aetideopsis* by 7.3-10.2% (with lower values in *A. carinata*), and from *C. obtusifrons* by 8.9-10.1%. Genetic differences between *Gaetanus* and *Aetideopsis* were 7.0-10.0%, with larger differences to *G. tenuispinus*. Within genera, genetic distances between *A. minor* and *A. rostrata* (0.8%), as well as *P. norvegica*, *P. glacialis*, and *P. polaris* (0.6 to 0.8%) were rather low (Table 3b). On the basis of COI, *Paraeuchaeta* differed from one another by 5.4-16.9% with lowest values for *P. rasa* and *P. polaris*. In Aetideidae, *Gaetanus* species differed from one another by 13.8% and *Aetideopsis* by 10.4-14.3%. *Paraeuchaeta* differed from *Gaetanus* by 13.7 to 18.2% and from *Aetideopsis*/*Chiridius* by 13.4-19.7%, while *Gaetanus* differed from *Aetideopsis*/*Chiridius* by 15.0-17.4%. High intra-specific variability was recorded for the mesopelagic *A. minor* (1.7%) and *G. tenuispinus* (5.0%), while in other species differences were ≤0.9% or even not existing.

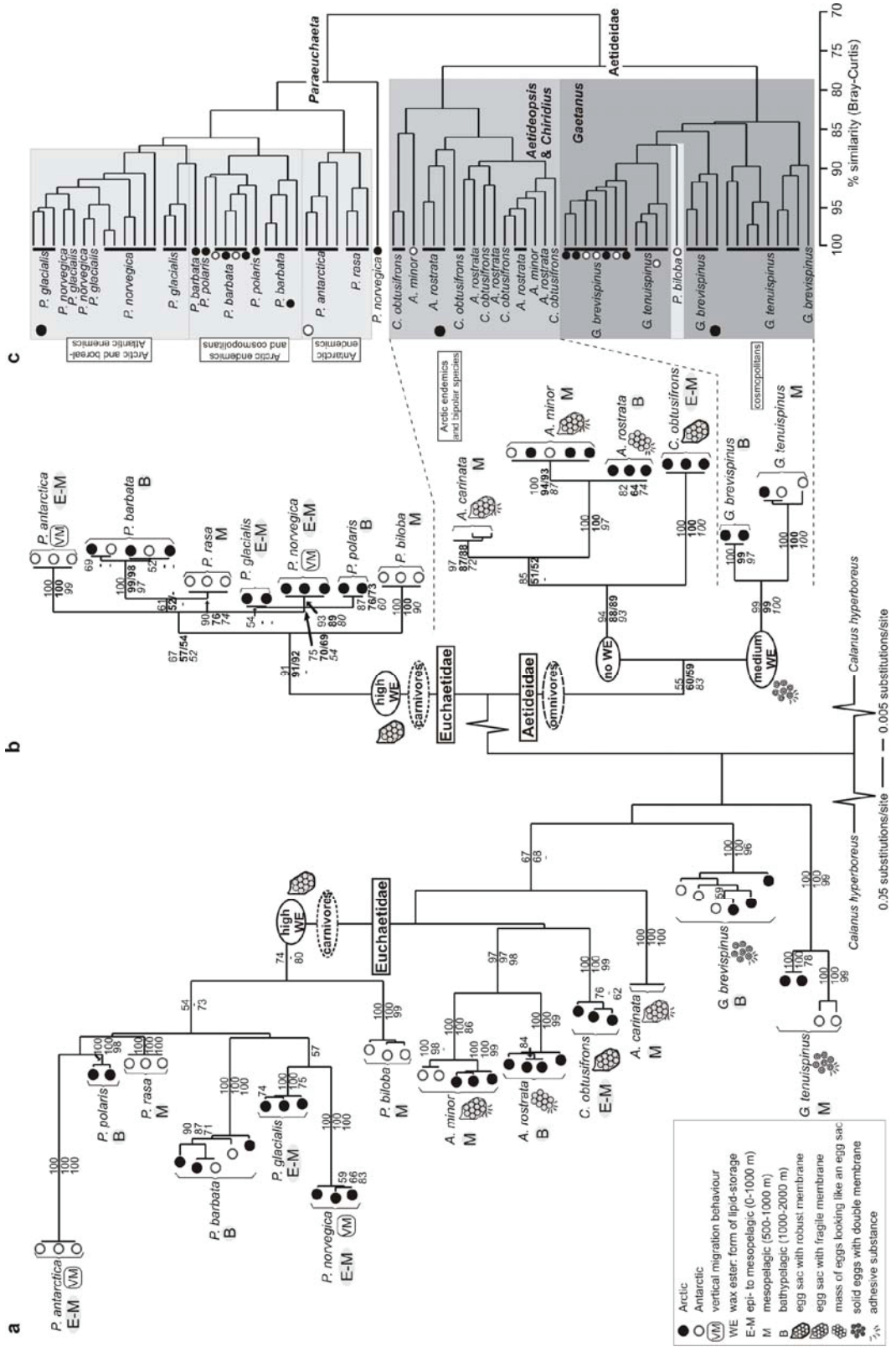
a
b
c
Arctic
Antarctic
vertical migration behaviour
wax ester: form of lipid-storage
E-M epi- to mesopelagic (0-1000 m)
M mesopelagic (500-1000 m)
B bathypelagic (1000-2000 m)
egg sac with robust membrane
egg sac with fragile membrane
mass of eggs looking like an egg sac
solid eggs with double membrane
adhesive substance
0.05 substitutions/site
0.005 substitutions/site
Calanus hyperboreus
Euchaetidae
Aetideidae
carnivores
omnivores
high WE
no WE
medium WE
Arctic endemics and bipolar species
cosmopolitans
Antarctic endemics
Arctic endemics and cosmopolitans
Arctic and boreal-Atlantic elements
Paraeuchaeta
Aetideopsis & Chiridius
Gaetanus
% similarity (Bray-Curtis)

Figure 2

Neighbour Joining (Distance) phylogram of a) COI and b) ITS2 of Euchaetidae and Aetideidae. Bootstrap values: regular figures (NJ), bold figures (MP/MP with gaps handled as 5th base) and italic figures (ML). P: *Paraeuchaeta*; G: *Gaetanus*; A: *Aetideopsis*; C: *Chiridius*. Information on: reproduction (Auel 1999, Kosobokova et al. 2007), wax ester content as well as vertical distribution (Laakmann et al. 2009a, b), vertical migration (Hopkins 1985, Williams 1988, Mauchline 1995, Kaartvedt et al. 2002, Skarra and Kaartvedt 2003) and on *Aetideopsis carinata* (Auel unpublished). c) Cluster analysis of lipid composition (fatty acids and fatty alcohols >4%) of Euchaetidae and Aetideidae

Cluster analysis of lipid composition

This analysis showed 70% similarity between Euchaetidae and Aetideidae and separated the two families with the exception of *P. biloba* clustered together with Aetideidae (Fig. 2c). In general, closely related species (congeners) clustered together, highlighting similar lipid composition, based on fatty acids and alcohols. Within Euchaetidae and Aetideidae species were similar in 79% and 77%, respectively (Fig. 2c). *Paraeuchaeta* showed a further diversification into Antarctic and Arctic species, each with two clusters. The Arctic cluster separated into a cluster of bathypelagic distributed *P. barbata* and *P. polaris* and into a cluster of epi- to mesopelagic distributed *P. norvegica* and *P. glacialis*. Aetideidae split into a cluster of *Gaetanus* spp. and *Aetideopsis* spp./*Chiridius obtusifrons*, respectively. A separation on species level was only evident between *P. antarctica* and *P. rasa*. The clusters of lipid composition were very similar to the three different clades supported in phylogeny based on ITS2.

DISCUSSION

Phylogenetic relationships

The application of different genetic markers allowed the analysis of phylogenetic relationships on different taxonomic levels. However, the ribosomal markers 18S and 28S did not provide a sufficient phylogenetic signal due to low mutation rates. In contrast, relationships of other calanoid copepod families and genera (e.g. species of Calanidae and Clausocalanidae) could be resolved using 18S as genetic marker (Bucklin et al. 2003). The non-resolution of Aetideidae and Euchaetidae and even their different genera might indicate a fast radiation. In this study, the nuclear non-coding marker ITS2 with its higher mutation rate than nuclear ribosomal genes and a lower one than the mitochondrial COI gene emerged as the most suitable for phylogenetic analysis. It resolved phylogenetic relationships between genera and species and to some extend supports the family of Aetideidae. No resolution of species relationships between *Paraeuchaeta* species compared to those of Aetideidae might be a result of a fast radiation within this genus, not detectable on the basis of this marker. ITS2 occurred as one copy within the genome of the copepods, since there was no interference during sequencing process and sequences for many individuals of the same species were identical.

Regarding the present taxonomy of Euchaetidae and Aetideidae, this study proved the closer relationship of the aetideid *Chiridius obtusifrons* to the genus *Aetideopsis* than to the genus *Gaetanus*, as this species was sometimes classified to both, the genus *Gaetanus* (Sars 1900, Seiler and Brandt 1997) and to the genus *Aetideopsis* (With 1915, Farran 1929). The closer relationship of *C. obtusifrons* to *Aetideopsis* is additionally reflected in their similar habitus, except of the lacking bifurcal rostrum in *C. obtusifrons* (Markhaseva 1996).

High diversity of mitochondrial COI provides species identification and is applicable for studying geographic variation within species (this study, Bucklin et al. 2003, Peijnenburg et al. 2004). Even for the identification of the two epi- to mesopelagic co-occurring *Paraeuchaeta norvegica* and *P. glacialis*, with young stages difficult to be distinguished based on morphological characters, COI emphasised as valuable tool for DNA barcoding. In Fram Strait, these two species are associated to the two opposing water currents, boreal-Atlantic *P. norvegica* with warm Atlantic water masses of the West Spitsbergen Current coming from the south and Arctic *P. glacialis* with cold Arctic water masses of the East Greenland Current coming from the north (Fig. 1a, Auel 1999). Compared to 1997, the boreal-Atlantic *P. norvegica* had a more broaden distribution and was more abundant in Fram Strait than in 2006 (Auel 1999, Laakmann et al. 2009a), as a result of a stronger inflow of warm Atlantic water masses and an temperature increase of more than 1°C compared to 1996 (Auel 1999, Schauer et al. 2008). Because of species´ association to the opposing water currents, they can be used as indicator species for global warming impacts. Furthermore, high variability of COI allowed the identification of geographic forms in the mesopelagic bipolar and cosmopolitan species and thus can be interpreted in phylogeographic contexts.

Feeding behaviour and lipid composition

The two synapomorphies in Euchaetidae, i.e. long setae on the first antennules used as mechanoreceptors and appendicular caudal setae used as balancing structure are regarded as an adaptation to their carnivorous tactile preying behaviour (Yen and Nicoll 1990, Park 1994a). Their carnivorous feeding mode, demonstrated in various studies (e.g. Yen 1991, Øresland and Ward 1993, Olsen et al. 2000, Laakmann et al. 2009a, b) is a major difference to the Aetideidae. Lurking in the water column to allow tactile prey detection is further approved by high levels of the lipid class wax esters (WE) (Fig. 2), which, due to their low density, maintain buoyancy at depths in these large and heavily built species (Laakmann et al. 2009a, b). This was also found in the genus *Euchaeta* as well as in euchaetid species from tropical to polar regions (e.g. Lee and Hirota 1973, Auel and Hagen 2005, Laakmann et al. 2009a, b). In general, WE are considered as long-term energy storage and an adaptation to seasonal food supply, since it was found in high amounts in epipelagic herbivorous copepods in polar regions (e.g. Lee and Hirota 1973, Kattner et al. 1994). However, carnivorous feeding mode of *Paraeuchaeta* spp., allows these species to feed all year-round (Øresland and Ward 1993),

resulting in WE to be rather involved in buoyancy aids in *Paraeuchaeta* species, as suggested for some zooplankton taxa (Visser and Jónasdóttir 1999). Aetideidae have the ability to synthesise WE and store moderate amounts (*Gaetanus* spp.) or only traces (*Aetideopsis* spp. and *C. obtusifrons*) (Fig. 2, Laakmann et al. 2009a, b). For these mixed feeders (e.g. Richter 1995, Auel 1999, Laakmann et al. 2009a, b) other lipid classes, like the short-term energy reserve triacylglycerol may play a more important role in lipid storage (Laakmann et al. 2009a, b).

Storage of fatty alcohols, which are the components of the WE, is the main reason for the three clusters in lipid composition, with *Paraeuchaeta* spp. storing high, *Gaetanus* spp. moderate amounts and *Aetideopsis* spp. and *C. obtusifrons* only traces (Laakmann et al. 2009a, b, Fig. 2). Furthermore, the clusters of Euchaetidae and Aetideidae are a result of fatty acid composition, with the domination of two fatty acids in Euchaetidae and a more diverse pattern in Aetideidae (Laakmann et al. 2009a, b). Coherence of phylogeny and biology thus demonstrates the similarity of closely related species in their lipid composition (Fig. 2). The non-resolution on species level, compared to phylogeny, is a result of fatty acids can be used as trophic biomarkers. Since certain fatty acids can only be synthesised *de novo* by specific organism, they can be used for tracing back recent assimilated food items (see Dalsgaard et al. 2003). Because the individuals are sampled in different seasons with different food availabilities and compositions as a function of high seasonality in polar regions, individuals of the same species do not always cluster together. They rather cluster together with specimens of similar feeding habits sampled during the same season, further indicating similar feeding habits of species within one cluster.

Reproduction

Congruence of phylogeny and biology like in lipid composition is also reflected in reproductive strategies of species (Fig. 2). Euchaetidae are uniform in having one gonad type (Niehoff 2007) and in carrying an egg sac with a robust membrane (Fig. 2, Mauchline 1988, Auel 1999, Kosobokova et al. 2007). In contrast, Aetideidae are more diverse, comprising three different gonad types (Niehoff 2007), carrying egg sacs with a robust (*C. obtusifrons*) or fragile membrane (*A. minor*, *A. carinata*), as well as being free-spawners (*A. rostrata*, *Gaetanus* spp.) (Fig. 2, Auel 1999, Kosobokova et al. 2007, Auel own observation). The free spawning *A. rostrata* produces a mass of eggs looking like an egg sac which disintegrated within 2h and *Gaetanus* eggs are composed of a double membrane of which the outer membrane enlarges just after egg deposition, enabling them to float (Fig. 2, Kosobokova et al. 2007). Both, eggs of *Aetideopsis* and *Gaetanus* species, are coated with an adhesive substance, which is generally a trait of benthopelagic copepods, in order to attach the eggs or clutches on substrates to prevent drifting (Matthews 1964 in Kosobokova et al. 2007). This substance is a typical feature of Aetideidae (Lindley 1997, Kosobokova et al. 2007). Development of an egg sac like in

Paraeuchaeta and *C. obtusifrons* is only found in a few calanoid copepods (Huys and Boxshall 1991) and was assumed as an adaptation to pelagic life (Kosobokova et al. 2007). Fragile and disintegrated egg sacs of *Aetideopsis* as well as floating characteristics of *Gaetanus* eggs may display a mode of junction between originally benthopelagic and pelagic life traits and several features of the Aetideidae suggest that the benthopelagic environment was first invaded in deep waters (Bradford-Grieve 2002, 2004).

Vertical distribution

In general, the closely related species are similar in biological terms, as demonstrated in lipid composition and reproductive strategy (Fig. 2, Laakmann et al. 2009a, b). To minimise or to avoid inter-specific competition they inhabit different depth strata as demonstrated in many aetideid and euchaetid species from both polar regions (Fig. 2, Ward and Wood 1988, Richter 1994, 1995, Seiler and Brandt 1997, Auel 1999, Laakmann et al. 2009a, b). Generally, *Paraeuchaeta* species inhabit meso- and bathypelagic depths (Park 1994a, b). In polar regions though, they also inhabit shallower depths, associated with the lower water temperatures which resemble those in deep waters. *P. norvegica*, *P. glacialis* and *P. antarctica* were very abundant in shallower depths, reflecting that the occupancy of these depth strata was successful for these species (Fig. 2, Laakmann et al. 2009a, b). Vertical partitioning of the water column is thus assumed to play an important role in speciation processes of these deep-sea copepods. Interestingly, this speciation is possible without physical isolation barriers in the three-dimensional pelagic deep-sea environment and is assumed as a sympatric speciation as a result of the exploitation of new niches, which automatically reduce gene flow among populations of the different niches/ depths.

Vertical migration was only observed for the epi- to mesopelagic distributed species *P. norvegica* and *P. antarctica* (Fig. 2) as well as in other epi- to mesopelagic Aetideidae (Ambler and Miller 1987, Falkenhaug et al. 1997, Schøyen and Kaartvedt 2004, Yamaguchi et al. 2004) and Euchaetidae (Hopkins 1985, Williams 1988, Mauchline 1995, Yamaguchi and Ikeda 2002, Kaartvedt et al. 2002, Skarra and Kaartvedt 2003). For the investigated meso- to bathypelagic Arctic Aetideidae, no vertical migration behaviour was observed (Richter 1995). We assume that vertical migration is not suitable for phylogenetic analysis, since it is only demonstrated in species from the mesopelagic zone.

In summary, species of one genus are similar in their general feeding behaviour, lipid composition and storage as well as reproductive strategies and the phylogenetic clades are reflected in these biological features (Fig. 2). The ecological and physiological characteristics of Euchaetidae can also be found in the species of the highly diverse family Aetideidae.

Geographic distribution

According to Park (1994b) dominant *Paraeuchaeta* species in highly productive areas belong to different *Paraeuchaeta* groups, which is true for *P. antarctica*, *P. rasa* and *P. biloba* in the Southern Ocean and to some degree for Arctic *Paraeuchaeta* species (Fig. 2). However, the poorly supported clade of boreal-Atlantic and Arctic endemics based on ITS2 (Fig. 2b) might suggest that these species separated later in geological times than endemic Antarctic ones, since the Antarctic is a geologically older polar habitat than the Arctic. An evidence for earlier colonisation of the Southern Ocean is the high number of endemic *Paraeuchaeta* species (Park and Ferrari 2009).

The endemic distribution of *Paraeuchaeta* species is suggested to be a result of their strong adaptation to regional eutrophic environmental conditions (Park 1994b, Park and Ferrari 2009). A wide-spread dispersal may be hindered by their low adaptation potential to differences in environmental physical and biological conditions. The same reasons were proposed for the epipelagic herbivorous copepod *Calanus pacificus* (Nuwer et al. 2008) with a stronger coupling to primary production than omnivorous or carnivorous zooplankton. In general, the spatial heterogeneity at macrogeographic scales is assumed to play an important role in plankton evolution (Kirby et al. 2007). In contrast, wide-spread species like cosmopolitans are assumed to be adapted to oligotrophic conditions and thus to low production, enabling them to disperse and survive on a large geographic scale (Park 1994b). In addition, the carnivorous or omnivorous feeding mode as well as the complete lifecycle in meso- or bathypelagic depths makes deep-water species somewhat independent from primary production and deep-water links allow the trans-equatorial exchange among populations (Machida et al. 2006).

In consequence, the cosmopolitans *P. barbata*, *G. brevispinus* and *G. tenuispinus* as well as the bipolar distributed *A. minor* (Park 1994a, Markhaseva 1996, Park and Ferrari 2009), might have a higher adaptation potential to varying abiotic and biotic factors than endemic species, that enables them to survive over a wide geographic range. However, the bipolar classification of *A. minor* is rather assumed as a cosmopolitan one as suggested for bipolar species in general (Park and Ferrari 2009). With lower sea surface temperature, this species occurs at shallower depths (polar emergence) as demonstrated in the Arctic (Markhaseva 1996, Kosobokova et al. 2007, Laakmann et al. 2009a) and Antarctic (Laakmann et al. 2009b compared to Schnack-Schiel et al. 2008), and it is supposed to be deeper distributed and/or less abundant in boreal and tropic regions.

Arctic and Antarctic individuals of these cosmopolitan and bipolar species differ in COI sequences and were almost identical in ITS2 (Fig. 2). These results suggest that ITS2 is not suitable for the identification of different geographic forms of one species in these copepods. Almost identical ITS sequences were also demonstrated for Arctic and Antarctic individuals of

the bipolar and cosmopolitan deep-sea benthic meiofaunal iso- and amphipods as well as foraminifera (Brandt et al. 2007a, Pawlowski et al. 2007). Since COI has the power to distinguish between copepods´ populations (e.g. Bucklin et al. 2000, Kirby et al. 2007, Nuwer et al. 2008, Winkler et al. 2008, Provan et al. 2009), the indications of low diversification in Arctic and Antarctic individuals of the bathypelagic cosmopolitans *P. barbata* and *G. brevispinus* suggest a single form on this wide geographic range. This is especially interesting for *P. barbata* because for this species a species complex with many geographic forms was proposed (Mauchline 1992). In contrast to the bathypelagic species, Arctic and Antarctic individuals of the mesopelagic cosmopolitan *G. tenuispinus* and bipolar *A. minor* clustered together, respectively, supported by high bootstrap values, indicating different geographic forms. The high intra-specific variations of COI between mesopelagic Arctic and Antarctic individuals (<2%) are common in ecologically distinct or geographically isolated populations (Bucklin et al. 2003).

For a variety of marine epipelagic plankton organisms, genetically distinct populations exist, comprising herbivorous copepods (Bucklin et al. 2000, Goetze 2003, Nuwer et al. 2008), euphausiids (Zane et al. 2000, Papetti et al. 2005) and chaetognaths (Peijnenburg et al. 2004). Similar results for deep-sea pelagic zooplankton, which do not directly depend on surface productivity, do not exist. However, for deep-sea nekton organisms, different genetic lineages were detected, for example in the circum-global monotypic deep-sea fish *Cyclothone* (Miya and Nishida 1997) and Greenland halibut with an extended pelagic phase (Knutsen et al. 2007).

In contrast to epipelagic zooplankton organism like copepods (Bucklin et al. 2000, Goetze 2005) and euphausiids (Papetti et al. 2005) for which ocean currents can play an important role in shaping the genetic structure, present day oceanic current systems may not play an important role for the dispersal abilities of cosmopolitan deep distributed zooplankton taxa. They are assumed to be dispersed from the Southern Ocean by the Antarctic Bottom Water, which represents a possible connection between Southern Ocean and the rest of the world oceans (Brandt et al. 2007b). In contrast to shallow distributed species, oceanic fronts like the Subtropical or the Antarctic Polar Front in the Southern Ocean do not represent dispersal barriers for mesopelagic and even deeper distributed zooplankton organisms (Deacon 1982 and references therein, Pakhomov et al. 2000). In the other parts of the deep sea, these organisms can be dispersed by the North Atlantic Deep Water which is formed in the Norwegian Sea and spread at abyssal depths throughout the world oceans (e.g. Mantyla and Reid 1983). However, hydrographic barriers in times of glaciations and variability of sea levels, which influenced global current systems including the deep-ocean currents, possibly structured dispersal of planktonic organisms leading to geographic forms.

It is suggested, that to a great extent, the ranges of many pelagic groups are limited by their ability to maintain viable populations over a wide geographic range, instead of any inability to disperse past hydrographic barriers for population exchange (Norris 2000). Recent studies support that species-specific ecological differences are likely to be involved in determining the population genetic structure of oceanic plankton species (Goetze unpubl. in Goetze 2005). Variability of environmental factors further implicates a higher possibility for unfavourable conditions, where species are unable to reproduce and maintain a viable population (e.g. Van der Spoel 1994, Goetze 2005). The indications of genetic homogeneity in bathypelagic and divergence in mesopelagic cosmopolitan and bipolar species are attributed to the variability of the respective depth zones with the mesopelagic species being more affected by large-scale variances in surface production. Decreasing variability of habitat with increasing depth is for example reflected in the largest part of net particulate matter consummation in the upper 500 m (epi- and mesopelagic zone) (Karl et al. 1988 and references therein). As a consequence, mesopelagic omnivores may have a stronger coupling to sea surface production than bathypelagic species. The stronger environmental variability at mesopelagic depths may result in a higher demand on adaptation potential and ecological tolerances for varying driving forces and strengthening factors as well as fluctuations in food availability, temperature or turbidity. In contrast, the more constant environmental conditions in the bathypelagic realm allow species a higher chance of survival on a wide geographic range and support a broad distribution with a possible constant gene flow within deep-water circulation systems. Furthermore, the evolutionary rate in deep-living species may be lower compared to shallower distributed ones as a function of a depth-related decline in metabolism (respiration rates) (Ikeda et al. 2006) and an assumed longer generation time (Mauchline 1994, 1995).

In summary, mesopelagic species seem to be more affected by abiotic and biotic variability. The establishment of different geographic forms may be a result of 1) allopatry caused by barriers, formed temporarily during earth history by inconstancy of ocean currents and an adaptation to these separated areas, 2) being more affected by spatial changes demanding a higher adaptation potential and ecological tolerance, and 3) the involvement of species-specific ecological characters in structuring populations in the open ocean.

Evolutionary background

It is suggested that the lineages of Calanoidea-Clausocalanoidea emerged in the Permian (251-299 million years ago) and survived the Cretaceous-Tertiary (K-T) boundary event by 1) remaining in the surface waters (during expansion of oxygen minimum zone), 2) adapting to low oxygen conditions, or 3) by retreating to deep-waters below the oxygen minimum zone (e.g. Aetideidae) (Bradford-Grieve 2002). After the K-T boundary event some Clausocalanoidea may have found refuges in deep water and from there reinvaded the water column (Bradford-Grieve 2002). These findings are consistent with the suggestion, that the ancestor of

the Aetideidae and Euchaetidae was a bathypelagic copepod (Braga et al. 1999), as well as that the benthopelagic habitats were first invaded in deep waters by Aetideidae (Bradford-Grieve 2002). Among Aetideidae hyper-benthic and benthopelagic lifestyle is common in some genera and species (Markhaseva 1996, Bradford-Grieve 2004). As well, the remains of benthopelagic life traits in some pelagic forms of Aetideidae, as demonstrated on reproductive strategies (Markhaseva 1996, Kosobokova et al. 2007) support these findings. In contrast, Euchaetidae are exclusively pelagic. The present results (low resolution within *Paraeuchaeta* genus) suggest a faster radiation of *Paraeuchaeta* species than of aetideid ones. Since it is suggested that Aetideidae re-radiated the pelagic from deep waters (Bradford-Grieve 2002), Euchaetidae may represent one form of these several radiation events. They are similar in morphology to Aetideidae and only differ by four synapomorphies, with two of them regarded as adaptations to their predatory feeding behaviour (Yen and Nicoll 1990, Park 1994a). Furthermore, they are uniform in reproductive strategy (Niehoff 2007, Kosobokova et al. 2007), lipid-storage strategy (Lee and Hirota 1973, Auel and Hagen 2005, Laakmann et al. 2009a, b) and feed carnivorously (e.g. Yen 1991, Øresland and Ward 1993, Olsen et al. 2000). All these characteristics can be found in varying degrees in the highly diverse group of Aetideidae.

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Eidesstattliche Erklärung

(Gem. § 6(5) Nr. 1-3 PromO)

Hiermit versichere ich, dass ich die vorliegende Arbeit:

1. Ohne unerlaubte, fremde Hilfe angefertigt habe
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Bremen, 28.Mai 2009

Silke Laakmann