Ecophysiology of Antarctic sea-ice meiofauna

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Abstract

Sea ice is permeated by small brine channels, which are characterised by sub-zero temperatures and varying salinities. Despite sometimes extreme conditions a relatively diverse fauna and flora thrives within these brine channels. *Stephos longipes*, *Paralabidocera antarctica* and *Drescheriella glacialis* are the dominant copepod species found within Antarctic sea ice. Their life-cycle strategies are well-established, but life cycles of other meiofauna (metazoans ≥ 50 µm) found within sea ice are little explored. Adaptation mechanisms allowing meiofauna species to survive within sea ice are largely unknown. In order to increase our knowledge of the Antarctic sea-ice meiofauna, different microhabitats of sea ice and their metazoan fauna were studied during two cruises with R/V "Polarstern" to the western Weddell Sea. The dominant sympagic copepod species found in the sub-ice layer was *Ectinosoma* sp., other sympagic copepod species occurring regularly were *D. glacialis/racovitzai*, *Diarthrodes* cf. *lilacinus*, *Idomene antarctica* and *S. longipes*. *Drescheriella glacialis/racovitzai* and *Stephos longipes* were the dominant members of the surface-layer meiofauna during late spring. Their populations consisted mainly of adults and early naupliar stages in this layer, which points to an active reproduction of these species within the surface layer. Other taxa found in the surface layer were undetermined turbellarians, the gastropod *Tergipes antarcticus*, a ctenophore and two amphipod species. Sampling records from the Bellingshausen Sea, the Weddell Sea, as well as from the Prydz and the Lützow-Holm Bay indicate that *T. antarcticus* is widely distributed in Antarctic sea ice. During this study, adults, juveniles, veliger larvae and egg clutches of *T. antarcticus* were found in sea ice. A thorough morphological and anatomical description of all life stages was performed and the developmental time from egg to veliger larvae was determined as being 31 days (range: 13 to 65 days) at 0 °C. The observed reproduction of *D. racovitzai*, *Idomene antarctica* and *T. antarcticus* within the habitat allows to assign also these species as true members of the sea-ice meiofauna. Adaptation mechanisms to changing salinities and ice formation were studied for *P. antarctica*, *S. longipes* and *T. antarcticus*. The haemolymph of the two copepod species is isosmotic to the medium at salinities from 25 to 45 g/kg (*S. longipes*) and 25 to 55 g/kg (*P. antarctica*). Thermal hysteresis (a non-colligative inhibition of ice growth) was found for *T. antarcticus* and *S. longipes*, but not for *P. antarctica*. These are the first reports of thermal hysteresis from gastropods and crustaceans, respectively. The acquisition of thermal hysteresis seems to enable *S. longipes* to exploit all available microhabitats within sea ice. In particular, *S. longipes* is found in high abundances in the surface layer, in which stronger temperature fluctuations can occur than in the lowermost centimetres of the ice. *P. antarctica* seems to be restricted physiologically to the lower layer. *T. antarcticus* was also found in the surface layer, but the importance of thermal hysteresis for habitat choice in *T. antarcticus* remains to be shown. Thermal hysteresis is probably also a prerequisite for the ability of *S. longipes* and *T. antarcticus* to spawn within the ice. Adaptations to low temperatures and elevated salinties on the transcriptional level were investigated for *S. longipes*. Two isoforms of a protein were found, which, if recombinantly expressed, confers thermal hysteresis. A high homology to a group of (putative) antifreeze proteins from a bacterium, a snow mold and several diatoms and, in contrast, no homologs in any metazoan lineage suggest that this protein was obtained through horizontal gene transfer. This seems to be a key event in the evolution of *S. longipes*.

Zusammenfassung

Meereis ist durchzogen von kleinen Solekanälen, die durch Temperaturen unter 0 °C und variable Salzgehalte gekennzeichnet sind. Trotz teilweise extremer Bedingungen ist das Solekanalsystem von einer relativ artenreichen Flora und Fauna besiedelt. *Stephos longipes*, *Paralabidocera antarctica* und *Drescheriella glacialis* sind die dominanten Copepodenarten des Antarktischen Meereises. Ihre Lebenszyklen sind gut untersucht, die Lebenszyklen anderer Meereis-Meiofauna Organismen (Metazoen $\geq$ 50 µm) hingegen nicht. Anpassungsmechanismen, die es Meiofauna-Organismen erlauben, im Meereis zu überleben, sind weitestgehend unbekannt. Um die Kenntnis der Antarktischen Meereis-Meiofauna zu verbessern, wurden während zweier Expeditionen mit dem Forschungsschiff „Polarstern“ in das westliche Weddellmeer verschiedene Mikrohabitate des Meereises und ihre Meiofauna untersucht. *Ectinosoma* sp. war die dominierende Copepodenart des Untereishabitats im späten Frühjahr. Weitere, regelmäßig vorkommende Arten waren *D. glacialis/racovitzai*, *Diarthrodes* cf. *lilacinus*, *Idomene antarctica* und *S. longipes*. *Drescheriella glacialis/racovitzai* und *S. longipes* dominierten in der Oberflächenschicht (engl.: surface layer). Ihre Populationen bestanden hauptsächlich aus Adulten und frühen Larvenstadien, was auf eine aktive Reproduktion dieser Arten in der Oberflächenschicht hindeutet. Andere in der Oberflächenschicht nachgewiesene Taxa sind Plathelminthen, die Nacktschnecke *Tergipes antarcticus*, eine Ctenophore und zwei Amphipoden. Nachweise aus dem Bellingshausen- und dem Weddellmeer, aus der Prydz- und der Lützow-Holm-Bucht weisen darauf hin, dass *T. antarcticus* im Antarktischen Meereis weit verbreitet ist. Im späten Winter wurden Adulte, Juvenile, Larven und Eiballen von *T. antarcticus* im Meereis gefunden. Eine eingehende morphologische und anatomische Beschreibung der Art wurde vorgenommen und die Entwicklungszeit vom Einzell-Stadium bis zum Schlüpfen der Veliger-Larve auf 31 Tage (Spannweite: 12 bis 65 Tage) bei 0 °C bestimmt. Die nachweisliche Reproduktion von *D. racovitzai*, *I. antarctica* und *T. antarcticus* im Meereis erlaubt es, auch diese Arten als echte Bestandteile der Meereis-Meiofauna einzustufen. Mechanismen der Anpassung an variierende Salzgehalte und Eisbildung wurden an *T. antarcticus*, *P. antarctica* und *S. longipes* studiert. Die Hämolymphe der beiden Copepodenarten ist isosmotisch zum Außenmedium bei Salzgehalten von 25 bis 45 g/kg (*S. longipes*) bzw. von 25 bis 55 g/kg (*P. antarctica*). Thermale Hysterese (die Behinderung von Eiswachstum) wurde bei *T. antarcticus* und *S. longipes* gefunden. Dies sind jeweils die ersten Nachweise von thermaler Hysterese für Gastropoden und Crustaceen. Diese physiologische Anpassung scheint es *S. longipes* zu ermöglichen, alle vorhandenen Habitate des Meereises zu besiedeln. Vor allem in der Oberflächenschicht des Eises, in welcher stärkere Temperaturschwankungen vorkommen können als in der Bodenschicht (engl.: bottom layer), ist *S. longipes* in hohen Abundanzen zu finden. *P. antarctica* scheint physiologisch auf die Bodenschicht beschränkt zu sein. *T. antarcticus* wurde ebenfalls in der Oberflächenschicht gefunden, aber die Bedeutung der nachgewiesenen thermalen Hysterese für die Habitatwahl dieser Art bleibt fraglich. Thermale Hysterese ist wahrscheinlich auch eine Voraussetzung dafür, dass *S. longipes* und *T. antarcticus* ihre Eier im Eis ablegen können. Anpassungen an niedrige Temperaturen und erhöhte Salzgehalte wurden auf Transkriptionsebene an *S. longipes* untersucht. Zwei Isoformen eines Proteins konnten identifiziert werden, welches rekombinant hergestellt thermale Hysterese bewirkt. In den verfügbaren Sequenzdatenbanken lassen sich keine tierischen Homologe finden. Dies und eine hohe Homologie der Proteine zu Gefrierschutzproteinen von einem Bakterium, einem Pilz und einer Meereis-Diatomee deuten darauf hin, dass das Protein durch horizontalen Gentransfer übertragen wurde. Dies scheint ein Schlüsselereignis in der Evolution von *S. longipes* gewesen zu sein.

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1. Introduction

1.1. Preface

Sea ice is a unique habitat from several points of view: it is a particularly dynamic habitat as it can form and melt away within one season and does normally not become older than a few years (Rigor and Wallace 2004). Pack ice, which makes up by far the largest amount of Antarctic sea ice compared to fast ice (Fedotov et al. 1998), is continuously displaced by winds and ocean currents (Schmitt et al. 2004). The temperature of sea ice is obviously always below the freezing point of water and temperatures as low as -15 °C have been measured within Antarctic sea ice (Bartsch 1989). Nevertheless, this unstable and cold environment is inhabited by a range of microscopic metazoan species which thrive within interstices of the ice (Schnack-Schiel 2003).

This work aims at the elucidation of adaptation mechanisms of meiofauna species (here defined as metazoans ≥ 50 µm) thriving within these interstices. Investigations on the levels of ecology, physiology and molecular biology were performed. The work is based on two cruises with R/V "Polarstern" to the Western Weddell Sea: ANT XXII/2 (Ice Station Polarstern, ISPOL, 6 November 2004 to 19 January 2005, Dieckmann et al. (2007)) and ANT XXIII/7 (Winter Weddell Outflow Study, WWOS, 24 August 2006 to 29 October 2006) (see Fig. 1 for a depiction of the investigation area).

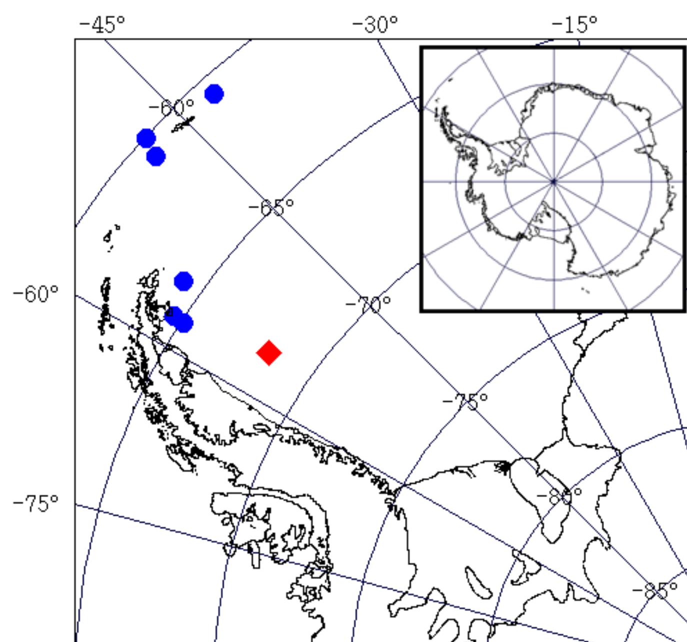

Fig.1. Map of the western Weddell Sea and the Antarctic Peninsula showing the locations of the sampling stations during ISPOL (red rhomboid) and WWOS (blue dots). The inset shows an overview of Antarctica with the Weddell Sea in the upper left corner.

This dissertation is mainly restricted to Antarctic sea ice and its inhabitants, and is divided into a general introduction, three stand-alone chapters and a general discussion. The three stand-alone chapters are divided into a special introduction regarding the respective topic of the chapter, material and methods, results and a special discussion of the results of the respective chapter. In the general introduction of this work, a short explanation is given why the sea-ice ecosystem is an especially fascinating and unique marine habitat. The different ice types, sea-ice drift patterns and formation processes of Antarctic sea ice are subsequently introduced. A description of abiotic factors setting limits to the survival of sea-ice organisms within the ice and the characteristics of small-scale habitats are described thereafter. The meiofauna community found within Antarctic sea ice is presented in the following section. Tolerance limits of sympagic (sea-ice associated) meiofauna species are the topic of the next section. The basic questions and goals of this work and the methods used to achieve these goals are presented in the last section of the introduction. Chapter two presents a comparison of the abiotic conditions and the meiofauna community of the sub-ice and surface layers in the western Weddell Sea during late spring. The third chapter deals with the distribution, morphology, anatomy, development and adaptation of the sympagic nudibranch *Tergipes antarcticus*, including the first report of thermal hysteresis in a gastropod. Chapter four describes and discusses adaptations of two sympagic calanoid copepods and presents the first report of thermal hysteresis in a crustacean. The protein responsible for this effect is described. A comprehensive discussion (chapter five) follows the three stand-alone chapters (chapters two to four) and covers the links identified between ecology, physiology and molecular biology. Finally, a short outlook is given, covering further planned and proposed experiments, in particular further potential applications of molecular-biological methods in sea-ice research.

1.2. Life in a freezer

Life on our planet depends on liquid water. Every place on earth where liquid water is occasionally present is occupied by at least some bacteria (Rothschild and Mancinelli 2001). Also within seemingly solid ice, liquid inclusions exist (down to at least -35 °C; Gilpin 1980) and there is some evidence that, even within ice crystals of deep glacial ice bacteria can survive for several thousand years (Rohde et al. 2007). Sea ice represents another interesting habitat. When seawater freezes, the contained salts are not incorporated into the crystal matrix but collect in between, forming a system of brine channels and pockets (Assur 1958). A

saline, liquid brine forms which can be teeming with life. Viruses, bacteria, fungi, algae and metazoans thrive in this system (for reviews see: Thomas and Dieckmann 2002, Schnack-Schiel 2003). The enormous extent of the Antarctic sea-ice ecosystem can be perceived from outer space (Fig. 2). In austral winter, an average sea-ice extent of $18*10^{6}$ km^2 can be observed in the Southern Ocean (Cavalieri et al. 2003). In austral summer, $3.5*10^{6}$ km^2 still persist. Minima and maxima are reached in the middle of February and September (see Fig. 2 for an example of the minimum and maximum Antarctic sea-ice extent in 2006). In extent, Antarctic sea ice is one of the largest ecosystems on earth. As a comparison, even the Antarctic summer sea-ice extent is about ten times larger than the area of Germany.

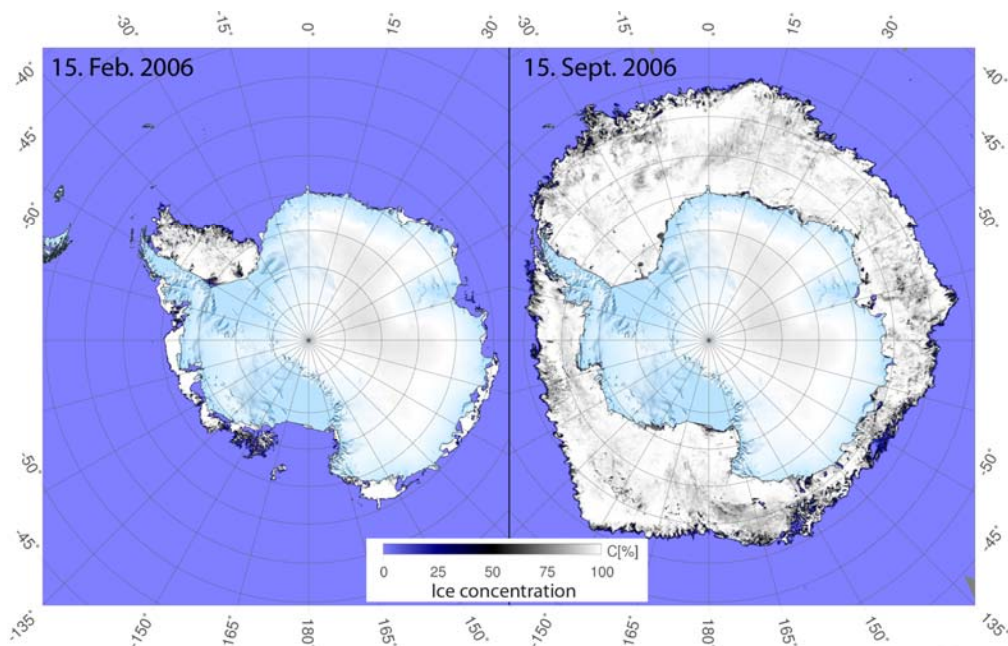

Fig. 2. Antarctic sea-ice extent and concentration in the middle of February (summer) and September (winter) 2006 as obtained by remote sensing (Spreen et al. 2008). Source of the satellite images: http://www.iup.uni-bremen.de:8084/amsr/amsre.html (last date accessed: 12 December 2008)

1.3. Antarctic sea ice: dynamics and ice types

1.3.1. Main ice types and drift patterns

Several different ice types can be distinguished in the Southern Ocean (Lange et al. 1989, Horner et al. 1992). These can be mainly categorised as multi-year ice or first-year ice, as well as fast ice or pack ice. As can be inferred from the large differences in ice cover in summer and winter (Cavalieri et al. 2003), most ice in the Southern Ocean persists for less than one year; this ice is therefore called first-year ice. Multi-year ice survives for at least one summer. Free-drifting ice is called pack ice, whereas ice attached to the land or to shelf ice is called fast ice. Ice formation around Antarctica takes place mainly in the open ocean or in coastal polynyas (Russian for open water) (Lange et al. 1989). The latter form when offshore winds drive away the pack ice from the coast, the shelf ice or the coastal fast ice in winter (Lemke 2001, Kern et al. 2007). The forming ice is displaced from the coast and incorporated into the pack ice. Pack ice is characterised by pressure ridges (Tin and Jeffries 2003) and the presence of more open water areas like cracks and leads in comparison to fast ice.

Ice drift influences the small- and medium-scale topology of sea ice through formation of pressure ridges or leads, but is also responsible for large-scale displacements of pack ice. Characteristic for the Southern Ocean are, first of all, the large, wind-driven circumpolar currents, the westward (counter-clockwise) Antarctic Coastal Current (driven by easterly winds) and the strong eastward (clockwise) Antarctic Circumpolar Current further offshore (Orsi et al. 1995). These result in a general westward drift of the remaining Southern Ocean pack ice in times of low ice extent (Fig. 3 A). In winter, the situation is more complex, with a westward component near the coast and an eastward component further offshore (Fig. 3 B). In the vicinity of the Drake Passage, the westward component is missing, mainly due to strong eastward ocean currents through the passage.

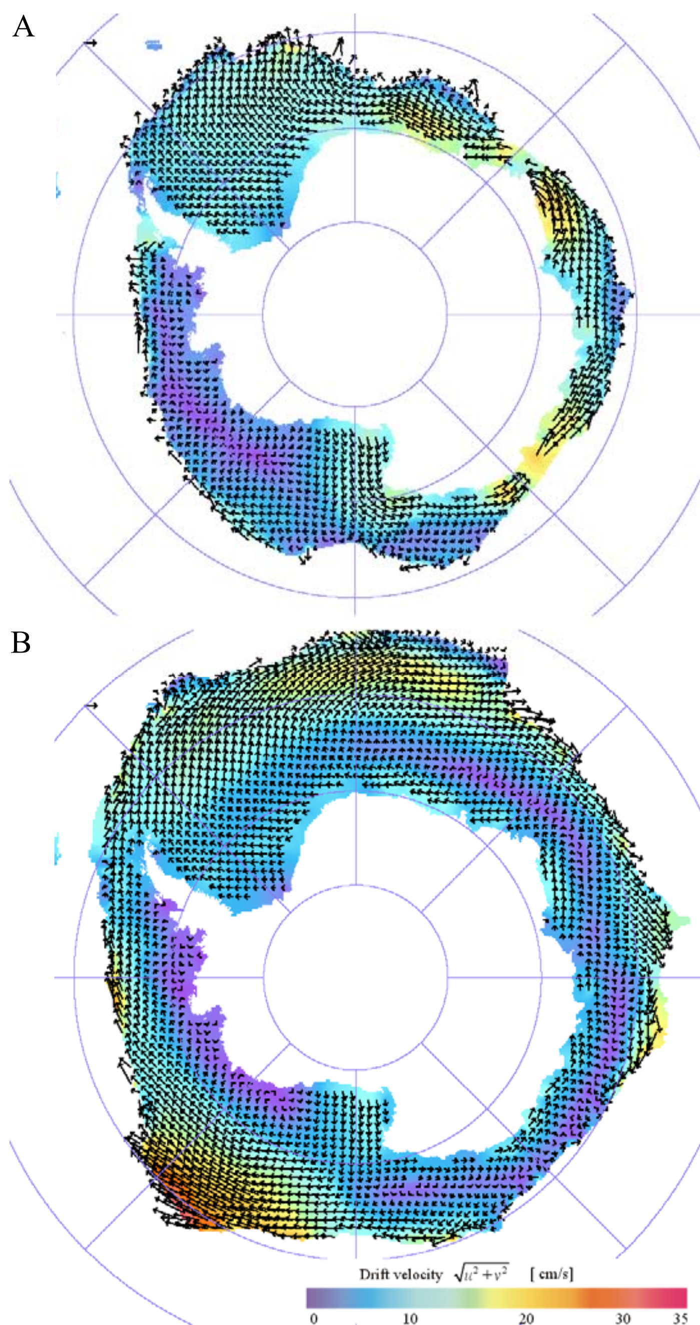

Fig. 3. Average ice drift in the Southern Ocean (1979 to 1997). (A) March, April and May (B) September, October and November. Gridded fields interpolated out of SSM/I point data with drift arrows from SSM/I. The background colours and arrows indicate the drift velocity; the length of the scale arrow (top left) indicates 10 cm/s drift velocity. Note: Interpolated values are only representative in the regions with drift arrows.

Source: http://imkhp7.physik.uni-karlsruhe.de/~eisatlas/HTML/eisatlas_means.html. (last date accessed: 12 Decembre 2008)

The two large embayments, the Weddell and the Ross Sea, are characterised by cyclonal gyres (Reid 1986) containing most of the multi-year ice present in the Southern Ocean. On a 20-year average from 1987 to 2007, the minimum ice area was 0.86 $\pm$ 0.21 $*10^6$ km² in the Weddell Sea and 0.32 $\pm$ 0.14 $*10^6$ km² in the Ross Sea; in the whole Southern Ocean, 1.80 $\pm$ 0.28 $*10^6$ km² of sea ice survives the summer (values calculated from data on http://www.nsidc.org/data/smmr_ssmi_ancillary/area_extent.html; obtained with the NASA algorithm; sea-ice area = sea-ice extent * concentration; last date accessed: 12 December 2008). The drift patterns in the Weddell and the Ross Sea are convergent (Eicken 1992). Floes

within the Weddell Sea can circulate for years in the Weddell Gyre. Nevertheless, as the Weddell and the Ross Sea are open towards the north, pack ice is finally displaced to the open Southern Ocean, where most of the ice melt takes place.

1.3.2. Mechanisms of ice formation

Ice formation around Antarctica takes place in winter, mainly under windy, turbulent conditions (Eicken and Lange 1989). Low atmospheric temperatures cause a decrease of the water temperature to values below the freezing point. Small ice crystals (frazil crystals) forming in the water column rise to the surface. An ice slurry (grease ice) forms and is compressed by wave action to so called pancake ice (Fig. 4 A). These pancakes grow in thickness, dampen the wave action and freeze together (Fig. 4 B). Such ice is genetically classified as frazil ice and can be structurally classified as granular ice (Fig. 4 A; inset), with large and irregular brine and pore spaces and an irregular orientation of the ice crystals (Eicken and Lange 1989). Further growth of the ice underneath the original ice sheet takes place under calm conditions, under which ice crystals grow downwards (columnar crystals). The forming ice is genetically classified as congelation ice and structurally as columnar ice (Fig. 4 C; inset), characterised by smaller brine channels, oriented parallel to the growth direction. Congelation ice also forms directly, if wind conditions are calm (Fig. 4 C). Besides this thermodynamic ice growth, dynamic growth takes place as well. Free-drifting floes can collide, leading to rafting and ridging of the floes. The forming pressure ridges (Fig. 4 D) can make up 50% of the total ice volume and can reach thicknesses of more than ten metres (Tin and Jeffries 2003).

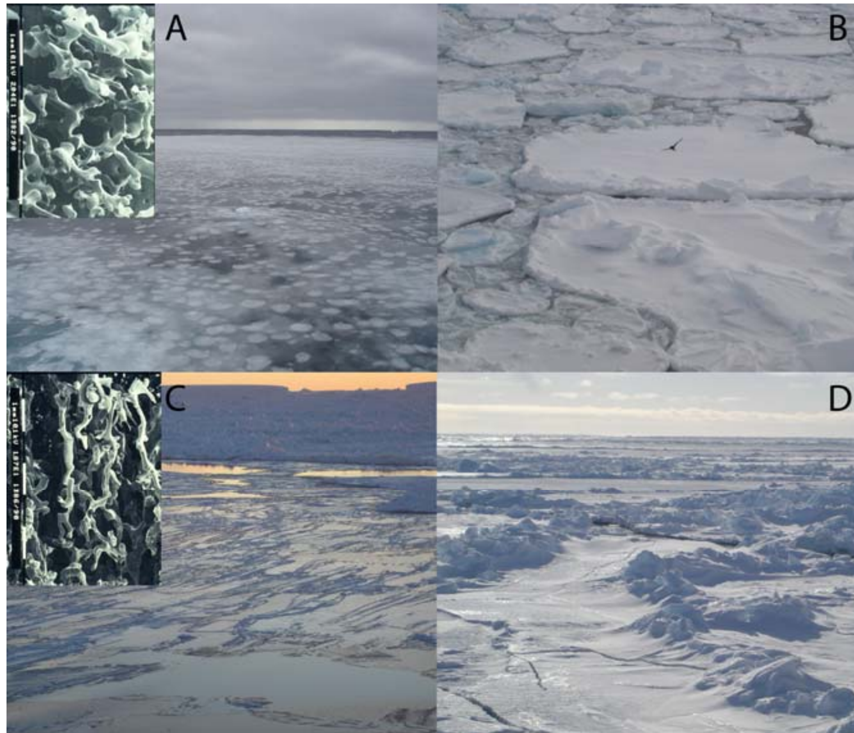

Fig. 4. Ice types found in the Western Weddell Sea. (A) pancake ice and grease ice; inset: resin cast of granular ice. (B) consolidated pancake ice. (C) nilas (very young congelation ice); inset: resin cast of columnar ice. (D) Pressure ridges. Approximate width of the lower picture boundary is five metres in B, ten metres in A and C and twenty metres in D. The pictures of the resin casts are from Weissenberger et al. (1992).

Two further ice types form around Antarctica. Heavy snow load or deformation processes can press down the ice and the snow on top of the ice underneath the water level. The snow is infiltrated by seawater, forming an infiltration layer, which, depending on temperatures, occasionally refreezes (Ackley and Sullivan 1994). A considerable amount of sea ice is formed through this process in the Southern Ocean (Haas et al. 2001, Massom et al. 2001). The infiltration layer is often grouped together with the gap layer, which develops from an infiltration layer if melting snow refreezes on top of the infiltration layer or develops in the interior of the ice at the freeboard level in spring and summer (Kattner et al. 2004). As both are characterised by similar abiotic conditions which comprise a large brine volume, enhanced exchange with seawater and a position at the surface of the ice column, both layers are summarised under the term surface layer in this work. Platelet layers form in the vicinity of shelf ice (Dieckmann et al. 1986). The melt of shelf ice at depth leads to the formation of pressurised low-salinity shelf water. If such a water mass is transported to the surface, the pressure decreases and the freezing point of the water mass is reached. Ice platelets form,

which rise to the surface and aggregate underneath sea ice as a platelet layer. If the original ice sheet grows, these platelets are incorporated into the solid ice. As these layers mainly form in the vicinity of ice shelves, they can normally be found underneath fast ice (Eicken 1992).

1.4. Abiotic factors within sea ice

Interstitial habitats of sea ice are distinguished not only by different formation processes and positions within the ice, but also through different abiotic conditions. These are related to position and formation history. Of special importance are ice temperature, brine salinity, and brine volume, which are tightly coupled (Assur 1958, Cox and Weeks 1983). During ice growth, salts contained within seawater are not included into the ice crystals. As more ice forms with lower temperatures, brine salinity is dependent on temperature. Lower temperatures will result in higher brine salinities and vice versa (see also Fig. 5). At low temperatures salts become saturated and precipitate, like ikaite ($CaCO_3 * 6H_2O$) at -2.2 °C (Assur 1958, Dieckmann et al. 2008) and mirabilite ($Na_2SO_4 * 10H_2O_{(s)}$) at -8.2 °C (Assur 1958). As salinity, in a strict sense, is only defined for seawater with a conserved composition of ions (Millero et al. 2008), we can no longer speak of salinity when different salts precipitate. Here the term "salinity" should be understood as a measure to relate ion contents of different liquids and not in the strict sense defined for oceanography. Salinities reported in the literature are transcribed to the actual definition of salinity (Millero et al. 2008).

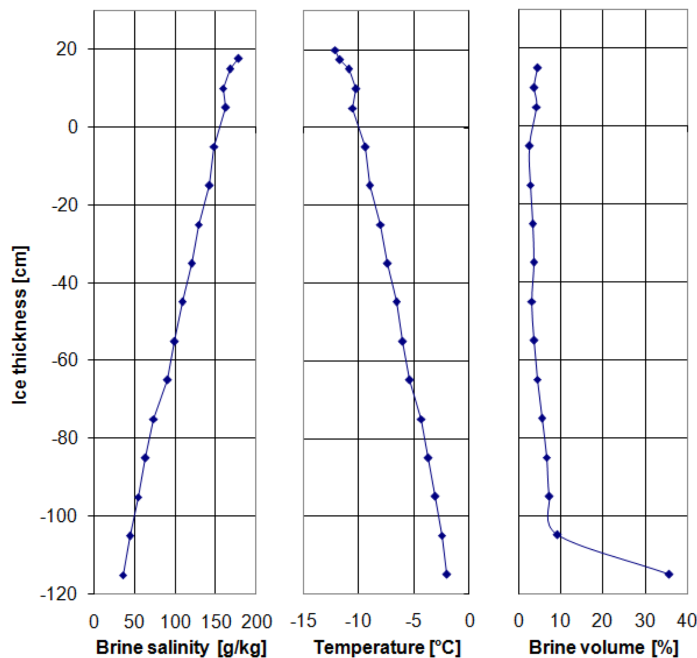

Fig. 5. Abiotic factors within sea ice. An example from 3.10.2006, Western Weddell Sea, Antarctica. Brine salinity was calculated according to Assur (1958) and Frankenstein and Garner (1967). Brine volume was calculated from temperature and bulk salinity (data not shown) according to Cox and Weeks (1983).

Temperature within the ice is, first of all, a function of air and ocean temperature. In winter, seawater underneath the ice is normally at the freezing point. With a salinity of around 34.6 g/kg, this corresponds to a temperature of -1.9 °C (Assur 1958). Air and ice-surface temperatures are generally lower in winter (down to at least -35 °C ice temperature, Maykut (1986)). Therefore, a temperature gradient from -1.9 °C at the bottom to very low temperatures at the top is found within the ice (see also Fig. 5). Correspondingly, brine salinities are close to seawater salinity at the bottom and very high at the top. Liquid brine within sea ice was reported at temperatures down to -25 °C (Krembs et al. 2002a) which corresponds to a salinity of 300 g/kg (Assur 1958). In summer, higher air temperatures can lead to a reversal of the temperature and brine salinity profile (Untersteiner 1968). Temperatures between 0 (the freezing point of freshwater) and -1.9 °C (the freezing point of seawater) are then typical for sea ice and brine salinities can vary between 0 and 35 g/kg.
The inhabitable space within sea ice - the pore space or brine volume - depends on bulk salinity (the amount of salt within a given mass of melted ice) and temperature (Cox and Weeks 1983). Therefore, at lower temperatures, less pore space is available. But, as bulk salinity can vary, brine volume can as well. Bulk salinity depends on formation and desalination processes and varies from ice type to ice type (Eicken and Lange 1989). Turbulent conditions during ice formation result in irregular ice growth. As a result, larger pore spaces are found within granular ice as compared to columnar ice grown under calm conditions. As more seawater is included during the formation of granular ice, bulk salinity of granular ice is also higher and, at a given temperature, granular ice contains more pore space than columnar ice (Weissenberger et al. 1992). This signal is often masked by later desalination processes (Eicken and Lange 1989). Brine drainage takes place in summer when sea ice warms up. In conclusion, multi-year ice contains less salt (lower bulk salinity) and less brine volume than first-year ice (Untersteiner 1968). Internal brine volume of sea ice varies between 0 and about 20 % (Untersteiner 1968, Eicken 1992). Infiltration layers consist of flooded snow and therefore contain large pore spaces (up to 81 %, see chapter two). A record of the liquid fraction of platelet layers was not found in literature, but the liquid fraction presumably is high, as the platelet layer is just a loose aggregation of ice. Diameters of brine channels do also depend on temperature, bulk salinity and formation history and can range from micro- to centimetres (Krembs et al. 2000, Haas et al. 2001, own observations). The bottom layer of sea ice is characterised by an especially large brine volume. In the transition area from ice to water, it can rather be a mushy layer than a solid substance (Wettlaufer et al. 1997, Feltham et al. 2006). Biological activity in isolated areas can lead to changes in oxygen

and carbon dioxide concentrations, pH and the accumulation of metabolic end products like ammonium (Schnack-Schiel et al. 2004, Papadimitriou et al. 2007).

Organisms thriving in the interstitices of sea ice have to cope with the described changes in temperature, salinity, space, oxygen and carbon dioxide concentration, pH and with ice formation. The quantity and quality of light might also be important for orientation or food identification, and is of fundamental importance for primary production, the basis of the sympagic food web.

1.5. The Antarctic sea-ice community

The sea-ice ecosystem is fuelled through primary production of mainly algae, particularly diatoms (Arrigo and Thomas 2004). Primary production – the build up of organic material – within the ice accounts for only 1 to 2 % of the annual primary production in the whole Southern Ocean (south of 50°S), but in areas covered by ice for at least a part of the year, it can make up 10 to 25 % of the total primary production (Arrigo and Thomas 2004). The chlorophyll *a* (chl *a*) concentrations found within the ice can be orders of magnitude higher (up to $10.1*10^3$ µg/L; Lizotte and Sullivan (1992)) than in the water column below (typical values 0 to 5 µg/L; Thomas and Dieckmann (2002)). Sea ice represents a physical support retaining algae within the euphotic zone, and within brine channels algae are protected from larger grazers (Krembs et al. 2000). Specific habitats which can be distinguished are, from top to bottom, the surface layer (Fig. 6 A), the internal habitat (Fig. 6 B), the bottom layer (Fig. 6 C) and the platelet layer (Fig. 6 D) (after Horner et al. (1992)).

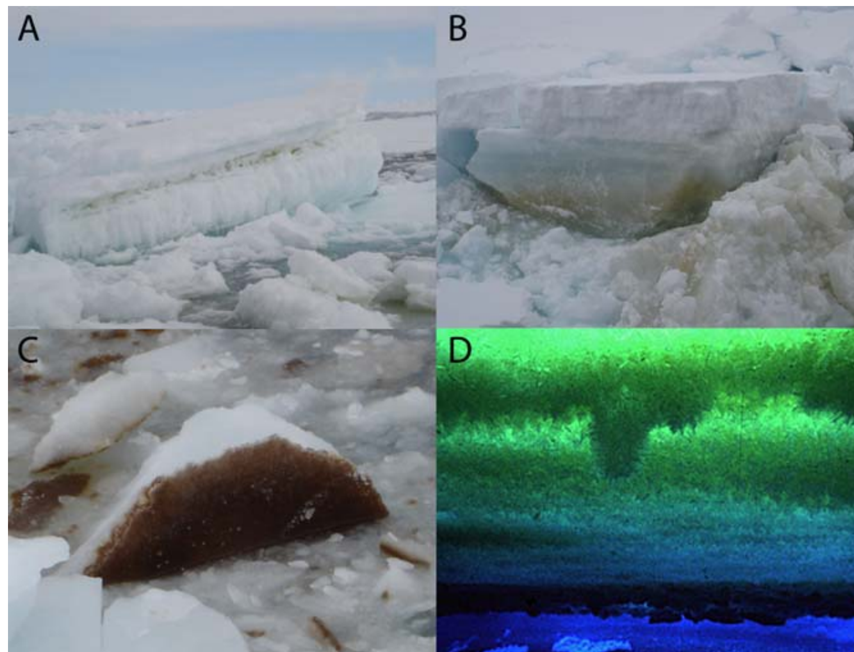

Fig. 6. Antarctic sea-ice habitats. (A) infiltration layer, (B) internal layer, (C) bottom layer and (D) platelet layer. The floe in A was uplifted out of its original position, the floes in B and C tilted. The floe in B is seen from the side, in C the bottom of the floe is visible. Approximate thickness of the floes in A, B and C is 1 m. The colouration of the respective layers is caused by high concentrations of brown and green algae. Picture of the platelet layer courtesy of C.W. Sullivan.

A replenishment of nutrients is, first of all, possible in the surface, bottom and platelet layers, allowing for a high algal biomass (Thomas and Dieckmann 2002). Internal communities do also develop if conditions are favourable (Kattner et al. 2004). Algal growth is depending on light and as a result, primary production also depends on factors like snow cover and day length and is hampered in polar winter (Gradinger et al. 1991, Mundy et al. 2005). Based on the primary production, a complex assemblage of viruses, bacteria, fungi, proto- and metazoans can thrive within the ice. These can reach abundances an order of magnitude higher in the ice than in the underlying water column (Thomas and Dieckmann 2002). Meiofauna organisms commonly reported from Antarctic sea ice are acoel plathyelminthes, as well as calanoid and harpacticoid copepods (Fig. 7 A - D) (Schnack-Schiel et al. 1995, Tanimura et al. 1996, Janssen and Gradinger 1999). Single reports of ctenophores (Dahms et al. 1990; Fig. 7 E), nudibranchs (Pelseneer 1903; Fig. 7 F), polychaete larvae, ostracods, amphipods, mysiids, appendicularians (Günther et al. 1999) as well as fish eggs and larvae (Vacchi et al. 2004) are also available. The life-cycle strategies of the dominant copepod species of Antarctic sea-ice communities, the calanoid copepods *Stephos longipes* (Fig. 7 A)

and *Paralabidocera antarctica* (Fig. 7 B) and the harpacticoid copepod *Drescheriella glacialis* (Fig. 7 C), are well-understood (Swadling et al. 2004, Schnack-Schiel et al. 1995, Dahms et al. 1990, Bergmans et al. 1991). The life-cycle strategies of other species are less well-studied.

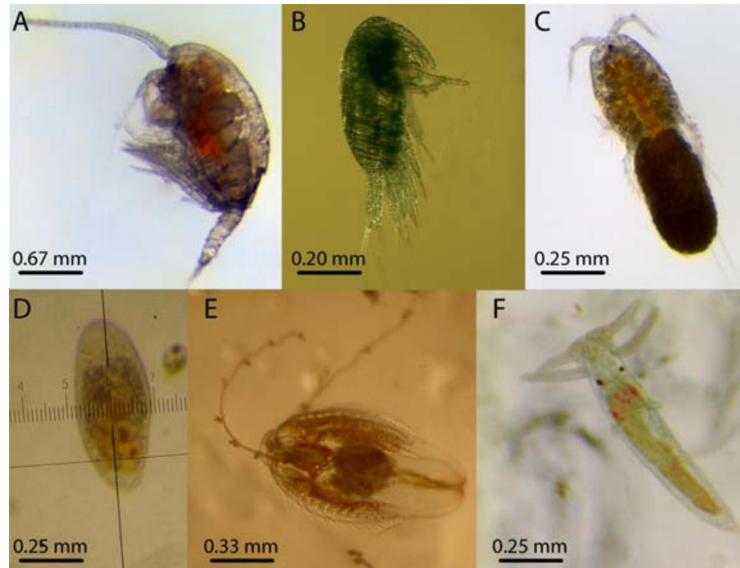

Fig. 7. Meiofauna organisms from the Antarctic brine-channel system. (A) calanoid copepod *Stephos longipes* (female with oocytes), (B) calanoid copepod *Paralabidocera antarctica*, (C) harpacticoid copepod *Drescheriella glacialis* (female with egg sack), (D) acoel plathyelminth, (E) ctenophore *Callianira antarctica* and (F) nudibranch *Tergipes antarcticus* (juvenile). Pictures A and C courtesy of J. Michels, B and E courtesy of M. Kramer.

1.6. Tolerance limits of sea-ice meiofauna

Sea ice is the only large marine habitat in which organisms live at temperatures below the freezing point of seawater. Therefore, it is a unique habitat to study adaptations of marine organisms to low temperatures, high salinities and ice formation. These factors are likely to have an important effect on the survival and growth of sympagic organisms in nature. Laboratory experiments show that sympagic meiofauna organisms are tolerant to salinity fluctuations. The harpacticoid copepod *Drescheriella glacialis* survives salinities from 18 to 90 g/kg at 0 °C (Dahms et al. 1990), and acoel Antarctic plathyelminths from 34 to at least 75 g/kg at 0 °C (Gradinger and Schnack-Schiel 1998). As temperature and salinity in sea ice are coupled (Assur 1958), tolerance to salinities of 90 and 75 g/kg might enable *D. glacialis* and the acoel plathyelminth to survive temperatures of -5.3 and -4.4 °C, respectively. Studies from

the Arctic have reported survival of *Tisbe furcata*, *Cyclopina scheideri*, *Cyclopina gracilis* and *Arctocyclopina pagonasta* at salinities from 20 to 70 g/kg (Grainger and Mohammed 1990). An acoel plathyelminth and the nematode *Theristus melnikovi* survived salinities from 5 to 65 g/kg and 5 to 100 g/kg, respectively (at 0 to 1 °C incubation temperature), furthermore they survived temperatures down to -6.0 and -6.8 °C at the respective equilibrium salinities of 90 and 120 g/kg (Friedrich 1997). The Arctic sympagic hydrozoan *Sympagohydra tuuli* survived 24 h of exposure to -4.6 °C at a salinity of 80 g/kg and also a salinity of 5 g/kg at 0 °C; movement ceased at these conditions, but was resumed when the animals were slowly brought back to a salinity of 33 g/kg (Siebert et al. in press). Apart from these descriptions of tolerance limits, investigations of adaptation mechanisms to changing salinities, low temperatures and freezing stress in sympagic meiofauna organisms are not available.

1.7. Objectives of this thesis

This work aims to yield a further understanding of the distribution and diversity of Antarctic sympagic meiofauna organisms and the characteristics of their habitat. The second major aim is the identification of adaptation strategies to the habitat with a special focus on the interrelated factors of temperature, salinity and freezing stress. Physiological und molecular-biological methods were applied in this study in order to get first insights into these strategies. These investigations should finally allow a further characterisation of traits which are characteristic for truly sympagic species. The general discussion summarises these investigations and discusses the probable effects of life-cycle and dispersal strategies, as well as niche separation on the distribution of sympagic meiofauna organisms. Furthermore, molecular-biological and physiological results and their links to the ecology of sympagic meiofauna species are discussed. A short outlook for future research is given.

Ecology

A long-term ice station in the Western Weddell Sea was used to study the characteristics of the surface and sub-ice layer habitats and their sympagic communities over one month in late spring 2004 (ISPOL, ANT XXII/2). This work was performed in parallel to work by Schnack-Schiel et al. (2008) on the internal habitat and the bottom layer and allowed the comparison of these sea-ice habitat types. Goals of this project were the identification of habitat preferences and life-cycle strategies of sympagic meiofauna species, with a focus on less abundant species. The results of this study are presented and discussed in chapter two.

A species found in the surface layer during ISPOL was the nudibranch gastropod *Tergipes antarcticus*. It was reported from sea ice for the first time in 1903 (Pelseneer 1903). During the second expedition to the Western Weddell Sea in austral winter 2006 (WWOS, ANT XXIII/7) large scale sampling efforts were undertaken in order to obtain samples from this species. Work on this species aimed at the first thorough description of the species and the first investigation of its distribution, abundance and life-cycle strategy. The results of this study are presented and discussed, together with work on the physiological adaptation strategies of this species, in chapter three.

Physiology

Classical physiological background parameters like osmolality of the haemolymph at different salinities, presence of thermal hysteresis and supercooling points were determined. As salinity changes occur within the brine channel system (Assur 1958) a major question was how species living inside sea ice regulate their internal osmolality in response to these external osmolality changes. The determination of haemolymph osmolalities also followed a second rationale. The freezing point of a liquid depends on its osmolality (DeVries 1982). Maintaining the osmolality within the body above or at the same level of the external medium keeps the freezing points of both compartments equal and therefore protects the animal from ice formation within brine channels. Another possibility to inhibit the freezing of body liquids is through thermal hysteresis, a non-colligative inhibition of ice growth (DeVries 1971). The determination of supercooling points (the temperature at which an organism freezes) can give a first hint, whether a species is freeze resistant and therefore remains unfrozen at usually encountered environmental temperatures or is freeze-tolerant (Sømme 1999). The results of these investigations, which aim at the understanding of freeze-protection mechanisms of three sympagic meiofauna species (the nudibranch gastropod *Tergipes antarcticus*, and the calanoid copepods *Stephos longipes* and *Paralabidocera antarctica*) are presented in the chapters three and four.

Molecular biology

Molecular-biological methods can yield a further insight into the genetic and molecular basis of adaptation mechanisms. Therefore, as a second strategy, gene-expression analysis was used to receive a first, unbiased insight into physiological responses to elevated salinities and low temperatures of the sympagic copepod *Stephos longipes*. This work revealed the presence of two isoforms of an antifreeze protein in this species. The detailed analysis of the antifreeze protein is presented in chapter four.

2. Living conditions, abundance and composition of the metazoan fauna in surface and sub-ice layers in pack ice of the western Weddell Sea during late spring

2.1. Introduction

Sea ice harbours a highly diverse fauna and flora that play an important role in the carbon cycle of the Southern Ocean (Arrigo and Thomas 2004). A total of 5–25% of the marine primary production in the Southern Ocean is associated with sea ice (Lizotte 2001, Arrigo 2003 and references therein). The high ice-algal standing stocks represent a potential food source for a diverse sympagic (= ice-associated) meiofauna community, but also for pelagic and benthic organisms after melting (Kurbjeweit et al. 1993, Werner et al. 2004, Lovvorn et al. 2005, Michels et al. 2008). Inside Antarctic pack ice, copepods and plathyelminths are the most abundant members of the sympagic meiofauna (Schnack-Schiel et al. 2001a). Three dominant species of copepods (*Stephos longipes*, *Paralabidocera antarctica*, *Drescheriella glacialis*) have been fairly well studied with respect to their life cyles (Dahms et al. 1990, Schnack-Schiel et al. 1995, Tanimura et al. 1996). However, much less is known about several other metazoan species (ctenophores, plathyelminths, other copepods, nudibranchs), which also have been found in Antarctic sea ice (Pelseneer 1903, Dahms et al. 1990, Schnack-Schiel 2003). According to Horner et al. (1992), four different sea-ice habitats and their respective communities can be identified in Antarctic pack ice: surface, interior, bottom and sub-ice layers and communities. Here I present studies on the environmental conditions and the metazoan meiofauna ($\geq$ 50 µm) in the surface and sub-ice habitats of Antarctic pack ice. Due to different formation processes, three different surface communities can be distinguished in detail: infiltration, deformation and melt-pond communities (Horner et al. 1992). They all are found in a semi-fluid layer on top of the ice proper, in which organisms from the seawater can occur. When superimposed ice forms on top of the surface layer, the latter is sometimes called a gap layer (Kattner et al. 2004). In the following, I do not discriminate between all these varieties and speak hereafter of the surface layer only. Surface layers are a common feature of Antarctic pack ice and well known for their high primary productivity (Garrison and Buck 1991, Kattner et al. 2004). Most studies investigating the biology of the surface layer have to date focussed on the microbial communities (Garrison and Buck 1991, Fritsen et al. 2001, Kattner et al. 2004). Very little is known about the

metazoan fauna in this habitat, from which only two species of copepods (*D. glacialis*, *S. longipes*) have as yet been described (Schnack-Schiel et al. 2001b). The sub-ice layer directly below the ice, which is physically connected to the brine channel system and the underside of the ice, is another habitat with a special community (Tanimura et al. 1984, Kurbjeweit et al. 1993, Schnack-Schiel et al. 1998, Werner 2006). Conditions in this habitat are governed by processes both in the overlying ice and in the underlying water column. Freezing conditions and probably scarce food supply during winter, as well as melting and enhanced release of food from the ice during summer characterise this habitat. Freshening of the sub-ice layer during summer seems to be an important factor, excluding truly pelagic, stenohaline species from this habitat (for the Arctic: Werner 2006). Studies on the composition of the sub-ice layer around Antarctica are scarce and were performed in areas of fast ice (Tanimura et al. 1984) or seasonal sea-ice cover (Kurbjeweit et al. 1993). Only one study from an area of multiyear pack ice exists from the Bellingshausen Sea (Schnack-Schiel et al. 1998). The Antarctic krill, *Euphausia superba*, a key member of the Southern Ocean food web, exploits the sub-ice layer, and distribution and feeding activity of this species is strongly correlated with the pack-ice cover (Loeb et al. 1997). Similar to the Arctic (Werner and Auel 2005), the ice–water interface can be inhabited by several species of amphipods; however, this is a relatively new observation for the Antarctic pack ice (Krapp et al. 2008). The goals of the present study were to (i) investigate the diversity and abundance of metazoan species in the surface and sub-ice layers in a region of perennial Antarctic pack ice, (ii) elucidate biotic and abiotic factors influencing the species' distribution in the different habitats, and (iii) deduce life-cycle strategies and niche separation of the species in, and between the respective habitats. Comparison of the data set presented in this publication with another data set on the abundances of copepods within the ice proper (Schnack-Schiel et al. 2008) allows identification of links and differences between the ice proper, sub-ice layer and surface-layer communities. It enables me to allocate and discuss life-cycle strategies of the poorly studied sympagic copepod species *Ectinosoma* sp., *Drescheriella racovitzai*, *Diarthrodes* cf. *lilacinus* and *Idomene antarctica* for the first time.

2.2. Material and Methods

2.2.1. Study area

This investigation of the surface and sub-ice layer habitats and their metazoan fauna took place in the western Weddell Sea during the ‘‘Ice Station POLarstern’’ (ISPOL; ANT XXII/2, 6 November 2004–19 January 2005 (Dieckmann et al. 2007). The research vessel Polarstern was moored to a floe from 28 November 2004 until 2 January 2005 and drifted with this floe mainly to the north from 68°15’S to 67°20’S at about 55°25’W. All investigations of the present study were performed on an initially 10 km * 10 km-sized floe, which broke up into several parts during the study period (Fig. 8). The floe consisted mainly of second-year ice (2 m thick, 0.8 m snow cover), interspersed by locally formed or advected first-year ice (0.9 and 1.8 m thick, respectively, 0.3 m snow cover). The area around the floe was covered to 9/10 by the same kind of pack ice. Flooding of the floe occurred at some places, and the establishment of surface layers with a brownish colour due to growing algae was observed at several sites. The floe composition was typical for an area of about 100 km * 500 km (north–south direction), a transition area from thick second-year ice located to the east to homogeneous first-year ice further west (C. Haas, personal communication). Dynamic ice conditions, which resulted in a considerable decrease of the mean floe size from 825 to 242 m^2 in the study area between 9 December and 1 January (Steer et al. 2008), were mirrored by a slowly progressing floe break-up, with a major break-up on 25 December. For further details on ice conditions, see Haas et al. (2008), Heil et al. (2008) and Steer et al. (2008). Besides shifting wind and drift directions, tidal movements, an increase in solar radiation (Bareiss and Görgen 2008) and a mean modelled heat flux of 15 Wm^{-2} from the ocean into the ice (McPhee 2008) were probably responsible for the reduction of floe size. Air temperatures (measured by the ship’s system) ranged between -7.0 and 2.7 °C, with an average of -2.6 °C. For further details on atmospheric conditions, see Bareiss and Görgen (2008). During the investigation period, a general thinning of the snow cover of 10–18 cm (-0.33 to -0.64 cm d^{-1}) occurred. Snowfall was observed only twice. Between 28 November and 2 December 7 cm and on the 27 December 2–4 cm of snow accumulated on the floe (Haas et al. 2008).

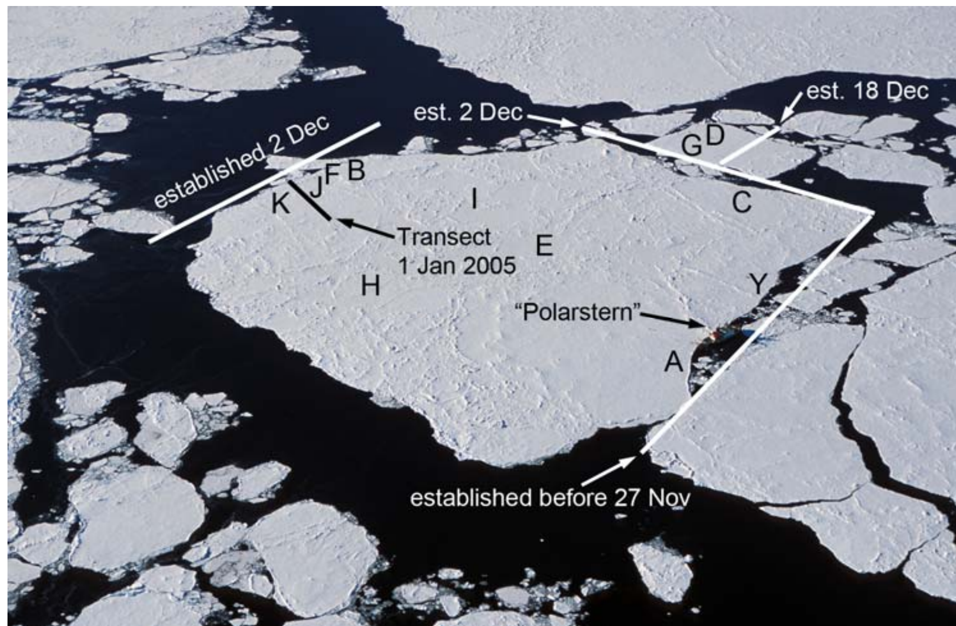

Fig. 8. Drifting sea-ice station in the western Weddell Sea during the ISPOL expedition. A - K denote sites for surface layer sampling; at site J a transect was sampled from the edge to the inner part of the floe, at site Y only qualitative samples were taken, and at sites C and G the under-ice habitat and fauna were sampled; white lines show locations (and dates of establishment) of cracks. Photo: Ingo Arndt.

2.2.2. Sampling and processing of samples from the surface layer

Different sites on the floe, chosen by their distance to the floe edge and the timing of crack formation (Table 1; Fig. 8) were sampled. Sampling took place between 12:00 and 19:00 h UTC (local noon was at 15:00 h UTC). If possible, sampling was performed either at 1 and 5 m distance to the floe edge or in the inner part of the floe (more than 35 m away from the floe edge). On 1 January, a transect from the floe edge to the inner part of the floe was sampled (distances to floe edge: 1, 2, 3, 4, 5, 6, 7, 8, 15 and 30 m). This sampling took place from 22:00 to 24:00 h UTC. At every site, snow and superimposed ice were removed carefully from above the surface layer. Thereafter, the semifluid layer on top of the solid ice proper was mixed with a shovel for homogeneous sampling representative of the whole layer. The thickness of this layer, the snow and the superimposed ice were measured with a ruler. A mesh was pushed inside the ice–water mixture, and temperature and salinity were measured inside the ice-free interior part of the mesh with a WTW microprocessor conductivity meter LF 196 (accuracy: T $\pm$ 0.1 °C, S $\pm$ 0.2) and S_R was calculated by multiplying the displayed values with 1.004715 g/kg as proposed by Millero et al. (2008). The measured salinity is

regarded to be the brine salinity of the surface layer. For the determination of chlorophyll *a* (Chl *a*) concentrations and bulk salinity, 1-L samples of the ice–water mixture were taken with a scoop, poured into acid-cleaned polyethylene-boxes and melted at 4 °C in the dark. Once melted, bulk salinity was measured with the WTW microprocessor conductivity meter LF 196. For determination of the Chl *a* concentration, melted ice samples were filtered on Whatman GF/F filters, extracted in 90% acetone, homogenised and analysed fluorometrically with a Turner Designs 10-AU digital fluorometer according to Evans and O'Reilly (1983). Chl *a* determination on directly melted ice samples involves the risk of organism losses during ice melting (Garrison and Buck 1986) and thus an underestimation of Chl *a* concentrations, but facilitates the determination of bulk salinity and Chl *a* concentration in the same ice sample. Furthermore, this method already has been applied in several Arctic sea-ice studies (e.g., Gradinger 1999a; Mock and Gradinger 1999, Krembs et al. 2001) and therefore allows a comparison of the results. As temperature and brine salinity in the surface layer were seldom in equilibrium (Fig. 9), brine volume was calculated as a function of bulk salinity and brine salinity according to the following formulae:

Brine volume = (bulk salinity * density of pure ice)/(brine salinity * brine density) (Cox and Weeks 1983), density of pure ice = $0.917 - (1.403 * 10^{-4}) * T$ (Pounder 1965), brine density = 1.0008 * brine salinity (Zubov 1945, Cox and Weeks 1975).

Chl *a* concentrations are given for the melted sample (Table 1) and related to brine volume (Fig. 10).

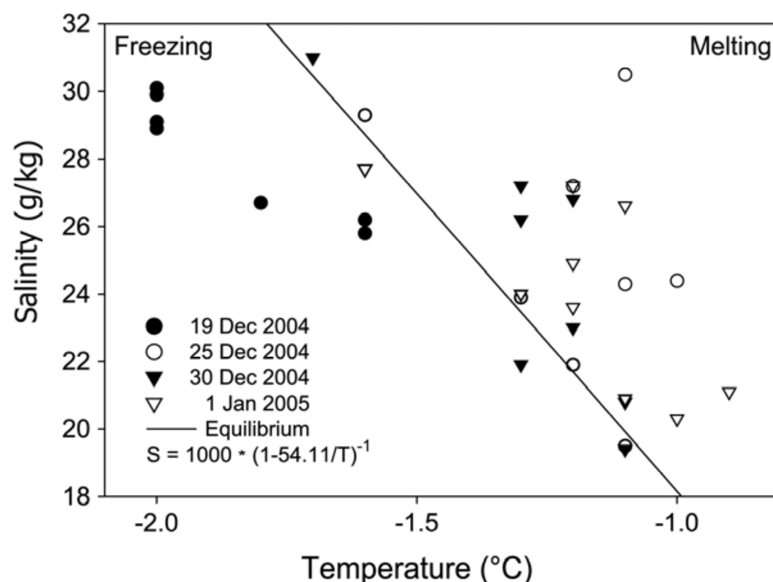

Fig. 9. Temperature and salinity in the surface layer of the drifting ice station from different sampling sites and on different sampling dates. The straight line depicts equilibrium conditions in sea ice calculated according to Assur (1958) and Frankenstein and Garner (1967).

For the determination of abundances and composition of the sympagic meiofauna, 1-L samples of the ice-water mixture were taken with a scoop and melted in the dark at 4 °C in a surplus of 0.2-µm-filtered seawater to avoid osmotic stress (Garrison and Buck 1986), especially for delicate forms such as plathyelminths. Once melted, the samples were concentrated over a 50-µm gauze and fixed with borax-buffered formalin in seawater (4% final concentration). For enumeration of metazoan species and developmental stages from the surface layer, samples were sorted under a stereomicroscope (10-100 x magnification). Samples were split into aliquots with a Folsom splitter to count small and numerous species, while large and scarce species were counted from the entire sample. In *Drescheriella glacialis* and *D. racovitzai*, only adults were identified to species level. Their naupliar and copepodite stages were combined. With one exception (nauplii indet.), all other harpacticoid nauplii were identified to species level, whereas the nauplii of cyclopoids and calanoids (except *Stephos longipes*) were not further identified. Species abundances are given for the melted sample. Exuviae were also identified and counted, but data are only presented for *Diarthrodes* cf. *lilacinus*. For dominant copepod species a mean developmental stage (MDS) was calculated, modified after Marin (1987). As nauplii were included in the calculation, a value of 1 means that all specimens found were nauplii of stage I, a value of 7 means that the copepodite stage I was the average stage found, and a value of 12 means that all animals found were adults. These indices also were calculated for nauplii and copepodids separately, if a bimodal stage distribution was obvious.

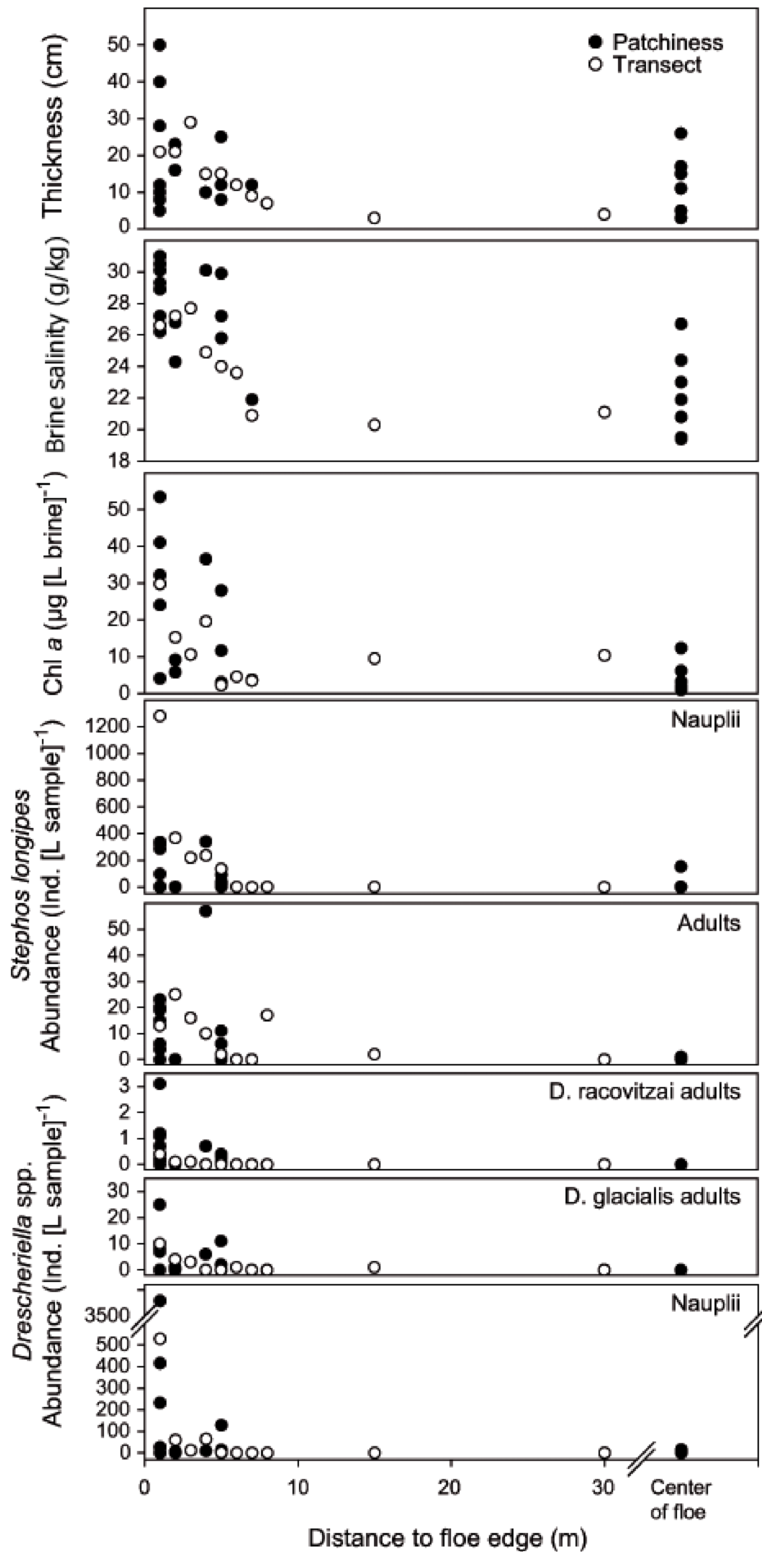

Fig. 10. Environmental parameters, algal biomass and dominant copepod abundances in the surface layer in relation to the distance to the floe edge. Data from different sampling sites of the drifting ice station (dark circles; A–K in Fig. 8) and different sampling dates (Table 1), as well as from the transect sampled on 1 January (open circles; J in Fig. 8; see also Table 1). Data from sampling sites more than 35 m away from the floe edge are presented together at the right side of the graph, denoted by ''centre of floe''. Note the high variability in salinity in the surface layer of the centre part of the floe, but the comparatively low Chl a concentrations and low meiofauna abundances there.

Furthermore, large volumes (more than 50 L of ice-water mixture) from the surface layer were filtered first through a 1-mm sieve, which retained the ice crystals, and then through a 50-µm sieve, which retained the organisms, in order to identify species which only occurred in low numbers in the infiltration layer. These samples were taken at the sites A, B and Y (Fig. 8).

Table 1: Dates of and environmental conditions at the different sampling sites for surface layer fauna

Sampling site	Date of sampling	Date of crack formation	Distance to floe edge (m)	Brine salinity (g/kg)	Bulk salinity (g/kg)	Temperature (°C)	Surface layer thickness (cm)	Super imposed ice (cm)	Snow thickness (cm)	Bulk Chl *a* ($\mu g\ L^{-1}$)	Brine volume (%)
A	19 Dec 04	b.a.	1	30.1	18.3	-2	10	0	30	29.1	54
A	19 Dec 04	b.a.	4	29.1	19.6	-2	10	0	50	22.1	60
B	19 Dec 04	2 Dec 04	1	28.9	14.7	-2	50	0	30	13.6	46
B	19 Dec 04	2 Dec 04	5	29.9	19.6	-2	12	0	30	1.8	59
C	19 Dec 04	2 Dec 04	1	26.2	14.8	-1.6	5	0	10	12.2	51
C	19 Dec 04	2 Dec 04	5	25.8	16.5	-1.6	8	0	10	6.7	57
D	19 Dec 04	/	30	/	15.9	/	/	/	/	3.1	/
E	19 Dec 04	/	30	26.7	21.5	-1.8	15	0	8	0.7	72
B	25 Dec 04	2 Dec 04	1	30.5	15.3	-1.1	40	6	22	14.5	45
B	25 Dec 04	2 Dec 04	5	27.2	11.7	-1.2	25	5	25	10.8	39
F	25 Dec 04	2 Dec 04	1	29.3	23.7	-1.6	12	7	12	32.6	72
D	25 Dec 04	18 Dec 04	2	24.3	10.3	-1.1	16	10	9	2.2	38
D	25 Dec 04	/	30	23.9	14.5	-1.3	15	4	11	1.8	55
G	25 Dec 04	/	30	21.9	14	-1.2	5	4	16	1.0	58
H	25 Dec 04	/	30	19.5	13.3	-1.1	3	4	12	1.0	62
I	25 Dec 04	/	30	24.4	22.6	-1	liquid	/	/	1.6	83
J	30 Dec 04	2 Dec 04	1	31	20.2	-1.7	33	20	21	23.9	58
J	30 Dec 04	2 Dec 04	7	21.9	13.2	-1.3	12	11	33	2.0	54
K	30 Dec 04	2 Dec 04	1	27.2	10.7	-1.3	8	22	17	1.4	35
D	30 Dec 04	18 Dec 04	2	26.8	14.8	-1.2	23	8	9	4.5	50
D	30 Dec 04	/	30	26.2	12.9	-1.3	26	1	11	0.8	44
G	30 Dec 04	/	30	20.8	12.6	-1.1	17	10	8	1.0	55
H	30 Dec 04	/	30	19.4	8.4	-1.1	/	/	/	2.4	39
I	30 Dec 04	/	30	23	15.8	-1.2	20	1	5	7.6	62
J	1 Jan 05	2 Dec 04	1	26.6	15.1	-1.1	21	29	18	15.2	51
J	1 Jan 05	2 Dec 04	2	27.2	18.6	-1.2	21	37	2	9.4	61
J	1 Jan 05	2 Dec 04	3	27.7	17.6	-1.6	29	10	31	6.1	57
J	1 Jan 05	2 Dec 04	4	24.9	14.2	-1.2	15	5	28	10.1	51
J	1 Jan 05	2 Dec 04	5	24	21.5	-1.3	15	24	19	1.8	81
J	1 Jan 05	2 Dec 04	6	23.6	13.8	-1.2	12	22	23	2.4	53
J	1 Jan 05	2 Dec 04	7	20.9	11.2	-1.1	9	34	14	1.7	48
J	1 Jan 05	2 Dec 04	8	/	15.5	-1.2	7	14	32	1.1	/
J	1 Jan 05	2 Dec 04	15	20.3	10.9	-1	3	4	43	4.6	48
J	1 Jan 05	2 Dec 04	30	21.1	9.9	-0.9	4	23	25	4.4	42
mean:				25.3	15.4	-1.4	16.2	10.2	19.8	7.5	54.4
sd:				3.4	3.8	0.3	11.0	10.8	11.4	8.5	11.5

For positions of sampling sites A–K and of cracks on the drift-ice station, see Fig. 8; sampling site J was used for a transect; b.a., before arrival of R.V. Polarstern at the drift-ice station; /, no data; S.D., standard deviation.

2.2.3. Sampling and processing of samples from the sub-ice layer

In order to document a time-series of the sub-ice fauna, sampling took place at position D, the same location as that chosen by Schnack-Schiel et al. (2008), except for two occasions (29 November and 14 December). On 29 November sampling took place on an area of the first-year ice near position D, which broke apart from the rest of the floe on 2 December. On 14 December sampling took place at position C (Table 1; Fig. 8). The morphology of the ice underside and the macrofauna in this habitat were recorded by a video camera lowered through a core hole (Werner and Lindemann 1997, Werner and Gradinger 2002). Temperature and salinity profiles in the sub-ice water layer (0–6 m below the ice underside) were measured *in situ* with the WTW microprocessor conductivity meter LF 196 lowered through a core hole. As variations in temperature and salinity were smaller than the accuracy of the conductometer used, only the ranges found are reported in the results. Discrete water samples for the analysis of Chl *a* concentrations were collected at 0 and 5 m depth below the ice with a polyethylene tube (4 cm internal diameter) with a valve at one end. The unequipped end of the tube was lowered into the water through a core hole with the valve closed. At the sampling depth, the valve was opened and closed again and the tube with the enclosed water sample was hoisted to the surface. Determination of Chl *a* concentrations was performed as described above for the ice samples. Organisms from the sub-ice water (0 and 5 m depth below the ice) were quantitatively sampled with an under-ice pumping system (Werner and Martinez Arbizu 1999) equipped with a standardised water meter (accuracy 0.1 L) and inserts of plankton gauze (mesh size 50 µm) to concentrate the organisms. Between 1.4 and 3.8 m^3 of sub-ice water were pumped at each station from each depth. Samples were fixed in borax-buffered formalin in seawater (4% final concentration). Enumeration of species and stages from the sub-ice layer and for dominant copepod species the calculation of a mean stage composition was performed as above.

2.3. Results

2.3.1. Environmental conditions in the surface layer

Surface-layer thickness varied between 3 and 50 cm, with generally higher values at the edge of the floe (Table 1; Fig. 10). The daily average surface-layer temperature increased from -1.9 °C (range: -1.6 to -2.0 °C) on 19 December to -1.2 °C (range: -0.9 to -1.6 °C) on 1 January. The average brine salinity decreased from 28.1 g/kg (range: 25.8–30.1 g/kg) to 24.0 g/kg (range: 20.3–27.7 g/kg) in the same period of time, as did the bulk salinity from 17.6 g/kg (range: 14.7–21.5 g/kg) to 14.8 g/kg (range: 9.9–21.5 g/kg). On most occasions, brine salinity was also higher at the edge of the floe than further inwards (Table 1; Fig. 10). Average brine volume of all samples was 54% (38–83%). The temperature–salinity relationship in the surface layer was seldom at equilibrium conditions (Fig. 9). A change from freezing to melting conditions occurred between 19 and 25 December. Chl *a* concentrations in the brine varied between 1.0 and 53.5 mgL^{-1} with higher values at the edge of the floe (Fig. 10). The trends of higher surface-layer thickness, bulk and brine salinities and Chl *a* concentration at the edge of the floe were clearly recognisable from the transect, sampled on 1 January (Fig. 10, open circles). Highest Chl *a* concentrations were found at stations with a long established floe edge.

2.3.2. Metazoan fauna in the surface layer

A total of at least 12 sympagic species of metazoans were found in the surface layer (Table 2). Copepods were the most diverse group consisting of the four harpacticoid species *Drescheriella glacialis*, *D. racovitzai*, *Idomene antarctica* and *Nitocra gracilimana* and the calanoids *Stephos longipes* and *Paralabidocera antarctica*. However, only the harpacticoids *D. glacialis/racovitzai* and the calanoid *S. longipes* were abundant. Other taxa found in the surface layer were undetermined plathyelminths, the gastropod *Tergipes antarcticus*, and, for the first time, the ctenophore *Callianira antarctica* and the amphipods *Eusirus antarcticus* and *Eusirus tridentatus*. The euphausid *Euphausia superba* occurred on rare occasions. The distribution of the dominant copepod species was very variable, with highest values generally at the floe edge (Fig. 10, see especially the transect sampled on 1 January, open circles). Copepods were only occasionally found in samples taken at the inner part of the floe (more than 35 m away from any floe edge). Abundances of the harpacticoids *Drescheriella* spp. ranged between 0 and 3792 $ind.L^{-1}$ (median: 2) for nauplii, and between 0 and 38 $ind.L^{-1}$

(median: 0) for copepodids and adults. The maximal abundance of *Drescheriella* spp. (3830 ind.L^{-1}), which was due to the high occurrence of nauplii (3792 ind.L^{-1}), was found at a station where the floe edge had been established more than 3 weeks before sampling (Fig. 8 (A)). Abundances of the calanoid *S. longipes* ranged between 0 and 1280 ind.L^{-1} (median: 2.5) for nauplii, and between 0 and 59 ind.L^{-1} (median: 0) for copepodids and adults. Maximum abundance of *S. longipes* (1293 ind.L^{-1}) was found at a site, where the floe edge had been established 1 month before the sample was taken (Fig. 8 (J)). As in *Drescheriella* spp., nauplii accounted for most of the specimens found (1280 ind.L^{-1}). A total of 5346 nauplii and 189 copepodids and adults of *Drescheriella* spp. and of 3960 nauplii and 275 copepodids and adults of *S. longipes* were found in all samples. The stage compositions of both the *Drescheriella* species and *S. longipes* were bimodal, with high numbers of nauplii and adults present. Copepodite stages I–V were found very seldom (15 of *Drescheriella* spp. and 10 of *S. longipes*). In *Drescheriella* spp., the mean developmental stage of the nauplii was 3.3, that of the copepodids 11.8. Adults of *D. glacialis* and *D. racovitzai* were encountered in similar numbers (89 and 85 individuals, respectively). Thirty percent of the 64 *D. glacialis* females and 47% of the 85 *D. racovitza*i females found carried egg sacks. In *S. longipes*, the mean developmental stage of the nauplii was 2.0, that of the copepodids 11.8. A total of 113 *S. longipes* males and 134 females were found, 25% of the females with attached spermatophores.

Table 2: Metazoan species found in the surface and sub-ice layers of the drift-ice station

Species/Taxa	Surface layer	Sub-ice layer	
		0 m	5 m
CTENOPHORA			
Callianira antarctica (Chun 1897)	+	+[a]	–
PLATHELMINTHES			
Plathyelminths indet	+	–	–
GASTROPODA, OPISTHOBRANCHIA			
Tergipes antarcticus Pelsener 1903	+	–	–
CRUSTACEA, COPEPODA			
HARPACTICOIDA			
Diarthrodes cf. *lilacinus* Pallares 1977	–	++	++
Drescheriella glacialis [a] Dahms & Dieckmann 1987	++	++	++
Drescheriella racovitzai [a] Giesbrecht 1902	++	++	++
Ectinosoma sp. Giesbrecht 1902	–	++	++
Idomene antarctica Giesbrecht 1902	+	++	++
Hastigerella antarctica Dahms & Schminke 1992	–	+	+
Nitocra gracilimana Giesbrecht 1902	+	+	+
Nauplii indet	-	++	++
CALANOIDA			
Calanoides acutus (Giesbrecht 1902)	–	+	++
Calanus propinquus Brady 1883	–	+	++
Ctenocalanus citer Bowman & Heron 1971	–	–	+
Metridia gerlachei Giesbrecht 1902	–	–	+
Paraeuchaeta sp.	–	–	+
Paralabidocera antarctica (IC Thompson 1898)	+	–	+
Stephos longipes (Giesbrecht 1902)	++	++	++
Nauplii indet.	–	++	++
CYCLOPOIDA			
Oithona spp.	–	++	++
Oncaea spp.	–	++	++
Nauplii indet.	-	++	++
CRUSTACEA, AMPHIPODA			
Eusirus antarcticus [b] Thomson 1880	+	+[a]	–
Eusirus tridentatus [b] Lowry & Bullock 1976	+	+[a]	–
CRUSTACEA, EUPHAUSIACEA			
Euphausia superba Dana 1850	+	+[a]	–

-, absent; +, present (single specimens); ++, abundant (mostly >1 ind.m^{-3}).
[a] Observed at the ice–water interface by under-ice video, but not collected by under-ice pump.
[b] Species not quantitatively separated.

2.3.3. Environmental conditions in the sub-ice layer

The morphological appearance of the ice underside changed from a smooth, level surface on 9 November to a more structured surface with many small holes and depressions on 30 December (Table 3).

Table 3: Dates of and environmental conditions at the different sampling sites for sub-ice layer fauna

	29 Nov	4 Dec	9 Dec	14 Dec	19 Dec	26 Dec	30 Dec
Sampling site	near D	D	D	C	D	D	D
Snow thickness (cm)	35	37.3	33.3	49.6	10.6	25	35
Ice thickness (cm)	78	83	78	203	90	81	78
Freeboard (cm)	-6	-14	-14	3	4	6	nd
Bulges and depressions at the ice underside	None	Very few	Very few	Few	Many	nd	Very many

For positions of sampling sites C and D on the drift-ice station, see Fig. 8; nd, no data.

Temperatures in the water column directly below the ice down to 6 m were always between -1.8 and -1.9 °C. On 29 November the salinity of the sub-ice water was between 34.2 g/kg and 34.4 g/kg, thereafter the salinity always varied between 34.4 g/kg and 34.6 g/kg. Chl *a* concentrations in the sub-ice water layer were very low (0.1–0.5 mgL^{-1}), except for 25 December when the Chl *a* concentration at 0 m depth was 2.3 mgL^{-1}. Chl *a* concentrations were always higher at 0 m than at 5 m depth below the ice. In general, Chl *a* concentrations in the sub-ice water layer increased slightly from the beginning of this study to the end at both depths.

2.3.4. Metazoan fauna in the sub-ice layer

A total of 23 species or higher taxa of sympagic and pelagic metazoans were found at the ice underside or in the sub-ice layer, 19 of which occurred directly below the ice at 0 m depth (Table 2). At five out of seven stations, amphipods (probably *Eusirus* spp.) were observed attached to, or crawling along, the ice underside, with abundances ranging from 0 to 70 ind.m^{-2}. Ctenophores (probably *Callianira antarctica*) occurred regularly at the ice–water interface, sometimes also attached to the ice, and single specimens of krill (*Euphausia superba*) were recorded at three stations below the ice. However, the dominant taxonomic group in the sub-ice layer were the copepods. All harpacticoids found in the sub-ice layer were sympagic species, whereas all but two, *Paralabidocera antarctica* and *Stephos longipes*,

of the calanoids were truly pelagic species, as were the cyclopoids. The pelagic calanoid copepods occurred with more species (Table 2) and in higher numbers at 5 m than at 0 m depth below the ice. At both sampling depths, cyclopoid nauplii were the most abundant group (median: 700 $ind.m^{-3}$, range: 58–2387 $ind.m^{-3}$). Besides the ubiquitous *Oithona* spp. (median: 28 $ind.m^{-3}$, range: 5–233 $ind.m^{-3}$), *Oncaea* spp. (median: 3, range 0–6 $ind.m^{-3}$), calanoid nauplii (median: 7 $ind.m^{-3}$, range: 1–28 $ind.m^{-3}$), and six species of sympagic copepods were abundant in the sub-ice layer at both 0 and 5 m depth. Within these six species, *Ectinosoma* sp. predominated in both depth layers with 69% (0 m) and 66% (5 m), followed by *Drescheriella glacialis/racovitzai* (14% and 16%), *Idomene antarctica* (10% and 11%), *Diarthrodes* cf. *lilacinus* (4% and 5%) and *Stephos longipes* (4% and 3%). Stage composition and abundance of each developmental stage of these six species in the course of the study period (4–30 December) are shown in detail in Fig. 11. All dominant sympagic copepods were always more abundant just below the sea ice at 0 m than at 5 m depth. At the beginning of this study, abundances of all species of copepods were very low in the sub-ice layer and increased in the course of time. All harpacticoid species occurred in highest numbers on 19 December, whereas the calanoid *S. longipes* was most abundant on 14 December.

A: *Ectinosoma* sp.

B: *Drescheriella glacialis/racovitzai*

Fig.11

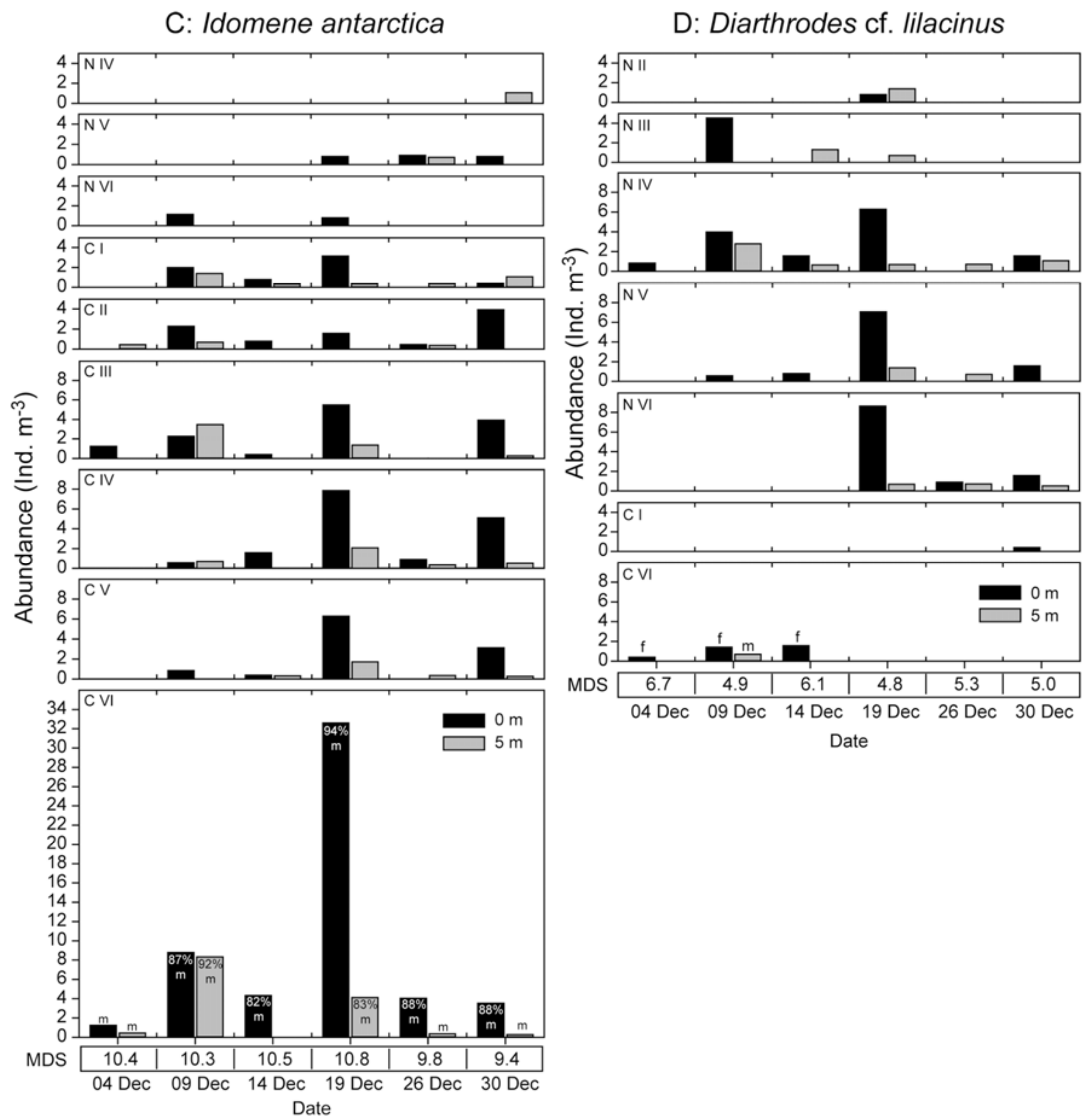

Fig. 11 continued

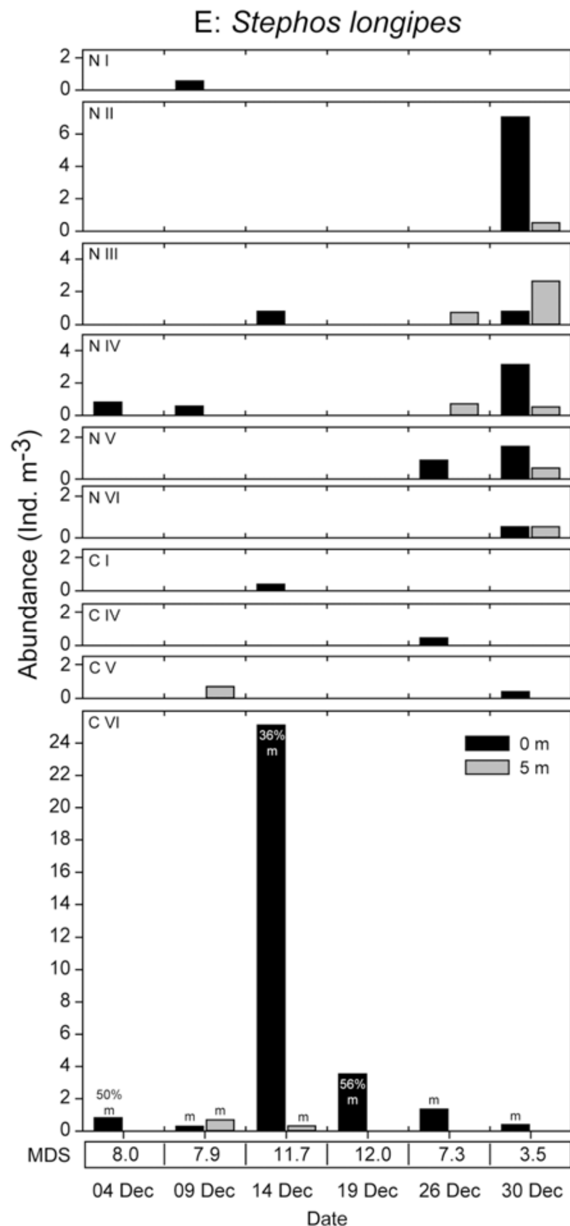

Fig. 11. Stage composition and abundances of dominant copepod species at 0 m and 5 m depth under the ice over the study period. N I - N VI = naupliar stages I - IV, C I - C V = copepodite stages I - V, C VI = adults, m = males, f = females

Fig. 11 continued

Ectinosoma sp.

Median abundances of *Ectinosoma* sp. over all developmental stages at 0 and 5 m depth were 59 ind.m^{-3} (range: 4–599 ind.m^{-3}) and 7 ind.m^{-3} (range: 1–118 ind.m^{-3}), respectively. Copepodids made up the largest fraction (89%), and within the copepodids, the stages C I–C III comprised between 72% and 100%. Copepodite stage V was found only during the last two sampling dates in low numbers, and adult specimens did not occur. Early nauplii (N I–N III) occurred only in very low numbers, whereas N V was the most abundant naupliar stage (Fig. 11 A).

Drescheriella glacialis/racovitzai

Median abundances of *Drescheriella glacialis/racovitzai* over all developmental stages at 0 and 5 m depth were 21 ind.m^{-3} (range: 7–67 ind.m^{-3}) and 8 ind.m^{-3} (range: 2–11 ind.m^{-3}), respectively (Fig. 11 B). At 0 m depth the population consisted mainly of copepodids (50%). Adults ranked second (36%), and nauplii made up 14%. At 5 m nauplii accounted for 46%, copepodids for 39% and adults for 15%. Only 15% of all adult *Drescheriella* specimens found were *D. glacialis*. Eighteen percent of all *D. glacialis* and 53% of all *D. racovitzai* adults were males. Nine percent of all *D. glacialis* and 19% of all *D. racovitzai* females carried egg sacks (Fig. 11 B).

Idomene antarctica

Median abundances of *Idomene antarctica* over all developmental stages at 0 and 5 m depth were 13 ind.m^{-3} (range: 3–58 ind.m^{-3}) and 3 ind.m^{-3} (range: 1–15 ind.m^{-3}), respectively. The *I. antarctica* population consisted mainly of adults which accounted for 46% of the total. Males always outnumbered females accounting for 90% of all adults. Twenty-five percent of the females carried egg sacks. Within the copepodids, the stages C III–C V predominated. Nauplii comprised <2% of the total, whereby only the stages N IV–N VI occurred (Fig. 11 C). Relatively high numbers of exuviae were found (3.5 and 1.5 exuviae m^{-3} at 0 and 5 m depth, respectively).

Diarthrodes cf. *lilacinus*

Median abundances of *Diarthrodes* cf. *lilacinus* over all developmental stages at 0 and 5m depth were 5 ind.m^{-3} (range: 1–23 ind.m^{-3}) and 2 ind.m^{-3} (range: 0–5 ind.m^{-3}), respectively. Naupliar stages dominated the population (92%). Adults, which made up 7%, were found only on the first three sampling days. Females were more numerous than males (>80%), but no female with an egg sack was found. Regarding only the naupliar stages, the mean population stage was one stage older (4.9) on 30 December than on 9 December (3.7) (Fig. 11 D).

Stephos longipes

Median abundances of *Stephos longipes* over all developmental stages at 0 m and 5 m depth were 3 ind.m^{-3} (range: 1–26 ind.m^{-3}) and 1 ind.m^{-3} (range: <1–5 ind.m^{-3}), respectively. On 14 and 19 December, highest abundances of adults were found when they clearly dominated the population (96% and 100%, respectively). Highest abundances of nauplii II–IV were found on 30 December (accounting for 96% of the population) (Fig. 11 E).

2.4. Discussion

2.4.1. General remarks

Several habitats associated with sea ice, which differ in their environmental conditions, are inhabited by sympagic metazoans in both polar regions (Schnack-Schiel 2003). The present study and that of Schnack-Schiel et al. (2008) show that different species or life-stages dominate in different habitats of the same ice floe, probably depending on their respective requirements for, and adaptations to environmental factors such as temperature, salinity, space and food resources. Gradients of mainly physical factors often control the distribution of sympagic metazoans (Swadling et al. 1997, Gradinger et al. 1999, Schnack-Schiel et al. 2001a, Werner and Gradinger 2002), especially related to the strong seasonal variations in the sea-ice ecosystem (Schnack-Schiel et al. 1995, Dieckmann et al. 1998, Gradinger 2001, Schünemann and Werner 2005, Werner 2006). Organisms living within or in close association with the ice have to survive the winter with reduced primary production, as well as freezing conditions and resulting ice growth. However, the reduction and destabilisation of the brine channel system during melting and the break-up of floes in spring and summer are probably a much larger challenge for sympagic species and their ice-associated life cycles (Werner 2006). The present dataset provides new insights into the life-cycle strategies of different sympagic species and their respective niche separation.

2.4.2. Habitat and community in the surface layer

The surface-layer habitat is a characteristic feature of Antarctic pack ice (Meguro 1962, Garrison 1991, Horner et al. 1992) formed by special processes and often characterised by high concentrations of algal biomass (Fritsen et al. 2001, Kattner et al. 2004). One methodological constraint during sampling of the surface layer was the disturbance of its vertical structure, resulting in features such as larger liquid-filled spaces not being properly resolved. Therefore, the large brine volumes calculated from bulk and brine salinity represent an average value for slush and liquid-filled spaces. Despite the coarse sampling procedure, some interesting trends can be deduced from the data. In terms of temperature and salinity, the ice-brine system of the surface layer, which is as a whole a dynamic system, was seldom at equilibrium conditions (Fig. 9). That means that either freezing or melting occurred at any time during the sampling period. This is probably due to changes in air temperature and irradiance, which can directly influence the surface layer closely connected to the atmosphere

(Eicken (1992) and references therein). The shift from freezing to melting conditions observed in this study occurred parallel to increases in air temperature (Bareiss and Görgen 2008). All physical and biological parameters in the surface layer vary primarily spatially with distance from the floe edge or crack. Largest surface-layer thicknesses as well as highest salinities, Chl *a* concentrations and copepod abundances were found at the floe edges. This is in accordance with the assumption that seawater infiltrating from the floe edge is responsible for the establishment of this surface-layer community (Meguro 1962, Garrison and Buck 1991). Chl *a* concentrations in the surface layer were in the same range (1–54 mgL^{-1} brine) as found by Garrison and Buck (1991) in the western Weddell Sea one month earlier in the season, but further north. In the present study, an influence of infiltrating seawater on the different parameters (higher values of salinity, Chl a concentration and abundance of copepods) was clearly evident up to a distance of 8 m from the floe edge, similar to the results of an earlier study by Garrison and Buck (1991). New crack formation (break-up of large floes) therefore means the establishment of new surface-layer habitats and the possibility for organisms to colonise them, making this sea-ice habitat particularly dynamic. Relatively high salinities in the inner part of the floe most probably resulted from brine expelled during freeze-up, and the surface layer here was then formed by thawing of ice and snow when air temperatures increased (Weeks and Ackley 1986, Martin et al. 1995 Rankin et al. 2002). The absence of copepods in the surface layer of inner parts of the floe supports the interpretation that at these sites surface-layer features developed independently of a direct exchange with seawater. Physical and biological parameters secondly varied with the timing of crack formation in relation to the sampling date. Highest Chl *a* concentrations and copepod abundances were found at places, where the floe edge had been established at least 1 month before sampling. About 3 weeks after the floe had broken up at sampling site F, Chl *a* concentrations of up to 44.9 mgL^{-1} brine were measured in the surface layer of this floe area. This can imply either a further enrichment of algal biomass by further infiltrating seawater with time, or production and growth in the newly formed habitat (Garrison and Buck 1991, Kattner et al. 2004). Internal production with a specific growth rate of 0.2 doublings d^{-1} (Garrison and Buck 1991), based on an initial concentration of 0.2 mgL^{-1} (as found in the sub-ice layer on 4 December), would have resulted in a Chl *a* concentration of 3.2 mgL^{-1} after 20 d. Therefore, internal production based on infiltrating nutrients is not sufficient to explain the Chl *a* concentrations found. Studies on algal species composition and light and nutrient levels within the surface layer, as well as on inflow rates of seawater into the surface layer are needed for a better understanding of the process of surface-layer development. Diversity of

sympagic metazoans in the surface layer was very low, with only three abundant and regularly occurring copepod species, *Drescheriella glacialis*, *D. racovitzai* and *Stephos longipes*. High abundances of adult males and females as well as of nauplii, but not of sub-adult copepodids, indicate that all three species probably use the surface layer as a breeding and nursery ground. The surface layer may be a suitable habitat, as brine volume, and thus space to colonise, is very large (in this study on average 54%) compared with the ice proper (maximum 20%; Schnack-Schiel et al. 2008). Algal biomass as a potential food source for the sympagic copepods (Hoshiai et al. 1987, Schnack-Schiel et al. 1995) also can be very high (up to 53.5 mg Chl *a* L^{-1} brine). *D. glacialis* adults accounted for 51% of all *Drescheriella* specimens in the surface layer, however, only for 15% in the sub-ice layer. This enrichment points to an active immigration into the surface-layer habitat and to a higher degree of specialisation towards a life in the surface layer compared to *D. racovitzai*, which rather exploits the sub-ice habitat. The absence or rare occurrence of pelagic or other sympagic copepod species in the surface layer, which were present in the sub-ice layer, implies that these species avoid entrainment into the surface layer. They might not be able to successfully inhabit the surface layer due to the variable and extreme environmental conditions and the consequent physiological constraints. Strong gradients and shifts in the temperature and salinity regimes as shown in the present study may set the limits, which only *S. longipes* and *D. glacialis/racovitzai* can cope with. This hypothesis is supported, e.g., by the fact, that *D. glacialis* is able to survive salinities from 18 to 90 g/kg for 72 h (Dahms et al. 1990) compared to the pelagic species *Metridia gerlachei* and *Calanus propinquus*, which only survive at a salinity of 35 g/kg but not at 45 g/kg (Gradinger and Schnack-Schiel 1998). For *S. longipes* and *D. glacialis*, the ability to reproduce in a very dynamic but widespread and productive habitat is very likely a key to their dominance in the Weddell Sea pack-ice communities (Kurbjeweit et al. 1993, Schnack-Schiel et al. 1995, 1998). There are to date no investigations on the physiological mechanisms which allow *S. longipes* and *D. glacialis* to survive at low temperatures and changing salinities.

This is the second report mentioning the occurrence of the nudibranch *Tergipes antarcticus* in the surface layer of Antarctic pack ice. It was first reported by Pelseneer (1903) from the Bellingshausen Sea. Due to its probably attached life style and its low abundances, the species was not observed in the sub-ice layer in this study. The retrieval of large volume samples was crucial for obtaining this comparatively rare species. More studies with a focus on large volumes will probably enlarge the number of known species from the sea-ice ecosystem and enable work on their general biology. All larger metazoan species found in the surface layer

probably immigrate from the water column and profit from an ample food supply (algae and/or copepods) in a protected habitat. The large brine volumes (on average 54%) allow for larger species and specimens, than normally found in the ice proper. *Eusirus antarcticus* and *Euphausia superba* exhibit mean lower lethal temperatures of -2.5 and -4.2 °C, respectively, and they are osmoconformers in the salinity ranges from 26 to 40 g/kg (*E. antarcticus*) and 25 to 45 g/kg (*E. superba*) (Aarset and Torres 1989). This allows them to survive in the dynamic surface-layer habitat.

2.4.3. Habitat and community in the sub-ice layer

The sub-ice water layer is a habitat which is both influenced by processes related to the sea ice, e.g., freezing or melting, and by processes taking place in the water column (e.g., currents) (Werner and Lindemann 1997, Krembs et al. 2002b). During the study period, slight melting occurred at the ice underside. This can be deduced from the changes in morphological appearance of the ice underside in the course of the time, showing clear melting structures (Poltermann 1997, Werner and Lindemann 1997). This melting probably resulted in a more or less continuous release of ice algae and sympagic copepods from the ice into the sub-ice water layer, a process which has also been described for the Arctic (Werner 2006). The most significant melting event probably took place around 19 December. On 25 December, Chl *a* concentrations at 0 m depth were 10-fold higher than on 9 December. Abundances of sympagic harpacticoid copepods were also clearly elevated on 19 December. Due to their occurrence in the ice (Dahms et al. 1990, Schnack-Schiel et al. 1998, 2008), the following harpacticoid copepod species can be defined as sympagic: *Drescheriella glacialis*, *D. racovitzai*, *Idomene antarctica*, *Diarthrodes* cf. *lilacinus*, *Nitocra gracilimana* and *Ectinosoma* sp.. *Hastigerella antarctica* is probably also a sympagic species; however, abundances were too low to allow a final conclusion about its life-cycle strategy. The decrease of abundances of all harpacticoid species after the 19 December to levels found before the melting event indicate that these species were either able to recolonise the ice, to colonise the surface layer, were preyed upon, or were distributed in a larger volume and/or area underneath the ice. Such a re-distribution in a freshened layer underneath the ice and resulting incorporation into newly forming ice or the colonisation of older ice is probably the mode of dispersal for sympagic species. Besides sympagic species entering the sub-ice water layer from the ice, pelagic species also occur in this habitat (this study; Fukuchi et al. 1985, Kurbjeweit et al. 1993, for the Arctic: Werner and Martinez Arbizu 1999). Very similar to

observations below Arctic sea ice (Werner et al. 2002), abundances of truly pelagic species were generally higher at 5 m than at 0 m below the ice, whereas abundances of sympagic species were higher at 0 m than at 5 m below the ice. This distribution points to the respective origins and preferred habitats of the different species found in the sub-ice water layer, and also to their adaptation capabilities, e.g., to variations in salinity (Gradinger and Schnack-Schiel 1998). The ctenophore *Callianira antarctica* (Ju et al. 2004) and the Antarctic krill *Euphausia superba* are pelagic species, which have, however, strong relationships to the sea-ice habitats at least during parts of their life cycles. It is well documented that *E. superba* uses the sub-ice layer as a crucial feeding ground during winter and spring (Loeb et al. 1997), but the function of the sea-ice habitats for *C. antarctica* is not yet known. The regular occurrence of several species of amphipods at the underside of Antarctic pack ice is a new observation, possibly due to new sampling techniques such as under-ice video (this study) and scientific under-ice diving (Krapp et al. 2008). Abundances in the present study were similar to those of autochthonous under-ice amphipod species below Arctic pack ice, which are well known for their important role in cryo-pelagic coupling processes (Werner and Gradinger 2002). Further studies on the ecology of Antarctic under-ice amphipods are thus necessary.

Stephos longipes occured in higher abundance at the underside of the ice on 14 December compared to other sampling days. Since abundances of this species inside the ice were generally low during the study period (Schnack-Schiel et al. 2008), they were probably not released from the ice at the station, but rather transported laterally or from deeper waters. Part of the *S. longipes* population overwinters as C IV in the water column and develops into adults during spring (Schnack-Schiel et al. 1995). The specimens observed in the sub-ice water layer on 14 December were nearly all adults. The abundant nauplii II–IV, found on 30 December, could then have been offspring from these adults, or could have been released from the surface layer where reproduction of this species probably took place. A study performed by Kurbjeweit et al. (1993) during January–February 1991 in the southeastern Weddell Sea found abundances of *S. longipes* in the sub-ice layer of up to 1100 ind.m^{-3} dominated by early copepodite stages ([S] = 8.0). Therefore, during this study reproduction of *S. longipes* in the sub-ice and sea-ice habitats had probably just begun, and higher abundances in the sub-ice layer could be expected during the forthcoming summer.

In *Drescheriella* spp., all developmental stages except the naupliar stage I were found. This indicates a year-round reproduction of probably both *Drescheriella* species within or directly below the ice as stated by Dahms et al. (1990) for D. glacialis. The higher percentage (85%) of *D. racovitzai* found underneath the ice as compared to *D. glacialis* points to a higher

specialisation of this species towards a life in the sub-ice water layer. The neutral buoyancy of diapausing copepodids IV and V of this species, as observed by Dahms et al. (1990), supports this interpretation.

The present study and the work by Schnack-Schiel et al. (2008) are the first published observations that deal in further detail with the sympagic occurrence, distribution and stage composition of *Ectinosoma* sp., *Idomene antarctica* and *Diarthrodes* cf. *lilacinus*. The presence of naupliar stages of all three species implies that they reproduce in the ice proper and/or in the sub-ice layer. The generally higher abundances at 0 m depth than at 5 m depth lead to the conclusion that these species are probably truly sympagic. The high percentage of *Ectinosoma* sp. found at 0 m depth below the ice (50% of all sympagic copepods) in contrast to low percentages of 6% within the ice proper (Schnack-Schiel et al. 2008) and 0% in the surface layer (this study) leads to the conclusion, that this species is mainly exploiting the ice–water interface and moves relatively freely within this confined layer. Adults and copepodite stage V of *Ectinosoma* sp. as well as naupliar stages I and II were virtually missing from the sub-ice water layer throughout the whole study. This points to an overwintering of *Ectinosoma* sp. within the ice as eggs or naupliar stages or an early reproduction of overwintering adults, which subsequently died and were not found in the samples. Naupliar stages I–III of *I. antarctica* were not found during most of the sampling period, but all other stages were present. Two different strategies seem to fit to this stage distribution, either overwintering as nauplii or eggs or overwintering of adults and an early reproduction. The presence of adult females throughout the whole sampling period favours the second interpretation. The relatively high amount of exuviae found in this species also indicates active development in the sympagic habitat. Stage distribution of *Diarthrodes* cf. *lilacinus* was bimodal at the beginning of this study with adult females and naupliar stages III–V being present. No adults were found after 19 December. This may indicate that *Diarthrodes* cf. *lilacinus* overwinters as adults and starts reproduction early. Further development of the nauplii within the sub-ice or the bottom-ice layer is obvious, as the mean developmental stage of nauplii increased by one stage during the study period.

2.4.4. Outlook for further research

The present study has shown that both, the surface layer and the sub-ice layer are important habitats for sympagic organisms in Antarctic pack ice, harbouring a special community and playing a crucial role for the life cycles of several dominant sympagic copepods. In order to further understand processes, which take place during the development of a surface layer, i would like to propose a field experiment with an artificially produced crack in a floe with a negative freeboard that is not flooded, and subsequent sampling of the forming surface-layer habitat and community at different times and distances from the crack. Such an investigation would be fundamental for the understanding of succession in the surface layer. Life-cycle strategies of three dominant Antarctic sympagic copepods (*D. glacialis*, *S. longipes* and *P. antarctica*) are comparatively well investigated in level sea ice with thicknesses of up to 3 m (Kurbjeweit et al. 1993, Schnack-Schiel et al. 1995, Swadling 2001). However, there is a large gap of knowledge about the ecological role of pressure ridges, which can account for up to 50% of the sea-ice volume in the Antarctic (Tin and Jeffries 2003), and thus be important for the distribution and life cycles of sympagic organisms. Furthermore, studies on the distribution of rare sympagic species within the sea-ice system are needed. An inspection of already existing sample sets with a taxonomical focus on harpacticoids would help to a better understanding of their role in the sympagic food web of the Southern Ocean. Another open question is how sympagic meiofauna species are adapted to low temperatures and variable salinities. Physiological and molecular-biological studies are needed to answer this question.

3. *Tergipes antarcticus* (Gastropoda, Nudibranchia): distribution, life cycle, morphology, anatomy and adaptation of the first mollusc known to live in Antarctic sea ice

3.1. Introduction

Antarctic sea ice is a habitat of enormous areal extent: on a monthly multiyear average (1979–2003), as much as $18*10^6$ km² of the Southern Ocean are covered by sea ice in September, while ice coverage can be as low as $3*10^6$ km² in March (Cavalieri et al. 2003). Different interstitial habitats are associated with the ice: a brine-channel system, developing within the ice during freeze-up, a platelet-ice layer underneath the ice in the vicinity of shelf ice, and a surface layer, developing when the ice surface is pressed underneath the water level and water infiltrates the snow on top of the ice (Ackley and Sullivan 1994 and references therein). The brine filled pores and channels vary in size from micro- to centimetres and are colonised by adapted sympagic (ice-associated) bacteria, algae, protozoans and metazoans (Horner et al. 1992 and references therein). Metazoan taxa described from these habitats include ctenophores (Dahms et al. 1990), plathyelminthes (Janssen and Gradinger 1999), nudibranchs (Pelseneer 1903), copepods (Schnack-Schiel et al. 2001b), amphipods and euphausids (chapter 2) and fish (Vacchi et al. 2004, Jin and DeVries 2006). The sea-ice habitat is characterised by varying temperatures (from 0 °C to at least -15 °C) and salinities (from 0 to at least 180 g/kg) (Bartsch 1989, Schnack-Schiel et al. 2008), as well as space restrictions (Weissenberger et al. 1992, Krembs et al. 2000) and the omnipresence of ice. These conditions are probably one of the reasons for the comparatively small number of metazoan taxa and species described for Antarctic sea ice. Another reason could be the relative scarcity of sea-ice studies focusing on Antarctic sea-ice metazoans and the comparatively small sample volumes obtained during these studies in relation to the extent of Antarctic sea ice. Most studies on sea-ice metazoans describe aspects of the biology of some abundant species, such as the copepods *Stephos longipes*, *Paralabidocera antarctica* and *Drescheriella glacialis* (Dahms et al. 1990, Schnack-Schiel et al. 1995, Tanimura et al. 1996, Swadling 2001, Guglielmo et al. 2007) and the euphausid *Euphausia superba* (Loeb et al. 1997). The nudibranch *Tergipes antarcticus* Pelseneer, 1903 (Nudibranchia, Aeolidoidea) has been

reported for Antarctic sea ice only by Pelseneer (1903) in the Bellingshausen Sea and for the Weddell Sea in chapter 2. Knowledge on *T. antarcticus* is restricted to a short species description and the two sampling locations. This study aims to close some gaps in the knowledge of this species. Using large-volume sampling methods, in addition to standard coring techniques (Horner et al. 1992), all life stages of *T. antarcticus* were obtained from the ice during the expedition ANT-XXIII/7 of R/V Polarstern in austral winter 2006. Analyses of samples from this and four other cruises [ANT-III/3, 1985 (Hempel 1985) ANT-V/3, 1986 (Schnack-Schiel 1987) ANT-VIII/2, 1989 (Augstein et al. 1991) ANT-X/3, 1992 (Spindler et al. 1993)] lead to the conclusion that *T. antarcticus* is widely distributed in sea ice throughout the Weddell Sea. Using DNA analysis, it is shown that egg clutches and veliger larvae found within the ice belong to this species. A description of egg development until hatching of the veliger larvae is given, including developmental times. A thorough morphological description of all life stages of *T. antarcticus* is furthermore given. Supercooling points of egg clutches and adults of *T. antarcticus* and the presence of thermal hysteresis activity in their body liquids is reported.

3.2. Material and methods

3.2.1. Study area, sampling and processing of samples

Various ice samples were taken in the north western Weddell Sea in austral winter 2006, during the expedition ANT-XXIII/7 of R/V Polarstern (24 August to 29 October 2006). The ice concentration in most parts of the study area was above 9/10. Modal ice thickness was 1.2–1.4 m, with secondary modes between 2.5 and 3.0 m indicating the presence of multi-year ice in the area; mean ice thickness was 2.1 m due to large amounts of ice thicker than 3 m (C. Haas pers. communication). To sample *Tergipes antarcticus*, large-volume samples of ice with a brown or green colour were taken at various locations along the cruise track: large pieces of ice were collected directly from the water using a cage with an opening in the floor. The cage was craned right above the surface of the water and the ice pieces were collected by hand through the opening. Level ice was sampled quantitatively in nine ice stations using an engine-powered KOVACS ice corer (internal diameter: 9 cm). On six stations the vertical *in situ* temperature profile was measured on one core directly after coring (Testotherm 720 thermometer, accuracy ±0.2 °C). Brine salinity was calculated from *in situ* temperature according to Assur (1958) and Frankenstein and Garner (1967). A second ice core was taken from these six stations and cut into vertical sections of 5–10 cm directly after coring. On all

nine stations, up to three replicate bottom ice sections (lowermost 5 cm) were taken. All ice samples were melted in the dark at +4 °C in a surplus of 0.2 µm filtered seawater (FSW; 200 ml FSW per 1 cm core length) in order to reduce salinity stress for the organisms (Garrison and Buck 1986). Within 24 h after complete melting of the ice, the samples were concentrated over a 20 µm gauze. Samples were then fixed with borax buffered formaldehyde (2% in FSW) for later analyses, or, in the case of the replicate bottom sections and large-volume samples, processed directly onboard at 0 °C. The different life stages of *T. antarcticus* were identified, counted and measured, using a stereomicroscope (Leica WILD MZ 12.5 and Leica MZ 16 F, 20 to 100x magnification, transmitted and impinging light). Identification was based on morphological characteristics, observations of egg development and DNA analyses. Vertical abundance profiles (individuals L^{-1} melted ice) and integrated abundances (individuals m^{-2}) were calculated for the full core. Samples and unpublished data from five other cruises to the Weddell Sea and adjacent regions (Integrated Soviet Antarctic Expedition, 1955–1958; ANT-III/3, 1985; ANT-V/3, 1986; ANT-VIII/2, 1989; ANT-X/3, 1992) have been included in this investigation for information on geographical distribution as well as for morphological and histological analyses. All analysed samples are listed in supplementary table S1.

3.2.2. Morphology

Morphological descriptions are based on the observations of living and fixed specimens, using a stereomicroscope. One animal preserved in 96% ethanol (ANT-XXIII/7) was dissected to analyse the jaws. For histological investigations, three animals preserved in 96% ethanol (Integrated Soviet Antarctic Expedition, ANT-XXIII/7) and one animal preserved in formaldehyde (ANT-III/3) were embedded in hydroxyethylmethacrylate (Kulzer). Serial sections (2.5 µm) were stained with toluidine blue and analysed by light microscopy (Olympus BX microscope).

3.2.3 DNA analysis

To prove the taxonomic identity, total RNA was isolated separately from two Tergipes antarcticus adults and two egg clutches, which had been stored at -80 °C, using Trizol® Reagent (Invitrogen) and chloroform/isopropanol extraction. Total RNA was transcribed into complementary DNA. Using these cDNAs as templates and the degenerated primers

RK_TE_COI_fw1 (5’-GGTGCTCCBGAYATRAGDTTYCC-3’) and RK_TE_COI_rev1 (5’-ARAACWGAYCAHACRAAYAAHCT-3’) (W = A or T, R = A or G, Y = Cor T, B = C or G or T, D = A or G or T, H = A or C or T), in a ‘‘Touch down’’- PCR, a 257 nt fragment of the cytochrome c oxidase I (COX I) was amplified. Cycling parameters during the ‘‘Touch down’’ procedure were: 94 °C for 4 min, followed by 12 cycles with 94 °C for 45 s, annealing for 45 s and 72 °C for 45 s. For each consecutive cycle, the annealing temperature was lowered by 1 °C, starting at 64 °C. Thereafter 33 cycles were performed with the following parameters: 94 °C for 45 s, 49.5 °C for 45 s and 72 °C for 45 s followed by a final extension step at 72 °C for 8 min. PCR products were separated on a 1% agarose gel. Single fragments were cut out, extracted from the gel, ligated, cloned and sequenced using standard molecular-biological techniques. Sequences were obtained by MWGBiotech (Ebersberg, Germany). Nucleotide BLAST engines at the NCBI server (http://www.ncbi.nlm.gov/BLAST) were used for homology searches in public databases. Alignments of nucleic acid sequences of the cytochrome c oxidase fragment obtained from *T. antarcticus* adults and egg clutches with those from *T. tergipes* (Aeolidoidea, Tergipedidae) (AY345032.1) and *Cuthona ocellata* (Aeolidoidea, Tergipedidae) (AY345043.1) were performed using the ClustalW tool in MacVector (Accelrys).

3.2.4. Development

A total of 32 *Tergipes antarcticus* egg clutches were isolated from large-volume ice samples (26 at 60°18’S, 50°45’W; 4 at 60°40’S, 42°09’W; 2 at 65°06’S, 57°24’W). To study the early development, 26 egg clutches were placed into separate dishes (diameter 3.5 cm) filled with FSW (8 ml) and kept in the dark at 0 °C for up to 1 month. The state of the eggs was monitored every 1–2 days, in case of cleavage stages 2–3 times per day. A total of 21 developmental stages were discerned (Table 4). In 21 egg clutches at least three consecutive stages were observed, with the highest numbers of observations for the later stages (after gastrulation); in two cases, development was observed from stage 1 (two cell stage) up to stage 9 (beginning gastrulation) or stage 12 (very early organogenesis). For each egg clutch, the developmental time $t_{x,x+1}$ was calculated for each stage x as $t_{x;x+1} = t_{x+1} - t_x$ where t_x is the (hypothetical) point of time when stage x – 1 develops into stage x. If the latest observation of stage x was at time t_{last} and the first observation of stage x + z ($1 \leq z \leq 2$) was at time t_{first}, it was assumed that $t_{x+z0} = t_{last} + ((t_{first} - t_{last})/(z + 1)) * z_0$; $1 \leq z_0 \leq z$.

The developmental times for the first and last stages observed for each clutch were not included, except for stage 1: here it was assumed that $t_1 = t^*$, t^* being the time when stage 1 was first observed. For each developmental stage, median, range and quartiles of the developmental times were determined. Length of different developmental phases and total developmental time from egg to veliger larvae were determined as the sums of the median developmental times of the single stages; the ranges were calculated by summing up the minima and maxima. Hatched veliger larvae were transferred into petri dishes (diameter 6 cm) filled with FSW, supplied with sympagic algae, kept at 0 °C and observed every few days.

3.2.5. Supercooling points

In order to measure the supercooling points (SCP) of egg clutches and adults of *Tergipes antarcticus*, a cooling apparatus was constructed analogous to that described by Sinclair and Sjursen (2001). At 0 °C ambient temperature, the apparatus could cool samples to -30 °C with an initial cooling rate of 5 K min^{-1} (0 to -10 °C) and thereafter at 1 K min^{-1}. Supercooling points were measured with a NiNiCr thermocouple, onto which the dry blotted egg clutches or animals were carefully transferred. Two adults and seven egg clutches were measured. The size of the egg clutches ranged from 20 to 100 eggs per clutch. Developmental stages from morula to intermediate organogenesis were measured. The adults had a length of 3.23 and 3.76 mm. The samples were placed into the cooling apparatus and the temperature was lowered. The temperature profile was recorded using a signal amplifier and a chart recorder. The supercooling point was taken as the temperature prior to the upward inflection caused by the release of the latent crystallisation heat. The cooling rate at the crystallisation point was determined. After determination of the SCP, samples were stored immediately at -80 °C for examination of thermal hysteresis and RNA extraction.

Table 4: Developmental stages of *Tergipes antarcticus* eggs.

stage			developmental time [h]			
no.	term	description	median	range	quartiles	n
1	1-cell-stage		nd	nd	Nd	
2	2-cell-stage, early	2 blastomeres (equal size, separated)	5	3–7	3–7	2
3	2-cell-stage, late	2 blastomeres (slightly merged)	8	3–12	3–12	2
4	4-cell-stage	4 macromeres (equal size)	19	9–29	9–29	2
5	8-cell-stage	4 macromeres, 4 micromeres	11	8–13	8–13	2
6	16-cell-stage	16 blastomeres	9	8–10	8–10	2
7	32-cell-stage	32 blastomeres	11	2–19	2–19	2
8	morula	slightly angular	9	9–9	9–9	2
9	blastula	round	13	12–14	12–14	2
10	gastrulation, beginning	vegetal pole slightly impressed	75	5–127	5–127	3
11	gastrulation, ongoing	distinct slit at vegetal pole	22	20–24	20–24	2
12	gastrula, late	round	97	12–157	12–157	3
13	organogenesis, very early	slightly angular	45	6–173	10-88	7
14	organogenesis, early	parts with differential transperancy	53	25–102	43–69	7
15	organogenesis, intermediate	body round, development of velum	30	25–96	26–63	8
16	organogenesis, late	body oval, velum equatorially	32	5–161	7–108	4
17	organogenesis, very late	velum moving towards pole	49	8–208	29–196	6
18	organogenesis, almost completed	velum moved to pole	24	16–42	16–46	3
19	organogenesis completed	cilia on velum motile	38	5–72	7–72	7
20	hatching, beginning	first larva has hatched	202	125–278	125–278	2
21	hatching completed	all larvae have hatched				

A description of each stage as well as the median, range and quartiles of developmental times for the respective stage are given.

3.2.6. Thermal hysteresis

Animals stored at -80 °C were thawed on ice, up to 1.0 µL distilled water was added and the samples were sonicated twice for 1 min in an ice-cooled ultrasonic bath, vortexed twice at maximum speed and centrifuged for 10 s at 13,000g at 2 °C. The overlying liquid was removed, transferred to a new microcentrifuge tube, and up to 0.2 µL of a protease inhibitor cocktail (fivefold concentrated) was added. Egg clutches, stored at -80 °C, were thawed on ice, vortexed for 1 min at maximum speed and centrifuged for 1 min at 13,000g at 4 °C. From both sample types, parts of the overlying liquid were transferred to the sample holder of a Clifton Nanoliter Osmometer (Clifton, Technical Physics, Hartford, New York) and presence of thermal hysteresis (the difference between the melting and freezing point of a liquid) was measured as described by Enevoldsen et al. (2003). As no measures against evaporation were taken and one sample was diluted, the melting points do not reflect *in situ* osmolalities.

3.3. Results

3.3.1. Taxonomy

Tergipes Cuvier, 1805 (type species Limax tergipes Forskål, 1775) (Tergipedidae Bergh, 1889, = Cuthonidae Odhner, 1934).

3.3.2. Diagnosis of genus

Summarising the diagnostic features described for *Tergipes* by Burn (1962), Miller (1977) and Schmekel and Portmann (1982), the following features define the genus: rhinophores and labial tentacles digitiform and smooth; anterior foot corners round; anus acleioproct; only one preanal digestive glandular branch present; only one ceras can be assigned to each digestive glandular branch; radula formula 0.1.0; teeth horseshoe-shaped with one prominent median denticle; edge of jaws with one row of denticles; penis usually with a penial gland.

3.3.3. Description of adults and juveniles

Morphology and colour of living and preserved specimens (Figs. 12, 13): adult length of living specimens up to 3.8 mm (juveniles 0.8–1.4 mm), width up to 0.4 mm (juveniles 0.1 mm); body elongate; foot tapering posteriorly; anterior foot without a notch and lateral edges slightly extended; rhinophores digitiform and smooth; oral tentacles of same length and shape as rhinophores (not present in very young juveniles); eyes lying directly behind rhinophores on lateral side; in adult specimens two pairs of cerata present; first pair of cerata lying after one-third of body length and second pair after two-third of body length; cerata inserting in opponent position; anterior pair is the first to be developed into juveniles; notum rim not present; anus papilla distinct and situated in front of the first, right ceras in acleioproct position. Overall body colour of living animals translucent white, digestive tract and especially digestive gland shining in brown, under transmitted light conditions red. In the proximate part of the cerata, distinct brown spots are visible in the digestive glandular branch.

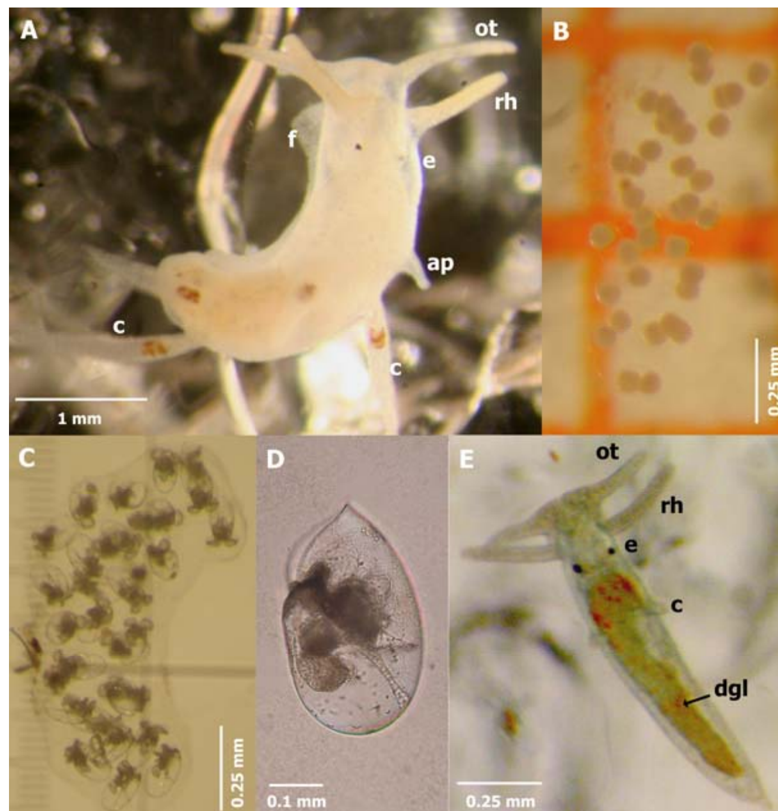

Fig. 12. Tergipes antarcticus (Pelseneer 1903). (A) Adult (B) egg clutch, stage 12: late gastrula (C) egg clutch, stage 17: organogenesis, very late (D) veliger larvae (E) juvenile with only one pair of cerata. ap anus papilla, c cerata, dgl digestive gland, e eye, f foot, ot oral tentacle, rh rhinophore.

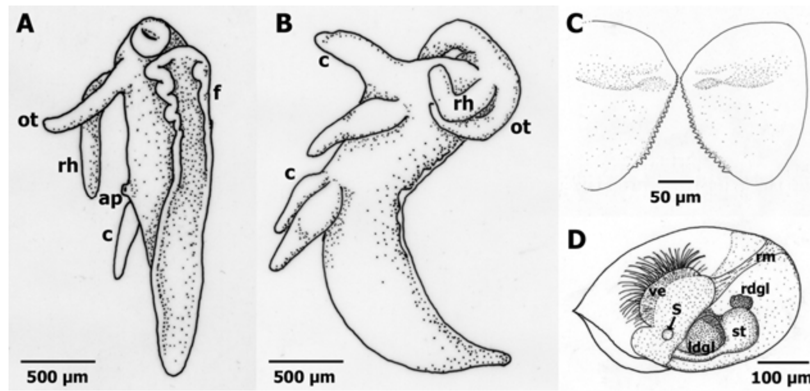

Fig. 13. *Tergipes antarcticus* (Pelseneer 1903). (A) Adult (ANT-XXIII/7), ventral side (B) Adult (ANT-III/3), dorsal side (C) Jaws (D) Veliger larvae. ap Anus papilla, c cerata, f foot, ot oral tentacle, rh rhinophore, ldgl left digestive gland, rdgl right digestive gland, rm retractor muscle, s statocyst, st stomach, ve velum with cilia.

Description of anatomy and histology of preserved specimens (Fig. 14): Epithelia: epidermis of main body with cuboidal cells with vacuoles, cells of the cerata with higher epithelium and more prominent vacuoles, representing a specialised vacuolated epithelium in the sense of Wägele (1998). Presence of spindles could not be verified. Cerata with subepithelial glandular cells containing granular secretion. Foot sole with long cilia and several large agglomerations of subepithelial lying glandular cells. Digestive system: oral tube short, with subepithelial glandular cells staining light violet indicating secretion of mucopolysaccharides. Pharynx very muscular and lined with a cuticle. Length of triangular shaped jaws about 300 µm, without any masticatory process, but with one row of prominent denticles (Fig. 13 C). Radula with one row of teeth each bearing about four lateral denticles on each side of the main cusp (reconstructed from histological slides). Large salivary glands present with cells containing violet granula and secreting acidic mucopolysaccharides. Oesophagus without a cuticular lining. Stomach not discrete from digestive gland, forming a large sac with broad transition into digestive gland. Stomachal epithelium with long cilia. Digestive gland large, filling most part of the visceral cavity especially in the smaller specimens. Digestive glandular epithelium composed of large cells containing many vacuoles staining blue-green (secretory or storage cells). Within cerata, digestive gland present as a single tube; no cnidosac could be detected in any specimen. Intestine opening into laterally lying anus; lined by a ciliated epithelium, without any glands. In the lumen of the digestive tract, a few small unicellular organisms could be identified. Nerve and sensory system: nerve ring lying around the anterior oesophagus. Cerebral and pleural ganglia fused, pedal ganglia distinct. Statocysts lying next to pedal ganglia, containing one statolith each (Fig. 14 C). Eye lying very close to statocyst,

half way between cerebral and pedal ganglion; with a pigmented cup and a small lense (Fig. 14 A, C). Circulatory and excretory system: Pericard large and a prominent ventricle present. Syrinx opening into pericard on lateral side, lined with long cilia. Kidney sac not very prominent. Genital system: In all specimens, the genital systems were still under development. A few undifferentiated tubes, without or with a genital opening could be identified. Few separate gonadal follicles were present in one animal. In two specimens, these follicles seemed fused. Spermatogenesis could be observed in different stages, but only one showed ripe sperm with distinct sperm head and a tail. The absence of oogonians in the gonad of all three histologically investigated specimens indicates a protandric life style, which is also typical for other opisthobranchs (Wägele unpublished data).

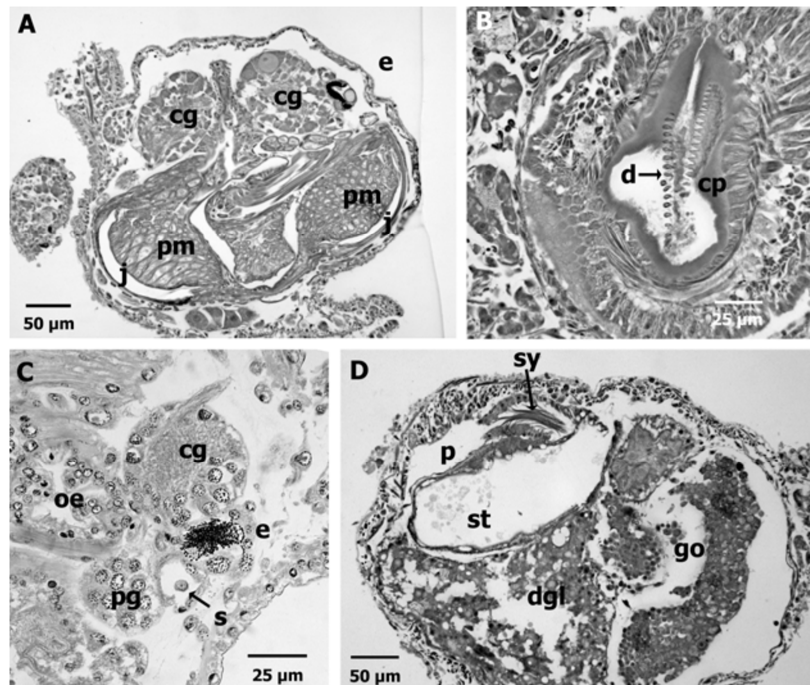

Fig. 14. *Tergipes antarcticus* (Pelseneer 1903), histology of adult organ systems. (A) Cross section in head area (B) Anterior pharynx with jaws sectioned (C) Cross section through nervous system of head region (D) Cross section through heart region. cg Cerebral ganglion, cp cuticle of pharynx, d denticles of jaws, dgl digestive gland, go gonad, j jaws, pg pedal ganglion, oe oesophagus, p pericard, pm pharynx muscles, s statocyst with otolith, st stomach, sy syrinx.

3.3.4. DNA analysis

The sequences of the COX I fragments isolated from *Tergipes antarcticus* adults (n = 2) and eggs (n = 2) were 98.4–99.2% similar to each other (GenBank entries: adults EU727250 and EU727251; eggs EU727252 and EU727253). In contrast, the same sequence fragment from *Tergipes tergipes* was 83.3–84.4% similar to these sequences. Additionally the sequence of the COX I fragment from *Cuthona ocellata* was 82.9–83.7% identical to the *T. antarcticus* sequences. As a comparison, the sequence fragments from *T. tergipes* and *C. ocellata* were 83.3% similar to each other.

3.3.5. Description of eggs and veliger larvae

Egg capsule: transparent, shape oval, a single white embryo situated at one pole; length of capsule 70–130 µm (median 100 µm, n = 8), width 60–80 µm (median 70 µm, n = 4; measured on formaldehyde-fixed eggs); egg capsules are held together by a mucoid gelatinous substance. In formaldehyde-fixed samples, only singular egg capsules were found, the shape of which had preserved well. Size of egg clutches: 5–160 eggs (median 30, n = 32), length 1.0– 5.7 mm (median 2.1 mm, n = 10), width 0.5–1.1 mm (median 0.7 mm, n = 10). The clutches did not float. Veliger larvae: 64–350 µm in length and 60–200 µm in width. Larval shell: transparent, egg-shaped, without distinct coils, shell type II (according to Thompson 1961); velum well-developed, with two ciliated lobes, which could be retracted separately into the shell; developed digestive system with prominent left and smaller right digestive gland; columellar muscle attached to the posterior part of the larval shell; otocyst present, eyes absent. A larval foot, as well as an operculum could not be detected in preserved nor living larvae. No nutritional contents (yolk) could be identified.

3.3.6. Development

Short descriptions of the stages of egg development are provided in Table 4 along with developmental times (median, range and quartiles) for each stage. Total developmental time from egg to larvae was calculated to be 31 days (range 13–65 days). The duration of the cleavage and morula stages was 71 h (range 42–99 h) and the duration of the blastula stage was 13 h (range 12–14 h). Gastrulation took 8 days (range 2–13 days) and organogenesis was completed within further 10 days (range 4–33 days), which was indicated by the onset of

movement of the cilia on the velum. Hatching began after further 38 h (range 5–72 h). The hatching procedure itself took only few minutes; the velum first broke through the shell of the egg, followed by the rest of the larval body. Although development in most cases was almost synchronous for all eggs of one clutch, the larvae did not hatch simultanously, but in intervals of several days. Only in two clutches did all larvae hatch, the interval from first to last hatching being 5–13 days. Having hatched, the larvae immediately started swimming by moving the cilia on the extended velum. The larvae grew during culturing, but growth rates were not determined. Metamorphosis of the larvae was not observed.

3.3.7. Geographic distribution and abundances

Tergipes antarcticus juveniles and adults were found in the Weddell Sea (ANT-III/3, ANT-V/3, ANT-X/3, ANT-XXIII/7) and the southern part of the Scotia Sea (ANT-XXIII/7) as well as in the areas of the Prydz and the Lützow-Holm Bay (Integrated Soviet Antarctic Expedition) (Fig. 15). Juveniles and adults were found within the ice from September to November (ANT-XXIII/7, ANT-V/3) and again in April (ANT-X/3). During ANT-XXIII/7, adults were only found in large-volume samples, while juveniles were found in both large-volume samples and bottom ice sections (one individual each in three cores taken at two stations). At the bottom of the ice, temperatures were slightly below the freezing point of seawater and brine salinities were close to seawater salinity. Veliger larvae and eggs were distributed throughout the whole ice column. Larvae occurred at temperatures down to -5.6 °C, with corresponding brine salinities of 94 g/kg [station 060921 (yymmdd), refer to Fig. 16 for sampling position, ice type, ice thickness and integrated abundance], and eggs were found at temperatures down to -8.0 °C and brine salinities as high as 130 g/kg (station 061003). Most eggs and veliger larvae were probably alive at these temperatures, since the destruction of morphological features through freezing or bacterial degradation would preclude their identification. At two stations (060921 and 060924), abundance maxima of veliger larvae were observed at the surface of the ice, with maximum abundances of 7 and 99 Ind L^{-1}, respectively. Egg abundances at one station (061003) exceeded 250 eggs L^{-1} within the lowermost 5 cm of the ice, and were as high as 61 eggs L^{-1} within the ice interior. At another station (060930) a surface maximum of 38 Ind L^{-1} was observed. Integrated abundances of eggs were highest at station 061003, exceeding 17 x 10^3 eggs m^{-2}, while abundances of veliger larvae were highest at station 060924, with more than 11 x 10^3 Ind m^{-2} (Fig. 16). Neither stages were found in the second-year ice of station 060919.

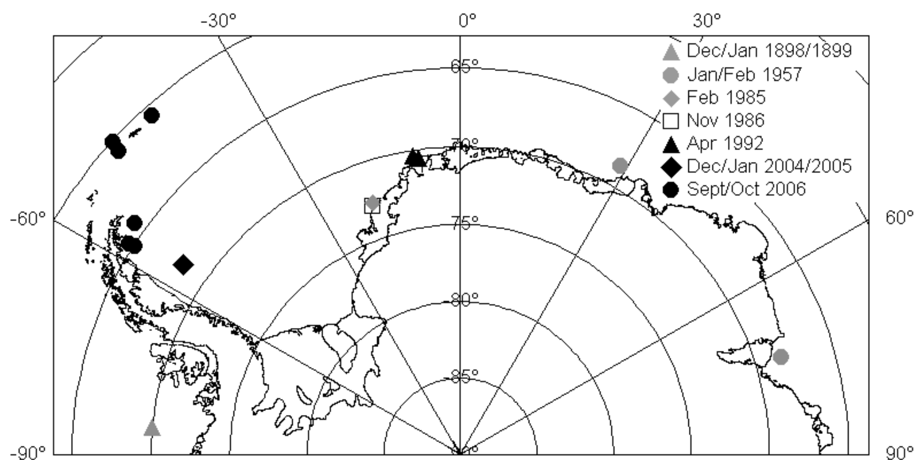

Fig. 15. Geographic distribution of *Tergipes antarcticus* in the Antarctic. All known sample locations of juveniles and adults. Samples from the cruises in 1957 and 1985 were retrieved from the water column, all other samples originate from the ice proper (1986, 1992 and 2006) or the surface layer (1898/1899 and 2004/2005). No animals were found in 1989. Sample locations from 1898/1899 and 2004/2005 were taken from Pelseneer (1903) and from chapter 2, respectively

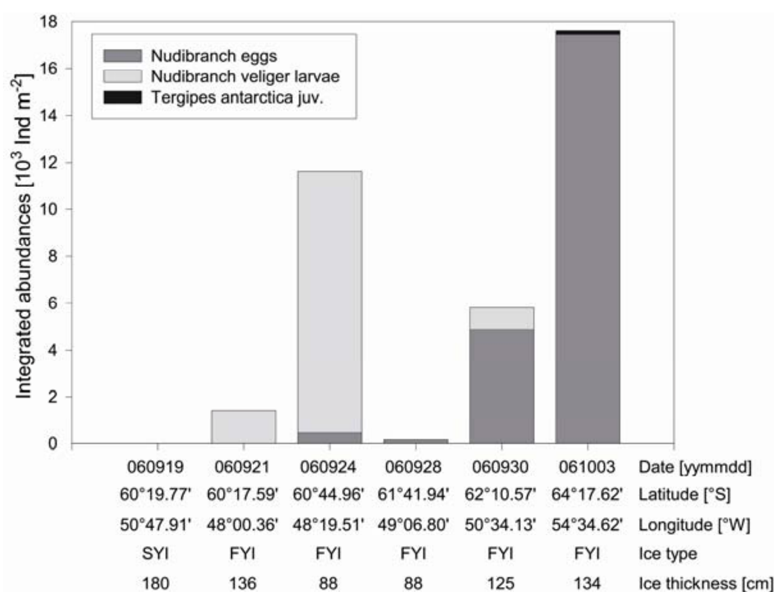

Fig. 16. Abundances of eggs, veliger larvae and juveniles of *Tergipes antarcticus* in Antarctic pack ice. Sampling date, position, ice type and ice thickness are given. SYI second-year ice; FYI first-year ice

3.3.8. Supercooling points

The mean SCP of *Tergipes antarcticus* egg clutches was -12.5 °C (standard deviation = 0.65 °C; n = 7). Cooling rates at the crystallisation point ranged from 0.16 to 5.29 K min^{-1}. SCP's of adults were -14.6 and -27.6 °C, and the cooling rate at the crystallisation point was 1.32 and 1.16 K min^{-1}, respectively.

3.3.9. Thermal hysteresis

The liquid withdrawn from egg clutches and adult *Tergipes antarcticus* showed clear signs of thermal hysteresis. In the liquid of the egg clutches, crystals took on a diamond shape in the hysteresis gap. After reaching the hysteresis freezing point, the crystals first grew to a rectangular shape. A thermal hysteresis of -0.16 to -0.19 K (n = 3) was observed for the egg clutches and melting points were recorded at -2.4 to -4.6 K. In the liquid of the adults, crystal forms could not be observed due to too many impurities. For the adults, a thermal hysteresis of -0.29 K (diluted sample) and -1.49 K was observed. Melting points were recorded at -0.59 K (diluted sample) and at -2.35 K.

3.4. Discussion

3.4.1. General remarks

Tergipes antarcticus is the only truly sympagic gastropod species identified so far. In the Arctic, juveniles of the pteropod *Limacina helicina* are a regular part of the sub-ice fauna (Herman and Andersen 1989, Werner 2006), but they have never been found within the ice. For *T. antarcticus*, I could demonstrate successful reproduction within Antarctic sea ice by means of laboratory observations and DNA analyses of egg clutches and adult specimens. I conclude that the entire life cycle of *T. antarcticus* is closely associated to sea ice. The classification of *T. antarcticus* as a truly sympagic species is supported by several facts: the occurrence in different ice habitats and ice horizons throughout the year and at various locations, in conjunction with the observation that adults are unable to swim actively and egg clutches of this species do not float; a comparatively fast developmental rate from egg to veliger larvae; physiological adaptation through thermal hysteresis and low supercooling points of egg clutches and adults. A change of food sources was probably necessary to exploit the sea-ice habitat.

3.4.2. Taxonomy

Tergipes antarcticus was first described by Pelseneer (1903) from a sample taken at the end of December 1898 near Charcot Island (70°S, 85°W) in pack ice, in between algae of a water hole, close to the surface where nearly fresh water was found. («Fin décembre 1898 à commencement janvier 1899, vers 70°S. et 85°W., dans la banquise, sur les Algues du trou à eau, près de la surface, où l'eau était presque douce.») I conclude from this description that the sample was taken from the surface layer. Since then it has been recorded one more time (chapter 2). The description provided by Pelseneer (1903) was very sketchy and based on a juvenile of 1.3 mm in length. The description of this species was extended considerably through this study although many details, especially concerning the genital system remain unknown. *T. antarcticus* exhibits a feature that clearly allows the assignment to the genus *Tergipes*: the single preanal branch of the right digestive gland reaches into one ceras. However, a combination of other characters indicates this relationship: the lack of lateral teeth, the shape of teeth and jaws and the shape of the anterior foot edge. Contrary to the only other congener, *T. tergipes* Forskål, 1775 (=*T. despectus*), the insertion of the cerata is not alternating, but opponent and only four cerata (instead of seven) are present.

3.4.3. DNA analysis

This investigation has shown that nudibranch egg clutches found within the ice belong to *T. antarcticus*. An intraspecific sequence divergency of 1.6–0.8% between egg clutches and adults was found for a 257 nt fragment of the COX I, whereas differences to other nudibranch species were approximately 16%. Wägele et al. (2006) describe a similar intraspecific sequence divergence for the opisthobranch *Umbraculum umbraculum* (partial COX I sequences). Other authors report intraspecific sequence divergencies of 1–4% for this region of the COX I sequence; interspecific sequence variations of 9–25% have been observed (Bucklin et al. 2003).

3.4.4. Distribution and abundances

Presented data indicate a widespread distribution of *Tergipes antarcticus* in the Weddell Sea and adjacent regions, where it was mainly found in the ice through most times of the year (Fig. 15). Animals found in the water column in January during the Integrated Soviet Antarctic Expedition and in February during ANT-III/3 were probably lost from nearby fast ice due to summer melting (stations situated less than 10 km from fast ice). This hypothesis is furthermore supported by the observation that *T. antarcticus* is not able to swim actively, but with its creeping movement is better adapted to live on surfaces. Adult *T. antarcticus* may thus live attached to the ice underside, within wider brine channels or within the surface layer. An occurrence in the platelet ice layers also seems possible. The unusually low number of cerata (only four in total) seems to be an adaptation towards an interstitial life style. It is comparable to interstitial aeolidoideans such as *Pseudovermis*. As egg clutches are not buoyant, the presence of egg clutches and high numbers of eggs within the ice proper can only be explained, if *T. antarcticus* adults lay their eggs directly into the ice. Occurrence of eggs in upper parts of the ice can be explained by thermodynamic or dynamic ice growth, which transports eggs laid in lower parts of the ice to upper parts. Distribution of sympagic species within the ice is generally very patchy (Spindler and Dieckmann 1986, Eicken et al. 1991, Swadling et al. 1997). This also seems to be the case for *T. antarcticus*, as revealed by comparison of replicate bottom ice sections. Furthermore, at some locations extremely high numbers of eggs and larvae, as well as several juveniles and adults were found in ice samples, while they were completely absent at other locations. It is probably due to this high patchiness, in combination with the low abundance of juveniles and adults in the ice and with the difficulty in identifying the small, and in part strongly deformed fixed eggs and larvae, that *T. antarcticus* has so seldom been reported. Due to the high individual biomass of adults, and the high abundance of larvae, being of the same order of magnitude as abundances previously reported for total sympagic proto- and metazoans (Gradinger 1999b), this species may nevertheless play an important role within the sea-ice ecosystem. Calculating a mean integrated abundance of individuals (mainly juveniles) found in all 88 ice cores (excluding replicate cores) taken for meiofauna studies during ANT-III/3, ANT-V/3, ANT-VIII/2, ANT-X/3 and ANT XXIII/7 results in 7 Ind m^{-2}. Multiplying this value with the absolute minimum summer sea-ice area of the Weddell Sea of 0.482×10^6 km^2 observed in 1998 (observation period 1987–2005; source:weddell.html), results in 3.44×10^{12} *T. antarcticus* juveniles. As animals were also found in areas, where sea ice does not persist through austral summer,

actual numbers in winter may even be higher and a considerable loss of biomass to the pelagic realm in summer in those areas is probable. I would like to emphasise that due to the small sampling area (0.56 m^2), compared to the large area of the Weddell Sea covered by summer sea ice, the error of this calculation is considerable. Nevertheless it shows that apparently low-abundant sympagic species can occur in high absolute numbers. Large-volume ice sampling enables work on more ‘‘rare’’ species and will probably result in the detection of new species within the sea-ice habitat.

3.4.5. Development

The here presented report of developmental time from egg to veliger of *Tergipes antarcticus* is the first for an Antarctic nudibranch with indirect development. With a developmental time of 31 days at 0 °C the development is fast, being only six times slower than the development of *T. tergipes*, with 5 days at 18–20 °C (Tardy 1964, Kress 1972). In comparison, egg development of other Antarctic nudibranchs with direct development, such as *Bathydoris hodgsoni* (more than 460 days at -1 to +1 °C) or *Austrodoris kerguelenensis* (more than 970 days at 0 °C) is 9–19 times longer than that in temperate species with the same direct development type like, for example, *Cadlina laevis* (50 days at 10 °C) (Thompson 1967, Wägele 1989, 1996). In general, lower temperatures are probably the main reason for the increase in developmental time (Clark and Goetzfried 1978, Thompson and Jarman 1986, Peck 2002). *T. antarcticus* egg clutches are light-built and can contain high numbers of rather small eggs per clutch (up to 160), indicating a low energetic investment per egg. Short life cycles and high reproductivity, as known from other nudibranchs (Clark 1975), may have been a preadaptation, allowing *T. antarcticus* to colonise the dynamic ice habitat. Such strategy is also known from the sympagic harpacticoid *Drescheriella glacialis*, which reproduces throughout the year (Dahms et al. 1990) and is characterised as an r-strategist, a life trait selected for by the dynamics of the sea-ice habitat (Bergmans et al. 1991). Taking the mean developmental time of 31 days and the stages observed directly after sampling into account, spawning took place at least from mid August to mid September. Whether *T. antarcticus* reproduces continuously or only in winter is an open question. Its congener *T. tergipes* reproduces throughout the year (Clark 1975), but reproduction in winter may be favourable for *T. antarcticus*, as loss of eggs to the water column due to melting is minimised, and eggs may serve as overwintering stages of the species. The hatching larvae resemble other larvae of opisthobranchs with a planktic developmental phase (Thompson 1967); in particular

it is similar to those of *Tergipes tergipes* (Tardy 1964). As in this species, the velum is large and allows swimming in the water column. Since most of the visceral cavity of the larvae seems to be filled with haemolymph, swimming in the water is facilitated. Veliger larvae may therefore play an important role for the dispersal of the species, in particular through bridging of ice free areas and times and thereby allowing the colonisation of distant or newly forming ice habitats. Whether a life phase in the water column is obligatory or facultative remains unclear. How long the veliger larvae can remain in the water column and what distances they can bridge is another open question. Larvae of *T. antarcticus* have not been reported from the water column so far, but this may be due to their small size and the difficulties in their identification. In this context, further analyses of subice water samples, as have been conducted by several authors (Tanimura et al. 1984, Kurbjeweit et al. 1993, Schnack-Schiel et al. 1998, chapter two), but with a focus on more fragile and smaller forms would be helpful. The use of DNA sequencing methods would help in the identification of larval forms of sea-ice species and therefore in the elucidation of life-cycle strategies and distribution mechanisms.

3.4.6. Physiology

Eggs, veliger larvae and adults of *T. antarcticus* are well adapted to low temperatures and ice formation, as well as high salinities within sea ice. Low temperatures within the ice during ANT-XXIII/7 enable the estimation of lowermost temperatures and uppermost salinities, which *Tergipes antarcticus* veliger larvae and eggs are able to survive, to be T = - 5.6 °C with S = 94 g/kg and T = - 8.0 °C, with S = 130 g/kg, respectively. Only few other investigations on temperature and salinity tolerance of Antarctic sympagic metazoans have been made: the amphipod *Eusirus antarcticus* and the krill *Euphausia superba* exhibit lethal temperature minima of -2.5 and -4.2 °C, respectively (Aarset and Torres 1989), the harpacticoid copepod *Drescheriella glacialis* survives salinities up to 90 g/kg (Dahms et al. 1990), and acoel plathyelminths survive salinities up to 75 g/kg (Gradinger and Schnack-Schiel 1998). Therefore, *T. antarcticus* eggs and veliger larvae exhibit an exceptionally high temperature and salinity tolerance, further supporting the hypothesis that these stages are overwintering stages of *T. antarcticus*. One *Tergipes antarcticus* egg clutch in gastrula stage was observed microscopically, before and after freezing. Freezing resulted in the death of the embryo, as within 1 h afterwards structural integrity of the embryo was lost, leaving slurry inside the egg capsule. Therefore embryos seem to be freeze intolerant, and freezing of egg clutches within

the environment has to be avoided. Freeze intolerance of mollusc egg clutches is also reported for *Cantareus aspersus* (a European land snail), and generally invertebrate eggs are mostly freeze intolerant (Ansart et al. 2007). A mean SCP of -12.5 °C was found for whole egg clutches of *T. antarcticus* and animals had even lower SCPs. Lowermost temperatures found within the ice during this study were -10.9 °C on 3 October 2007 in the uppermost section of the ice; hence egg clutches and adults are protected from freezing down to temperatures occuring *in situ*. Liquids withdrawn from *T. antarcticus* adults and egg clutches show thermal hysteresis. This is the second report of thermal hysteresis for a marine mollusc: however, a report of thermal hysteresis in *Mytilus edulis* (Theede et al. 1976) was not confirmed on a different population of this species (Loomis 1995). Furthermore, this is the third report of thermal hysteresis for a marine invertebrate, the only other report being about an Antarctic nemertean (Waller et al. 2006). In bacteria, fungi, plants and in both vertebrate marine and invertebrate terrestrial animals, a thermal hysteresis is diagnostic of antifreeze proteins being present (DeVries 1982, Duman 1982, Duman and Olsen 1993). These proteins protect the organism from internal ice growth, which in most cases destroys cellular integrity and causes death. When such proteins are present, ice crystals forming within the liquid grow preferentially along the c-axis (Matsumoto et al. 2006); as also observed for *T. antarcticus*. We conclude that one protection mechanism from ice growth within *T. antarcticus* is probably via antifreeze proteins. Whether these proteins are produced by *T. antarcticus* itself or obtained via the food remains an open question. At least ice-active proteins, causing recrystallisation inhibition, are available in the habitat, for example in sea-ice algae (Raymond and Knight 2003). Further investigations on temperature and salinity tolerance and adaptation of *T. antarcticus* and other sympagic species are clearly needed.

3.4.7. Food web

Most members of the Aeolidoidea are known to be extreme food specialists, feeding almost exclusively on cnidarians, especially hydrozoans (Tergipedidae, Flabellinidae, Glaucidae), octocorals (several members of the Favorinidae) and hexacorals (Aeolidiidae) (Clark 1975, McDonald and Nybakken 2006 (http://people.ucsc.edu/~mcduck/nudifood.htm; last date accessed: 2 Feb 2008)). In particular, *Tergipes tergipes* feeds on hydroid polyps (Lambert 1991). However, recent studies have shown that some aelididoidean species commonly considered as food specialists appear to be more generalistic (McDonald and Nybakken 2006; Megina et al. 2007). As there are no references of cnidarians within Antarctic sea ice, the

typical hydrozoan food for tergipedids is probably not available in this habitat. The absence of a cnidosac for incorporation of cnidocysts furthermore supports the view that *T. antarcticus* does not feed on cnidarian prey and thus has to exploit a different food source. The structure of radula and jaws gives no hint on an alternative food source. It is possible that *T. antarcticus* feeds on the highly abundant sympagic algae, as cultivated animals survived for up to 1 month when supplied with a diet of mixed sympagic algae. Single uni-cellular algae were found in the pharynx, and one dinoflagellate could be identified in the digestive gland of the specimen found in the water column (ANT-III/3). The brown spot at the tip of the digestive glandular branches reaching into the cerata may represent algal remnants. It is also possible that *T. antarcticus* feeds on small individuals of the ctenophore *Callianira antarctica*, which has been reported recently from Antarctic sea ice (chapter 2). The absence of eyes in hatched veliger larvae is an indication for planktotrophic development, since in these forms the intracapsular development is shorter than in lecithotrophic larvae and eye development is rather late (Thompson 1976). The lack of yolk in the digestive system furthermore indicates the necessity to feed after hatching to ensure future metamorphosis. Veliger larvae probably feed on small algae or on bacteria which are abundant within sea ice (Thomas and Dieckmann 2002) and in the water column. The larvae could be reared for several weeks on a diet of mixed sympagic algae and grew during this period. The small interstices of sea ice seem favourable for *T. antarcticus*, as known nudibranch predators like fish or pycnogonids are virtually absent. Nevertheless animals living in larger channels in the ice and crawling at the underside of sea ice may serve as food source for fish and maybe also for amphipods which exploit these habitats (Krapp et al. 2008). *T. antarcticus* is protected from visual predators through the white appearance of the body. Egg clutches are probably well protected within the ice from larger predators. A possible predator of *T. antarcticus* within the ice is the ctenophore *Callianira antarctica*. Pelagic *C. antarctica* feed primarily on copepods and krill larvae (Ju et al. 2004, Scolardi et al. 2006), but it has generally been observed that ctenophores are opportunistic feeders (Purcell 1997 and references therein). Predation of *C. antarctica* on juvenile or larval *T. antarcticus* thus seems possible, but as discussed above, also feeding of juvenile and adult *T. antarcticus* on smaller individuals of *C. antarctica* can not be excluded. Studies on the food web within sea ice, including food-choice experiments with different food sources, gut content analysis and the use of lipid biomarkers, are obviously needed to elucidate the role of *T. antarcticus* within the food web.

Supplementary table S1**:** *Tergipes antarcticus* samples obtained and analysed for this study.

Life stage	Expedition	Sampling position	Sampling date	Sample type	Analysis
EC 1	ANT-XXIII/7	60°40' S 42° 9' W	Sep 10, 2006	LVS	Dev
EC 2	ANT-XXIII/7	"	Sep 10, 2006	LVS	Dev
EC 3	ANT-XXIII/7	"	Sep 10, 2006	LVS	Dev
EC 4	ANT-XXIII/7	"	Sep 10, 2006	LVS	Dev
EC 5	ANT-XXIII/7	"	Sep 10, 2006	LVS	Dev, SCP, TH
EC 6	ANT-XXIII/7	"	Sep 10, 2006	LVS	Dev, SCP, TH
EC 7	ANT-XXIII/7	"	Sep 10, 2006	LVS	Dev
EC 8	ANT-XXIII/7	"	Sep 10, 2006	LVS	Dev
EC 9	ANT-XXIII/7	"	Sep 10, 2006	LVS	Dev
EC 10	ANT-XXIII/7	"	Sep 10, 2006	LVS	Dev, SCP
EC 11	ANT-XXIII/7	"	Sep 10, 2006	LVS	Dev
EC 12	ANT-XXIII/7	"	Sep 10, 2006	LVS	Dev
EC 13	ANT-XXIII/7	"	Sep 10, 2006	LVS	Dev, THP
EC 14	ANT-XXIII/7	"	Sep 10, 2006	LVS	Dev
EC 15	ANT-XXIII/7	"	Sep 10, 2006	LVS	Dev, SCP, RNA
EC 16	ANT-XXIII/7	"	Sep 10, 2006	LVS	Dev, SCP, TH
EC 17	ANT-XXIII/7	"	Sep 10, 2006	LVS	Dev
EC 18	ANT-XXIII/7	"	Sep 10, 2006	LVS	Dev
EC 19	ANT-XXIII/7	"	Sep 10, 2006	LVS	Dev
EC 20	ANT-XXIII/7	"	Sep 10, 2006	LVS	Dev
EC 21	ANT-XXIII/7	"	Sep 10, 2006	LVS	Dev
EC 22	ANT-XXIII/7	"	Sep 10, 2006	LVS	Dev
EC 23	ANT-XXIII/7	"	Sep 10, 2006	LVS	Dev
EC 24	ANT-XXIII/7	"	Sep 10, 2006	LVS	Dev
EC 25	ANT-XXIII/7	"	Sep 10, 2006	LVS	Dev
EC 26	ANT-XXIII/7	"	Sep 10, 2006	LVS	Dev
EC 27	ANT-XXIII/7	60° 18' S 50° 45' W	Sep 19, 2006	LVS	Dev
EC 28	ANT-XXIII/7	"	Sep 19, 2006	LVS	Dev
EC 29	ANT-XXIII/7	"	Sep 19, 2006	LVS	Dev
EC 30	ANT-XXIII/7	"	Sep 19, 2006	LVS	Dev
EC 31	ANT-XXIII/7	65° 06' S 57° 24' W	Oct 8, 2006	LVS	SCP
EC 32	ANT-XXIII/7	"	Oct 8, 2006	LVS	SCP, RNA
Juv 1	ANT-V/3	72° 52' S 19° 20' W	Nov 17,1986	Ice core	-
Juv 2	ANT-V/3	"	Nov 17,1986	Ice core	-

Juv 3	ANT-X/3	70°31'S 7°59'W	Apr 7, 1992	Ice core	-
Juv 4	ANT-XXIII/7	60° 40' S 42° 9' W	Sep 10, 2006	LVS	LO
Juv 5	ANT-XXIII/7	"	Sep 10, 2006	LVS	LO
Juv 6	ANT-XXIII/7	"	Sep 10, 2006	LVS	LO
Juv 7	ANT-XXIII/7	"	Sep 10, 2006	LVS	LO
Juv 8	ANT-XXIII/7	"	Sep 10, 2006	LVS	LO
Juv 9	ANT-XXIII/7	"	Sep 10, 2006	LVS	LO
Juv 10	ANT-XXIII/7	60° 06' S 47° 53' W	Sep 23, 2006	LVS	LO
Juv 11	ANT-XXIII/7	64° 18' S 54° 35' W	Oct 3, 2006	Ice core	-
Juv 12	ANT-XXIII/7	60° 45’ S 48° 20’ W	Sep 24, 2006	Ice core	-
Juv 13	ANT-XXIII/7	60° 45’ S 48° 20’ W	Sep 24, 2006	Ice core	-
Ad 1	ISAE	68° 18' S 73° 06' E	Jan 29, 1957	Conical net	Histology
Ad 2	ISAE	"	Jan 29, 1957	Conical net	Histology
Ad 3	ISAE	68° 38' S 29° 03' E	Feb 18, 1957	Conical net	Radula preparation
Ad 4	ISAE	"	Feb 18, 1957	Conical net	-
Ad 5	ANT-III/3	72° 37' S 19° 02' W	Feb 14, 1985	Bongo net	Morphology
Ad 6	ANT-X/3	70° 18' S 8° 54' W	Apr 16,1992	Sackhole	-
Ad 7	ANT-XXIII/7	60° 40' S 42° 9' W	Sep 10, 2006	LVS	LO
Ad 8	ANT-XXIII/7	"	Sep 10, 2006	LVS	LO
Ad 9	ANT-XXIII/7	"	Sep 10, 2006	LVS	LO
Ad 10	ANT-XXIII/7	64° 43' S 57° 20' W	Oct 6, 2006	LVS	SCP, TH, RNA
Ad 11	ANT-XXIII/7	"	Oct 6, 2006	LVS	SCP, TH, RNA
Ad 12	ANT-XXIII/7	"	Oct 6, 2006	LVS	-
Ad 13	ANT-XXIII/7	65° 06' S 57° 24' W	Oct 8, 2006	LVS	Morphology, Histology

Abbreviations: Ad = adult, Dev = development, EC = egg clutch, Juv = juvenile, LO = live observation, LVS = large volume sample, RNA = RNA isolation, SCP = supercooling point, TH = thermal hysteresis.

4. Acquisition of freeze protection through horizontal gene transfer in a crustacean

4.1. Introduction

Sea ice is porous and houses a fascinating interstitial flora and fauna (Thomas and Dieckmann 2002). Three interstitial habitats, characterised by different formation processes, are associated with Antarctic sea ice. From top to bottom these are the surface layer, the brine-channel system within and at the bottom of the ice as well as the platelet layer accumulating underneath the ice in the vicinity of ice shelves (Horner et al. 1992). Besides viruses, bacteria, unicellular algae (mainly diatoms) and protozoans, several metazoan species thrive within these interstitial systems (Thomas and Dieckmann 2002). Temperature changes within the ice result in changes of brine volume and salinity, as only water molecules are incorporated into the growing ice matrix and the ions remain in the liquid brine. Lower temperatures result in lower volumes and higher brine salinities (Assur 1958). Temperatures below 0 °C (down to –22 °C) and a wide salinity range (from 0 to 220 g/kg) (Schünemann and Werner 2005), as well as space restrictions (Krembs et al. 2000) and the omnipresence of ice characterise the interstitial systems. Whereas sea-ice bacteria and algae seem to survive *in situ* temperatures down to -25 °C (Krembs et al. 2002a), metazoans are restricted to less extreme conditions. Veliger larvae of the nudibranch *Tergipes antarcticus* were found intact within sea ice at a temperature of -5.6 °C (chapter 3). Also the large scale dynamics of ice formation and melting within the Southern Ocean make this habitat challenging for sympagic species (Eicken 1992). In winter large areas are covered by sea ice (up to 18 million km²; Cavalieri et al. (2003); see also http://nsidc.org/data/smmr_ssmi_ancillary/regions/total_antarctic.html for recent data; last date accessed: 12 December 2008) but, as solar irradiation is low, primary production (Arrigo et al. 1997) and therefore food availability for metazoans is poor. In summer comparatively small areas (3.5 million km²; Cavalieri et al. (2003)) remain ice covered. In areas where sea ice does not persist, species must be able to survive periods within the open water column or at the sea floor.

Stephos longipes and *Paralabidocera antarctica* are the two dominant calanoid copepod species of Antarctic sea ice (Arndt and Swadling 2006). *S. longipes* dominates in the central parts of the Weddell Sea, in the Bellingshausen, Amundsen and Ross Sea and is found mainly in pack ice (Kurbjeweit et al. 1993, Schnack-Schiel et al. 1995, 1998, Guglielmo et al. 2007,

Schnack-Schiel et al. 2008). In contrast, *P. antarctica* is mainly found in coastal shelf regions of the Indian and Pacific Sectors of the Southern Ocean and is strongly associated with fast ice (Tanimura et al. 1996, Swadling et al. 1997, Swadling 2001, Tanimura et al. 2002). Two different life cycle strategies enable *Stephos longipes* and *Paralabidocera antarctica* to survive the large-scale dynamics of sea ice. *S. longipes* survives the winter as copepodite IV stage in deeper water layers. In late winter/early spring these animals ascend to the ice-water interface, develop into adults (Schnack-Schiel et al. 1995), thereafter colonise the surface and bottom layer of sea ice and reproduce within these layers in the productive spring and summer season (Schnack-Schiel et al. 1995, chapter 2). Naupliar and copepodite stages develop in the surface and bottom layer and spread through the whole ice column when the ice is warm and porous enough to allow this (from spring to autumn) (Schnack-Schiel et al. 2008). The copepodids are released to the water column when the ice melts or may actively leave the ice in autumn. In winter copepodite stage IV retreats to deeper water layers. Some naupliar stages seem to overwinter within the ice, mainly in the bottom layer (Schnack-Schiel et al. 1995).

Paralabidocera antarctica inhabits the bottom part of fast ice during winter as naupliar and early copepodite stages (Swadling et al. 2004). Later stages leave the ice in summer and dwell in the sub-ice habitat (Tanimura et al. 1996). Reproduction takes place during a short period in summer and the eggs sink to the sea floor (Swadling et al. 2004). From this egg bank recruitment takes place in late January and the pelagic naupliar stages I and II colonise the bottom part of the ice in late February (Swadling et al. 2004). The need to recruit from these egg banks is probably one factor restraining *P. antarctica* to coastal shelf regions.

Mechanisms enabling metazoans thriving in the interstitices of sea ice to survive the presence and formation of ice are unknown. Formation of ice crystals within body compartments is detrimental to most organisms, as it disrupts cell membranes (Duman 2001). Freeze avoidance can be reached through osmoconforming to the external medium, thereby colligatively reducing the freezing point of body liquids. Another possibility is the non-colligative reduction of the freezing point through thermal hysteresis (TH), which is the difference between the freezing and melting point of a substance. TH was first discovered in Antarctic fish (DeVries 1982) and is also known from Artic fish as well as from terrestrial arthropods (Duman 2001), bacteria, fungi and plants (Duman and Olsen 1993) and from three marine invertebrates (Theede et al. 1976, Waller et al 2006, chapter 3).

In bacteria, fungi, plants, insects and fish TH is traced back to the presence of antifreeze(glyco)proteins (AF(G)Ps) (Duman 2001, Duman and Olsen 1993). These proteins evolved several times independently (Cheng 1998) indicating the importance and efficiency

of freeze protection through TH. A group of proteins, unrelated to other AF(G)Ps on sequence level, was identified from bacteria and algae inhabiting icy habitats and termed ice binding proteins (IBPs) (Raymond et al. 1994). IBPs secreted by sea-ice diatoms to the interstitial water of the platelet layer provided no TH at *in situ* concentrations (Raymond et al 1994). To my knowledge higher IBP concentrations were never tested for TH, and IBPs identified from bacteria and other diatoms were analysed only for their ability to provide recrystallisation inhibition (RI) (Raymond 2000, Raymond and Fritsen 2000, 2001, Raymond and Knight 2003, Janech et al. 2006, Raymond et al 2007). To assess for RI, a liquid sample is shock frozen and maintained at a temperature significantly below the freezing point. Initially, the sample will consist of very many, very small ice crystals, but without a recrystallisation inhibitor slightly bigger crystals will grow at the cost of smaller ones. A recrystallisation inhibitor will prevent this growth. It should be noted that probably all AF(G)Ps also possess RI activity (Mueller et al. 1991). A protein from *Typhula ishikariensis*, homologous to diatom IBPs was termed AFP as it provided a maximum TH of 1.0 °C at a concentration of 20 mg/ml (Hoshino et al. 2003). Furthermore, data presented in this chapter also show that a protein with high homology to diatom IBPs has considerable TH activity. Such measurements are missing for IBPs and might reveal that these IBPs provide TH activity at concentrations found inside or in the direct surrounding of bacterial and algal cells, like the extracellular matrix or the extracellular polysaccharide coat which at extreme conditions surrounds algal cells (Krembs et al. 2002a). In consequence, the functional discrimination of AFPs and IBPs, which is based on differences in analysis, may be obsolete. To avoid further confusions I will use the term antifreeze protein (AFP) to account for both groups and the initially given protein name to account for a single protein.

In order to increase our knowledge about adaptation mechanisms of *Stephos longipes* and *Paralabidocera antarctica* to salinity changes and ice formation, haemolymph osmolality and TH were measured after incubation at different medium salinities. The presence of TH in *S. longipes*, but not in *P. antarctica* is to some extent responsible for the different abilities of these species to colonise microhabitats of sea ice.

Screening for genes expressed at low temperatures and high salinities with a suppression subtractive hybridisation (SSH) approach (Diatchenko et al. 1996) revealed two isoforms of a secreted antifreeze protein (St-AFPa and b) in the transcriptome of *Stephos longipes*. The recombinant protein possesses TH activity. The protein is homologous to an IBP identified in a bacterium (Raymond et al. 2007), an AFP from a snow mold (Hoshino et al. 2003) and a

diatom IBP (Janech et al. 2006), suggesting that it was obtained by *S. longipes* through horizontal gene transfer (HGT).

4.2. Material and methods

4.2.1. Sampling and cultivation

Copepods were obtained during two cruises to the western Weddell Sea, Antarctica, with "RV Polarstern" (ANT-XXII/2, 2004/2005 and ANT-XXIII/7, 2006). Large volume samples from the surface layer and the ice proper were melted in the dark at +4 °C in a surplus of 0.2 µm filtered seawater (FSW) in order to reduce salinity stress for the organisms (Garrison and Buck 1987). After complete melting of the ice, the samples were enriched over a 20 µm gauze. Further material was obtained from sub-ice and surface plankton catches. Animals were sorted alive at 0 °C under a stereomicroscope and maintained at 0 °C in 1 L beakers filled with FSW and supplied with a mix of sea-ice algae or a culture of *Fragilariopsis cylindrus*. A *S. longipes* culture could be maintained for half a year in the home laboratory. The osmolality and TH measurements were done with these animals.

4.2.2. Osmolality and thermal hysteresis of the haemolymph

Adult *Stephos longipes* were transferred to different salinities (S_R = 25 to 45 g/kg) at 0 °C and incubated in the final medium for 48 hours. *Paralabidocera antarctica* were transferred to T = -1.2 °C; S_R = 25 g/kg, T = -1.8 °C; S_R = 35 g/kg as well as to T = -3.1 °C; S_R = 55 g/kg. FSW was adjusted to the respective salinities by diluting with MilliQ®-water or by adding artificial sea salt (Tropic Marin®). Salinities were measured with a WTW microprocessor conductivity meter LF 196 (accuracy: S = ±0.2) and S_R was calculated by multiplying the displayed values with 1.004715 g/kg as proposed by Millero et al. (2008). After the incubation period copepods were transferred to a microcentrifuge cap in a small water volume and covered with Cargille immersion oil type b. The water was withdrawn from the animals and thereafter the oil droplet with the contained animal was transferred onto a small glass slide. The rest of the retained water was withdrawn with a capillary. The haemolymph was withdrawn from the animals by inserting a pointed, oil filled glass capillary dorsally into the heart region of the animals. The liquid was transferred to the sample holder of a Clifton Nanoliter Osmometer and the osmolality of the haemolymph and presence of thermal hysteresis (the difference between the melting and freezing point of a liquid) was measured as

described previously (Enevoldsen et al. 2003). The osmolality was determined as the melting point of the last ice crystal of the sample. Wescor® osmolality standards (290 and 1000 mOsm) as well as MilliQ®-water were used to calibrate the osmometer according to manufacturers instructions. To assess TH the size of the last, single ice crystal was reduced to 2 to 5 µm, thereafter the temperature was lowered with a cooling rate of ca. 1.3 K/min and the osmolality reading determined at which the crystal grew again. Measurements were repeated at least three times and the mean value was calculated. Osmolality values were converted to temperature by multiplying by -0.001858 °C/mOsm (DeVries 1982).

Evaporation of the samples was carefully avoided, but it occurs sometimes and therefore the osmolality data is shifted to slightly higher values. This effect is especially prevalent when working with very small samples like *S. longipes* haemolymph. I also attribute the high variability of *S. longipes* TH measurements to the extreme difficulty in extracting haemolymph from these mm-sized animals. Sometimes tissue was injured while extracting the haemolymph. This could result in a contamination of the sample, e.g. with proteinase, which nevertheless has no effect on the osmolality of the sample. Differences in the size of the extracted lipid drop also have an effect on the observed TH, as in larger drops the size of the last crystal is more difficult to adjust.

4.2.3. Nucleic acid isolation

Prior to molecular-biological experiments, animals were kept for one day in FSW in order to allow gut evacuation. Thereafter, animals were isolated, fixed with Trizol® (Invitrogen) and samples were stored at -80 °C. Total RNA was isolated from these samples using a standard chloroform/isopropanol extraction according to the manufacturer protocol. Messenger RNA (mRNA) was isolated using the Qiagen Oligotex kit according to the manufacturer protocol. Genomic DNA was isolated from the organic phase of Trizol® RNA extractions: the organic phase was isolated and an equal volume back extraction buffer (4 mol l^{-1} guanidine thiocyanate, 50 mmol l^{-1} sodium citrate, 1 mol l^{-1} Tris (free base)) was added. After mixing and centrifugation at 12,000 g for 30 min at 20 °C, the aqueous phase was transferred to a new tube. An equal volume of isopropanol was added and the DNA was precipitated by centrifugation at 12,000 g for 15 min at 4 °C.

4.2.4. Suppression subtractive hybridisation

For the establishment of SSH-libraries 500 *Stephos longipes* copepods (adults and CV) were transferred stepwise to temperatures of -1.2 °C and salinities of 25 g/kg (Driver), as well as to -3.1 °C and 55 g/kg (Tester) and kept for two days at the respective conditions in 50 mL vials. SSH was performed with mRNA isolated from these groups using the BD PCR-Select™ cDNA Subtraction Kit (BD_Biosciences Clontech) according to the manufacturer protocol. Resulting subtracted complementary DNA (cDNA) was size selected with a cDNA size selection column (Invitrogen), ligated into the pGEM-T easy Vector (Promega), and *Escherichia coli* JM109 cells were transformed with the ligation mix. Plasmids were isolated from insert bearing clones (indicated through blue/white selection) and each sequenced once (984 clones sequenced; average insert length 583 nucleotides). TIGR Indices Clustering Tools (Pertea et al. 2003) were used for clustering the sequences. Seqtools program (S.W. Rasmussen, www.seqtools.dk) was used for batch sequence analysis. Nucleotide, protein, and translated BLAST engines at the NCBI server were used for homology searches in public databases.

4.2.5. Sequence retrieval and analysis

Stephos longipes RNA was transcribed into cDNA using Superscript III Reverse transcriptase (Invitrogen). To obtain 5' and 3' end sequences the FirstChoice RLM RACE Kit (Ambion) was used according to previous descriptions (Mark et al. 2006). Full length sequences of St-AFPa and b were obtained with the primers St_AFPb_full_fw1 5'-ACA TGA GTT TCA CCA KAA WTC ATC-3', St_AFPa_full_fw1 5'-CTG TGA ACA GCG AAG AAG TCA T-3' and St_AFP_full_rev1 5'-TGA TCA GCA CTT TAG CTT GTC-3' and using standard PCR conditions (annealing temperatures 60 °C). Contamination with cDNA from photosynthetic organisms was tested with a PCR using conserved primers for Rubisco (RUBISCO_K1 5'-AAR CCW AAR YTA GGK YTW TC-3' and RUBISCO_V1 5'-CYT CYA RYT TAC WAC DAC-3' from Xu and Tabita (1996) without the restriction site added there). *Fragilariopsis curta* cDNA was used as positive control for the Rubisco PCR. TMpred and SignalP (Bendtsen et al. 2004) programs were used for further protein characterisation. AFP sequences were aligned using the Clustal W tool in MacVector (Accelrys).

4.2.6. Whole mount *in situ* hybridisation

Methods for whole mount *in situ* hybridisation were adapted from previous work (Augustin et al. 2006). Briefly, digoxygenin-labeled RNA probes were synthesised as follows: a PCR product was generated with M13 forward and reverse primers from a pGEMT-easy plasmid containing the conserved region of St-AFPa/b. Sense and antisense probes were synthesised from these products using Sp6 and T7 RNA Polymerase (Invitrogen). After fixation animals were cut longitudinally to enable probe penetration. *In situ* hybridisation with uncut animals was not successful. Hybridisation was performed over night at 57 °C.

4.2.7. Semiquantitative real time PCR

Semiquantitative real time PCR was performed on cDNA from pooled animals incubated at -1.2 °C; S = 25 and -3.1 °C, S =55 using primers from the conserved regions of St-AFPa and b (St-AFP_fw1 5'-TGC AGA TCG GAG CAG GTT TA-3' and St-AFP_full_rev1 5'-TGA TCA GCA CTT TAG CTT GTC-3'). eEF-1α was amplified via PCR from *Stephos longipes* cDNA using degenerated primers and subsequently ligated, cloned and sequenced. Specific primers for St-eEF-1α (GenBank Accession No. FJ538310) were designed (EEF1a_ST_fw1: 5'-CAT GGT TGT CAC CTT TGC C-3' and EEF1a_ST_rev3: 5'-CGA CGA TCA ACC TTC TCC TT-3') and used for the control reaction in the semiquantitative real time PCR. A water control was included in every experiment, as well as a control for contamination with Rubisco (Data not shown).

4.2.8. Genomic dot blot

Digoxygenin (Dig)-labelled DNA probes were synthesised in a PCR containing alkali labile DIG-dUTP and a plasmid containing the full St-AFPb sequence together with the primers St-AFPb_full_fw1 5'-ACA TGA GTT TCA CCA KAA WTC ATC-3' and St_AFP_full_rev1 5'-TGA TCA GCA CTT TAG CTT GTC-3'. Heat shock protein 70 (HSP70) was amplified via PCR from *Stephos longipes* cDNA using degenerated primers and subsequently ligated, cloned and sequenced. The identity of the sequence as a copepod HSP70 was confirmed through Blast searches and a ClustalW alignment. Using this template a DIG-labelled St-HSP70 (GenBank Accesion No. FJ538309) probe was generated using the primers HSP70b-ST-R1 5'-GCC AGT GCT TCA TAT CAG CC-3' and HSP70b_ST_fw1 5'-GAA GTC AAG

TCC ACT GCT GGT-3'. Genomic DNA from *Stephos longipes* (concentration: 1.5 µg/µL and 6 µg/µL) and plasmids containing the tested probe sequence were spotted onto two Hybond N+ membranes. After UV-crosslinking, membranes were subjected to a standard Southern hybridisation protocol using the generated probes separately on the two different membranes. Anti-Dig alkaline phosphatase was used as detection antibody and NBT/BCIP for the colour reaction.

4.2.9. Recombinant expression

cDNA coding for the mature St-AFP (without signal peptide) was cloned between the *BamH*I and *Hind*III sites within the multiple-cloning site of the pET28a vector (Novagen) providing His_6-Tags at the N- and C terminal end. *Escherichia coli* Rosetta pLys cells were transformed with the construct. Recombinant protein was induced in *E. coli* with 1 mM isopropyl-b-D-thiogalactoside for 5 h at 37°C. After harvesting, cells were lysed under native conditions using lysozyme and a sonicator to disrupt cells as well as RNase A and DNase I digestion. Cleared lysate of bacteria containing either the expression construct or the empty vector were checked for TH activity. To analyse the concentration dependency of TH activity, samples were diluted with MilliQ® water and osmolality and TH determined at least three times per sample. A dilution factor was determined by multiplying the osmolality value with 1/2953 mOsm, the osmolality value of the original sample.

4.2.10. Calculation of alien indices

Alien indices were calculated according to Gladyshev et al. (2008) with e-values from BlastP searches using the following formula: AI = log((Best e-value for member of a fungi, stramenopile or metazoan, respectively) + e-200) – log ((Best e-value for the member of a bacteria or archaeon) + e-200). If no hits were found, the e-value was set to 1.

4.3. Results

4.3.1. Haemolymph osmolality and thermal hysteresis

The haemolymph of *Stephos longipes* was isosmotic in the tested salinity range from 25 to 45 g/kg, and the haemolymph of *Paralabidocera antarctica* was isosmotic in the tested salinity range from 25 to 55 g/kg (Fig. 17). Compared to *P. antarctica*, the haemolymph of *S. longipes* was very viscous, which made a measurement at S = 55 g/kg impossible. A TH of 0 to 3.8 K (median 1.0 K; n = 13) was found for the haemolymph of *S. longipes*. A correlation with incubation salinity was not observed, due to a high variability of single TH measurements. A distinct shape of the crystal in the hysteresis gap was not observed. Growth of the crystal after the hysteresis gap was very fast, and the whole liquid sample froze almost instantaneously. A distinct shape of the growing crystal was therefore not observed. TH was not observed for *P. antarctica*.

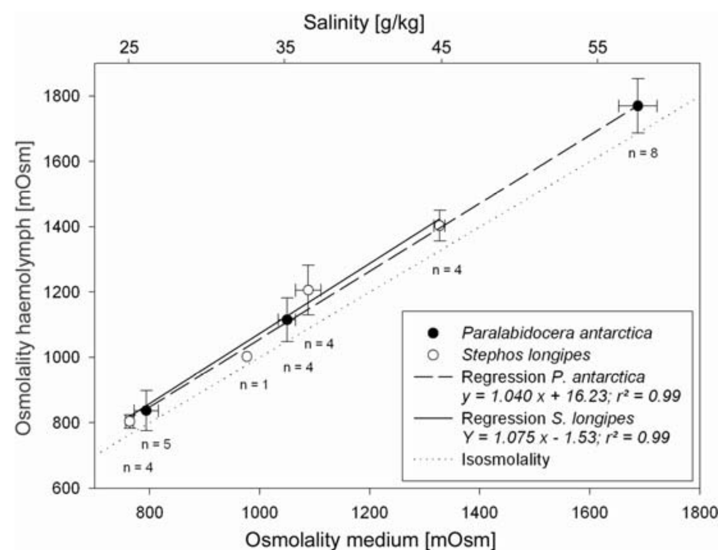

Fig. 17. Osmolality of the haemolymph of *Stephos longipes* and *Paralabidocera antarctica* in relation to the osmolality of the medium after incubation at different salinities for 48 hours. Values are means +/- standard deviation, n = number of animals measured.

4.3.2. *Stephos longipes* antifreeze proteins

An expressed sequence tag with high homology to IBPs from the sea-ice diatom *Navicula glaciei* was identified in a SSH cDNA library established for *Stephos longipes*. Full length sequences were obtained using 5’ and 3’ rapid amplification of cDNA ends. Two isoforms were found and confirmed through amplification and sequencing of the respective PCR

products. A BlastP search with the full length sequences at the NCBI server identified several ice-active IBPs from the sea-ice diatom *Navicula glaciei* as best hits with e-values ranging from 1e-63 to 5e-38. An AFP from the snow mold *Typhula ishikariensis* (e-value 2e-44) and an IBP from the sea-ice bacterium *Colwellia* sp. (e-value 9e-41) were also identified. Similarity of both copepod isoforms to these AFPs was high on the aminoacid (aa) level: 64 to 65 % to *N. glaciei* IBP (Genbank Accession no. AAZ76254); 57 to 58 % to *T. ishikariensis* K1-A (BAD02895) and 54 to 55 % to *Colwellia* sp. IBP (ABH08428) (Fig. 18).

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StAFPrec MGSSHHHHHHSSGLVPRGSHMASMTGGQQMGRGSDGPSRVNLLKAGKFALLTKTGVTTTG  60
-------------------------------------------------------------------
St-AFPa                MSFSIIQLLATAMVLGVTMADGPSRVNLLKAGKFALLTKTGVTTTG  46
St-AFPb            MSFTRNHQTALLLATAMVLGVTMADGPSRVNLLKAGKFALLTKTGVTTTG  50
NgIBP                  MMFLAKTVTLLVALVASSVAAEQSAVDLGTAGDFAVLSKAGVSTTG  46
TiAFP                  MFSSTYLLAIIALAVSSVFAAGPTAVPLGTAGNYAILASAGVSTVP  46
C sp IBP        MKTLISNSKKVLIPLIMGSIFAGNVMAAGPYAVELGEAGTFTILSKSGITDVY  53
                              .           *     * *  ** . .*. .*..

StAFPrec ITEVKGDMGTSPIARAALTGFSLVADSTNEFSGSPFVTGNVYASNDAVPTPQLLTDAVLD 120
-------------------------------------------------------------------
St-AFPa  ITKVKGDMGTSPIARAALTGFTLVADSTNEFSESPFVTGNVYASNDAVPTPQLLTDAVLD 106
St-AFPb  ITKVKGDMGTSPIARAALTGFSLVADSTNEFSGSPLVTGNVYASNDAVPTPQLLTDAVLD 110
NgIBP    PTEVTGDIGTSPIASTALTGFALIKDSSNTFSTSSLVTGKIYAADYTAPTPSKMTTAISD 106
TiAFP    QSVITGAVGLSPAAATFLTGFSLTMSSTGTFSTSTQVTGQLTAADYGTPTPSILTTAIGD 106
C sp IBP PSTVTGNVGTSP-----ITGAALLLN-CDEVTGAMYTVDSAGPLPCSINSPYLLELAVSD 113
          . . * .* **     .** .*    .   . .               .*  .  *. *

StAFPrec MQAAYTDAAGRPDPDHLNFGAGSIEGETLVPGLYKWDGGVSFTDVVTFDGTSTDIWILQI 180
-------------------------------------------------------------------
St-AFPa  MQAAYTDAAGRPDPDHLNFGAGSIEGETLLPGLYKWDAGVSFTDGVTFEGSSTDIWILQI 166
St-AFPb  MQAAYTDAAGRPDPDHLNFGAGSIEGETLLPGLYKWDAGVSFTDGVTFNGSSTDIWILQI 170
NgIBP    MSTAFTDAAGRSDPDFLELGAGSIEGETLVAGLYKWGTDVSFTSSLVFDGSATDVWILQV 166
TiAFP    MGTAYVNAATRSGPNFLEIYTGALGGKILPPGLYKWTSPVGASADFTIIGTSTDTWIFQI 166
C sp IBP MGIAYNDAAGRVPADHTELGTGEIGGLTLEPGVYKWSSDVNISTDVTFNGTMDDVWIMQI 173
         *  *.  ** *         .* . *  *  *.*** . *  .      *.  * ** *

StAFPrec GAGLNVGSNARVVLKGGAKVKNIFWQIVGPAVLGTGSHVQGVFLCKTNMVFQTGSSLKGA 240
-------------------------------------------------------------------
St-AFPa  GAGLNVGSGAKVKLAGGAKVKNIFWQIVGPAVLGTGSHVQGVFLCKTNMVFQTGSSLKGA 226
St-AFPb  GAGLNVGSGAKVKLAGGAKVKNIFWQIVGPAVLGTGSHVQGVFLCKTNMVFQTGSSLKGA 230
NgIBP    AKDFIVGNGAQMYLTGTAKAENIFIQVSGAVNIGTTAHVEGNILSATAIALQTGSSLNGK 226
TiAFP    AGTLGLAAGKKIILAGGAQAKNIVWVVAGAVSIEAGAKFEGVILAKTAVTLKTGSSLNGR 226
C sp IBP SGNLNQANAKRVTLTGGALAKNIFWQVAGYTALGTYASFEGIVLSKTLISVNTGTTVNGR 233
                   .. * * *   *.   . *     .   .*  *. * .   **... *

StAFPrec VLAQTAVTLDSARITKRSFCDITVGCETSAAALEHHHHHH                     278
-------------------------------------------------------------------
St-AFPa  VLAQTAVTLDSARITKRSFCDITVGCETS                                255
St-AFPb  VLAQTAVTLDSARITKRSFCDITVGCETS                                259
NgIBP    ALSQTAITLDSVTIVS                                             242
TiAFP    ILSQTAVALQKATVVQK                                            243
C sp IBP LLAQTAVTLQKNTINAPTEQYEEAPL                                   259
          *.***..*    .
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Fig. 18. Alignment of antifreeze proteins (AFPs) with known antifreeze or ice binding activity including the construct for recombinant expression of Stephos-Antifreeze protein (StAFPrec). The following sequences were taken from the NCBI server: NgIBP, *Navicula glacei* (AAZ76254); TiAFP, antifreeze protein *Typhula ishikariensis* (BAD02895); C sp IBP, ice-binding protein *Colwellia* sp. (ABH08428). Gray shade: consensus match, stars: 100 % identity, dots: similar amino acids; italics: pet28a Vector; italics bold: His Tag; gray shaded sequence of StAFPrec is identical to St-AFPa and b; StAFPrec was not included in the alignment analysis.

As the recombinant protein exhibits thermal hysteresis (see below), the two isoforms were designated as Stephos-antifreeze protein a and b (St-AFPa and b, GenBank Accession No. FJ483935 and FJ483936). Homology of St-AFPa and b to each other was high, with 97 % at nucleotide and 94 % at aa level (Clustal W similarity score). St-AFPa and b differed significantly in possessing two different signal peptides (according to SignalP analysis) of different length (St-AFPa 20 aa, St-AFPb 24 aa; total length: St-AFPa 255 aa, St-AFPb 259 aa) suggesting a secretion of the two proteins to different cellular or extracellular compartments. The mature proteins were nearly identical (99 % identity on nucleotide level and 98 % on aminoacid level). *In situ* hybridisation revealed the expression of *St-AFPa/b* in the whole body in every cell, including oocytes (Fig. 19 A, B). A discrimination of the isoforms was not possible with the method applied. Histologically, oocytes are free of foreign cells (Fig. 19 B). *St-AFPa/b* was expressed constitutively at high and low temperatures in amounts similar to *eEF-1α* (*eukaryotic elongation factor 1 alpha*) (Fig. 20).

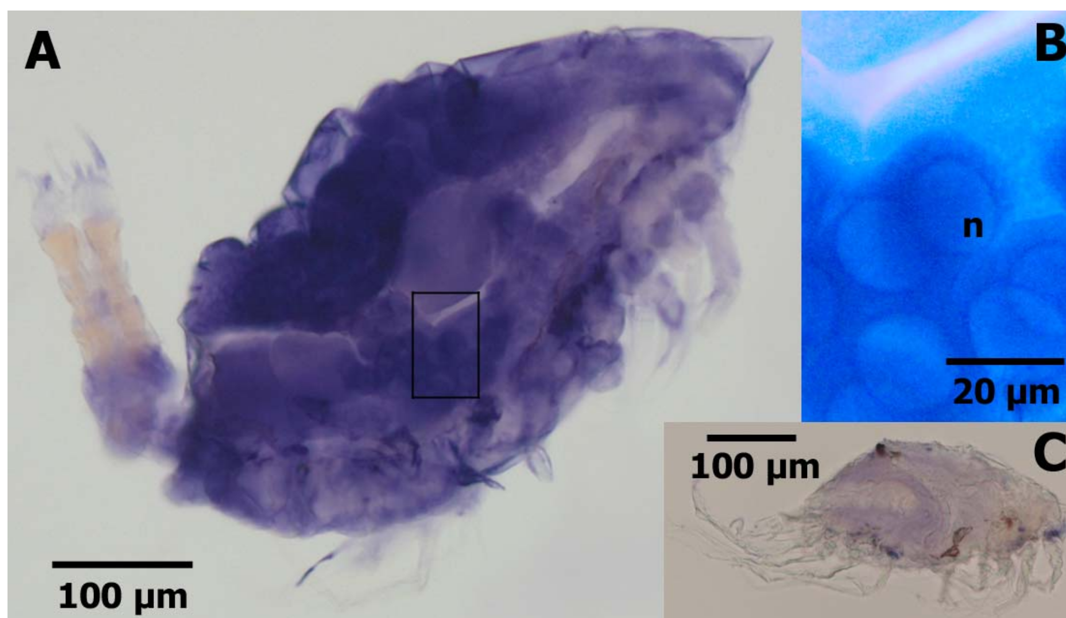

Fig. 19. Whole mount *in situ* hybridisation of *St-AFPa/b*. (A) female with oocytes, head of the animal in the upper right corner, tail to the left, the animal was cut before the hybridisation procedure along the sagital plane, the view is onto the opened body cavity, inset enlarged in B (B) oocytes are *St-AFPa/b* positive, oocytes are visible as dark stained circles with a prominent nucleus, n = nucleus of oocyte, (C) sense control gives no staining.

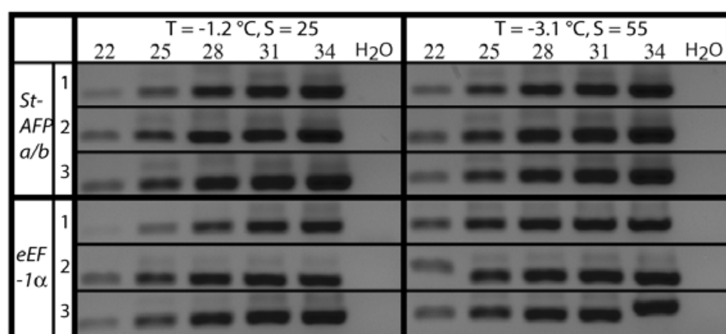

Fig. 20. Semiquantitative real time PCR. *St-AFP a/b* and *eEF-1α* are expressed in similar amounts at S = 25; T = -1.2 °C and S = 55; T = -3.1 °C. Numbers above columns indicate cycle number in PCR.

Contamination of cDNA preparations with *Rubisco* (*Ribulose-1,5-bisphosphate carboxylase/oxygenase*) was not found using conserved primers, which were shown to be suitable for cDNA of the sea-ice diatom *Flagillariopsis curta* (Data not shown). Dot blots (analogous to a Southern hybridisation) confirmed the presence of St-AFPa/b in genomic DNA of *S. longipes*. Positive staining with a *S. longipes* HSP70 probe and with a ST-AFPb probe was observed for genomic DNA and control plasmids. The St-AFPb probe gave a slightly stronger signal than the HSP70 probe. In conclusion, St-AFPa and b are an actively transcribed part of the genome of *S. longipes* and not a contamination of ingested sea-ice diatoms.

4.3.3. Recombinant Stephos-Antifreeze protein shows thermal hysteresis activity

Recombinant Stephos-Antifreeze protein (StAFPrec) was expressed in *Escherichia coli*. Cleared lysate was ice active, showing recrystallisation inhibition (Fig. 21) and a concentration dependent TH of 0.1 to 0.58 K (Fig. 22). The maximum TH of StAFPrec was not determined, as the contaminating proteins of the cleared lysate did not allow such measurements. A distinct shape of the crystal in the hysteresis gap was not observed. The shape of the growing ice crystal was irregular to hexagonal (Fig. 21) and sometimes, in the case of fast growth, a six pointed star was formed (data not shown). Cleared lysate of *E. coli* containing the expression vector without insert showed no TH activity or recrystallisation inhibition, and the shape of the growing ice crystal was round (Fig. 21).

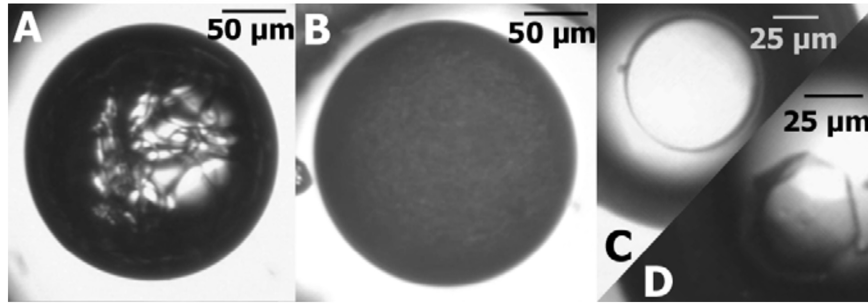

Fig. 21. Cleared lysate of *E. coli* expressing recombinant St-AFP is ice active. (A, B): Recrystallisation of a deep-frozen sample after incubation at -3.7 °C for 20 min without (A) and with (B) recombinant St-AFP. Few large micrometre sized crystals form if the recombinant protein is absent (A). Initially formed crystals of nano- to micrometre size persist if recombinant St-AFP is present, resulting in the opaque appearance of the sample (B). (C, D): Ice crystals grown from a minute crystal in the absence of recombinant ST-AFP are round (C), whereas in its presence they are hexagonal (D).

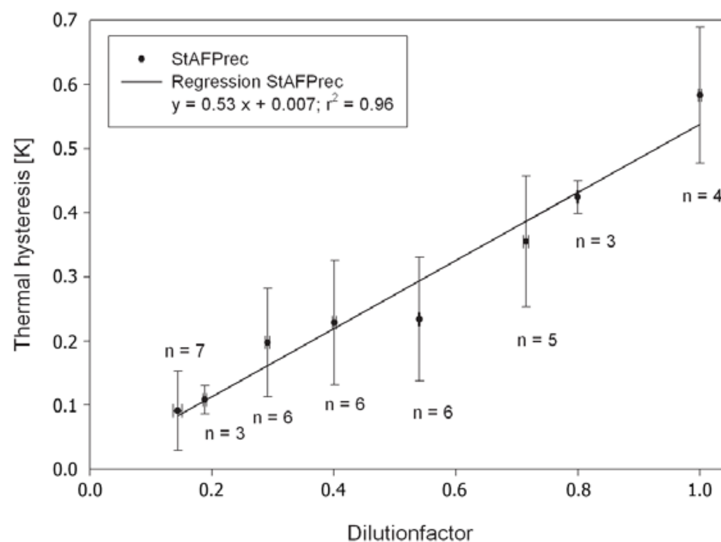

Fig. 22. Thermal hysteresis activity of recombinant ST-AFP (StAFPrec) is concentration-dependend. Values are means +/- standard deviation. n = number of measurements. Cleared lysate of *E. coli* expressing recombinant ST-AFP was diluted with water and osmolality and thermal hysteresis measured of the diluted samples measured. The dilution factor was calculated by multiplying the osmolality value with 1/2953 mOsm, the osmolality value of the original sample.

4.3.4. Phylogenetic analysis of (putative) antifreeze proteins

A BlastP search using St-AFPb as query sequence identified several other (putative) antifreeze proteins from a variety of bacteria, archae and another fungus (e-values < 1e-5). A phylogenetic analysis of all sequences available revealed four larger clades (Fig. 23). Proteins of clade one and four were only distantly related to the others and are found in two bacteriodetes (clade one) and a proteobacterium (clade four). Members of clade two were mainly found in Actinobacteria. To my knowledge, none of the proteins from this group has been tested for ice activity until now. Clade three comprises four subgroups. Group 3a was found in an archaeon, group 3b exclusively in proteobacteria and group 3c in two bacteriodetes and a snow mold. Diatom IBPs and St-AFPa and b constituted group 3d to which the *Flagillariopsis cylindrus* protein probably also belonged. The subdivision of groups 3c and 3d was only weakly supported. Blast searches of available protein and nucleotide data sets did not give any hits to metazoan sequences.

Alien indices, calculated according to Gladyshev et al. (2008) for St-AFPa and b with the *N. glaciei* e-value of 1e-63, were 143 and 145, respectively. Alien indices for *N. glaciei* IBP (calculated with *Rhodoferax ferrireducens*; YP_523138*) T. ishikariensis* AFP (with *Cytophaga hutchinsoni*; YP_676864), and the putative *Phaeosphaeria nodorum* IBP (with *Streptomyces sviceus*; ZP_03196126) were 151, 102 and 47, respectively. All values are above the value of 45, from which genes are classified as foreign (Gladyshev et al. 2008). The respective proteins are marked in Fig. 23.

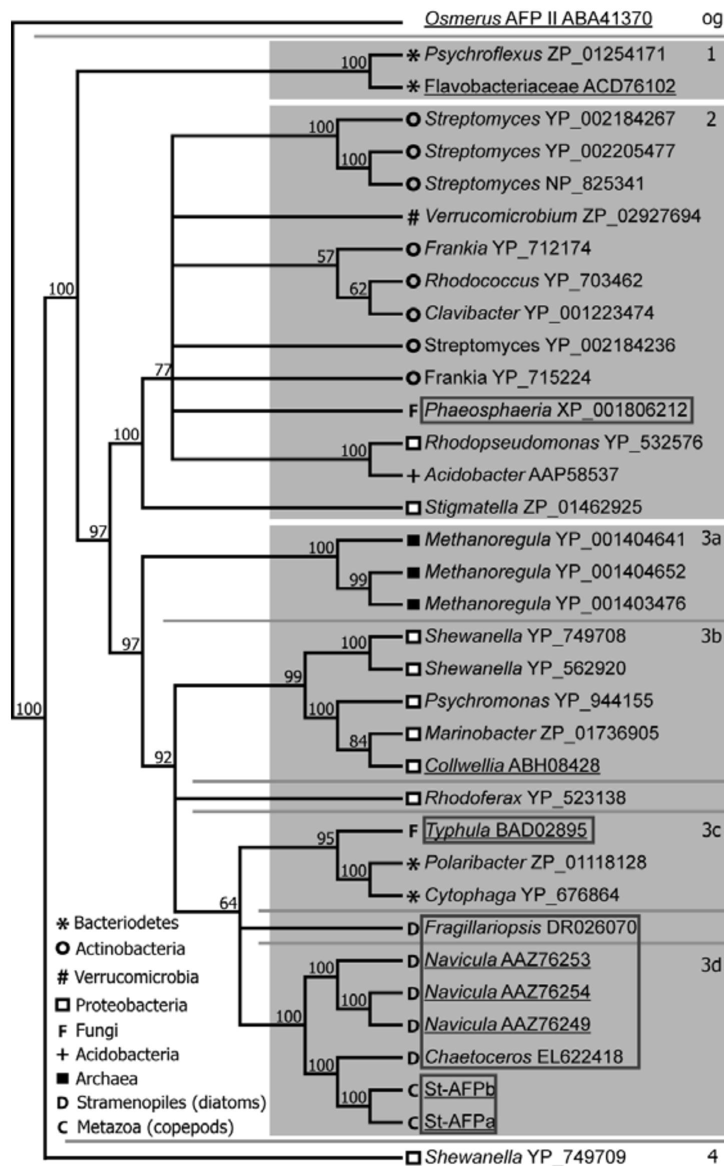

Fig. 23. Neighbour-joining tree showing the phylogenetic relationships among (putative) antifreeze proteins that matched St-AFPa and b with expect thresholds of 1e – 05 or less in a BlastP search. An IBP from a Flavobacteriaceae with known ice binding activity and translated ESTs from *Fragilariopsis cylindrus* and *Chaetoceros neogracile* were also included. Sequence alignment and phylogenetic analysis were performed with MacVector. Numbers at nodes indicate bootstrap values for 1000 replicate runs. Nodes with a bootstrap value below 50 are not resolved (distance: uncorrected "p", gap sites ignored). Genus name and GenBank accession numbers are shown. Fish AFP II from *Osmerus modax* was used as an outgroup (og) to root the tree. Underlines indicate proteins with known ice activity. Boxed proteins were probably obtained through horizontal gene transfer. Numbers behind larger branches indicate phylogenetic groupings mentioned in results.

4.4. Discussion

4.4.1. Thermal hysteresis activity explains small and large scale distribution patterns

Stephos longipes and *Paralabidocera antarctica* both inhabit the interstitices of Antarctic sea ice during parts of their life cycles. They are found in different areas of the Southern Ocean. Where they co-occur, they seem to exclude each other on small scales (Günther et al. 1999). This exclusion can be a result of competition for the same food resource or the preference of different microhabitats but finally also a result of their different physiological capabilities.
Life within sea ice is challenging. Salinity changes must be endured and ice formation in the habitat poses another threat for life within the ice. *P. antarctica* and *S. longipes* are both euryhaline, surviving short term exposure to salinities from 25 to 55 g/kg. In response to salinity changes, both regulate the haemolymph osmolality isosmotically. Conforming to the ambient salinity is advantageous, as the freezing point of body liquids remains the same as that of the ambient medium. As a consequence both *S. longipes* and *P. antarctica* are protected from internal ice formation. The presence of TH in *S. longipes*, which is to my knowledge the first report of TH activity for a crustacean, therefore seems surprising. Nevertheless, the presence of TH in *S. longipes* and the absence of TH in *P. antarctica* may explain the different habitat preferences of both species. *P. antarctica* inhabits only the bottom part of fast ice (Swadling et al. 2004), where temperature and salinity changes are buffered by the ocean below and through insulation which the ice column and snow above provide. Therefore freezing stress in this microhabitat rarely occurs. In contrast, *S. longipes* colonises the surface layer of pack ice from spring to autumn (Schnack-Schiel et al. 1995) and spawns within this layer (chapter 2). The surface layer forms, when heavy snow load or deformation processes press down the ice and the snow on top of it to below water level, causing seawater to infiltrate the snow (Ackley and Sullivan 1994). As the surface layer is in closer contact to the atmosphere, strong changes in air temperature of up to 20 K per day can result in fast temperature changes in the surface layer of up to 5 K per day (Massom et al. 2001). Such strong atmospheric temperature changes occur from autumn until early spring (Vihma et al. 2002). Even smaller temperature changes lead to conditions where temperatures below the freezing point of the brine can occur (chapter 2). Antifreeze proteins secreted by bacteria and algae may also contribute to this supercooling. The described conditions make the surface layer especially challenging and TH therefore provides a selective advantage. The ability of *S. longipes* to colonise and reproduce within the highly productive surface layer (Kattner et al. 2004) is recognised as one key to the dominance of this species in pack ice of

the Weddell Sea (chapter 2). The lack of TH activity in *P. antarctica* helps to explain why this species does not colonise or reproduce within the surface layer.

In *Stephos longipes*, St-AFPa and b provide thermal hysteresis activity. *St-AFPa/b* is expressed in high amounts in all cells and the recombinant St-AFP possesses considerable TH activity, as does *S. longipes* haemolymph. The constitutive expression of ST-AFP after incubation at -1.2 and -3.1 °C and the presence of TH even after half a year of incubation at 0 °C without ice indicate that a continous background protection is established in *S. longipes*. Whether only St-AFPa and b are responsible for the observed maximum TH of 3.8 K remains an open question, as concentration measurements of St-AFP in the haemolymph were not possible. The high viscosity of the haemolymph could be a result of high protein concentrations. The presence of other AFPs or antifreeze potentiating proteins as observed for the Antarctic notothenioid fish *Pagothenia borchgrevinki* (Jin and DeVries 2006) can not be excluded. Furthermore, posttranslational modifications of St-AFPa/b in *S. longipes* may result in higher activities of the natural proteins than those found for recombinant St-AFP. As St-AFP a/b is also expressed in oocytes, it is highly probable that eggs are also protected from ice formation through TH. This protection may be mandatory for the release of eggs within the interstitices of sea ice and was also observed for the nudibranch *Tergipes antarcticus* (chapter 3).

In conclusion, the different life cycle strategies of the two dominant sympagic calanoid copepods in Antarctic sea ice are most probably to a large extent a result of their different physiological capacities. Other mechanisms like the competition for food resources might contribute to the differences in life-cycle strategies. Further investigations of the freeze tolerance of both species and the variability of TH with environmental conditions in *S. longipes* are desirable.

4.4.2. Acquisition of ice binding proteins through horizontal gene transfer

Horizontal gene transfer is discussed in several cases as a mechanism for the retrieval of genetic information from one organism by another. The endosymbiont theory (Margulis 1970) which states that during the evolution of higher organisms the internalisation of prokaryotes resulted in the establishment of mitochondria and chloroplasts is generally accepted. The closer integration of the endosymbiont by the host cell occurred through transfer of genetic information (Martin et al. 1998, Gray et al. 1999). HGT to metazoans seems less probable (Nikoh et al. 2008). Relatively few examples of HGT to metazoans exist (see Andersson

(2005) for review), but numbers are increasing (Dunning Hotopp et al. 2007). HGT is normally identified through BLAST searches of the genes under investigation. High similarity to genes from unrelated organisms is taken as an indication for HGT, as is the case for St-AFPa and b. In contrast, no homologues to St-AFPa and b exist in any metazoan lineage. TH or ice binding activity is not reported from any other crustacean that has been examined. Several under-ice amphipods and the krill *Euphausia superba* (Aarset 1991, Kiko et al. in press), as well as the epipelagic calanoid copepod species *Calanoides acutus* (Antarctic) and *Calanus glacialis* (Arctic) do not show signs of TH (Kiko, unpublished). Ice active proteins are therefore not present in these crustaceans. The Alien indices of 143 and 145 for St-AFP a and b, respectively, are clearly above the threshold of 45 above which genes are identified as horizontally transferred (Gladyshev et al. 2008). St-AFPs group within diatom IBPs, and all other related (potential) AFPs stem from bacteria, archae and fungi. An alternative scenario to HGT which could explain this phylogenetic distribution would be a common source of all AFPs in a prokaryote and the loss of this gene in nearly all phylogenetic lineages except for bacteria, diatoms, snow molds and *Stephos longipes*. This is highly improbable. In summary, the data indicate that St-AFPa and b were obtained through horizontal gene transfer. The phylogenetic grouping suggests that a sea-ice diatom was the donor of the St-AFP coding sequence. The patchy phylogenetic distribution of the discussed protein family and the calculated alien indices even suggest that several events of HGT occurred (already discussed in Raymond et al. (2007)). The ancestor of this protein family might have evolved in one prokaryotic lineage and was then obtained by other prokaryotes, fungi and diatoms through HGT. The proposed HGT from a diatom to *S. longipes* therefore could be a secondary transfer. Further investigations of the phylogeny of this protein family are needed to identify the direct source of the St-AFPs, as well as to elucidate the timing of the horizontal transfer of St-AFP. This could provide an independent estimate for the minimum existence time of the Antarctic sea-ice ecosystem. The probable occurrence of at least three seemingly independent HGT events in this protein group could be a hallmark of the positive selective advantage, which the protein confers. In this context it would be interesting to investigate whether the presence of TH activity in the sympagic nudibranch *Tergipes antarcticus* (chapter 3) is also a result of HGT.

4.4.3. Potential mechanisms for horizontal gene transfer

A prerequisite for HGT to occur is the close proximity of donor and recipient. This is now clearly established for St-AFP, as sea-ice diatoms and bacteria as well as *Stephos longipes* inhabit the confined interstitial system of sea ice. The nature of the transferred material might be bulk DNA, mRNA or cDNA (possibly virus-mediated; Henze and Martin 2001).

Three different transfer ways are possible. *S. longipes* is known to feed on sea-ice diatoms (Kurbjeweit et al. 1993) and during early life stages probably also on sea-ice bacteria. Therefore uptake of foreign DNA material through the gut is one possibility, but absence of active phagocytosis and germ-soma separation in eukaryotes make the establishment of this material within the germ line improbable (Kondo et al. 2002). A second possibility would be a transfer from endosymbionts, as observed for the transfer of genetic material from bacterial *Wolbachia* endosymbionts to the beetle *Callosobruchus chinensis* (Kondo et al. 2002). Other examples, where a transfer from an (endo)symbiont is discussed come from *Hydra viridis* (Habetha and Bosch 2005) and plantparasitic nematodes (McKenzie Bird and Koltai 2000). In these three cases the transferred gene was shown to be active. To enable an establishment in the germ line, endosymbionts should be transferred in oocytes (Kondo et al. 2002). The presence of endosymbionts within oocytes of *S. longipes* seems unlikely. *Rubisco*, a key enzyme in photosynthetic organisms, was not amplified from the cDNA preparations and oocytes were free of any visible cells. A third way for the retrieval of foreign DNA has been discussed for bdelloid rotifers (Gladyshev et al. 2008). This way could be described as a natural transformation procedure. Bdelloids often experience a dehydration of their habitat. This or the accompanying increase of salt concentrations could lead to a punctuation of cell membranes of donor and recipient and the transfer of DNA. Reconstitution of the membrane after rehydration would complete the transformation. A similar scenario is possible within the sea-ice habitat. Eggs of the ancestor of *S. longipes* could have been entrapped in growing sea ice, as described for algae (Garrison et al. 1989). A fast change in temperature or salinity (analogous to transformation procedures used in molecular biology) during the growth or melting of sea ice could have increased the permeability of the membrane. Alternatively, ice crystals growing into the membrane could have led to a puncture of the egg analogous to a microinjection and enable the acquisition of foreign DNA. Amelioration of environmental conditions would enable a reconstitution of the cell membrane and the completion of the natural transformation.

Summing up, HGT events to metazoans seem to occur in two situations, first as a transfer of genetic material from an endosymbiont to the host, and second in extreme habitats, where desiccation and rehydration or fluctuations in temperature or ion concentration provide natural transformation conditions (sea ice, hydrothermal vents, tide pools, temporary ponds). The transfer of genetic material in the second case is probably dependant on the presence of liquid water. Therefore, in terrestrial animals, the HGT from endosymbionts to their hosts should be prevalent, if not exclusively present, whereas in extreme habitats both mechanisms seem possible.

The proposed mechanism of natural transformation is obviously not restricted to metazoans. As discussed above, IBPs may have been transferred several times. This could also be due to an increased probability of natural transformations within icy habitats. Investigations of other genes and organisms will show, if icy habitats represent “hot spots” for HGT.

5. General discussion

5.1. Preface

The work presented here is the first approach to link questions in and methods of ecology, physiology and molecular biology of sympagic meiofauna organisms. The preferential use of different sea-ice habitats by several copepod species has been identified (chapter two). Thermal hysteresis (the inhibition of ice growth) as an adaptation strategy to the freezing conditions encountered in the habitat has been found in two sympagic species, the calanoid copepod *Stephos longipes* and the gastropod *Tergipes antarcticus* (chapters three and four). Both represent the first reports of thermal hysteresis for the respective phylum. Thermal hysteresis in *S. longipes* could be traced back to the acquisition of an antifreeze protein through horizontal gene transfer (chapter four). A mechanism of "natural" transformation is proposed for sea ice and other extreme habitats.

We can classify organisms found inside sea ice into two groups: one group obligatorily spends a part of its life cycle within sea ice and is adapted to the habitat (truly sympagic). Another group is just occasionally found within sea ice and can easily do without it. All three stand-alone chapters of this work are concerned with this topic and present data, which support the grouping of several species as truly sympagic. In the following, I will summarise these findings and try to identify the necessary adaptations which allow truly sympagic organisms to colonise sea ice temporarily or to maintain a population within sea ice continuously. Particularly, the adaptations to seasonality, ice dynamics, habitat dimensions, salinity variations, as well as temperature variations and freezing stress will be covered. Finally, as an outlook I will summarise options which molecular-biological techniques offer to answer some questions of sea-ice biology.

5.2. Overwintering strategies and life cycles

The overriding factor influencing life-cycle strategies of most polar organisms is seasonality of solar radiation and the accompanying changes in primary production and, therefore, food availability (Peck et al. 2006). An important question is how sympagic meiofauna organisms have adapted to this seasonality and whether the dynamics of the sea-ice habitat select for special life-cycle strategies. Many polar metazoans have a life cycle of more than one year, are inactive or less active in winter (Barnes and Peck 2005, Peck et al. 2006) and accumulate energy reserves like wax esters to survive the winter (Schnack-Schiel 2001, Hagen and Auel 2001). Some sympagic species also show highly adapted seasonal life-cycle strategies. The main overwintering stage of calanoid copepods is a late copepodite stage (Arndt and Swadling 2006). Such a strategy has been proposed also for *Stephos longipes* (Schnack-Schiel et al. 1995), which seems to survive the winter in deeper water layers as copepodid, ascends to the surface in spring, colonises sea ice, reproduces and grows therein (Schnack-Schiel et al. 1995, chapter two). The life cycle is completed through a retreat to deeper water layers in autumn (Schnack-Schiel et al. 1995). *Paralabidocera antarctica* shows a rather different strategy as it survives the winter as naupliar stages in the bottom layer of fast ice, leaves the sea ice in spring and develops further in the sub-ice layer (Hoshiai and Tanimura 1986). Eggs are spawned in summer and sink to the sea floor (Swadling et al. 2004). From the hereby established egg bank (Swadling et al. 2004), recruitment takes place in late January (autumn) and the nauplii ascend to the surface in late February, where they colonise the bottom layer (Swadling et al. 2004). The life cycles of the harpacticoids *Ectinosoma* sp., *Diarthrodes* cf. *lilacinus* and *Idomene antarctica* also seem to be synchronised with season, as only specific life stages (mainly nauplii) were found in the ice in late spring during the ISPOL expedtion (chapter two). Further investigations are necessary to scrutinise this hypothesis and to establish the life-cycle strategies of these species.

But the sea-ice ecosystem seems also to select for another strategy which is unusual for polar marine organisms, but is also found in some intertidal species (Peck et al. 2006). Flexible and fast exploitation of temporarily beneficial conditions through short life cycles and high reproductivity seems to be the second strategy (r-strategy) which allows the exploitation of the sea-ice interstitices. All life stages of *Drescheriella glacialis* are found in sea ice during different seasons (Dahms et al. 1990). High reproductivity and a minimum life cycle of about four and a half months led Bergmans et al. (1991) to the conclusion that *D. glacialis* is an r-strategist. This stands in contrast to minimum life-cycle lengths of Antarctic sediment-

dwelling harpacticoids of at least seven months, but more commonly one year (Bergmans et al. 1991). A relatively short median developmental time of 31 days from egg to veliger in *Tergipes antarcticus* and the observation that all life stages of this species were present in late winter/early spring 2006 hint to a similar strategy in this species (chapter 3). This again stands in contrast to the strategy of two benthic Antarctic ophistobranchs, for which relatively long developmental times (more than 100 days) of few yolky eggs were observed (Wägele 1996).

5.3. Habitat stability and dispersal

Seasonality of solar radiation does not only cause seasonality in food supply, but also in habitat stability. Most of the Antarctic sea ice melts away during summer (Cavalieri et al. 2003) and therefore does not provide a constant colonisation platform. Also, most of the multi-year pack ice will finally be exported to the open Southern Ocean (Eicken 1992). These large-scale variations of the ice cover are probably difficult to cope with for microscopic animals. The ability to colonise newly forming ice habitats and/or to reproduce within the ice are therefore necessary for truly sympagic species. Different dispersal strategies and capacities could possibly explain the large- and small-scale distribution patterns of sympagic meiofauna species. These hypotheses will be discussed in the following.

A simple classification of sea-ice types in terms of habitat stability and predictability would rank multi-year fast ice as most stable, multi-year pack ice and seasonal fast ice as intermediate and seasonal pack ice as unstable. As parts of the multi-year pack ice cover are lost every year in the Southern Ocean (Eicken 1992, Steer et al. 2008), the colonisation of newly forming habitats also in these areas seems important. The ISPOL floe consisted of second-year ice, as well as of two types of first-year ice (Haas et al. 2008). The colonisation of newly-forming ice from persisting older ice in this case seems a likely mechanism. Small- and medium-scale (metres to kilometres) dispersal could allow the colonisation of new habitats in a confined ice-covered area through sea-ice meiofauna species. Such dispersal could take place to a minor extent within the ice or to a larger extent in the sub-ice layer. Friedrich and Hendelberg (2001) observed that Arctic acoel plathyelminths moved with an approximate speed of 14.4 m/d through the brine-channel system. As this movement is probably not directed due to the mazelike structure of the brine-channel system (Weissenberger et al. 1992, Krembs et al. 2000), dispersal distances per day will be considerably smaller. The sub-ice layer is probably very important for the dispersal of sympagic species. During the melt season sympagic species are found within a low-salinity

layer directly underneath the ice, whereas pelagic species are excluded from this layer (chapter two, Werner 2006). The establishment of a halocline could restrain the dispersal of sympagic species vertically and result in prevailingly horizontal transport in close vicinity to the ice underside. Such a mechanism would link different ice types like multi-year and first-year ice or fast and pack ice within an area on scales of at least tens to hundreds of metres. This mechanism seems restricted to ice-covered areas, and whether such a mechanism could be effective on larger scales remains to be shown. Species which are mainly found within the ice like *Drescheriella glacialis* and *Diarthrodes* cf. *lilacinus* probably have a lower dispersal potential compared to those found predominantly in the bottom and sub-ice layer like *D. racovitzai* and *Ectinosoma* sp. (chapter two, Schnack-Schiel et al. 2008, Fig. 24).

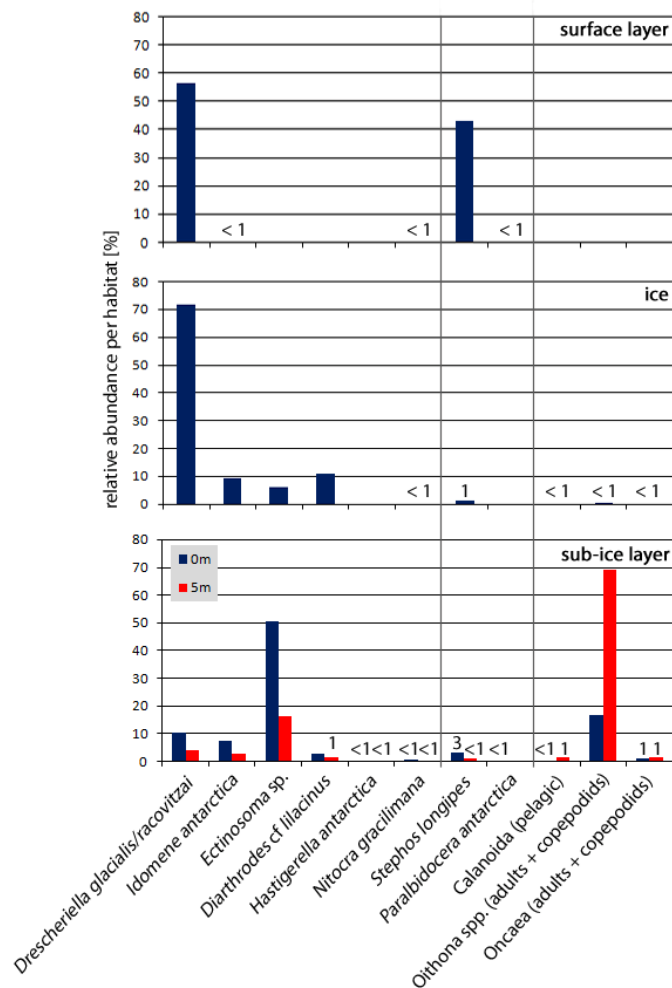

Fig. 24. Relative abundance of sympagic and pelagic copepods in different habitats of the sea-ice ecosystem during late spring in the western Weddell Sea (ISPOL). Left part of the figure: sympagic harpacticoid copepods; middle part: sympagic calanoid copepods; right part: pelagic copepods. A compilation of data presented in chapter 2 and by Schnack-Schiel et al. (2008).

Another mechanism which displaces sympagic organisms is ice drift itself (Eicken 1992). Therefore, the species composition found within an area might mirror the species composition of the area of ice formation (Eicken 1992). During the formation of new ice in polynyas in the vicinity of (multi-year) fast ice a colonisation of pack ice might occur. The ISPOL cruise took place on a floe which also contained ice originating from the Rønne Polynya (Haas et al. 2008). The occurrence of *Diarthrodes* cf. *lilacinus* at this site (chapter two, Schnack-Schiel et al. 2008) and its absence in the central Weddell Sea (Schnack-Schiel et al. 2008) could be a consequence of ice origin. A low dispersal capacity of *Diarthrodes* cf. *lilacinus* could possibly explain its restricted occurrence in this area and its absence in the central Weddell Sea. High abundances of *Paralabidocera antarctica*, a typical fast-ice species (Tanimura et al. 1996, 2002, Swadling et al. 1997, 2001) in pack ice of the Western Weddell Sea, which probably originated from the southern Larsen Polynya (C. Haas pers. communication) were also interpreted as a result of ice origin (Kramer 2007).

The proposed dispersal mechanisms are random and are likely to result in a dilution of the population within a given area. To compensate for this, reproduction within the habitat or constant immigration from an outside source seems necessary to colonise multi-year pack ice permanently. Reproduction within the sea-ice habitat is already proven for acoel plathyelminthes (Janssen and Gradinger 1999), *Stephos longipes* (Schnack-Schiel et al. 1995) and *Drescheriella glacialis* (Dahms et al. 1990). Furthermore, an oovigerous female of *Microsetella norvegica* was isolated from the ice by Dahms et al. (1990). *S. longipes* and *D. glacialis* also reproduce within the ice (chapter two). Furthermore, oovigerous females and early naupliar stages from *D. racovitzai* and *Idomene antarctica* (chapter two, Schnack-Schiel et al. 2008) as well as eggs and veliger larvae of *Tergipes antarcticus* (chapter three), indicate reproduction in the habitat. Successful reproduction within the sea-ice habitat could be judged as a high degree of adaptation to the habitat. Direct evidence for reproduction within the habitat was not found for *Ectinosoma* sp. and *Diarthrodes* cf. *lilacinus*. Whether these species reproduce within sea ice or whether they reproduce in another habitat and later colonise the ice remains to be shown. Growth of *Diarthrodes* cf. *lilacinus* was observed during ISPOL, as the mean developmental stage of nauplii increased by one stage during the study period. The relatively high amount of *Ectinosoma* sp. exuviae found indicates development also of this species in the sympagic habitat.

Seasonal sea ice needs to be recolonised every year, and a prolonged ice-free time must be bridged. Bridging of ice-free times is possible in the pelagic or benthic zone. *Stephos longipes* is found in the water column during ice-free times (Schnack-Schiel et al. 1995). The veliger

larvae of *Tergipes antarcticus* seem to be the pelagic life stage of this species (chapter 3). The life cycle of *Paralabidocera antarctica* is an example for the retreat to the benthos during ice-free conditions in summer (Swadling et al. 2004). This probably restricts this species to more coastal and therefore fast-ice areas, an argument already discussed in chapter four. One benefit of such a retreat is that an export with the ice to the open ocean or into other areas is restricted.

Colonisation of seasonal pack ice formed over deep water seems more difficult for species without a pelagic phase. Comparative work on meiofauna of the Pacific sector, where largely seasonal ice dominates (Comiso 2003), and the Western Weddell Sea, where largely multi-year ice dominates (Eicken 1992, Haas et al. 2008), indicates that the abundance of sympagic meiofauna organisms is lower in seasonal pack-ice areas (A. Scheltz and M. Kramer, pers. communication). Similarly, abundances of sympagic organisms were lower in first-year ice than in multi-year ice in the Arctic (I. Werner, pers. communication). The observation that also few harpacticoid copepods and plathyelminthes were found in Antarctic seasonal pack ice (A. Scheltz and M. Kramer, pers. communication) shows that this ice type is to some extent accessible for poor swimmers. Further work on different ice types and colonisation processes are necessary in order to understand the links between different ice regimes and the impact of dispersal strategies on the ecology of the sea-ice meiofauna.

Summing up, seasonality of food supply, ice formation and melt, as well as the drift dynamics of sea ice are important factors influencing the life-cycle strategies of sympagic metazoans. Seasonally adapted life cycles of one-year duration with a pelagic or benthic phase seem to allow for the colonisation of seasonal ice, as well as multi-year ice. Shorter life cycles seem less well-suited for seasonal ice and are more likely to be found in multi-year ice or areas influenced by multi-year ice. A high dispersal capacity and the ability to exploit newly forming habitats seem further important traits to colonise dynamic sea-ice habitats. Different habitat preferences of sympagic meiofauna species could result in different dispersal capacities, which again may find a reflectance in different degrees of patchiness. These hypotheses need to be tested in future. Based on the finding of oovigerous females and early naupliar stages of the harpacticoid copepods *Drescheriella racovitzai* and *Idomene antarctica* (chapter two), and based on the observation of viable egg clutches of the nudibranch *Tergipes antarcticus* within sea ice (chapter three), a high degree of adaptation to the system in these species is inferred.

5.4. Niche separation

The observed differences in habitat preference could result in an avoidance of interspecific competition on temporal or spatial dimensions or be, in turn, the result of interspecific competition. In general, *Stephos longipes* exploits the ice during summer, whereas *Paralabidocera antarctica* thrives in the sub-ice layer. *P. antarctica* is more restricted to coastal fast ice, whereas *S. longipes* also colonises pack ice (Schnack-Schiel et al. 1995, Tanimura et al. 1996, 2002 Swadling et al. 1997, 2001). Thereby, a competition for food is largely avoided in *S. longipes* and *P. antarctica*. The differences in large scale distribution patterns have been traced back to the restricted presence of thermal hysteresis activity only in *S. longipes* and the resulting ability of this species to colonise the surface layer of sea ice (chapter 4). The ability to spawn and reproduce within sea ice is another likely result of thermal hysteresis in *S. longipes* and also in *Tergipes antarcticus*. This topic will be covered in more detail below. While calanoid copepods feed on suspende food items (Koehl and Strickler 1981, Malkiel et al. 2003), harpacticoid copepods mostly graze on surfaces (Hicks and Coull 1983) and therefore occupy a different niche. Harpacticoid copepods themselves were found in different microhabitats of the ice during ISPOL and therefore in different niches (Schnack-Schiel et al. 2008, chapter 2, Fig. 24).

Apart from seasonal dynamics of food supply and ice formation, a restricted size of the brine channels, changing temperatures and salinities as well as freezing conditions characterise the habitat. Analysis of adaptation strategies towards these factors allows a further interpretation of life-cycle strategies and can yield further arguments for the classification of a species as highly adapted to the system.

5.5. Habitat dimensions

Most organisms are excluded from the brine-channel system because the dimensions of the brine channels are smaller than their body size (Krembs et al. 2000). Within the highly porous infiltration and platelet layers, centimetre-sized organisms like amphipods have been found (Günther et al. 1999, chapter two), but most organisms inside sea ice are considerably smaller (< 4 mm in length; < 1 mm in width, *Tergipes antarcticus*, chapter 3). The structural similarity of the brine-channel system to the interstices of sandy and muddy substrates can explain the high similarity of benthic and sympagic meiofauna assemblages (Krembs et al.

2000, Bluhm et al. 2007). Wormlike (vermiform) species with small appendages dominate in both habitats. For the nudibranch *Tergipes antarcticus* a reduction in number and size of cerata compared to *T. tergipes* has been described (chapter 3). Other beneficial traits might be the ability to move in a confined space and/or the ability to change body shape (Krembs et al. 2000, Siebert et al. in press). Internalised development was recognised as a special trait in the Arctic sympagic cnidarian *Sympagohydra tuuli* (Siebert et al. in press). A positive phototactic response and strong thigmotactic behaviour (the reflex to stick to surfaces) appear beneficial to remain in contact with the substrate (Tanimura et al. 2002).

Temperature changes of the ice will result in changes of brine salinity, brine volume and brine channel diameters (Cox and Weeks 1983, Krembs et al. 2000). The effects of brine volume reduction will be covered below, in conjuction with the effects of freezing stress (chapter 5.7). Both effects are difficult to discuss separately and also need to be considered in combination with the effects of salinity variations.

5.6. Salinity variations and osmotic stress

Brine-salinity values in Antarctic sea ice can range from nearly 0 g/kg (the melting point of pure ice) to at least 180 g/kg (Bartsch 1989). All sympagic meiofauna organisms tested until now exhibit a comparatively high tolerance to salinity changes, as already described in the general introduction of this thesis. *Stephos longipes* and *Paralabidocera antarctica* tolerate exposure to salinities from 25 to 55 g/kg (chapter four). In addition, all Antarctic and Arctic under-ice amphipods studied until now are euryhaline (Aarset 1991, Kiko et al. in press), as well as the Antarctic krill *Euphausia superba* (Aarset and Torres 1989), which is known for its close association with sea ice (Loeb et al. 1997) and even occurs in the surface layer (chapter two). In contrast, the pelagic copepods *Calanus propinquus* and *Metridia gerlachei*, which are only rarely found in sea ice, show a low tolerance towards low and high salinities (Gradinger and Schnack-Schiel 1998).

In particular, the tolerance to low salinities could be important for sympagic species. High atmospheric temperatures in summer lead to ice melt and a decrease of brine salinity, sometimes significantly below the salinity of seawater (Untersteiner 1968). Furthermore, a low-salinity layer often establishes underneath the ice (Werner 2006, chapter two). The exclusion of pelagic organisms from this layer was shown for the Arctic (Werner 2006) and first results from the Antarctic indicate that this is probably also the case for Antarctic sea ice (chapter two). The Arctic pelagic amphipod *Themisto libellula*, which is in trophic interaction

with the ice (Auel et al. 2002), is stenohaline, and it was concluded that, as a consequence, it is excluded from the low-salinity layer (Aarset and Aunaas 1987).

Salinity changes within the brine-channel system are equal to changes in medium osmolality. Osmolality changes are challenging, as they can disturb the ion homeostasis of an organism (Hochachka and Somero 2002). A decrease of external salinity (and therefore osmolality) can result in an inflow of water into the organism. An increase of external salinity can lead to an outflow (Mantel and Farmer 1983). Thereby, the concentration of dissolved contents in body liquids is changed. Marine animals have evolved different strategies to cope with this problem. In general, osmoregulators and osmoconformers can be identified (Mantel and Farmer 1983).

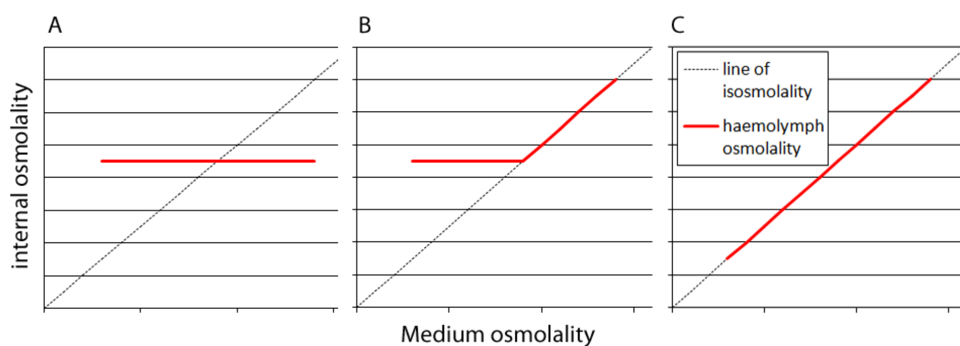

Fig. 25. Relationship between internal and external osmolality in marine organisms. (A) hyper- hyposmotic regulator, (B) hyper- isomotic regulator and (C) osmoconformer (after Mantel and Farmer (1983)).

Osmoregulators control the osmolality of the haemolymph (Fig. 25 A and B). Different osmoregulatory strategies exist. Some organisms maintain a steady osmolality at low and high medium osmolalities (hyper- hyposmotic regulators, Fig. 25 A), whereas other organisms keep up high osmolalities at low-medium osmolalities but are isosmotic at elevated medium osmolalities (hyper- isosmotic regulators, Fig. 25 B) (Mantel and Farmer 1983). The in- and outflow of water is usually controlled in some semipermeable parts of the body, whereas other parts are more impermeable. In crustaceans, the gills, the gut and the antennal or maxillary gland are the main osmoregulatory organs (Mantel and Farmer 1983). Osmoconformers do not regulate the osmolality of their haemolymph. The osmolality of the haemolymph is then always the same as that of the medium. As cell membranes are semipermeable, intracellular osmolality follows the haemolymph osmolality (Mantel and Farmer 1983). Cells of osmoregulators are, therefore, better protected from external osmolality changes than those of osmoconformers. The haemolymph of the two species tested in this study is isosmotic to the medium in the tested range from 25 to 45 g/kg (*Stephos longipes*) and 25 to 55 g/kg

(*Paralabidocera antarctica*) (chapter four). The Antarctic amphipod *Eusirus antarcticus* and the euphausid *Euphausia superba*, which were found in the surface layer of sea ice (chapter 2), are also osmoconformers (Aarset and Torres 1989). Arctic under-ice amphipods were found to be hyperosmotic at low salinities and isosmotic at seawater salinities of 30 g/kg and above (Aarset and Aunas 1987, Kiko et al. in press). In conclusion, all sea-ice metazoans tested until now are isosmotic to the medium at salinities above normal seawater salinity. As the freezing point of a liquid depends on its osmolality, maintenance of the haemolymph osmolality at or above the osmolality of the brine will furthermore protect the haemolymph and the cells of these organisms from ice formation (Rakusa-Suszczewski and McWhinnie 1976). In consequence, all sympagic crustaceans tested until now are protected from organismal freezing, if the medium temperature is equal to the equilibrium freezing point.
If the osmolality is not regulated, the concentrations of sodium and chloride in the haemolymph are usually not regulated and therefore equal to seawater (Mantel and Farmer 1983, Normant et al. 2005). High ion concentrations at elevated salinities are harmful to enzyme functioning (Hochachka and Somero 2002). In particular, high intracellular sodium concentrations need to be avoided. In temperate organisms, isosmolality within cells is reached through the accumulation of compatible osmolytes like free aminoacids and polyols (Burton and Feldmann 1982, Hochachka and Somero 2002, Willett and Burton 2002). Investigations of such adaptation mechanisms are missing for sympagic metazoans.

5.7. Temperature variations and freezing stress

Several meiofauna species have been found in upper layers of the sea-ice habitat in late spring (chapter two) and also during late winter (Kramer 2007). Metazoans thriving in these habitats are exposed to more atmospheric conditions and have to survive lower temperatures (Kramer 2007) and stronger temperature changes than within lower layers (chapter two, Massom et al. 2001). A decrease in air temperature can result in a non-equilibrium "supercooling" of the surface-layer brine, as observed during ISPOL (chapter two). The presence of antifreeze proteins with thermal hysteresis activity in the brine could prolong the duration of "supercooling" and inhibit ice growth. The presence of ice-active proteins was reported for brine retrieved from the platelet layer (Raymond et al. 1994). While no records of thermal hysteresis activity for sea-ice algae have been published as yet, investigations presented in chapter four show that a protein with high homology to proteins identified in sea-ice diatoms does possess thermal hysteresis. Similarly, a homologous protein from a snow mold confers

thermal hysteresis activity (Hoshiai et al. 2003). Furthermore, a recombinantly expressed antifreeze protein from the sea-ice diatom *Fragilariopis cylindrus* showed thermal hysteresis (M. Bayer, pers. communication). In consequence, supercooled brine could possibly be explained through secreted sea-ice diatom antifreeze proteins. As colligative freeze-protection is not effective under such non-equilibrium conditions, isosmotic organisms thriving within "supercooled" brine need other mechanisms of freeze protection. A reduction of the freezing point of body liquids through thermal hysteresis was found in *Stephos longipes* (chapter four) and seems to enable this species to thrive in the surface layer of sea ice (chapter two). Thermal hysteresis was also found for *Tergipes antarcticus* (chapter three), and it seems possible that its ability to colonise the surface layer (chapter two) is a result of freeze protection. The osmoregulatory strategy of this species remains to be studied, to allow further conclusions on the importance of thermal hysteresis activity in this species. In *S. longipes*, the acquisition of thermal hysteresis seems to be the result of a horizontal gene transfer of an antifreeze protein from a diatom (chapter four). This could be a remarkable example for co-evolution in sea-ice algae and a metazoan. The likely importance of thermal hysteresis for the large- and small-scale distribution pattern of *S. longipes* has been discussed above (chapter four).

A reproduction strategy, which includes the free release of eggs into growing ice, is probably also depending on thermal hysteresis. If antifreeze proteins are not present in the brine, a decrease in temperature will result in ice growth (DeVries 1971, chapter four). Once released within the ice, eggs probably get into contact with ice crystals. *Tergipes antarcticus* egg clutches are not floating (chapter three), and Kurbjeweit et al. (1993) report that *S. longipes* eggs are extremely sticky and they speculate that the eggs are actively attached to ice crystals. In contrast to larvae and adults, which can move away, active evasion of an advancing ice front seems impossible for eggs. Both egg types are larger than 1 µm (chapters three and four), a size below which an advancing ice front repulses a particle (Rempel and Worster 1999). Therefore, also passive evasion is unlikely and free eggs within brine channels are probably encased into solid ice when ice growth takes place. Encased within ice, eggs are no longer protected through colligative freezing-point reduction of circumfluent brine. In this case, either an internal increase in osmolality through synthesis of osmolytes (Hochachka and Somero 2002) or thermal hysteresis could protect the eggs from freezing. At least for *T. antarcticus* eggs freezing is detrimental (chapter 3) and invertebrate eggs are mostly freeze-intolerant (Ansart et al. 2007). Thermal hysteresis was observed for egg clutches of *Tergipes antarcticus* (chapter three), and the expression of an antifreeze protein in oocytes of *S.*

longipes indicate that these are also protected through thermal hystersis (chapter four). As a consequence, thermal hysteresis seems necessary for the survival of free eggs in growing ice. The eggs of the Antarctic silverfish *Pleuragramma antarcticum* were found within the platelet layer underneath two-metre-thick fast ice (Vacchi et al. 2004). Although the blood of adults possesses thermal hysteresis activity, the eggs showed no such activity. The platelet layer was isolated from the atmosphere through the overlying ice. Therefore, strong temperature changes and ice growth in this area were very unlikely to occur and freeze protection through thermal hysteresis seems not necessary. The hypothesis that freeze protection is beneficial or maybe even necessary for a reproduction strategy which includes the free release of eggs into growing ice remains to be tested further with other species. Several egg types found within the ice (Kramer 2007) need to be assorted to the respective species and tested for thermal hysteresis to answer this question. Furthermore, the amount of freeze protection through thermal hysteresis needs to be established and conditions tolerated *in situ* determined and compared.

5.8. Conclusions

Whether a truly sympagic sea-ice meiofauna exists, was under debate about twenty-six years ago (Carey and Montagna 1982). Data accumulated since then by many other studies in the Arctic and Antarctic, as well as the data shown and discussed in this thesis, indicate that truly sympagic species with unique adaptations to the system exist. Life-cycle strategies are adapted to seasonality of ice dynamics and niche separation is obvious. Furthermore, tolerance to low temperatures and changing salinities, isosmotic regulation of the haemolymph, as well as small size characterise all sympagic meiofauna species investigated until now. At least two species (*Tergipes antarcticus* and *Stephos longipes*) even acquired freeze protection through thermal hysteresis. Physiological and molecular-biological investigations improved and will further improve the understanding of abiotic constraints on the ecology of sympagic species.

5.9. Outlook: Molecular biology and sea-ice biology

In several parts of this work molecular-biological techniques were successfully applied and further experiments were proposed to allow the answer of specific questions in sea-ice research. I will briefly sum up these remarks and provide an outlook for future research. The identification of species and particularly the assignment of eggs and larvae to respective adults can be facilitated through DNA barcoding techniques. This will allow the identification of life-cycle strategies of plathyelminths and will possibly even result in the detection of yet undescribed or cryptic species. The life cycle of *Tergipes antarcticus* was successfully elucidated with this technique (chapter 3). Such work could furthermore add value to phylogenetic discussions as demonstrated for the Arctic sympagic hydrozoan *Sympagohydra tuuli* (Siebert et al. in press). Furthermore, it would be interesting to determine the exchange rate of organisms between the Weddell and the Ross Sea via population genetics (Held and Leese 2007) in order to understand the influence of the large-scale dynamics of ice drift on species distribution. Several sympagic species like *Stephos longipes*, *Paralabidocera antarctica* and *Harpacticus furcifer* are found in both areas (Schnack-Schiel et al. 1998, Guglielmo et al. 2007), but it is unclear whether they form one common or two discrete populations and on what time scales an exchange takes place. A hypothesis already stated by Carey and Montagna (1982) for Arctic fast-ice meiofauna is that the sympagic meiofauna is derived from pelagic and mainly from benthic origin and that colonisation takes place in coastal areas. Similar abiotic conditions as those found for the sea-ice ecosystem, in particular changing temperatures and salinities are known from coastal and intertidal ecosystems (Aarset and Zachariassen 1988, Waller et al. 2006). Intertidal organisms are therefore likely invaders of the sea-ice ecosystem and this leads to the hypothesis that organisms found in sea ice mainly evolved from ancestors which thrived in coastal ecosystems. The comparison of both communities, particularly using phylogenetic methods, could help to test this hypothesis and maybe also help to elucidate on what time scales an exchange between these two communities takes place. The age of the Antarctic sea-ice ecosystem can only roughly be estimated. These estimates range from 14 million years (the time when ice sheets developed on Antarctica for the first time (Lear et al. 2000), to 120 thousand years (the time when atmospheric temperatures after the last interglacial dropped below levels of the current interglacial (Petit et al. 1999)), with a more likely range from 3 million years (the time since when the Western Antarctic ice sheet persists (Zachos et al. 2001)) to 120 thousand years. The use of molecular clocks (Allegrucci et al. 2006) seems a promising method to answer this question independent

from geological observations. Determining the timing of the horizontal transfer of St-AFP could yield an estimate for the minimum age of the Antarctic sea-ice ecosystem, independent from geological observations.

The work on *Stephos longipes* presented in chapter four indicates the success of unbiased molecular-biological methods to uncover special adaptation mechanisms. The detection of a gene of algal origin providing freeze protection in a copepod was unexpected. The implications of this work for the whole group of antifreeze proteins identified in sea-ice bacteria and algae and also for the understanding of horizontal gene transfer have been discussed in chapter four. In particular, this work yields new hypotheses on the mechanisms of horizontal gene transfer. The established cDNA library will be analysed in further detail in future using microarray techniques and bioinformatic tools. This will help to understand the adaptation mechanisms of *Stephos longipes* to changing salinities and temperatures. This approach might even yield enzymes, proteins and strategies apt for the application in biotechnological areas. Another long-term goal should be the establishment of one or several model organisms for comparative research on adaptation mechanisms to specific stresses dominating in the sea-ice ecosystem. A comparison of Arctic, temperate, tropic and Antarctic species seems promising. Harpacticoids, in particular *Drescheriella glacialis* or *Tisbe prolata* (Antarctic sea ice; Günther et al. 1999), *Tigriopus angulatus* (South-Georgia; Waller et al. 2006), *T. californicus* (west coast of North America; Willett and Burton 2002) or *T. japonicus* (east coast of Asia; Lee et al. 2005) and *Tisbe furcata* (Arctic sea ice and benthos; Grainger 1991) are, in my view, promising candidates for such a project. In this context, the establishment of cultures is needed to allow laboratory experiments. Studies e.g. on developmental rates, respiration rates, freeze tolerance, thermal hysteresis and changes in transcription, translation and protein activity should be performed and will enable a further understanding of the ecophysiology of sea-ice meiofauna.

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Jan Michels, Elke Mizdalski, Sigrid B Schnack-Schiel and Iris Werner
Living conditions, abundace and composition of the metazoan fauna in surface and sub-ice layers in pack ice of the western Weddell Sea during late spring

Maike Kramer, Michael Spindler and Heike Wägele
Tergipes antarcticus (Gastropoda, Nudibranchia): distribution, life cycle, morphology, anatomy and adaptation of the first mollusc known to live in Antarctic sea ice

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8. Eidesstattliche Erklärung

Hiermit erkläre ich an Eides statt, dass ich die vorliegende Dissertation selbständig und nur mit den angegebenen Hilfsmitteln angefertigt habe. Es wurde bislang noch kein Promotionsversuch unternommen. Die Arbeit wurde entsprechend den Regeln guter wissenschaftlicher Praxis durchgeführt.

Kiel, 17.12.2008 Rainer Kiko

9. Curriculum vitae

Personal details

Name:	Rainer Kiko
Adress:	Auf den Häfen 106 28203 Bremen
Birthday:	14. November 1977
Birthplace:	Werl
Marital status:	Unmarried
Citizenship:	German

Education

1. 8. 1984 - 15. 6. 1988	Primary school: St. Josefs-Grundschule Werl-Westönnen
1. 8. 1988 - 13. 6. 1997	Grammar school: Marien-Gymnasium Werl
1. 7. 1997 - 1.5. 1998	Military service
1. 10. 1998 - 30. 11. 2004	Biology studies at Christian-Albrechts-University, Kiel Degree: Diplom biologist
1.10. 1999 - 6. 9. 2005	Chemistry studies at Christian-Albrechts-University, Kiel Degree: Bachelor of science in Chemistry

Since 1.8. 2005	PhD-Thesis at the Institute for Polar Ecology, Kiel and at the Alfred-Wegener-Institute for Polar and Marine Research, Bremerhaven Topic: „Ecophysiology of Antarctic sea-ice meiofauna“
Languages:	English, fluent in written and spoken French, school knowledge

Expeditions

28. 2. 2003 - 24. 4. 2003	ARK XIX/1; “WARPS – Winter Arctic Polynya Study“ with R/V “Polarstern” to Storfjord, Svalbard and the central Arctic Ocean Position: Graduate assistant Working group: Sea-ice biology, Institute for Polar Ecology, Kiel Principal investigator: Dr. Iris Werner
6. 11. 2004 - 19. 1. 2005	ANT XXII/2; “ISPOL- Ice Station Polarstern“ with R/V “Polarstern” to the western Weddell Sea Position: Graduate assistant Working group: Sea-ice biology, Institute for Polar Ecology, Kiel Principal investigator: Dr. Henrike Schünemann
24. 8. 2006 - 29. 10. 2006	ANT XXIII/7; „Winter Weddell Outflow Study“ with R/V “Polarstern” to the western Weddell Sea Position: Principal investigator Working group: Sea-ice biology, Institute for Polar Ecology, Kiel

28.7.2007 - 7.10. 2007	ARK XXII/2 with R/V Polarstern to the central Arctic ocean Position: Principal investigator Working group: Sea-ice biology, Institute for Polar Ecology, Kiel
31.5.2008 – 6.6.2008	Expedition with R/V Lance to St. Johns Fjord and Kongsfjord, Svalbard Position: Consultant of a film project

10. Publications

Articles

Werner I, Auel H, Kiko R (2004) Occurrence of *Anonyx sarsi* (Amphipoda: Lysianassoidea) below Arctic pack ice: an example for cryo-benthic coupling? Polar Biology 27:474–481

Augustin R, Franke A, Khalturin K, Kiko R, Hemmrich G, Bosch TCG (2006) Dickkopf related genes are components of the positional value gradient in Hydra. Developmental Biology 296:62–70

Kiko R, Michels J, Mizdalski E, Schnack-Schiel SB, Werner I (2008) Living conditions, abundace and composition of the metazoan fauna in surface and sub-ice layers in pack ice of the western Weddell Sea during late spring. Deep Sea Research Part II 55:1000–1014

Kiko R, Kramer M, Spindler M, Wägele H (2008) *Tergipes antarcticus* (Gastropoda, Nudibranchia): distribution, life cycle, morphology, anatomy and adaptation of the first mollusc known to live in Antarctic sea ice. Polar Biology 31:1383–1395

Kiko R, Werner I, Wittmann A (2009) Osmotic and ionic regulation in response to salinity variations and cold resistance in the Arctic under-ice amphipod *Apherusa glacialis*. Polar Biology 32:393-398

Siebert S, Anton-Erxleben F, Kiko R, Kramer M (in press) *Sympagohydra tuuli* (Cnidaria, Hydrozoa) – first report form sea ice of the central Arctic Ocean and insights into histology, reproduction and locomotion. Marine biology DOI:10.1007/s00227-008-1106-9

Oral presentations (international conferences and invited only)

Kiko R. Ecophysiology of sea-ice metazoans. Annual meeting of the Society for Experimental Biology (SEB) Glasgow, United Kingdom, 5.4.2007.

Kiko R. How to survive within sea ice? First insights from Antarctic nudibranchs and copepods. Colloquium at the Institute for Evolutionary Biology and Ecology, Bonn, Germany, 18.6.2007.

Kiko R. Sea ice meiofauna and molecular biology. International Sea-ice Summer School, Longyearbyen, Svalbard, 13.7.2007.

Kiko R. Ershova A. Kramer M. Krapp R. Preobrazhenskaya O. Schünemann H. Siebert S. Werner I. News from the sea-ice biological research in the Arctic and Antarctic. 23. International Polar Symposium of the German Polar Research Association 2008, Münster, Germany, 13.3.2008.

Kiko R. Kramer M. Lucassen M. Schnack-Schiel S.B. Werner I. Antarctic sea ice: Habitat characteristics, metazoan fauna, and adaptations to low temperature. SCAR/IASC IPY Open Science Conference 2008, St. Petersburg, Russia, 9.7.2008.

Kiko R. Kramer M. Lucassen M. Schnack-Schiel S.B. Ecophysiology of metazoans living inside sea ice. 15th International Symposium on Polar Sciences 2008, Incheon, Republik of Korea, 24.9.2008.

Posters

Kiko R. Werner I. Auel H. Occurence of *Anonyx sarsi* (Amphipoda: Lysianassoidea) below Arctic pack ice: an example for cryo-benthic coupling? The First International Symposium on the Environmental Physiology of Ectotherms and Plants Roskilde, Denmark, 2005

Kiko R. Lucassen M. Schnack-Schiel SB. Werner I. Ecophysiology of the sympagic copepod *Stephos longipes*. International Sea-ice Summer School, Longyearbyen, Svalbard, 2.7. - 13.7. 2007.

Kiko R. Lucassen M. Schnack-Schiel S.B. Werner I. Antarctic sea ice: habitat characteristics, metazoen fauna, and adaptations to low temperature. Annual meeting of the Society for Experimental Biology (SEB), Glasgow, United Kingdom, 31.3. - 5.4. 2007.