

Trophic positioning, diel vertical migration and physiological constraints in euphausiid species of the Namibian upwelling system

*- Implications for krill as indicator of environmental
conditions -*

Dissertation

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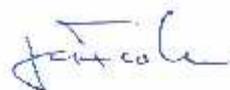
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DEDICATED TO MY FAMILY

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Abbreviations

DVM – diel vertical migration

dw – dry weight

EBUE – Eastern Boundary Upwelling Ecosystem

fw – fresh weight

GDP – gross domestic product

IMP – intermoult period

INC – size increment at moult

LDH – lactate dehydrogenase

MS – moult stage

NBC – Northern Benguela Current

nBus – northern Benguela upwelling system

OMZ – oxygen minimum zones

P_c – critical oxygen pressure

RQ - respiratory quotient

SDS – sexual developmental stage

SST – sea surface temperature

TL – trophic level

ww – wet weight

„Das derzeitige Wissen über die überaus komplexen Zusammenhänge zwischen Klima, physikochemischen Meeresbedingungen, marinen Ökosystemen und Fischerei reicht nicht aus, um verlässliche Prognosen über die Reaktion der marinen Systeme auf Klimaveränderungen abzugeben“ (Sondergutachten der WBGU 2006)

„The current knowledge about the complex interactions between climate, physicochemical marine conditions, marine ecosystems and fisheries are insufficient to make reliable predictions about the response of marine systems to climate change.“ (special report WBGU 2006; translated from German)

Deutsche Zusammenfassung

Euphausiiden, auch Krill genannt, sind in fast allen Meeresgebieten der Erde anzutreffen und dort teilweise mit sehr hohen Individuenzahlen vertreten. Durch ihre Biomasse, ihre physiologische Plastizität und ihre vermittelnde Stellung zwischen Primärproduktion und höheren trophischen Ebenen nehmen diese Tiere eine Schlüsselposition in marinen Ökosystemen ein. Kenntnisse über ihre Verteilung, ihre Häufigkeit, ihre Lebenszyklus-Strategien, ihr Wachstum, sowie Anpassungen der Physiologie und ihres Verhaltens an kurz- und längerfristige Veränderungen ihrer Umwelt können genutzt werden, die hohe Variabilität in den Krillbeständen zu erklären und Aussagen über den Einfluss von Umweltveränderungen auf die Krillbestände zu ermöglichen. Ein Ökosystem, in dem die Zooplanktonbiomasse von Euphausiiden dominiert wird, ist der nördliche Benguelastrom bzw. das nördliche Benguelaauftriebsgebiet.

Der nördliche Benguelastrom ist ein hochproduktives und stark stratifiziertes Auftriebsgebiet, welches durch vielfache Planktonblüten im Jahresverlauf charakterisiert ist (poly-gepulst). Hohe Fischereierträge und große Populationen an verschiedenen Fisch-, Vogel- und Robbenarten veranschaulichen die wirtschaftliche und ökologische Bedeutung dieses Gebietes. Während der letzten Jahrzehnte kam es zu tiefgreifenden Veränderungen der physikalischen und biologischen Zustände in diesem Gebiet. In diesem Zusammenhang können Euphausiiden, bedingt durch ihre zentrale Position im Ökosystem und als Schlüsselarten in Nahrungsnetzen, als kennzeichnende Indikatoren für die stattfindenden Veränderungen in ihrer Umwelt genutzt werden. Krill sind in der Lage sich schnell an biotische und abiotische Bedingungen anzupassen und Euphausiiden beeinflussen sowohl niedere als auch höhere trophische Ebenen. Für ein besseres Verständnis der Prozesse im Ökosystem ist deshalb eine Erforschung dieser Schlüsselarten, ihrer Biologie und Lebenszyklusstrategien, sowie Anpassungen ihrer Physiologie und ihres Verhaltens an die vorherrschenden Umweltbedingungen von grundlegender Bedeutung. Der Schwerpunkt dieser Arbeit liegt dabei auf den Anpassungen der Krillarten an die stark schwankenden abiotischen Parameter (Temperatur und Sauerstoffgehalt) und biotische Faktoren (Nahrungsverfügbarkeit). Ein besseres Verständnis des Einflusses dieser Faktoren kann dabei helfen, die variable Verteilung und Biomasse dieser Tiere im nördlichen Benguelasystem zu verstehen und mögliche klimabedingte Veränderungen der Ökosystemstruktur und Funktion vorherzusagen. Bis zum heutigen Tage haben ökophysiologische Anpassungen von Krillarten, trotz der großen Relevanz dieser Tiere im Benguelaökosystem, wenig Aufmerksamkeit erhalten. Das Ziel dieser Arbeit ist es deshalb, einige dieser Lücken zu schließen, wobei das

Deutsche Zusammenfassung

Hauptaugenmerk auf dem Einfluss der Temperatur, des Sauerstoffgehaltes und der Nahrungsverfügbarkeit auf die Physiologie und das Verhalten der dominanten Arten, besonders *Euphausia hanseni* (Zimmer 1915), liegt.

Ein Vergleich der physiologischen Anpassungen des Krills an dieses poly-gepulste System mit Anpassungen an einfach gepulste Systeme, wie der Nordsee, soll helfen, den Einfluss des Nahrungsregimes, sowohl in Bezug auf dessen Länge als auch die zeitliche Koordinierung, auf die Physiologie von Zooplankton im Allgemeinen zu verstehen. Diese Doktorarbeit besteht aus fünf Studien, die die Temperaturgrenzen (2), das Vertikalwanderungsverhalten (3), die physiologischen Leistungsfähigkeit (4) sowie die Position der Krillarten im Nahrungsnetz (5) des Benguela-Auftriebsgebietes untersuchen. Ein weiteres Kapitel beschäftigt sich mit der anaeroben Kapazität von *E. hanseni* (6).

Der Einfluss der Temperatur auf die metabolische Aktivität, also Atmung und Exkretion, von *E. hanseni* und *Nematoscelis megalops* (G.O. Sars 1883) wurde mittels bordgestützter Laborexperimente untersucht. Sowohl die Respirations- als auch die Exkretionsraten beider Arten nahmen mit steigenden Temperaturen zu, wobei sich die Arten allerdings hinsichtlich ihrer Anpassungsfähigkeit unterschieden (**Kapitel 1**). *N. megalops* ist besonders gut an niedrige und *E. hanseni* an höhere Wassertemperaturen adaptiert. Unterschiedliche Respirationsraten zusammen mit unterschiedlichen Stoffwechselsubstraten führen bei *E. hanseni* zu einem höheren Energie- bzw. Kohlenstoffbedarf im Vergleich zu *N. megalops*. Die ermittelten Temperaturgrenzen beider Arten entsprechen dabei ihrer vertikalen Position in der Wassersäule.

Das Vertikalwanderungsverhalten von Tieren trägt erheblich zu dem Kohlenstofftransport in die unteren Wasserschichten bei (Tarling and Johnson 2006). Der ausgeprägte Vertikalwanderer *E. hanseni* spielt in diesem Zusammenhang, im Gegensatz zu dem schwachen Wanderungsverhalten von *N. megalops*, eine bedeutende Rolle. Beide Arten nutzen die Sauerstoffmangelzone als ein Refugium um sich vor Räubern zu schützen. Allerdings mied *N. megalops* die Temperatursprungschicht wohingegen *E. hanseni* diese regelmäßig durchquerte. Andere Krillarten, z.B. *Euphausia americana* (Hansen 1911), hielten sich meist oberhalb der Sauerstoffsprungschicht auf und mieden Wassermassen mit geringen Sauerstoffgehalten. Entsprechend scheinen sich die Krillarten im Benguelastrom in zwei ökologische Gruppen zu unterteilen und zwar eine, die die Sauerstoffmangelzone nutzt und eine, die diese weitestgehend meidet. Saisonale Unterschiede des Wanderungsverhaltens im Bezug zur Temperatur, zum Sauerstoffangebot und zur Nahrungsverfügbarkeit konnten festgestellt werden und Berechnungen des Energiebedarfes zeigten, dass die Temperatur das

Vertikalwanderungsverhalten bestimmt, das durch das Nahrungsangebot weitergehend beeinflusst wird (**Kapitel 2**).

Das Nahrungsangebot im nördlichen Benguelastrom wird durch Auftriebsereignisse bestimmt, welche für einen erhöhten Nährstoffeintrag in die oberen Wasserschichten sorgen und somit Phytoplanktonblüten induzieren. *E. hanseni* stellt seine Fortpflanzungs- und Häutungsaktivitäten auf diese Ereignisse ein (**Kapitel 3**). Sexuell reifere Weibchen weisen einen höheren Sauerstoffbedarf auf und dementsprechend kann der physiologische Zustand von *E. hanseni* als Indikator für die vorherrschenden Nahrungsbedingungen bzw. Auftriebsereignisse genutzt werden. Der physiologische Zustand der Tiere ist dabei durch kurzzeitige Veränderungen der Umweltbedingungen (Auftrieb) stärker beeinflusst als durch saisonale Unterschiede. Ein Vergleich der metabolischen Anpassungen von *E. hanseni* an die schwankende Nahrungsverfügbarkeit mit Anpassungsstrategien anderer Krillarten, wie *Meganyctiphanes norvegica* (Sars 1857) und *Euphausia superba* (Dana 1850) zeigte, dass der physiologische Zustand von Euphausiiden als zuverlässiger Indikator auch für kurzfristige und längerfristige Umweltveränderungen dienen kann.

Um die Interaktionen verschiedener Komponenten des Nahrungsnetzes zu verstehen und räumliche und zeitliche Variationen in der trophischen Position der Euphausiiden beurteilen zu können, kamen stabile Isotopenanalysen zur Anwendung. Krillarten des nördlichen Benguela-Auftriebsgebietes unterschieden sich hinsichtlich ihrer trophischen Position, diese lag im Untersuchungszeitraum zwischen 2 und 4 (**Kapitel 4**). Die deutlichen Unterschiede weisen auf regional verschiedene funktionelle Rollen im Nahrungsnetz und Verschiebungen in den Nahrungspräferenzen hin. Alle untersuchten Arten sind demzufolge omnivor. Wiederum waren Unterschiede in der trophischen Position durch unterschiedliche Auftriebssituationen ausgeprägter, als saisonale. Die Arten der Gattung *Euphausia* nahmen innerhalb der Euphausiiden die niedrigste Position im Nahrungsnetz ein, wohingegen die Arten *Thysanopoda monacantha* (Ortmann 1893), *N. megalops* und überraschenderweise die kleine, filtrierende Art *Nyctiphanes capensis* (Sars 1883) deutlich höhere Positionen besetzten.

Die ausgeprägte und in ihrer Ausdehnung noch zunehmende Sauerstoffmangelzone hat aller Voraussicht nach einen deutlichen Effekt auf die vertikale Verteilung und den Lebenszyklus von Krill. Arten, die regelmäßig in dieser Mangelschicht anzutreffen sind, sollten folglich morphologische, physiologische und Verhaltensanpassungen an die niedrigen Sauerstoffgehalte entwickelt haben. Die stark migrierende Art *E. hanseni* zeigt sich in diesem Zusammenhang als ausgeprägter Regulierer, also fähig, unabhängig von der Menge an

Deutsche Zusammenfassung

Sauerstoff im Atemmedium, eine konstante Respiationsleistung aufrecht zu erhalten. In den Experimenten war sie in der Lage bis zu einem Sauerstoffgehalt, der deutlich unter $0.5 \text{ mg O}_2 \text{ L}^{-1}$, zu überleben (**Kapitel 5**). Ihr kritischer Sauerstoffpartialdruck lag dabei zwischen $1.3 \pm 0.3 \text{ mg O}_2 \text{ L}^{-1}$ und $2.0 \pm 0.4 \text{ mg O}_2 \text{ L}^{-1}$, abhängig von der Jahreszeit und Temperatur. Der kritische Sauerstoffpartialdruck wurde durch Veränderungen der Wassertemperatur beeinflusst, wobei höhere Temperaturen zu einer Erhöhung des kritischen O_2 -Partialdruckes führten. Dies kann möglicherweise, vor allem im Zusammenhang mit der beschriebenen Erderwärmung, einen erheblichen (negativen) Einfluss auf die vertikale und horizontale Verteilung dieser Art im nördlichen Benguela-Auftriebsgebiet haben.

Zusammenfassend lässt sich sagen, dass die Euphausiidengemeinschaft des nördlichen Benguelasystems, in Bezug zu den artspezifischen Anpassungen des Verhaltens und der Physiologie an die stark schwankenden Umweltbedingungen in diesem System, sehr divers ist. Entsprechend dieser artspezifischen Anpassungen an die Temperatur, den Sauerstoffgehalt und die Nahrungsverfügbarkeit zeigen die dominanten Arten deutlich unterschiedliche autökologische Strategien, welche die Krillgemeinschaft des nBus klar strukturieren. Physiologische- und Verhaltensanpassungen erfolgten dabei an kurzfristige, meist auftriebsbedingte, Veränderungen in der Umwelt und nicht an saisonale Unterschiede. Krillarten des nördlichen Benguelastroms eignen sich als Indikatoren für die vorherrschenden Umweltbedingungen und darüber hinaus zeigen sie sowohl kurzfristige als auch längerfristige Veränderungen in der Umwelt an. Diese Indikatorfunktion ermöglicht es Euphausiiden als Modellorganismen für den Vergleich zwischen einfach gepulsten und mehrfach gepulsten Systemen in Schelfmeeren zu nutzen.

English summary

Euphausiids are distributed ubiquitously across the globe and many krill species are represented in extraordinarily high numbers in various ecosystems. Their biomass, physiological plasticity and importance as a trophic link between primary production and higher trophic levels enable these animals to fill key positions in marine ecosystems. Knowledge about their distribution, abundance, life-history traits, growth patterns, physiological and behavioural adaptations to short- and long-term changes in their environment will help to understand both spatial and temporal variability found in krill populations. Changes in krill distribution and abundance may significantly impact the food web and ecosystem structure and functioning. One ecosystem, where euphausiids play a pivotal role and can dominate the zooplankton biomass, is the Northern Benguela Current (NBC) also called the northern Benguela upwelling system (nBus).

The NBC is a nutritionally poly-pulsed, highly productive and stratified coastal upwelling system. High fishery yields and large populations of fish, birds and seals point to the economic and ecological importance of this area. Several physical and biological changes have occurred over the last decades in this Eastern Boundary Upwelling Ecosystem (EBUE). As a component keystone species in the food web of the NBC the dominant krill species may serve as indicators for ongoing changes in the environment since they are known to react quickly to changes in abiotic and biotic conditions. It is therefore crucial to enhance our understanding of the biology and life-history of the dominant krill species in this area and to investigate their physiological constraints and behavioural adaptations to the prevailing conditions. Most notable and important areas for further investigations are adaptations to highly fluctuating abiotic parameters, such as temperature and dissolved oxygen, as well as metabolic and life-history adaptations to short-term upwelling pulses and seasonal differences in terms of food availability. A better understanding of how these parameters impact the variable distribution and biomass of euphausiids in the challenging northern Benguela upwelling system will help to predict possible climate induced changes in ecosystem structure and functioning. To date, ecophysiological adaptations of euphausiids from the NBC have received little attention, despite their important role for this ecosystem. The aim of this thesis is to fill some of these gaps and to elucidate the influence of temperature, oxygen and food availability on the physiology and behaviour of the dominant krill species, principally *Euphausia hanseni* (Zimmer 1915).

Furthermore, a comparison between the physiological adaptations of krill to this nutritionally poly-pulsed system and those to mono-pulsed systems, such as the North Sea, will help to

English summary

understand the impact of the productive regime (timing and duration of pulses) on physiological adaptations of zooplankton in general. In this regard, euphausiids may serve as useful model organisms with a great capacity for applying general concepts to other similar organisms. This thesis compiles five chapters focusing on (1) thermal constraints, (2) diel vertical migration behaviour, (3) physiological performance and (4) trophic position of krill species in the nBus. Furthermore, a chapter concerning the anaerobic capacity of *E. hanseni* is included (5).

The impact of temperature on the metabolic activity, respiration and excretion rates, of *E. hanseni* and *Nematoscelis megalops* (G.O. Sars 1883) was determined through laboratory experiments on board research vessels. Respiration rates and excretion rates of both species increased with rising temperatures (**Chapter 1**). However, the impact of temperature on metabolic activity differed significantly between species. *N. megalops* showed adaptations to low water temperatures whereas *E. hanseni* was better adapted to warmer temperatures. These different thermal constraints correspond to their respective vertical positions in the water column. Each species uses a different predominant metabolic substrate. Thus the energy demand, as measured by carbon demand, is much greater for *E. hanseni* compared to *N. megalops*.

Diel vertical migration (DVM) of animals indirectly contributes to downward transport of carbon (Tarling and Johnson 2006). In this regard, the pronounced long range vertical migrant *E. hanseni* significantly contributes to the carbon transfer from the upper water layers to deeper waters, relative to the weak migrant *N. megalops*. While both species used the oxygen minimum zones (OMZ) as a refuge from predation, *N. megalops* avoided the thermocline whereas *E. hanseni* regularly crossed it. Other krill species, such as *Euphausia americana* (Hansen 1911), remained usually above the oxycline and avoided low dissolved oxygen concentrations. Accordingly, euphausiids in the nBus are separated into two ecological groups; one that uses and one that avoids the OMZ. Seasonal behavioural differences, due to differences in temperature, dissolved oxygen levels and food availability were apparent and energetic analyses suggest that temperature was a controlling factor, with food supply further modifying the DVM behaviour of krill (**Chapter 2**).

Food supply in the NBC is determined by upwelling events which draw up deep-water nutrients and favour subsequent phytoplankton blooms. Favourable feeding conditions are known to enhance growth and reproductive effort (Buchholz 2003). Consequently, reproductive effort and moult activity of *E. hanseni* are determined by upwelling (**Chapter 3**). Oxygen demand of *E. hanseni* females at later sexual developmental stages was elevated.

Differences in physiological performance were more apparent on short time-scales than between seasons. A comparison between the metabolic adaptations of *E. hanseni* and the adaptations of *Meganyctiphanes norvegica* (Sars 1857) and *Euphausia superba* (Dana 1850) to the fluctuating trophic environment indicated that, despite different adaptive strategies, the physiological performance of euphausiids can be a useful indicator of the ongoing short- and long-term changes in the environment.

To understand interactions between different components of the food web and to assess spatial and temporal variation in the trophic position of the euphausiid community, stable isotope analyses were conducted. The trophic position of krill species in the NBC varied both in space and time between levels 2 and 4 (**Chapter 4**). These high variations suggested regionally divergent functional roles and dietary shifts. All krill species are considered omnivores, with species-specific variation in dietary emphases. However, upwelling mediated differences in the trophic level (TL) of euphausiids between seasons were less pronounced than regional ones. The species of the genus *Euphausia* occupied the lowest trophic levels whereas *Thysanopoda monacantha* (Ortmann 1893), *N. megalops* and surprisingly the small filter-feeding species *Nyctiphanes capensis* (Sars 1883) filled significantly higher TL.

The pronounced and expanding oxygen minimum zones (OMZ) in the nBus may severely affect the vertical distribution and life history of krill species. Yet, adaptations to low oxygen levels should have evolved within species that regularly inhabit these areas. The pronounced vertical migrant *E. hanseni* showed a high degree of regulation capacity and was able to survive at an oxygen level well below $0.5 \text{ mg O}_2 \text{ L}^{-1}$ (**Chapter 5**). The assessed critical oxygen pressure (P_C) varied between $1.3 \pm 0.3 \text{ mg O}_2 \text{ L}^{-1}$ and $2.0 \pm 0.4 \text{ mg O}_2 \text{ L}^{-1}$, dependent on temperature and season. Rises in water temperature elevated the P_C and thereby the oxygen concentration at which the onset of the anaerobic metabolism takes place. This may severely affect the vertical and horizontal distribution of *E. hanseni*, especially in conjunction with the ongoing climate change.

In summary, the nBus euphausiid community is highly diverse with respect to physiological and behavioural adaptations to prevailing and fluctuating environmental conditions. Species-specific adaptations to temperature, oxygen and food availability show that the predominant species have distinct “autecological strategies”, leading to a highly structured euphausiid community in the NBC. Physiological and behavioural adaptations were driven by short-term differences in environmental conditions mediated by upwelling and non-upwelling events rather than seasons. Furthermore, krill species in the NBC are useful indicators of the prevailing environmental conditions, both on short-term and long-term scales. This may allow

English summary

euphausiids to be used as suitable model organisms for comparison between mono- and poly-pulsed systems.

Key findings

I. Thermal constraints on the respiration and excretion rates of krill, *Euphausia hanseni* and *Nematoscelis megalops*, in the northern Benguela upwelling system off Namibia

- Respiration and excretion rates of *E. hanseni* and *N. megalops* were positively correlated with temperature.
- The impact of temperature on the respiration rates of both species differed significantly.
- *N. megalops* showed adaptations to low temperatures between 5°C and 10°C, whereas *E. hanseni* showed adaptations to higher water temperatures between 10°C and 20°C.
- Physiological adaptations of both species corresponded to their preferred ambient temperatures as displayed by their different vertical migration behaviour.
- *E. hanseni* predominantly metabolized proteins whereas *N. megalops* predominantly metabolized lipids.
- Carbon demand of *N. megalops* was lower than in *E. hanseni*.

II. Diel vertical migration behaviour of euphausiids of the northern Benguela Current: Seasonal adaptations to food availability and strong gradients of temperature and oxygen

- Euphausiids were divided into two different ecological groups: species that enter and species that avoid the oxygen minimum zone.
- Energy budgets for *E. hanseni* and *N. megalops* indicated that the vertical position of krill species was controlled by temperature and further influenced by food abundance. This was apparent from seasonal differences in their vertical position due to variations in temperature, oxygen- and food availability.
- *E. hanseni* is a strong vertical migrator, regularly crossing the thermocline and leaving the OMZ for short feeding excursions.
- In contrast, *N. megalops* showed marginal diel vertical migration, avoiding the thermocline and staying in the OMZ at all hours of the day.

Key findings

III. Regional and seasonal differences in physiological performance of *Euphausia hanseni* in the northern Benguela ecosystem, influenced by upwelling

- Upwelling pulses, which induce sudden changes in the trophic environment, appeared to control reproduction and moult activity in *E. hanseni*.
- Metabolic rates were more influenced by events at short-time scales rather than differences between seasons.
- Females of *E. hanseni* with high sexual developmental stages (SDS) showed higher respiration rates than those with low SDS.
- Moulting stages did not affect oxygen consumption rates in *E. hanseni*.
- Euphausiids were identified to be useful indicators of the current hydrographic and trophic conditions and indicators for short-term changes in the environment.

IV. Trophic position of euphausiid species of the northern Benguela upwelling system determined by stable isotopes – a regional and seasonal comparison

- Regional differences in the trophic position of euphausiids in the nBus were more pronounced than seasonal ones, leading to regionally divergent roles in the food web.
- All krill species showed omnivorous feeding.
- *N. capensis*, a comparably small filter-feeding species, occupied an unexpectedly high trophic level, apparently due to feeding on micro-plankton.
- Trophic levels of euphausiids ranged from 2.1 to 4.1, characteristic for primary to tertiary consumers.

V. First results on the anaerobic capacity of *Euphausia hanseni* in the NBC

- *E. hanseni* maintained a constant oxygen uptake irrespective of the ambient oxygen levels over a broad range of oxygen saturation which was demonstrated by a high regulation index (RI) of 0.7 ± 0.2 .
- The critical oxygen pressure (P_C) which marks the onset of the anaerobic metabolism varied between 1.3 ± 0.3 mg O₂ L⁻¹ and 2.0 ± 0.4 mg O₂ L⁻¹, dependent on temperature and season.
- The P_C was influenced by temperature, illustrated by a shift towards a higher P_C at higher water temperatures.

Outline of publications and personal contributions

This cumulative PhD thesis consists of four manuscripts that contributed to the GENUS project (Geochemistry and Ecology of the Namibian Upwelling System) and one supplementary chapter concerning adaptations of *E. hanseni* to low oxygen levels. Sampling and on board respiration measurements were conducted during three cruises with the *FRS Africana* (2009), *RRS Discovery* (2010) and *RV Maria S. Merian* (2011). Further analyses were done in laboratories at the Alfred-Wegener-Institute for Polar and Marine Research in Bremerhaven, Germany between 2009 and 2012. The general concept was developed by Prof. Dr. F. Buchholz as part of the project proposal for subproject 7 of the GENUS project (BMBF-03F0497F). The specific approaches were autonomously developed and realized. Below the manuscripts are listed and the contributions of all authors are specified.

- I. **Werner, T.**, Huenerlage, K., Verheye, H. and Buchholz, F. (2012) Thermal constraints on the respiration and excretion rates of krill, *Euphausia hanseni* and *Nematoscelis megalops*, in the northern Benguela upwelling system off Namibia. *African Journal of Marine Science* **34** (3) (accepted for publication in the *African Journal of Marine Science*)

The ideas and concepts for this manuscript were compiled by T. Werner and F. Buchholz. All analyses were done by T. Werner assisted by K. Huenerlage. The manuscript was prepared and written by T. Werner with contributions by all co-authors listed.

- II. **Werner, T.** and Buchholz, F. (2012) Diel vertical migration behaviour in Euphausiids of the northern Benguela Current: Seasonal adaptations to food availability and strong gradients of temperature and oxygen. *Journal of Plankton Research* (in revision).

The ideas and concepts for this manuscript were compiled by T. Werner and F. Buchholz. Sampling was done by T. Werner and F. Buchholz during winter 2010 and by F. Buchholz in 2004/2008. Enumeration of preserved krill samples was conducted by R. Schumacher. The applied conceptual model was designed by T. Werner. The manuscript was prepared and written by T. Werner with contributions by F. Buchholz.

Outline of publications

- III. **Werner, T.** and Buchholz, F. (2012) Regional and seasonal differences in physiological performance of *Euphausia hanseni* in the northern Benguela ecosystem, induced by upwelling. *Marine Biology* (submitted)
The ideas and concepts for this manuscript were compiled by T. Werner and F. Buchholz. The moult stages and the sexual developmental stages of *Euphausia hanseni* were determined by F. Buchholz and C. Buchholz, assisted by T. Werner. Metabolic measurements were conducted by T. Werner. The manuscript was prepared and written by T. Werner with contributions by F. Buchholz.
- IV. **Werner, T.** and Buchholz, F. (2012) Trophic position of euphausiid species of the northern Benguela upwelling system determined by stable isotopes – a regional and seasonal comparison.
The ideas and concepts of this manuscript were compiled by T. Werner and F. Buchholz. Preparation of samples was done by T. Werner. The manuscript was prepared and written by T. Werner with contributions by F. Buchholz.
The data shown in this manuscript will be used for a modeling approach with ECOPATH/ECOSIM in order to describe the trophic interactions and energy fluxes in the pelagic food web. This will be done in combination with other GENUS sub-projects.
- V. **Werner T.** First results on the anaerobic capacity of *Euphausia hanseni* in the NBC
The idea and concept of this chapter were compiled by T. Werner and F. Buchholz. Respiration measurements and calculations were conducted by T. Werner. The chapter was prepared and written by T. Werner.



Plankton sampling using a Tucker Trawl during a cruise with the FRS *Africana* in 2009.

Introduction

Coastal waters and environmental changes

Coastal and shelf waters play an important role in nutrient cycles as well as the uptake and discharge of climate relevant gases. Approximately 90% of organic carbon runoff from land is stored in the sediments of coastal oceans. Due to a high biodiversity and rich fishery yields, these areas are of great ecological and socio-economic importance. Although coastal (shelf) areas account for only 7% of the ocean surface, they contribute up to 90% of global marine fish catches and contain the bulk of primary and secondary production. Moreover, they are often rich in natural resources, such as ore, gas and oil. Coastal marine ecosystems have therefore been greatly exploited by humans and are threatened by the ongoing anthropogenic induced climate change (IPCC 2001 and 2007). Recently, there has been an increasing scientific effort to evaluate the impact of changing environmental conditions on the physiological state and population dynamics of organisms, which will permit more accurate predictions of changes to ecosystem structure and functioning. Such research has been largely motivated by preserving the commercial resources and nutrition security that these ecosystems provide to adjacent countries.

The combined effects of anthropogenic disturbances and climate related changes affect marine ecosystems, primarily through their higher trophic levels, and these effects may cascade down to lower trophic levels (Casini et al. 2008). In turn, lower trophic levels, e.g. zooplankton, may channel the environmental changes to higher trophic levels, like commercially important fish species (Möllmann et al. 2005). Accordingly, zooplankton play a decisive role in the impact of changing environmental conditions on pelagic ecosystems (Möllmann et al. 2003 and Chiba et al. 2006). However, changes in environmental conditions can alter the strength of interactions between species in the food web of pelagic ecosystems at all trophic levels and lead to alterations in the abundance of species and changes in community compositions. (Kraberg et al. 2011) These effects may be direct, e.g. through the expansion of oxygen minimum zones, elevated water temperatures, ocean acidification and changes in hydrodynamic conditions or indirect through alterations in food web dynamics (Pörtner and Peck 2010). In this regard, the success of an individual, and by extension a species, is determined by its physiological plasticity in responses to changes in abiotic conditions such as temperature, ocean carbon dioxide levels and oxygen availability. The occurrence, distribution patterns and survival rates of pelagic species are determined by

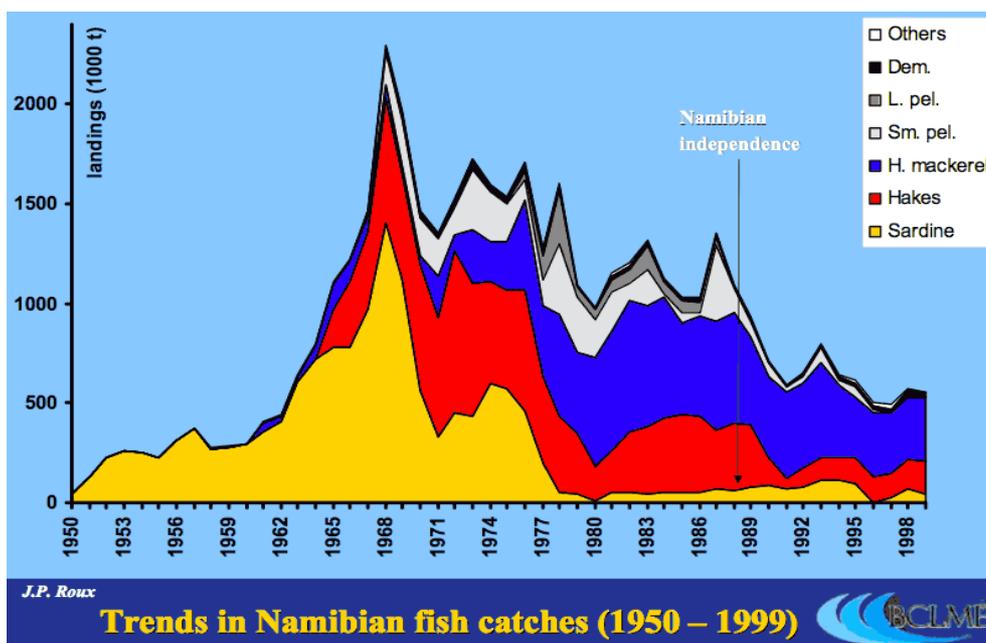
environmental conditions (Bakun and Broad 2003). If closely interlinked species differ in their plasticity and sensitivity to changes in the physical environment, the interactions between them will also be affected (Walther et al. 2002) and indirectly alter interactions through the entire food web (Edwards and Richardson 2004). Taylor and Rand (2004) provided evidence that hypoxia can lead to a decoupling of predator-prey interactions and change trophic fluxes within the food web. In general, the warming of water masses is associated with latitudinal displacements of populations, particularly phyto- (Richardson and Schoeman 2004) and zooplankton (Richardson 2008). Sudden shifts in structure and functioning of a marine ecosystem from one alternate state to another and physical driven shifts in biological systems are referred to as regime shifts (Kraberg et al. 2011). Recently, several anthropogenic and climate related changes in various marine ecosystems were detected (cf. Walther et al. 2002, Cury and Shannon 2004, Smetacek and Nicol 2005, IPCC 2007, Pörtner and Peck 2010, Kraberg et al. 2011).

For example, a trend towards a greater number and abundance of warm water species has been observed in the North Sea (Beaugrand 2004) and accordingly, species abundance and composition had changed (Alheit 2009). Rising water temperatures affected timing and abundance of plankton species and thereby reduced the survival of young cod (Beaugrand et al. 2003). In the Baltic Sea, temperature and circulation changes are assumed to be responsible for diatoms no longer dominating the spring bloom, having largely been replaced by dinoflagellates (Wasmund and Uhlig 2003). In Arctic waters, declines in sea ice coverage and warming water temperatures affect the current primary production regime, timing of phytoplankton blooms and functioning of sea-ice communities (Søreide et al. 2010). Changes within the composition of the euphausiid community in a high Arctic fjord, with more species from an Atlantic origin (Buchholz et al. 2010), and the first observation of krill spawning in this area (Buchholz et al. 2012, see attachment) are indicative of on-going (temperature) changes, which may affect the Arctic food web and therefore, influence ecosystem structure and functioning. In the Antarctic, the abundance, distribution and life cycle of Antarctic krill (*Euphausia superba*) are most likely negatively affected by declines in sea ice coverage and warming of water (Flores et al. 2012, see attachment). Moreover, changes in krill abundance and distribution drastically affect food-web interactions (Atkinson et al. 1999, Reid and Croxall 2001, Atkinson et al. 2004) and alter predator foraging ecology (Fraser and Hoffmann 2003). In the Humboldt Current periodical regime shifts from sardines to anchovies appear to be driven by temperature and oxygen availability (Bertrand et al. 2011). The highly productive coastal upwelling system of the Northern Benguela Current (NBC) has

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experienced a decline in sardine and anchovy stocks, largely replaced by gobies, horse mackerel and jellyfish (Cury and Shannon 2004), which incurred severe costs to the pelagic fisheries of Namibia (Fig. 1). Euphausiids often dominate zooplankton communities, show high abundances and biomasses throughout the world's oceans and form a central component of many food webs (Ekau et al. 2010). Thus, they may serve as indicators for changing environmental conditions in pelagic systems.

This thesis focuses on the impact of fluctuating abiotic (temperature and oxygen supply) and biotic (food availability) parameters on euphausiids' physiology and behaviour, and assesses the trophic position of krill species in the commercially important marine ecosystem of the northern Benguela off Namibia. Furthermore, differences in the physiological and behavioural adaptations of euphausiids between nutritionally poly-pulsed systems, like the northern Benguela, with mono-pulsed systems, like the North Sea will be elaborated. The following chapter summarizes the physical and biological characteristics of the nBus along with anthropogenic and climate related changes on-going in the ecosystem. A general description of krill biology and a detailed description of the predominant krill species of the nBus are also provided.



↑ **Figure 1** Trends in Namibian fish catches (1950-1999) (J.P. Roux, BCLME, published by van der Lingen et al. 2006)

The northern Benguela upwelling system (nBus)

The Benguela upwelling ecosystem located off the west coast of South Africa, Namibia and Angola is one of the four major Eastern Boundary Upwelling Ecosystems (EBUE) in the world's oceans. EBUE form less than 1% of the world oceans by area, but provide approximately 11% of the global new production (= all primary production associated with newly available nitrogen (Chavez and Toggweiler 1995)), and up to 20% of the global fishery catches (Pauly and Christensen 1995). The Northern Benguela Current (NBC) is divided in two sub-systems by the strong permanent upwelling cell at Lüderitz around 26°S (Boyer et al. 2001), namely the Northern Benguela Current (NBC) off Namibia and Angola and its southern counterpart, the southern Benguela Current off South Africa (Fig. 2). The northern Benguela upwelling system (nBus) extends from the Angola-Benguela front (16°-17°S) southwards to the strong Lüderitz upwelling cell (Hutchings et al. 2009), is characterized by cool, nutrient-rich upwelled waters and a high phytoplankton biomass (500-600 g C m⁻² year⁻¹ Lalli and Parsons 2006), which largely determines the yield of local and commercial fisheries. Primary production is mainly dependent upon the inshore upwelling of cold, nutrient-rich waters because of the enduring SO trade winds, which drive the seaward advection of surface waters due to strong Ekman transports. These nutrient-rich upwelled waters induce intensive primary production, characterized by diatoms, favouring short food chains.

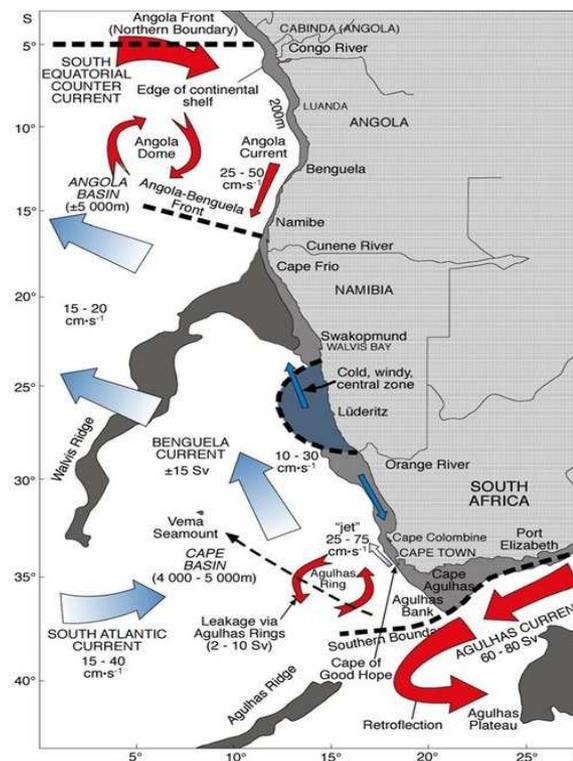


Figure 2 Map of the Benguela Current (from Shannon, L.V. and O'Toole, M.J. 2003)

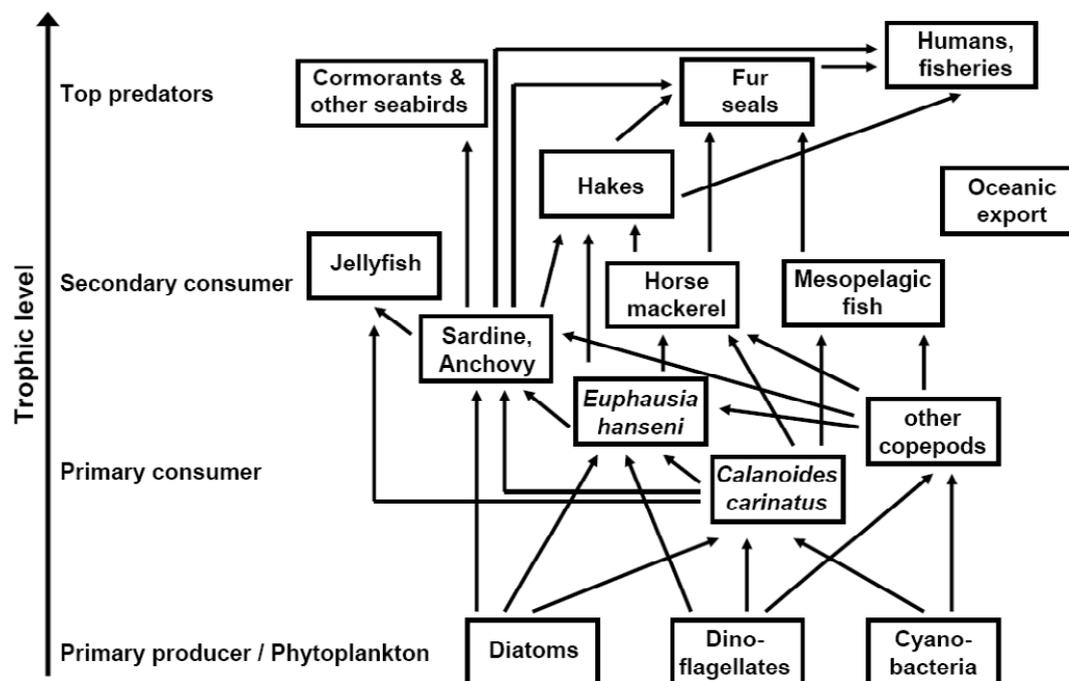
Introduction

Every year, warm, highly saline water is advected from the Angolan Dome into the NBC (Boyd et al. 1987). These southward intrusions may sometimes range as far as 25°S and are called Benguela Niños, analogous to Pacific El Niño events (Shannon et al. 1986). However, these events are less frequent and less intense than their Pacific counterparts (Shannon et al. 1986).

The nBus is a nutritionally poly-pulsed and highly stratified environment characterized by perennial upwelling and strong winds, which make it one of the most productive areas in the world's oceans. Accordingly, interest in this area from commercial fisheries is high. Fisheries in the nBus provide Namibia with a gross annual profit of US\$ 592 million (FAO 2004-12). Fisheries contributed approximately 7% to the GDP and approx. 15% to total export earnings for Namibia in 2005 (FAO 2004-12). However, during the last decades substantial changes have been observed in the nBus. From the 1970s a drastic decline in sardine biomass caused a shift from a sardine-dominated system to a system where a suite of planktivorous fishes, like horse mackerels and pelagic gobies, prevail (Boyer and Hampton 2001). Historically, sardine fisheries have always been important to Namibia, with up to 1.4 million tons caught in 1968 (Boyer and Hampton 2001), however, yields of small pelagics have declined and have remained below 100 000 tons since the early 1980s (van der Lingen et al. 2006, Fig. 1). Accordingly, the importance of small pelagics, like sardines (*Sardinops sagax* (Jenyns 1842) and anchovies (*Engraulis capensis* (Gilchrist 1913)), for fisheries has decreased and larger fishes, like horse mackerels (*Trachurus trachurus* (Linnaeus 1758)) and hakes ((*Merluccius capensis* (Castelnau 1861) and *Merluccius paradoxus* (Franca 1960)), have become more important (van der Lingen et al. 2006). Overexploitation is believed to be accountable for the decline in sardine stocks and physical environmental anomalies, such as the intensification of hypoxic conditions and strong Benguela Niños are believed to be responsible for poor sardine recruitment (Cury and Shannon 2004). Changes in hydrodynamics can directly influence oxygen levels over the shelf. Oxygen levels are a result of several processes, including the decay of organic material on the shelf, the influx of oxygen-depleted water masses from the Angolan dome and well-oxygenated waters from the Cape Basin (cf. Monteiro et al. 2008). Moreover, upwelled low oxygen waters on the inner shelf may interact with naturally or eutrophication-induced hypoxic waters (Stramma et al. 2010). However, Cury and Shannon (2004) showed that climatic driven changes in the environment can lead to alterations at the base of the food web and affect higher trophic levels by favoring e.g. gelatinous organisms. The two jellyfish species *Chrysaora hysoscella* (Linnaeus 1766) and *Aequorea forskalea* (Forsskal 1755, previously *A. aequorea*) became noticeably more abundant (Boyer et al.

2001) and jellyfish biomass is now estimated to exceed the biomass of once abundant fish species (Lynam et al. 2006). Clearly the ecosystem structure and functioning of the NBC has changed over the last three decades (Heymans et al. 2004). The removal of certain species and age classes, due to a overfishing may further shorten the food chain and reduce ecosystem resilience (Hutchings 2000).

In the northern Benguela ecosystem, omnivore euphausiids form a pivotal component of the mesozooplankton community (Barange et al. 1991) and can dominate the zooplankton biomass. They are an important food source for top predators such as fish (Macpherson and Roel 1987, Roel and Macpherson 1988) and facilitate the energy flow between primary production and higher trophic levels (Ekau et al. 2010). Thus, euphausiids may be useful indicators of changes in food web composition and environmental conditions over short- and long-term periods. The lower trophic level of the food web in the nBus is mainly dominated by diatoms, which are preferentially eaten by copepods, like *Calanus carinatus* and euphausiids, like *E. hanseni* and *N. capensis*, which in turn are eaten by horse mackerel and clupeoid fishes (Fig. 3). Changes in krill populations may thus have dramatic impacts on the ecosystem and vice versa, ecosystem changes may seriously affect krill populations.



↑ **Figure 3** Conceptual model of the northern Benguela food web and its trophic interactions (Figure taken from the project proposal of the GENUS project)

Introduction

Krill biology

Euphausiids, or so called krill, are relatively large shrimp-like holoplanktonic, eucaridean crustaceans. The word `krill` refers to the Norwegian word “*krill*” for `small fish` and was first introduced by Norwegian whalers who found these `small fishes` in huge amounts in the stomachs of baleen whales (Nicol 1994). There are 86 species included in the order *Euphausiacea*, distributed between two families and 11 genera (Gibbons 1997). Euphausiid life spans vary with the latitude; subtropical and tropical species have a **life span** of approx. 6-8 months, whereas high latitude species like *E. superba* can live for more than 6 years (Siegel 2000). Krill are exclusively marine and distributed worldwide in high abundances, ranging from tropical to high latitude polar regions (Siegel 2000). While they inhabit various ecosystems with marked differences in environmental conditions, krill are able to quickly react and adapt to changes in their environment (Buchholz 2003). Euphausiids comprise major proportions of the zooplankton biomass in the North Pacific (*Euphausia pacifica* (Hansen 1911)), North Atlantic (*M. norvegica*), Antarctic Ring Ocean (*E. superba*) and NBC (*E. hanseni* and *N. capensis*). A better understanding of krill population parameters will help to assess their importance in the energy flow between trophic levels and to predict the impact of climate variability on euphausiid populations (Siegel 2000). Spatial distribution patterns of euphausiids are influenced by several factors, such as circulation patterns, temperature, recruitment success and behaviour. As **intense vertical migrators**, krill play an important role in the downward transport of carbon. Migrating euphausiids have to cope with large physical variations in their environment, especially temperature, food and oxygen availability. Despite their ecological importance for marine ecosystems, euphausiids have become a target for human exploitation (Siegel 2000) and are mainly processed for the diets of farmed fish (Lalli and Parsons 2006). Euphausiids are generally **omnivorous**. Their diet consists largely of detritus, phytoplankton and a variety of zooplankton species. Larger species, like *E. hanseni*, are also capable of feeding on fish larvae. However, different genera are prone towards either herbivory or carnivory (Pillar et al. 1992).

The general **life cycle** of euphausiids has been studied intensively (e.g. Gurney 1942, Mauchline and Fisher 1969, Iguchi et al. 1993, Pakhomov 2000) and despite minor differences between species, is relatively well understood. Krill are gonochoric. During the mating process a spermatophore is transferred from the male via modified 1st pleopods to the thelycum of the female. The fertilized eggs are either carried in an ovigerous sac (sac spawners) or are directly released into the water column (broadcast spawners). Euphausiids

have a discontinuous development through several larval stages from the first nauplius stage to the last furcilia stage before they reach the juvenile phase, each stage being sub-categorized further. The exact sequence and number of larval stages vary within species and depend on the prevailing environmental conditions. The first larval stages lack developed mouthparts and a digestive tract and are therefore considered “non-feeding” stages. The earliest development of mouthparts can occur at calyptopsis (or furcilia) stages. It would be evolutionary favourable to time these developmental stages to occur during favourable feeding conditions. Krill can have **multiple broods** during a year. The timing and length of the spawning season vary interspecifically but depend strongly on environmental conditions, like temperature and food availability (Siegel 2000, Tarling and Cuzin-Roudy 2003). Euphausiids therefore adapt their reproductive cycles to local feeding conditions and the recruitment success of krill species depends both on adequate condition of the females prior to spawning and favourable trophic conditions during larval development (Tarling and Cuzin-Roudy 2003). According to Mauchline (1980) observing a diversity of larval forms at any given time is an indicator of suboptimal or varying environmental conditions. **Growth** and moulting in euphausiids species under various conditions has been intensively studied (e.g. Mauchline 1985, Buchholz 1985 and 1991, Buchholz et al. 1996, Iguchi and Ikeda 1995, Atkinson et al. 2006 and Pinchuk 2007). Temperature and food availability are the main drivers (Huntley and Boyd 1984). In some euphausiids even shrinking has been observed under adverse conditions (Buchholz 1985, Shaw et al. 2010). Krill are able to quickly react to changing environmental conditions, particularly feeding conditions, by adjusting both their size increment at moult (INC) and the intermoult period (IMP) (Buchholz 2003). Synchronization of moulting within a krill swarm may be a common strategy (Buchholz et al. 1996, Tarling and Cuzin-Roudy 2003), possibly triggered by favourable feeding conditions. A **cyclical coupling of moult and reproduction** typically occurs (Cuzin-Roudy 1993 and 2000) with both processes being influenced by external factors like temperature and nutrition (Cuzin-Roudy and Buchholz 1999). **Metabolic adaptations** in euphausiids have been identified in relation to several internal and external stimuli, including temperature (e.g. Hirche 1983), pressure (e.g. Teal and Carey 1967), light (e.g. Pearcy et al. 1969, Teschke et al. 2007), and nutrition (Buchholz and Saborowski 2000). Thus, metabolism is species-specific and influenced by numerous processes.

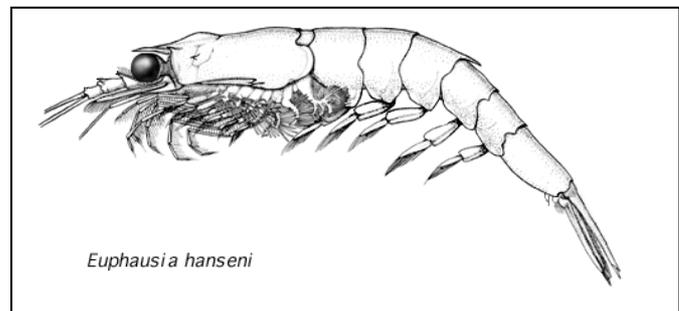
Introduction

Euphausiids of the northern Benguela upwelling ecosystem

In the nBus, krill occupy an important niche as a food source for top predators such as fish (Pillar et al. 1992). For example, *N. capensis* is the dominant food source of young Cape hake (*M. capensis*) and adult horse mackerels (*T. trachurus*) feed almost exclusively on euphausiids (Andronov 1983, Konchina 1986). Anchovies feed predominantly on large zooplankton including euphausiids (James 1987). As omnivores euphausiids are capable of using fluctuating food conditions, characteristic of the NBC, to their advantage (Pillar et al. 1992). They can switch from one food source, such as phytoplankton, to another, such as zooplankton, and thus exploit the NBC plankton community efficiently. However, despite their ecological importance in most of the world's oceans, research attention has not been distributed evenly (Siegel 2000) and knowledge about euphausiids of the nBus is relatively scarce. For example, detailed information on growth, reproduction and metabolism as a function of temperature and oxygen levels are lacking. Of the eight species regularly occurring in this ecosystem, three of them, *Euphausia hanseni*, *Nematoscelis megalops* and *Nyctiphanes capensis* show high abundances and biomasses (Barange and Stuart 1991, pers. obs.) and will be described in more detail below.

Euphausia hanseni (Zimmer 1915):

Despite its high abundance and biomass in the northern Benguela upwelling ecosystem, relatively little is known about this species. The only publications about adults of *E. hanseni* (Fig.4) concern its reproductive potential (Stuart and Nicol 1986), diet and feeding (Barange et al. 1991), distribution patterns (Barange and



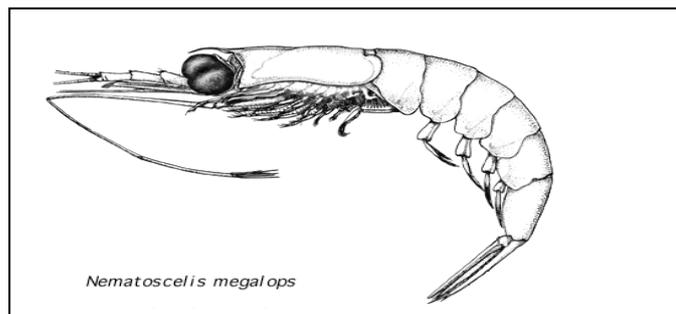
↑ **Figure 4** Lateral view of *Euphausia hanseni* (Baker, Boden and Brinton 1990)

Stuart 1991, Barange et al. 1992), maintenance mechanisms (Barange and Pillar 1992), and diel vertical migration behaviour (Barange 1990). *E. hanseni* is a comparably large species (adults: 19-27mm and up to 33mm in the nBus (pers. obs.)) with a life span of at least 6 months (Barange and Stuart 1991, own observations: > 1 year). *E. hanseni* attains a maximum body size in September, coinciding with the maximum intensity of upwelling, and maximum densities coincide with seasonal warming (Barange and Stuart 1991). It lives in the coastal south-east Atlantic from 26°N to 33°S. In the nBus, females reach a larger size than males. *E.*

hanseni is an omnivorous species, with swarms strongly localized around the shelf break and most animals occurring at depths of 200-1000m (Barange and Stuart 1991). Feeding appendages (mouthparts) in *E. hanseni* are well adapted for filter feeding (Mauchline and Fisher 1969) mainly on copepods, dinoflagellates and tintinnids. However, prey selection in *E. hanseni* is basically passive (Barange et al. 1991). It performs pronounced diel vertical migration (up to 400m) with different night-time vertical distributions differing between sexes (Barange 1990). Accordingly, this species encounters a wide range of ambient temperatures and oxygen levels, which can strongly affect its metabolism, physiological performance and behaviour. *E. hanseni* shows continuous breeding with broods comprised of relatively large eggs, which are broadcast after fertilization into the water column (Barange and Stuart 1991). Its larval development is comparable to that of other neritic and shelf species (Barange 1990).

Nematoscelis megalops (G.O. Sars 1883):

Possibly due to its wide distribution range, *N. megalops* (Fig. 5) is comparably well studied. Various papers about its abundance and distribution patterns (e.g. Mauchline and Fisher 1969, Lindley 1982, Wiebe and Boyd 1979, Cartes et al. 1994, Gibbons et al. 1995), its dietary preferences (e.g. Barange et al. 1991, Gurney et al. 2001 and 2002, Richoux



↑ **Figure 5**
Lateral view of *Nematoscelis megalops*
(Baker, Boden and Brinton 1990)

2011) and its biochemical (elemental) composition (e.g. Gorsky et al. 1988, Lindley et al. 1999) have been published. However, data about *N. megalops* in the nBus and physiological adaptations to environmental conditions, in general, are still missing.

N. megalops occurs in the warm-temperate belts of the north and south Atlantic. It has been sampled in the Indian Ocean, south Pacific, and it appears also in the Mediterranean Sea. Migration across the Gulf Stream has led to isolated populations in northwestern Atlantic cold-core rings (Wiebe and Flierl 1983). Recently, *N. megalops* was observed in a high Arctic fjord in West Spitsbergen (Buchholz et al. 2010). The horizontal distribution of *E. hanseni* and *N. megalops* in the nBus largely overlaps (Barange et al. 1991). Adults of *N. megalops* achieve a length of 22-26mm and several publications report a high degree of carnivory in this species (e.g. Barange et al. 1991, Gurney et al. 2002), which is further supported by their

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extremely elongated 2nd thoracic leg equipped with a group of long, straight, distal spines (Fig. 6).

These elongated limbs and into two lobes divided eyes are supposed to represent adaptations to carnivorous feeding (Mauchline 1980). In contrast to their feeding ecology their DVM behaviour is still unclear (Wiebe et al. 1982, Roe et al. 1984, Barange 1990), but preliminary reports regard *N. megalops* as a weak migrant. Larval development consists of four different phases: metanauplius, calyptopsis, furcilia and juvenile (Gopalakrishnan 1975).

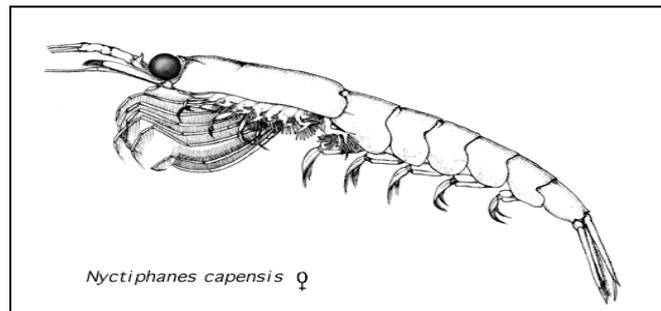


↑ **Figure 6**

View of distal end of the 2nd thoracic leg of *N. megalops* equipped with spines

Nyctiphanes capensis (Hansen 1911):

N. capensis (Fig. 7) shows extraordinarily high abundances over the Namibian shelf. However, as in the case for *E. hanseni*, knowledge of its autecology is relatively poor. Most publications to date studied its distribution patterns (Barange and Stuart 1991, Barange and Pillar 1992, Barange and Boyd 1992, Cornew et al. 1992), life-history (Barange and Boyd 1992, Pillar 1985, Stuart and Nicol 1986), trophic



↑ **Figure 7**

Lateral view of *Nyctiphanes capensis* female (Baker, Boden and Brinton 1990)

ecology (Pillar et al. 1992) and DVM (Barange and Pillar 1992). This coastal species regionally dominates the NBC zooplankton community and occurs in shelf and slope waters to about 250m. *N. capensis* abundance considerably drops near the shelf-break (Pillar and Stuart 1988, Barange and Stuart 1991, Barange and Pillar 1992). It can be encountered in the South African coastal region, Cape Verde Island, Andreu and Cape Blanc. Adults reach a size of 12-21mm in the NBC, which is in contrast to other areas, where the maximum size recorded was only 13mm total length (Barange and Stuart 1991). Their life span is estimated to be approximately 6 months (Barange and Stuart 1991). Males are larger than females, suggesting different growth rates or a different life span. Maximum body sizes in *N. capensis*, like in *E. hanseni*, coincide with the maximum intensity of upwelling and maximum densities

with seasonal warming (Barange and Stuart 1991). Distribution patterns remain constant throughout the year and biomass is compared to other euphausiid species in temperate regions, exceptionally high (Barange and Stuart 1991). *N. capensis*, like *E. hanseni*, displays continuous breeding in the nBus. However, in *N. capensis* the spawning frequency is lower and spawning takes place in neritic waters with eggs carried in a brood pouch until they are released as metanauplii into the water column (Barange and Stuart 1991). As suggested by these authors *N. capensis* females may use cross-shelf transport for spawning to maintain high abundances in this area. DVM behaviour is still uncertain. Due to its high biomass this species is an important diet for several fish species that occur in this area.

Introduction

Objectives

The results and conclusions of this thesis are presented in the following chapters and in the form of four manuscripts and one additional chapter dealing with seasonal adaptations of *E. hanseni* to low oxygen levels. This is the first study focusing on the impact of environmental conditions on the physiology of euphausiids in the nBus. Emphasis is laid on the influence of the nutritionally poly-pulsed system on the behaviour and the physiology of *E. hanseni*. Further attention was turned on the assessment of the position of krill in the food web of the NBC. Attached are the abstracts of two additional papers concerning the impact of climate change on krill species in the Arctic and Antarctic. These papers are not principal parts of this thesis; however, they highlight the importance of krill species in various ecosystems and point out the impact of climate change on euphausiids. Both papers were written during my PhD studies.

The central aims of this thesis are:

- **Evaluation of the effect of temperature on the metabolic activity of krill species**
- **Description of the vertical migration behaviour of euphausiids as a function of temperature, oxygen and food availability**
- **Determination of physiological adaptations to nutritionally poly-pulsed systems**
- **Assessment of the trophic position of krill species in the NBC**
- **Estimation of the anaerobic capacity of *E. hanseni***

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Nematoscelis megalops female with eggs, caught during a cruise with the RRS *Discovery* during winter 2010.

Thermal constraints on the respiration and excretion rates of krill, *Euphausia hanseni* and *Nematoscelis megalops*, in the northern Benguela upwelling system off Namibia

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Abstract

Rates of respiration and ammonia excretion of *Euphausia hanseni* and *Nematoscelis megalops* were determined experimentally at four temperatures representative of conditions encountered by these species in the northern Benguela upwelling environment. The respiration rate increased from 7.7 $\mu\text{mol O}_2 \text{ h}^{-1} \text{ g}_{\text{ww}}^{-1}$ at 5 °C to 18.1 $\mu\text{mol O}_2 \text{ h}^{-1} \text{ g}_{\text{ww}}^{-1}$ at 20 °C in *E. hanseni* and from 7.0 $\mu\text{mol O}_2 \text{ h}^{-1} \text{ g}_{\text{ww}}^{-1}$ (5 °C) to 23.4 $\mu\text{mol O}_2 \text{ h}^{-1} \text{ g}_{\text{ww}}^{-1}$ (20 °C) in *N. megalops*. The impact of temperature on oxygen uptake of the two species differed significantly. *N. megalops* showed thermal adaptations to temperatures between 5 °C and 10 °C ($Q_{10}=1.9$) and metabolic constraint was evident at higher temperatures ($Q_{10}=2.6$). In contrast, *E. hanseni* showed adaptations to temperatures of 10-20 °C ($Q_{10}=1.5$) and experienced metabolic depression below 10 °C ($Q_{10}=2.6$). Proteins were predominantly metabolized by *E. hanseni* in contrast to lipids by *N. megalops*. Carbon demand of *N. megalops* between 5 and 15 °C was lower than in *E. hanseni* versus equal food requirements at 20 °C. It is concluded that the two species display different physiological adaptations, based on their respective temperature adaptations, which are mirrored in their differential vertical positioning in the water column.

Chapter 1

Introduction

The Benguela Current off the west coast of southern Africa is one of four major upwelling systems in the world with the northern Benguela off Namibia (16 - 26° S) being characterized by strong upwelling events throughout the year, showing a maximum in austral winter and a minimum in summer (Hutchings et al. 2009). It is a typical coastal upwelling system with equatorward winds, cool nutrient-rich water, high plankton biomass and moderate to high fish stocks (Hutchings et al. 2009). Substantial environmental changes, such as the intensification of hypoxic conditions (Ekau et al. 2010) are currently taking place. Within the Benguela Current system euphausiids form a pivotal component of the zooplankton and an important food source for top predators such as fish (Macpherson and Roel 1987, Roel and Macpherson 1988). Changes in this ecosystem may have far-reaching effects on fish-stock dynamics (Barange and Stuart 1991). Euphausiids can play a crucial role in regions affected by Oxygen Minimum Zones (OMZ), including the northern Benguela Current system, because some species can tolerate hypoxic conditions (Antezana 2009).

In terms of biomass and abundance, the euphausiid *Euphausia hanseni* (Zimmer 1915) is the major krill species in the northern Benguela and partly shares its habitat with another euphausiid species, *Nematoscelis megalops* (Sars 1883) (Barange 1990, Barange et al. 1991). Both species show pronounced seasonal and interannual fluctuations in abundance and biomass in this region (Buchholz pers. obs.). *E. hanseni* is associated with the shelf break whereas *N. megalops* is indicative of the transition between the shelf and the open ocean. According to Barange (1990) and Barange et al. (1991), horizontal distributions largely overlap and they display vertical space partitioning that is likely to reduce competition. *E. hanseni* shows pronounced diel vertical migration (DVM) with an amplitude of up to 400 m, *N. megalops* displays only limited DVM with the thermocline as the upper migration limit (Barange 1990, Buchholz and Werner unpublished data). During descent and ascent, *E. hanseni* experiences a wider temperature range (approx. 8-20 °C, depending on the upwelling phase) than *N. megalops* (approx. 8–10 °C). In addition, *E. hanseni* is reported to be a filter feeder in the upper mixed layer, whereas *N. megalops* is an active hunting species below the thermocline (Barange et al. 1991). The differential migratory behaviours of the two species are reflected in their cyclic feeding behaviours and food preferences (Barange et al. 1991).

Temperature is one of the most important factors affecting the respiration rate of zooplankton in general (Ikeda 1974, Ikeda and Motoda 1978, Vidal 1980, Ikeda et al. 2000, Castellani et al. 2005), particularly euphausiids (Buchholz and Saborowski 2000). Knowledge about the

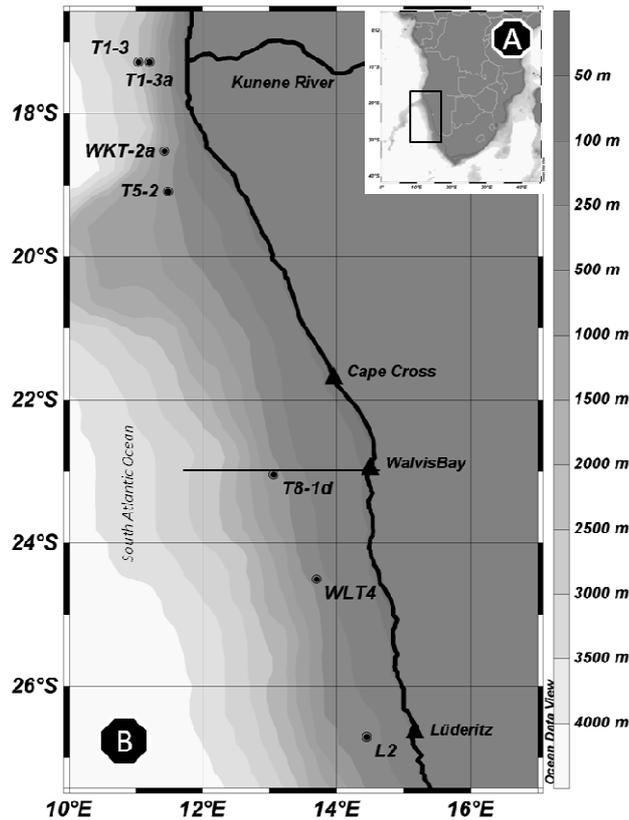
respiration rate of a species at different temperatures is important because it provides basic indications of its metabolic requirements in different environments (Castellani et al. 2005). Euphausiids may encounter both spatial (seasonal) and temporal (during DVM) differences in temperature (Spicer and Saborowski 2010). Accordingly, it is not surprising that the impact of temperature on metabolism (i.e. oxygen uptake) and excretion rates of many euphausiids has been intensively studied. However, few studies have been conducted over a wide temperature range (Mayzaud 1973, Sameoto 1976, Strömberg and Spicer 2000, Saborowski et al. 2000 and 2002) and data on the metabolism of *E. hanseni* and *N. megalops* are lacking.

In this study the effects of temporal temperature variations on oxygen uptake, ammonia excretion rate (NH₄-N) and O:N ratio of these two euphausiids were measured experimentally to determine their metabolic level and to discern differences in acclimation and adaptation strategies within their respective ambient temperature regimes. Furthermore, we calculated their Q₁₀ values and their energy and carbon requirements and related these to their respective migration behaviour.

Material and Methods

Field sampling - Krill was collected during Cruise MSM17-3 on board the RV *Maria S. Merian* between Lüderitz (26.6° S) and Kunene (17.25° S) in late austral summer of 2011 (30 January – 7 March) under low to moderate upwelling conditions. The animals were caught at different depths at six stations (Fig. 1) during nighttime with a 1-m² MOCNESS (Multiple Opening and Closing Net with Environmental Sensing System) with a large mesh size (2000 µm) fitted with a large, soft, closed cod-end bucket to reduce stresses on the experimental animals.

Immediately after capture the animals were gently transferred into aerated 20 l plastic aquaria filled with sea water at *in situ* temperatures (8-10 °C). After approximately 12 h of acclimation, animals in good condition were selected for respiration measurements.



↑ **Figure 1** Location of the northern Benguela upwelling system in the Southwest Atlantic Ocean (inset A) and the station positions sampled for *E. hanseni* and *N. megalops* (B). Circles denote locations of sample stations, triangles geographic landmarks. The black line indicates the position of the hydrographic transect.

Metabolic measurements – On-board respiration measurements were performed using a closed respirometry system with oxygen-microsensors (PreSens, Germany) and a 4-channel micro-fiber optic oxygen transmitter (Oxy-4-micro, PreSens, Germany). Small tube-shaped chambers (volume 20 ml) were used as respiration chambers optimized for krill; they were specially designed to measure the respiration rate of a single specimen and to reduce possible differences in individual swimming activity. Whereas the length of the chambers (60 mm) allowed to some extent back- and forward movements of the animals (15 - 28 mm) their small diameter (20 mm) prevented appreciable up- and downward movements. Trial measurements showed that substantial differences in activity of the krill were avoided without causing stress to the animals. These results were considered to be the normal metabolism of the animals, i.e. `the oxygen consumption rate measured with uncontrolled but minimum motor activity of the specimens`, according to Ikeda et al. (2000).

To assess the metabolic rates of the two krill species under ambient thermal conditions, respiration measurements were conducted for 3-6 h, down to approx. 60% oxygen saturation, at four temperatures (5, 10, 15 and 20 °C), covering the range of average water temperatures in the northern Benguela between 400 m depth and the sea surface. Before starting an experiment, the specimens were acclimated to the experimental temperature for 1-4 h. The acclimation period chosen was a compromise between their natural migration behaviour (in the case of *E. hanseni*) on the one hand and avoidance of a temperature shock (*N. megalops*) on the other. In order to minimize bacterial oxygen consumption and/or nutrient excretion, filtered seawater (0.2 µm Acropak™ 1000 Capsule, Pall Filtersystems GmbH, Germany) was used in all experiments, which were conducted in the dark to standardize the measurements and to avoid activity differences due to changing light conditions. Three animals and one control (without animal) were tested in each experiment. In addition, all measurements were performed under constant thermal conditions in a temperature-controlled room. A water bath controlled by a laboratory cooler (Julabo F25, Germany) provided stable temperatures. The sampling stations, experimental temperatures and the number of replicates for *E. hanseni* and *N. megalops* are given in Table 1. A comparison of respiration rates of animals from different parts of the study area, showed no regional affect on the rates (Mann-Whitney U-test or ANOVA, depending on the number of groups (stations)). Given that *N. megalops* is a weak migrator and avoids the thermocline and remains resident close to 200 m during the day, whereas *E. hanseni* exhibits pronounced vertical migration, typically between a depth of 30 m at night and 200 m during day time, the two species therefore experience different water temperatures (Werner and Buchholz, pers. obs.). Accordingly, temperatures at depth vary by ≤ 3.0 °C between stations, but vertical excursions of *E. hanseni* result in a regional temperature differential of max. 5.6 °C for half of the day. Therefore, to avoid acclimatory differences in respirations rates of krill from different regions, specimens were maintained at temperatures around 10 °C for 12h. This ensured similar conditions for all animals tested. In addition, the animals were acclimated to the experimental temperatures for another 1-4 h.

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↓ **Table 1** Experimental temperatures and the number of replicates at each of the sampling stations of *E. hanseni* and *N. megalops* in the northern Benguela system (see Figure 1). The water column temperatures at approximate daytime residence depths of the two species are also given.

	Sampling stations				
<i>E. hanseni</i>	L2	WLT 4	T8-1d	T1-3a	n
5°C	4	9	7		20
10°C		7	5		12
15°C		8			8
20°C		5		10	15
Temperature at 30 m	15.2	15.5	18.5	20.8	
Temperature at 200	10.5	11.4	11.7	13.5	
<i>N. megalops</i>	WKT- 2a	T5-2	T1-3	T1-3a	
5°C			6		6
10°C	4	4	5	6	19
15°C		5	6	6	17
20°C				6	6
Temperature at 200	12.6	12.2	13.0	13.5	

After each experiment, specimens were frozen at -80 °C for subsequent determination of their size, sex and fresh weight in order to examine possible allometric and/or sex-dependent differences. To allow a comparison between different size or weight classes the respiration rates were normalized to a 'standard krill' of 1 g wet weight. To describe the relationship between respiration rate and temperature the van't Hoff rule (van't Hoff 1884) was used, which is described by the following Q_{10} approximation:

$$Q_{10} = (k_1/k_2)^{10/(t_1-t_2)}$$

where respiration rates k_1 and k_2 correspond to temperatures t_1 and t_2 (Prosser 1961). Subsamples of 2 ml were taken from the same respiration chambers, using a small plastic pipette for ammonia ($\text{NH}_4\text{-N}$) determination, providing simultaneous measurements of respiration and excretion rates were possible. The samples were immediately frozen in liquid nitrogen for subsequent laboratory analysis on land using the phenol-hypochlorite method according to Solorzano (1969). The respiration and excretion rates were used to calculate the atomic O:N ratio in order to distinguish between protein- and lipid-oriented metabolism (Mayzaud and Conover 1988). The carbon and calorific units were then calculated according to Gnaiger (1983) in order to assess the carbon and energy demands of each species at

different temperatures. Minimum food requirements were calculated by converting oxygen uptake from metabolic rates to units of carbon (Ikeda et al. 2000), using the equation:

$$\text{ml O}_2 (\text{g}_{\text{ww}} \text{ h})^{-1} \times \text{RQ} \times 12/22,4 = \text{mg C} (\text{g}_{\text{ww}} \text{ h})^{-1}$$

where RQ represents the `respiratory quotient`, the molar ratio of carbon dioxide produced to oxygen consumed (0.71 for lipids, 1.0 for carbohydrates and 0.84-0.97 for proteins, depending on the excretory end-product) and where 12/22,4 is the weight of carbon in one mole (22,4 l) of carbon dioxide. These data were multiplied by 24 to standardize the carbon demand per 24 h day. The nitrogen losses per day (N_{adj}) were calculated using the following equation:

$$N_{\text{adj}} (\mu\text{g g}_{\text{ww}}^{-1} \text{ d}^{-1}) = E_{\text{adj}} (\mu\text{mol NH}_4\text{-N h}^{-1} \text{ g}_{\text{ww}}^{-1}) * 24 * 14$$

where E_{adj} is the excretion rate measured, multiplied by 24 to consider the daily loss and multiplied by 14 (the molar mass of N) to convert μmol to μg . Assuming a dry weight (dw) of 25% wet weight (ww) in both species, these nitrogen losses can be related to the nitrogen content of the animals (N %dw) and expressed as N_{adj} (% body N d^{-1}).

Chemical composition - In the land-base laboratory the cephalothorax and the first abdominal segment of the frozen specimens were weighed on a microbalance (Sartorius LA 230 S, Germany; $d=0.1$ mg) and freeze-dried for 24 h using an ALPHA 1-4 LSC freeze-drier (Christ GmbH, Germany). The other parts of the animal's body were used for biochemical analyses (data not shown here). The samples were ground with a tapered glass pestle. For C/N analysis (Euro EA CHNS-O elemental analyzer, HEKAtech GmbH, Germany), 1-2 mg of powdered samples were used. For comparison between species, the nitrogen (N %dw), carbon content (C %dw) and the ratio of carbon to nitrogen by mass (C:N) was calculated. After 12h of acclimation time, the stomach and gut contents of the specimens were evacuated, ensuring that the C/N measurements were not biased.

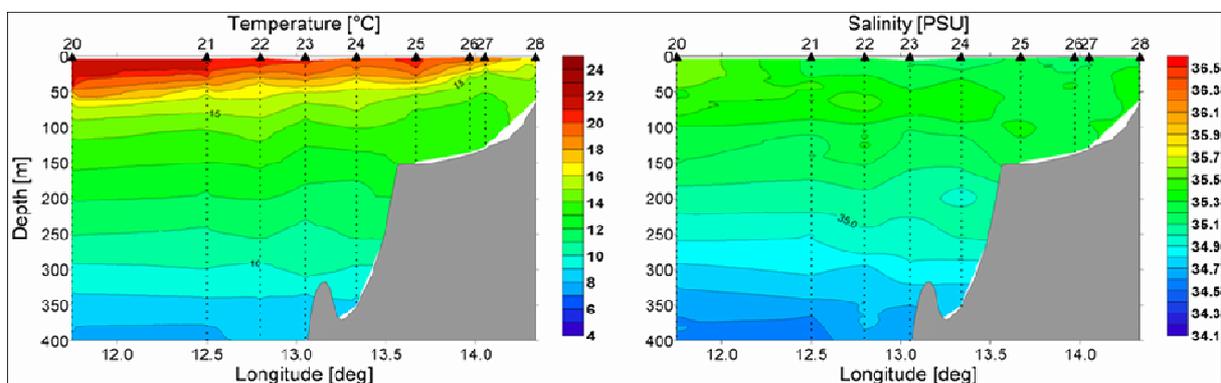
Statistical treatment of data - For statistical analyses, a t-test or a one-way analysis of variance (ANOVA) with Tukey's multiple comparison *post hoc* test was performed. Data were tested for normality using a D'Agostino and Pearson omnibus normality test and/or a Shapiro-Wilk normality test. If the data failed these tests, they were normalized using log-transformation. If it was not possible to normalize the data, a Mann-Whitney U-test was used. For analysing the effect of two factors a two-way ANOVA was performed. Analysis of

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covariance (ANCOVA) was used to test interspecific differences in the effect of temperature on respiration rate. The significance level was generally set at $p < 0.05$ and the SD of the mean is used throughout.

Results

Hydrography – A detailed description of the hydrographic conditions during the cruise is provided in Lahajnar et al. 2011. In general, surface water temperatures showed a west-east gradient, with cooler water masses occurring along the coast indicating weak coastal upwelling. In addition, there was a north-south gradient due to the transition from tropical conditions in the north, near Kunene (17.25° S; approx. $18-24^{\circ}$ C), to subtropical waters south of the area of investigation (26.6° S; approx. $15-19^{\circ}$ C). The off-shore waters north of 20° S had higher seasonal temperatures than normal (Lahajnar et al. 2011). The vertical distributions of temperature and salinity along the Walvis Bay transect (22.57° S) during 5-9 February 2011 are shown in Fig. 2. Sea surface temperature (SST) ranged from approximately 22° C in the open ocean to 18° C near the coast. The depth of the mixed layer decreased eastward from 50 m to 20 m. Only weak active upwelling cells were observed near the coast along this transect in a thin layer of about 40 m (Lahajnar et al. 2011).

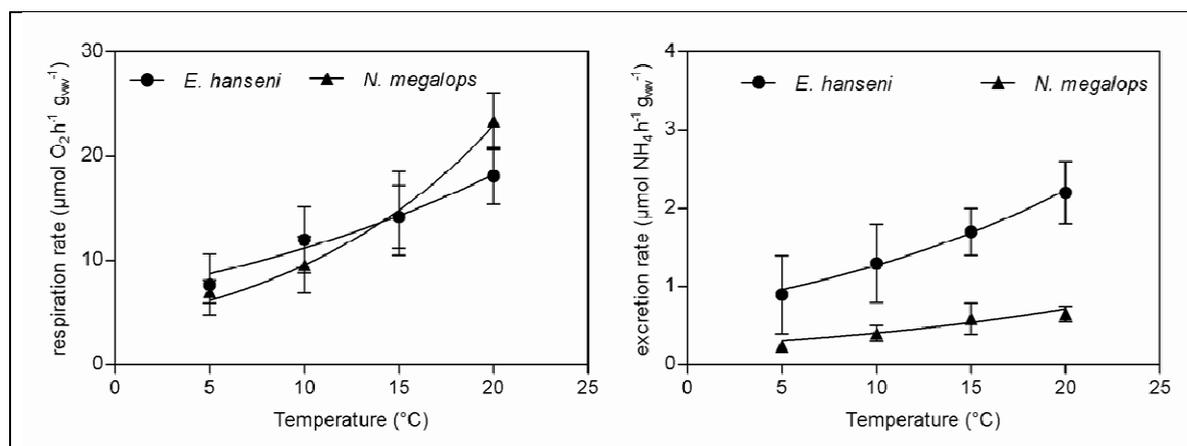


↑ **Figure 2** Temperature and salinity sections along the Walvis Bay transect. Image is based on CTD data (Lahajnar et al. 2011).

Allometric effects and sex differences – There were no significant differences between sexes in terms of body mass, oxygen uptake, excretion rate and O:N ratio (Mann Whitney U-test, $p > 0.05$). Male and female data were therefore pooled for subsequent analysis.

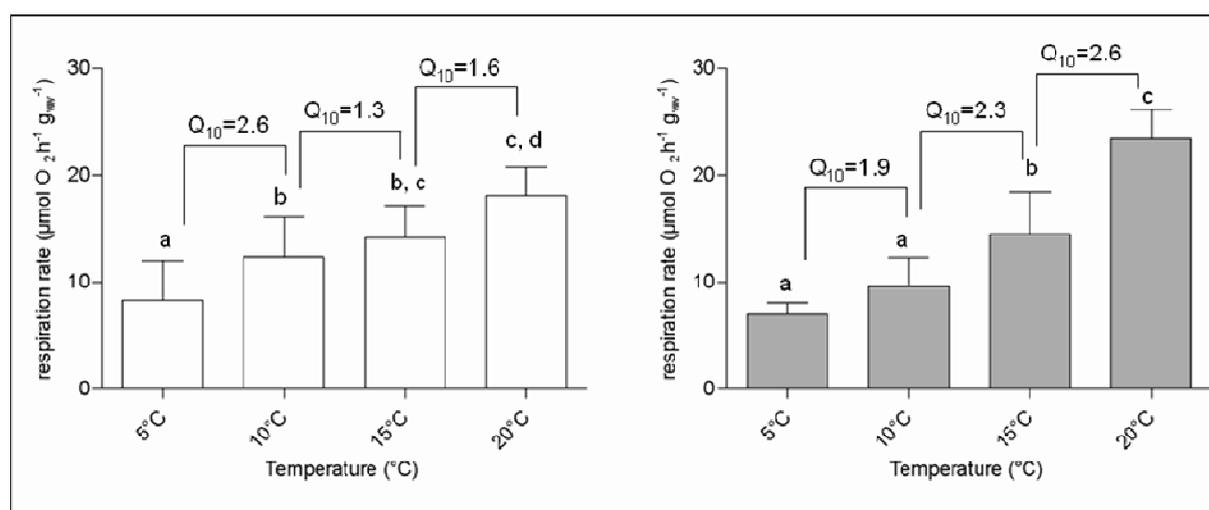
Temperature effects on the oxygen uptake and ammonia excretion rate – There were no regional differences in the respiration rates in animals from different sampling stations (Mann-Whitney U-test or ANOVA, $p > 0.05$). Accordingly, the data were pooled. In both

species there was a pronounced effect of temperature on respiration and excretion rates (Fig.3).



↑ **Figure 3** Non-linear regressions of oxygen uptake ($\mu\text{mol O}_2 \text{ h}^{-1} \text{ g}_{\text{ww}}^{-1}$, means \pm SD, left) and excretion rates ($\mu\text{mol NH}_4 \text{ h}^{-1} \text{ g}_{\text{ww}}^{-1}$, means \pm SD, right) of *E. hanseni* (n=8-20) and *N. megalops* (n=6-19) at different experimental temperatures. The relationships presented here are based on mean values. Error bars denote SD.

In *E. hanseni* the oxygen uptake increased from a mean of $7.7 \pm 2.9 \mu\text{mol O}_2 \text{ h}^{-1} \text{ g}_{\text{ww}}^{-1}$ at 5 °C to $18.1 \pm 2.7 \mu\text{mol O}_2 \text{ h}^{-1} \text{ g}_{\text{ww}}^{-1}$ at 20 °C (ANOVA, $F=18.96$, $df=55$, $p<0.001$). In *N. megalops* the respiration rate increased from a mean of $7.0 \pm 1.1 \mu\text{mol O}_2 \text{ h}^{-1} \text{ g}_{\text{ww}}^{-1}$ (5 °C) to $23.4 \pm 2.7 \mu\text{mol O}_2 \text{ h}^{-1} \text{ g}_{\text{ww}}^{-1}$ (20 °C) (ANOVA, $F=26$, $df=46$, $p<0.001$) (see Fig. 4). The Q_{10} value was 1.8 for *E. hanseni* and 2.2 for *N. megalops* between 5 °C and 20 °C respectively (Figure 4).



↑ **Figure 4** Weight-specific respiration rates ($\mu\text{mol O}_2 \text{ h}^{-1} \text{ g}_{\text{ww}}^{-1}$) of *E. hanseni* (left) and *N. megalops* (right) versus temperature (T, °C). Groups were tested by one-way ANOVA. Different letters indicate significant differences between temperatures (ANOVA with Tukey's Multiple Comparison Test). Q_{10} values are given for each temperature pair, error bars denote SD.

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Generally, the oxygen uptake in *N. megalops* was slightly lower compared with that of *E. hanseni* at temperatures between 5–10 °C. At 10 °C, oxygen uptake was significantly lower in *N. megalops* than in *E. hanseni* (t-test, $t=2.528$, $df=37$, $p<0.05$). At 15 °C, both species had the same oxygen consumption (t-test, $t=0.0198$, $df=23$, $p>0.05$). The oxygen uptake at 20 °C was significantly higher in *N. megalops* than in *E. hanseni* (t-test, $t=0.023$, $df=23$, $p<0.01$). The influence of temperature on the oxygen uptake differed between species, the steeper slope in *N. megalops* indicating a stronger influence at increasing water temperatures (ANCOVA, $F=10.12$, $df=98$, $p=0.0002$; Figure 3). A summary of the respiration and excretion rates and O:N ratios at different temperatures is given in Table. 2.

↓ **Table 2** Respiration rates (R_{adj}), excretion rates (E_{adj}) and O:N ratios of *E. hanseni* and *N. megalops* at different experimental temperatures. Given are the means \pm SD and the number of replicates (n) in parentheses. ww = wet weight. NS: not significant. Asterisks indicate significant differences between species (*, $p\leq 0.05$; **, $p\leq 0.01$; ***, $p\leq 0.001$).

Temperature (°C)	<i>E. hanseni</i>			<i>N. megalops</i>		
	R_{adj} ($\mu\text{mol O}_2 \text{ h}^{-1}$ $\text{g}_{\text{ww}}^{-1}$)	E_{adj} ($\mu\text{mol NH}_4\text{-N h}^{-1}$ $\text{g}_{\text{ww}}^{-1}$)	O:N	R_{adj} ($\mu\text{mol O}_2 \text{ h}^{-1}$ $\text{g}_{\text{ww}}^{-1}$)	E_{adj} ($\mu\text{mol NH}_4\text{-N h}^{-1}$ $\text{g}_{\text{ww}}^{-1}$)	O:N
5	$7.7\pm 2.9^{\text{NS}}$ (12)	$0.9\pm 0.5^{**}$ (8)	22 (8)**	7.0 ± 1.1 (6)	0.3 ± 0.03 (6)	59 (6)
10	$12.4\pm 3.7^*$ (20)	$1.3\pm 0.5^{***}$ (20)	22 (20)***	9.6 ± 2.7 (19)	0.4 ± 0.1 (19)	53 (19)
15	$14.2\pm 3.0^{\text{NS}}$ (8)	$1.7\pm 0.3^{***}$ (8)	17 (8)***	14.5 ± 4.0 (17)	0.6 ± 0.2 (17)	56 (17)
20	$18.1\pm 2.7^{**}$ (15)	$2.2\pm 0.4^*$ (6)	15 (6)**	23.4 ± 2.7 (6)	0.7 ± 0.1 (6)	73 (6)

There was an increase in the ammonia excretion with increasing temperature in both species (Figure 3). The excretion rate fluctuated between a mean of $0.9 \mu\text{mol NH}_4\text{-N h}^{-1} \text{g}_{\text{ww}}^{-1}$ (SD 0.5) and $2.2 \mu\text{mol NH}_4\text{-N h}^{-1} \text{g}_{\text{ww}}^{-1}$ (SD 0.4) in *E. hanseni* at 5 and 20 °C respectively (ANOVA, $F=9.54$, $df=39$, $p<0.001$). In *N. megalops*, the values ranged between 0.24 (SD 0.03) and $0.65 \mu\text{mol NH}_4\text{-N h}^{-1} \text{g}_{\text{ww}}^{-1}$ (ANOVA, $F=12.58$, $df=46$, $p<0.01$). There was a significantly lower overall excretion in *N. megalops* than in *E. hanseni* (unpaired t-test, $t=3.597$, $df=6$, $p<0.05$). The Q_{10} value for excretion is 1.8 in *E. hanseni* and 1.9 in *N. megalops*. There was no effect of temperature on the atomic O:N ratio; however, significant differences were found between the two species (2-way ANOVA, $F=105.1$, $df=80$ (row

factor), $p < 0.0001$, Table 2). A mean O:N ratio of 20 (SD 9) was calculated for *E. hanseni* and 57 (SD 21) for *N. megalops*. A high variability of the O:N ratio was found in both species. This ratio ranged between 9 and 49 in *E. hanseni* and between 29 and 118 in *N. megalops*.

Elemental composition – Both species had the same N content, but differed in both C content (unpaired t-test, $t=3.511$, $df=46$, $p=0.001$) and C:N ratio (unpaired t-test, $t=3.086$, $df=46$, $p=0.0034$, Table 3).

↓ **Table 3** Carbon (C) and nitrogen (N) content and C:N (by mass) ratios of *E. hanseni* and *N. megalops* in the northern Benguela during February and March 2011. Given are the means \pm SD and the number of replicates (n) in parentheses. ww = wet weight. NS: not significant. Asterisks indicate significant differences between species (**, $p \leq 0.01$).

	<i>E. hanseni</i>	<i>N. megalops</i>
C (%dw)	40.4 \pm 1.5** (24)	41.8 \pm 1.1 (24)
N (%dw)	11.2 \pm 0.6 ^{NS} (24)	11.3 \pm 0.3 (24)
C:N	3.6 \pm 0.1** (24)	3.7 \pm 0.1 (24)

The nitrogen content in *E. hanseni* ranged between 10.4 and 12.6 %dw (mean \pm SD = 11.2 \pm 0.6) and between 10.8 and 11.9 %dw (11.3 \pm 0.3) in *N. megalops*. The carbon content differed significantly between species (unpaired t-test, $t=3.511$, $df=46$, $p=0.001$), ranging from 39.5 to 43.2 %dw (40.4 \pm 1.5) in *E. hanseni* and from 38.5 to 43.8 %dw (41.8 \pm 1.1) in *N. megalops*. The C:N ratio by mass also differed significantly between species (unpaired t-test, $t=3.086$, $df=46$, $p=0.0034$) ranging from 3.3 to 3.8 (3.6 \pm 0.1) in *E. hanseni* and from 3.6 to 3.8 (3.7 \pm 0.1) in *N. megalops*.

Diel variation in energy demand – Oxygen consumption was converted to carbon and calorific units according to Gnaiger (1983), based on a RQ of 0.97 in *E. hanseni*, with an O:N ratio of <24 , indicative of a protein-dominated metabolism (Ikeda 1974) with ammonia as the excretory end product, and a RQ of 0.72 in *N. megalops*, with an O:N ratio of >24 , indicative of a lipid-oriented metabolism. The energy demand in *E. hanseni* varied from 81.6 J d⁻¹ g_{ww}⁻¹ at 5 °C to 194.4 J d⁻¹ g_{ww}⁻¹ at 20 °C and in *N. megalops* from 74.4 J d⁻¹ g_{ww}⁻¹ to 249.7 J d⁻¹ g_{ww}⁻¹. Expressed in calorific units, this was 19.2 cal d⁻¹ g_{ww}⁻¹ for *E. hanseni* at 5 °C up to 45.6 cal d⁻¹ g_{ww}⁻¹ at 20 °C and 16.8 cal d⁻¹ g_{ww}⁻¹ to 59.1 cal d⁻¹ g_{ww}⁻¹ respectively for *N. megalops*.

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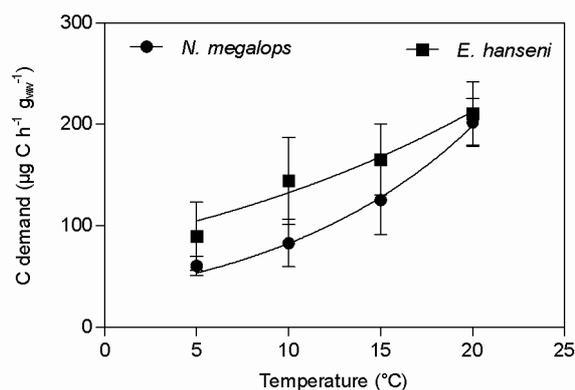
A summary of the energy demand expressed in joules (J) of the two species is given in Table 4.

↓ **Table 4** Energy demand (ED_{adj}), carbon requirements (CA_{adj}) and nitrogen losses (N_{adj}) of *E. hanseni* and *N. megalops* at different experimental temperatures ($^{\circ}C$).

Temperature ($^{\circ}C$)	<i>E. hanseni</i>			<i>N. megalops</i>		
	ED_{adj} ($J d^{-1} g_{ww}^{-1}$)	CA_{adj} ($mg C d^{-1} g_{ww}^{-1}$)	N_{adj} (% body N d^{-1})	ED_{adj} ($J d^{-1} g_{ww}^{-1}$)	CA_{adj} ($mg C d^{-1} g_{ww}^{-1}$)	N_{adj} (% body N d^{-1})
5	81.6	2.2	1.1	73.8	1.5	0.3
10	132.0	3.5	1.6	101.1	2.0	0.5
15	151.2	4.0	2.1	153.0	3.0	0.7
20	194.4	5.1	2.7	247.4	4.9	0.8

The migratory behaviour of *E. hanseni* and *N. megalops*, as observed by Barange (1990) and Barange et al. (1991), served as a pattern to assess the diel variability in the energy demand of the two species, assuming a temperature profile depicted in Figure 2 with a hypothetical SST of around $20^{\circ}C$. Our observations showed that *E. hanseni* descended within 2 h from the surface to 400 m at dawn where they spent the day. It was assumed that during the migration period euphausiids had a 2.7 times higher respiration rate than at rest (Torres and Childress 1983, Buskey 1998). Our calculations were based on the assumption that *E. hanseni* remains for 12 h at 400 m depth at a temperature close to $10^{\circ}C$ during the day and that it takes another 2 h to migrate to the surface where they remain during the the night (8 h) at a temperature of around $20^{\circ}C$. As proposed by Geller (1986), a major advantage of diel vertical migration (DVM) is to gain energy that is not available in the cold, deep waters, e.g. to avoid delayed development at lower temperatures. If *E. hanseni* were to stay permanently in the upper layers at approx. $20^{\circ}C$, it would consume $434 \mu mol O_2 d^{-1} g_{ww}^{-1}$ or $45.6 cal d^{-1} g_{ww}^{-1}$. The daily costs for migration would amount to $447 \mu mol O_2 d^{-1} g_{ww}^{-1}$ or $47 cal d^{-1} g_{ww}^{-1}$. According to Barange (1990) and Buchholz (pers.obs.) the migration behaviour in *N. megalops* is not as clear as in *E. hanseni* and appears to be irregular at times. The migration amplitude of *N. megalops* appears to be around 100 m with the thermocline as the upper limit of their vertical distribution (Barange 1990). Accordingly, we assumed a residence time of 22 h at depth at a temperature of about $10^{\circ}C$ and a migratory time of 2 h. The energy demand for migration was calculated to be $263 \mu mol O_2 d^{-1} g_{ww}^{-1}$.

Additional energetic considerations – To assess minimum food requirements we converted oxygen uptake from metabolic rates to units of carbon (Ikeda et al. 2000). Accordingly, daily losses of body nitrogen were estimated by combining our data of metabolic rate with those of elemental body composition (C, N). Over the temperature range examined, *E. hanseni* thus requires 2.2 to 5.1 mg C d⁻¹ g_{ww}⁻¹ and *N. megalops* 1.5 to 4.9 mg C d⁻¹ g_{ww}⁻¹. Hence, daily nitrogen losses due to ammonia excretion were 1.1-2.7% body N d⁻¹ in *E. hanseni* and 0.3–0.8% body N d⁻¹ in *N. megalops* at temperatures ranging between 5 and 20 °C (Table 4). At 5 °C, the oxygen uptake in *E. hanseni* was 10% higher than that in *N. megalops*. At that temperature, minimum food requirements of *E. hanseni*, in terms of their carbon demand, were 48 % higher compared with *N. megalops*. At 10 °C the respiration rate of *E. hanseni* was 29 % higher and its carbon demand 75 % higher than at 5 °C. At 15 °C the oxygen uptake of both species was similar, but the carbon requirements in *E. hanseni* were 32 % higher than in *N. megalops*. At 20 °C, *E. hanseni* had a lower oxygen uptake but the carbon requirements for both species were comparable (4 %; Figure 5).



↑ **Figure 5** Non-linear regressions of the mean carbon demand of *E. hanseni* and *N. megalops* at different experimental temperatures. The relationships presented here are based on mean values. Error bars denote SD

Discussion

Differences in physiological adaptations, with respect to temperature constraints and food requirements of *E. hanseni* and *N. megalops*, are discussed in terms of the differences in their behaviour and vertical distribution in the northern Benguela upwelling system.

Respiration and excretion - Variation in environmental conditions and swimming activities affect the metabolic level of animals (Hirche 1983). However, the respiration rate of zooplankton is strongly influenced by temperature (Vidal 1980, Ikeda et al. 2000). Whereas

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respiration rates have been measured previously in several krill species at various temperatures, to our knowledge, this study is the first to examine effects of temperature constraints on the respiration and excretion rates of *E. hanseni* and *N. megalops*.

Whereas our data may well compare with, for instance, those of Saborowski et al. (2002) who examined effects of temperature over the same range, as in our study, on oxygen uptake in *Meganyctiphanes norvegica*, as well as other studies and species, a direct comparison between species is often difficult due to differences in their nutritional status, ecology and/or life history.

Several studies have shown that seasonality has an impact on the respiration and excretion rates of zooplankton (cf. Meyer et al. 2010), but this is beyond the scope of the present study. However, euphausiids in the northern Benguela upwelling system are subject to frequent upwelling pulses that lead to rich plankton blooms. Consequently, changes in metabolism in a nutritionally multi-pulsed and stratified environment should be related to upwelling intensity and thus to strongly variable food availability rather than to seasonality alone.

The strongly migrating species *E. hanseni* showed a near-linear correlation between temperature and metabolism between 5 and 20 °C. In contrast, the weaker migrating species *N. megalops* exhibited a steep exponential correlation, so that the influence of temperature on the metabolism of both species differed significantly. According to Barange (1990), and supported by our own observations, *N. megalops* spends day and night in deeper waters at low temperatures of between 7 and 10 °C, thus avoiding warmer conditions above the thermocline. Different krill species adjust their vertical position according to the structure of the water column (Barange 1990), showing a preference for specific temperature regimes (Wiebe and Boyd 1978). Thus, in *N. megalops*, a Q_{10} value of 1.9 between 5 and 10 °C suggests an adaptation to lower temperatures whereas a Q_{10} of 2.6 between 15 and 20 °C indicates possible energy constraints at higher temperatures (Figure 4). In *E. hanseni*, however, the Q_{10} values suggest a metabolic depression at temperatures <10 °C ($Q_{10} = 2.6$ between 5 and 10 °C) and thermal adaptation between 10 and 20 °C ($Q_{10} = 1.5$). But, contrary to several other invertebrate species, e.g. copepods (Hiromi 1988, Gaudy 2000), neither *E. hanseni* nor *N. megalops* were able to maintain constant metabolic rates at different temperatures.

Both, *E. hanseni* and especially *N. megalops* have to adapt to periods of low oxygen conditions prevailing in their environment. This may be facilitated by a shut-down of

metabolic activity in *E. hanseni* or low oxygen demand in *N. megalops*. Other processes, like a high anaerobic capacity, should be considered. Furthermore, a highly variable system such as the northern Benguela Current, with substantial seasonal and interannual variability in atmospheric forcing, water mass properties, upwelling intensity, sea surface temperatures and food availability may not allow for narrowly pre-determined thermal adaptations. However, both species appear to be capable of adjusting to some extent their overall metabolic processes to the prevailing ambient temperature, as has been shown also for *M. norvegica* (Saborowski et al. 2002).

A comparison of Q_{10} values of *E. hanseni* and *N. megalops* from this study with those of other euphausiid species shows higher Q_{10} values in the latter species, e.g. 4.3 in *T. longipes* (Iguchi and Ikeda 2005), 2.0-2.4 in *E. pacifica* (Iguchi and Ikeda 1995) and 2.0-3.5 in a number of species (Teal and Carey 1967). Q_{10} values at temperatures below 10 °C support the concept of energy conservation in *E. hanseni* ($Q_{10}=2.6$; 5–10 °C), whereas temperatures above 15 °C in *N. megalops* ($Q_{10}=2.6$; 15–20 °C) lead to a possibly deleteriously high energy demand (Figure 4). In general, Q_{10} values <2 indicate a weak influence of temperature on metabolism or rather a temperature-independent metabolism over the temperature range measured. Accordingly, *E. hanseni* and *N. megalops* can be considered eurythermal species.

Considerations of the O:N ratio - Seasonal changes in chemical composition of an animal are linked to food availability, which in turn affect its respiration and excretion rates and thus the O:N ratio (Conover and Corner 1968). *Euphausia hanseni* showed an increase of the $\text{NH}_4\text{-N}$ excretion rate with increasing temperature, but *N. megalops* had a significantly lower overall excretion rate than *E. hanseni*. *N. megalops* indicated reduced use of nitrogen-rich compounds (e.g. proteins) or alternatively, an elevated demand of essential amino acids (Mayzaud and Conover 1988). The high abundance and biomass of *E. hanseni* together with their more intensive excretory activities may be indicative of the possible role of this species in nitrogen regeneration in the northern Benguela upwelling system. Further quantitative investigations would be useful to assess this fraction of nitrogen recycling, e.g. in relation to nitrogen demand of phytoplankton (cf. Ikeda and Motoda 1978, Cockcroft and McLachlan 1993, Kim et al. 2010).

Moreover, the O:N ratio reflects changes in the biochemical composition of zooplankton that store large energy (lipid) reserves (Mayzaud and Conover 1988). In contrast, in zooplankton with low energy reserves, as reported for *N. megalops* (Cartes 2011) and *E. hanseni* (W.

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Hagen, Marine Zoology, University of Bremen, Germany pers. comm., K. Huenerlage, unpubl. data), this ratio rather reflects the quality and quantity of available food (Mayzaud and Conover 1988). Carbohydrates account for only a very small fraction in krill (Buchholz and Prado-Fiedler 1987). Although significant differences in carbon content and the C:N ratio of the two species were detected, both species do not store considerable amounts of lipids (8-9% dw in *E. hanseni*; K. Huenerlage pers. obs. and 7-11% dw in *N. megalops*; Cartes 2011). Therefore, simultaneous measurements of oxygen uptake and ammonia excretion allowed the calculation of the O:N ratio as an indicator of the predominant substrate metabolized. According to Conover and Corner (1968) and Mayzaud and Conover (1988) a phytoplankton bloom accelerates both respiration and excretion in copepods, where the oxygen uptake is more enhanced than the ammonia excretion, because most of the ingested food is utilized in somatic growth or reproduction. However, lower O:N ratios are typical of herbivorous zooplankton (copepods) during a phytoplankton bloom, owing to a low lipid content of algae. Saborowski et al. (2002) found the same relation in *Meganyctiphanes norvegica* from the oligotrophic Ligurian Sea showing highest excretion rates and lowest O:N ratios during the short phase of high food supply in spring. In contrast, Ikeda and Kirkwood (1989) showed that during a phytoplankton bloom in the Southern Ocean the O:N ratio of *Euphausia superba* increased as a result of depressed ammonia excretion. These authors argued that *E. superba* do not store large lipid reserves and rather use the ingested food to replenish body protein, which had been catabolized during the previous winter. Kim et al. (2010) confirmed this (seasonal) pattern for the euphausiids *Euphausia pacifica* and *Thysanoessa inspinata* but failed to find significant relationships between metabolic rates and environmental parameters such as chlorophyll_a and temperature. However, in our study the average O:N ratio of 20 in *E. hanseni* indicated a protein-oriented metabolism, whereas an O:N ratio of 57 found in *N. megalops* indicated a lipid-oriented metabolism, according to Ikeda (1974). In both species there were strong fluctuations in the O:N ratio; between 9 and 49 in *E. hanseni* and between 29 and 118 in *N. megalops*. Indications for rapid changes in the ammonia excretion rate, which were likely due to the different quality and quantity of the available food, support the idea of opportunistic feeding in both species. Krill may shift their diet according to changing proportions of prey types, as has been shown for *E. lucens* in the southern Benguela upwelling region (Stuart and Huggett 1992). An accurate assessment of the trophic position of euphausiids is often difficult, because they adopt different feeding strategies to adapt to changes in food composition (Gurney et al. 2001). *N. megalops* is described as a carnivorous

feeder (Barange et al. 1991, Gurney et al. 2001 and Cartes 2011), in contrast to *E. hanseni*, which appears to be more omnivorous.

In summary, the differences in the O:N ratio of the two species suggested different food preferences and dissimilar metabolic pathways. The high fluctuations in O:N ratio suggested that the metabolism of the two species was closely related to ambient temperature and the immediate food supply. Therefore, they were strongly affected by environmental changes.

Energetic considerations – By integrating the results of the respiration measurements with the O:N ratios, some estimations of the energy and carbon demands of both species under different temperature conditions were made that can be related to their migration behaviour. At 5 °C, the carbon demand of *E. hanseni* was about 10% higher than in *N. megalops*, assuming they used the same metabolic substrate. This difference increased substantially to 48% when considering different substrate metabolites, which was evidenced by the significantly different O:N ratios of the two species. This highlights the importance of simultaneously measuring the oxygen uptake and the nitrogen excretion rate in order to identify the substrate predominantly used and to calculate minimum food requirements.

The carbon demand of *N. megalops* at 5, 10 and 15 °C equated to slightly lower respiration rates at 5 and 10 °C than in *E. hanseni*, possibly indicating an adaptation to their natural environment in deep waters where temperature and oxygen content are low and feeding conditions are less favourable compared to the upper water layers. According to Teal and Carey (1967) DVM allows euphausiids to conserve energy, and food availability was identified as a possible trigger for migration.

N. megalops showed lower minimum food requirements than did *E. hanseni*. Accordingly, the latter species had a higher carbon demand and thus had more need to migrate to the upper layers where food conditions are more favourable. In contrast, owing to their low carbon demand *N. megalops* could benefit from low water temperatures in the deep. According to Barange et al. (1991), the two species are separated by vertical space partitioning. Considering their temperature constraints and energy demands, physiological adaptations are likely explanations for their differential vertical positioning in the water column.

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MOCNESS used for stratified sampling during a cruise with the FRS Africana during summer 2009 (Foto: F. Buchholz)

Diel vertical migration behaviour in Euphausiids of the Northern Benguela
Current: Seasonal adaptations to food availability and strong gradients of
temperature and oxygen

Thorsten Werner and Friedrich Buchholz

Abstract

The Northern Benguela upwelling system is the second most productive upwelling system of the world where euphausiids can dominate the rich mesozooplankton community and may contribute substantially to the vertical flux of organic carbon. The diurnal vertical distribution of four euphausiid species was observed over three seasons from different years. The most abundant *Euphausia hanseni* showed pronounced long range vertical migration, regularly crossing the thermocline and retreating again to the oxygen minimum zone (OMZ). *Nematoscelis megalops* was a weak migrant, avoiding the thermocline and persisting in the OMZ throughout 24h. Euphausiids were divided into different ecological groups using or avoiding the oxygen minimum zones. Diel vertical migration (DVM) behaviour differed due to seasonal variations in water temperature, oxygen- and food availability. The energetic costs for migration, as part of the total daily carbon demand, were $24\pm 6\%$ in *E. hanseni* and $6\pm 2\%$ in *N. megalops*. Further energetic considerations showed that temperature acted as a controlling factor but did not alone determine the seasonal vertical positioning of the krill species. Food abundance further modified the DVM behaviour, but the vertical distribution of krill did not stringently coincide with food maxima throughout the water column.

Chapter 2

Introduction

The Northern Benguela upwelling system (NBus) located at the west-coast off Namibia is one of the four major eastern boundary upwelling regions (Hutchings et al. 2009) and characterized by high productivity. It is characterized by strong temperature and oxygen gradients in the water column and a high seasonal and interannual variability in atmospheric forcing and water mass characteristics (Hutchings et al., 2009). Euphausiids and copepods can dominate the mesozooplankton communities in this region and are an important trophic link between primary producers and higher trophic levels throughout the world oceans (Ekau et al. 2010). Eight different euphausiid species regularly occur in the NBus with *Euphausia hanseni* and *Nematoscelis megalops* showing highest biomass and abundance (Barange, 1990). Due to different diel vertical migration (DVM) behaviour different euphausiid species occupy divergent vertical strata (Barange, 1990). Accordingly, krill species in this system encounter various water masses during their ascent and descent and have thus to deal with a wide range of temperatures, pronounced oxygen minimum zones (OMZ) and different trophic environments. Euphausiids, or in general zooplankton, have to adapt to these variations to some extent or have to avoid conditions which are not suitable for them. Vertical migration in krill can substantially contribute to the vertical flux of organic carbon to deeper layers.

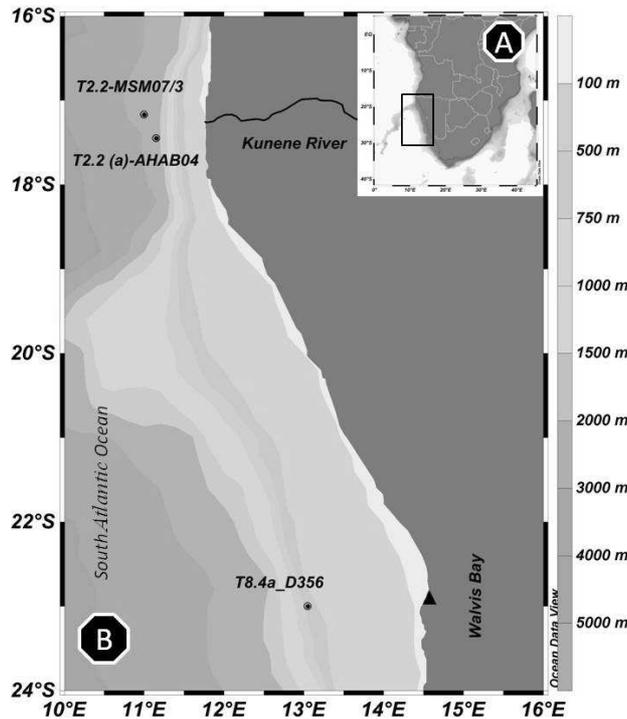
The typical pattern of the diel vertical migration of zooplankton consists of an ascent during dusk and descent during dawn with shallow residence at night and deep residence by day (Andersen et al., 1992; Timonin, 1997; Taki, 2008). However, also several observations of a `reversed` migration were reported (Ohman et al., 1983). To explain the benefits of vertical migration, competing hypotheses like metabolic and demographic advantages, energy conservation, resource related hypotheses and light-related mortality were proposed (Lampert, 1989). Predator avoidance, food availability and metabolic capacity modify patterns of DVM in many zooplankton taxa (Buchholz et al., 1995). Trade-offs between these different aspects, e.g. maximum energy input versus maximum protection, have to be considered. However, a general concept or unifying theory is still lacking (Lampert, 1989) and the possibility to build such a unifying theory is doubtful because of the high adaptive flexibility in behaviour and physiology of species. Thus each species shows quite distinct DVM patterns when inhabiting different environments. The DVM of euphausiids, in particular, was targeted by several studies (Brinton 1967; Barange, 1990; Buchholz et al., 1995; Tarling et al., 2000; Yoon et al., 2007; Taki, 2008). Some krill species migrate intensively while others migrate only to a small extent. In some krill species, e.g. *N. megalops*, the migration behaviour is irregular (Barange,

1990). In the current study the DVM behaviour of four different krill species, *Euphausia americana*, *Euphausia hanseni*, *Euphausia recurva* and *Nematoscelis megalops* of the Northern Benguela upwelling system, was examined. Stratified net catches were conducted during three different cruises in summer 2004, autumn 2008 and winter 2010 at two locations over a period of 36h. The data were related to the concurrent temperature and salinity profiles and the oxygen content of the water column. Furthermore, metabolic rates at different temperatures were measured during shipboard experiments to assess the energy demand in krill and the trophic environment was observed to demonstrate possible food induced changes in the DVM behaviour of these species.

The aim of this work was to investigate the diel vertical migration behaviours of various, co-occurring euphausiids in the NBus and to relate these DVM behaviours to seasonal differences of vertical gradients of food availability, temperature and oxygen constraints. Furthermore, energetic calculations were conducted in order to evaluate possible trade-offs between different proximate factors. The vertical migration studies conducted at three seasons and at two different stations were evaluated within the GENUS project (Geochemistry and Ecology of the Namibian Upwelling System), over a period of six years, with concurrent data of hydrographic and trophic conditions and subsequent calculation of energetic trade-offs in krill.

Material and Methods

Vertical distribution and migration behaviour of euphausiids were investigated over a time period of 36h during three different cruises in the NBus during (late) Austral summer, autumn and (late) winter. Sampling was carried out at three different locations from RV *Alexander von Humboldt* in 2004 (Cruise AHAB04, Kunene-Transect at T2.2 (a); 03.-04. February 2004), RV *Maria S. Merian* in 2008 (Cruise MSM07/3, Kunene-Transect at T 2.2; 01.-02. April 2008) and RRS *Discovery* in 2010 (Cruise D356, Walvis Bay-Transect at T8.4 (a); 07.-08. October 2010; Fig.1).



↑ **Figure 1** Location of the northern Benguela upwelling system in the Southwest Atlantic Ocean (inset A) and the station positions sampled (B). Circles denote locations of sample stations, triangles geographic landmarks.

Net catches targeting euphausiids were conducted using a 1 m² MOCNESS (Multiple Opening and Closing Net and Environmental Sensor System, Wiebe et al. 1985) with a large mesh size of 2000 µm and a soft cloth cod-end to increase the catchability of the net and to reduce damage to specimens at the same time. The MOCNESS was deployed every six hours close to the bottom then hauled obliquely to the surface while opening and closing nets in a pre-set sequence (Table 1). Zooplankton samples for description of the trophic environment were further taken with a double MOCNESS (mesh size 330 µm) at fixed stations every 6h over a 36h time period during autumn 2008 and winter 2010, always starting at dusk. Sampling was done day and night at six different strata in the water column: 350-300 m; 300-250 m; 250-200 m; 200-150 m; 150-100 m; 100-50 m; 50-25 m. The towing speed for both nets was 2 knots and after catch the samples were preserved in 4% buffered formalin and shipped to the laboratories in Bremerhaven, Germany. An overview of the sampling stations, strata and times are given in Table 1.

↓ **Table 1** Sampling stations, strata (depth (m)) and times (local time; LT) at different stations with a MOCNESS in the northern Benguela system during three cruises in 2004, 2008 and 2010 including astronomic times for dusk and dawn.

	AHAB 04	MSM07/3	D356
Dates	03.02.2004- 04.02.2004	01.04.2008- 02.04.2008	07.10.2010- 08.10.2010
Season	summer	autumn	winter
Location	17°10 S; 11°18 E	17°46 S; 11°00 E	23°00 S; 13°02 E
Dusk/dawn (LT)	20:35/07:36	18:28/06:09	20:04/05:47
<i>Dusk 1</i> (LT)	19:33-21:29	18:06-19:51	18:32-19:33
<i>Midnight</i> (LT)	00:12-01:51	00:00-01:26	01:05-01:54
<i>Dawn</i> (LT)	07:06-08:55	06:01-07:24	06:44-07:34
<i>Noon</i> (LT)	11:24-13:11	12:02-13:35	12:41-13:39
<i>Dusk 2</i> (LT)	-	18:05-19:26	18:38-19:39
Strata sampled (m)	600-400; 400-300; 300-200; 200-120; 120-80; 80-40; 40-0	600-400; 400-300; 300-200; 200-150; 150-100; 100-50; 50-25; 25-0	380-350; 350-300; 300-200; 200-100; 100-50; 50-25; 25-0

In the laboratory, juveniles and adults of euphausiids were separated, counted and their lengths were measured between the front of the eyes to the tip of the telson using a millimeter scale under a stereomicroscope. The wet weight was determined on a microbalance (Sartorius LA 230 S). The relation between length and weight was characterized by regression analysis (Table 2). Biomass of each species was calculated using species-specific weight to body length correlations. The abundances of the krill species were standardized to a volume of 1000 m³ (Ind. 1000 m⁻³). In order to describe the vertical migration behaviour of euphausiids the weighted mean depths (WMD) were estimated for each tow after Barange (1990):

$$\text{Equation 1: } \text{WMD} = \frac{\sum(n_i \times d_i)}{N}$$

where d_i corresponds to the mean depth of the sample stratum; n_i corresponds to the number of individuals at that depth and N is the sum of individuals over all sampling depths. WMD estimation was done for each species and for five different size classes (5-10 mm, 11-15 mm,

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16-20 mm, 21-25 mm and >26 mm) in order to reveal possible differences between different size classes.

▀ **Table 2** Species-specific length-weight regressions for different krill species in the Northern Benguela upwelling system.

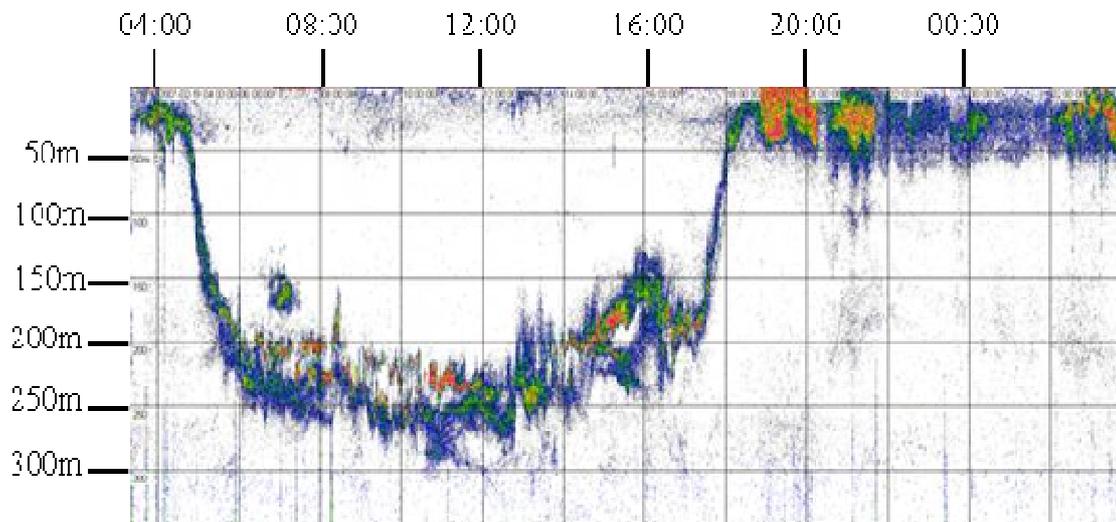
species	regression	R ²	n
<i>E. hanseni</i>	$y=0.0084x^{2.9447}$	0.939	110
<i>N. megalops</i>	$y=0.0103x^{2.847}$	0.929	58

Before the nets were deployed the hydrographic conditions, i.e. temperature (°C), salinity (PSU), oxygen (mL O₂ L⁻¹) and fluorescence (Chl_a; mg m⁻³), were recorded with a CTD-probe (Sea-Bird). In parallel to the 36h stations, except AHAB04, the vertical distribution and abundance of the zooplankton community was investigated. Copepod biomass was determined in order to further describe the vertical trophic environment during the D356 cruise (Karolin Bohata, Hamburg, pers. comm.). These datasets were related to the DVM of krill species. Samples were preserved in 4% buffered formalin for further analysis. The zooplankton samples were divided into four size classes: <0.5 mm; 0.5-1mm; 1-2 mm and 2-5 mm. Furthermore the abundance of five dominant copepod species during D356 in the NBus was calculated: *Calanoides carinatus*, *Eucalanus hyalinus*, *Metridia lucens*, *Pleuromamma sp.* and *Rhincalanus nasutus*.

Chl_a data (mg m⁻³) were taken from CTD casts and converted with a conversion factor of F=70 (Carbon Chl_a⁻¹) into carbon content (mg C m⁻³). In the literature the conversion factor F varies between 10 and 150 in general and between 10 and 130 in the Benguela region (Pitcher 1988). However, medium conversion factors were given for diatoms which prevailed in the NBus between Walvis Bay and Kunene River (N. Wasmund, Rostock, pers. comm.). Accordingly, a conversion factor of F=70 was used.

Temperature/oxygen consumption equations for *E. hanseni* and *N. megalops* given by Werner et al. (2012, in press) were used to calculate the oxygen consumption at a specific temperature. It was assumed that during migration euphausiids had a 2.7 times higher respiration rate than at rest (Torres and Childress, 1983). Euphausiid swimming speed is about 0-10 body lengths (bl) s⁻¹, with most of the observations at the lower end of this range (de Robertis et al., 2003). Multi-beam acoustic data (Jens-Otto Krakstad, IMR – Bergen, RV Dr. F. Nansen, cruise report) showed a clear diurnal pattern most probably due to the vertical migration of *E. hanseni* as indicated by control net catches. *E. hanseni* descended within 1h from 30 m depth to 170 m depth at dawn (Fig. 2, used with permission). From the migration

pattern recorded at the krill-relevant frequency, 120 kHz, descent and ascent speeds were assessed at 2.3 bl s^{-1} , assuming a body length of 22 mm.



↑ **Figure 2** Multi-beam acoustic backscatter profile (120 kHz) during a cruise with the RV Dr. F. Nansen in March 2007 (BENEFIT Survey, 23/07, 7.2. - 23.3.2007)

We developed a conceptual model to investigate the effect of temperature and food availability on the migration behaviour of *E. hanseni* and *N. megalops*, accordingly. Temperature is known to have a strong impact on metabolic rates. Therefore, temperature may influence the DVM behaviour of krill species (Lampert, 1989), due to different thermal adaptations of species, different energy expenditures (Werner et al., 2012 (in press)), e.g. swimming costs and a variety of other physiological processes, e.g. moult activities. Accordingly, the different temperature profiles at the sampling stations must be considered to calculate the energy demand or more specifically the oxygen uptake of euphausiids during DVM. Using the respiration rates, O:N ratios and estimated minimum food requirements, published in Werner et al. (Werner et al., 2012 (in press)), we assessed the costs of DVM in *E. hanseni* and *N. megalops*. Accordingly, different temperature regimes at the sampling stations were used as references for calculating the carbon demand of different DVM behaviour. It was assumed that krill species migrated with a swimming speed of 2.3 bl s^{-1} in one “step” between their shallowest and deepest residence depths. Furthermore, a swimming speed of approx. 1 bl s^{-1} was assumed to represent the routine metabolism of each species (Torres and Childress, 1983). Although Price had shown that the euphausiid species *Thysanoessa raschii* doubled its swimming speed within an algal patch (Price, 1989), it was further assumed that a swimming speed of approx. 1 bl s^{-1} was fast enough to perform their regular feeding activities. Price stated that the observed increased swimming speed contradicted optimal foraging

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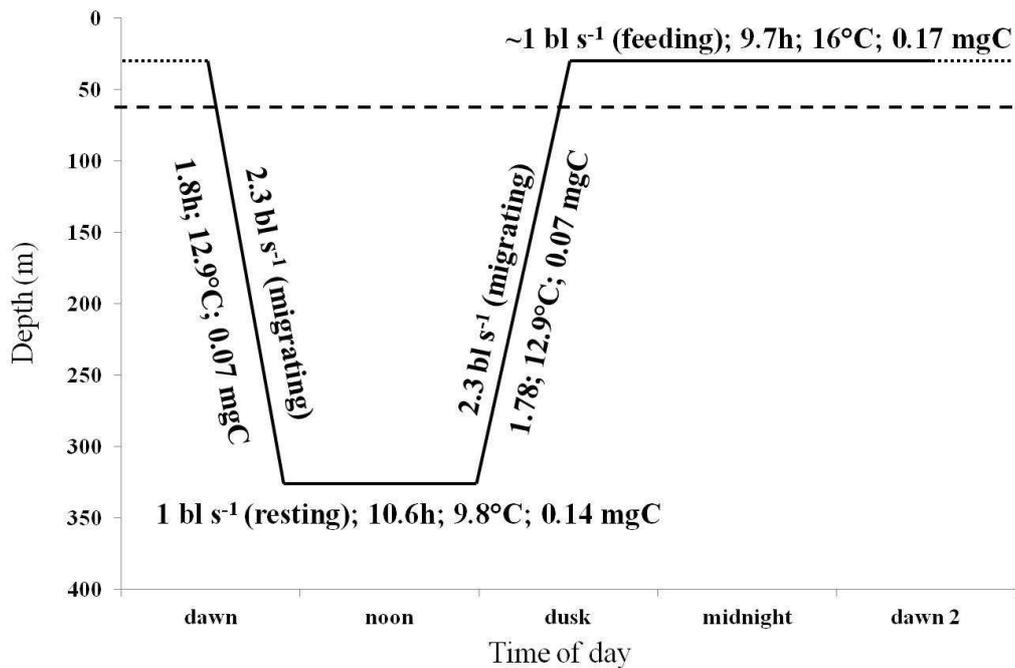
considerations and that, following the assumptions of Kils (Kils, 1982), the increased swimming velocity observed, may be a consequence of the change in swimming path orientation (Price, 1989) rather than increased pleopod beating. “Migration time” was estimated by dividing the differences between WMD at time 1 (average of dusk1+2 and midnight) and time 2 (noon) by a swimming speed of 2.3 bl s^{-1} (Fig. 3). The oxygen uptake and carbon demand was calculated over 24h in order to estimate the energetic costs of a whole vertical migration cycle. The calculation was based on the following equation:

$$\text{Equation 2: } R_{\text{DVM}} (\mu\text{mol O}_2 \text{ g}_{\text{ww}}^{-1}) = R_{\text{Temp}} (\mu\text{mol O}_2 \text{ h}^{-1} \text{ g}_{\text{ww}}^{-1}) \times h (\times 2.7)$$

where R_{Temp} corresponds to the oxygen demand at a specific temperature, where “h” is the amount of time spent at that temperature/depth (Fig. 3) and 2.7 corresponds to the behaviour whether the animals showed “normal” swimming speed (1 bl s^{-1}) or “increased” swimming speed (2.3 bl s^{-1}). These calculations were repeated until a whole migration cycle was completed (24h) and then the different R_{DVM} were summed up. These results were converted from μmol to mL and then the carbon demands (CA_{adj}), as indices for minimum food requirements, were calculated using:

$$\text{Equation 3: } CA_{\text{adj}} (\text{mg C g}_{\text{ww}}^{-1} \text{ d}^{-1}) = R_{\text{DVM}} (\text{mL O}_2 \text{ g}_{\text{ww}}^{-1} \text{ d}^{-1}) \times \text{RQ} \times 12/22.4$$

where RQ represents the ‘Respiratory Quotient’, the molar ratio of carbon dioxide produced to oxygen consumed (0.97 for *E. hanseni* and 0.72 for *N. megalops*; see Werner et al. in press) and where 12/22.4 is the weight of carbon in one mole (22.4 L) of carbon dioxide. Assuming a dry weight (DW) of 25% wet weight (WW) and a carbon content of 40% DW in *E. hanseni* and 42% DW in *N. megalops* (Werner et al. in press), we calculated the daily ratio of carbon losses due to vertical migration expressed in % body C d^{-1} .



↑ **Figure 3** Migration behaviour of *E. hanseni* (100 mg weight) depicted as migration model. Given are the assumed swimming speeds (bl s^{-1}), residence time (h), temperatures ($^{\circ}\text{C}$), according to CTD data from the D356 cruise, and calculated carbon demands (mg C). Broken line indicates position of the oxycline during the 36h station T8.4a, Walvis Bay-Transsect winter 2010.

The minimum food requirements, necessary to cover metabolic energy demands, were related to the trophic environment at the different stations in order to assess energetic trade-offs between temperature and food availability. To assess possible (dis-)advantages, e.g. energetic trade-offs, from the oxygen demand during different DVM behaviour and different temperature profiles, the minimum carbon uptake was calculated according to the assumption that assimilation efficiency is similar to that of *Euphausia pacifica*, thus 0.8 for phytoplankton and 0.9 for carnivorous diets (Ross, 1982). Due to the high variability of filtration rates for euphausiids given in the literature (Morris, 1984) and the uncertainty of (maximum) ingestion rates in krill (Pakhomov et al., 1997; Atkinson et al., 2006), filtration rates of *E. hanseni* were assessed assuming phytoplankton feeding to meet 75% and 50% of the daily metabolic requirements during nighttime. One calculation was based on the average amount of Chl_a in the upper 50m of the water column during winter. Another approach using the highest concentration of Chl_a found in the upper 50m during which was applied assuming that euphausiids were capable to detect rich algal patches (Price, 1989). Filtration rates were assessed using the equation:

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$$\text{Equation 4: } F = CA_{\text{adj}} \times 0.75 \times (h \times C_{\text{Phyto}} \times 0.8)^{-1}$$

where F represents the filtration rate (L h^{-1}), CA_{adj} the daily carbon demand (mg d^{-1}), the value 0.75 describes the fraction of phytoplankton feeding when this meets 75% of the daily metabolic requirements. This value is set 0.5 when phytoplankton feeding meets 50%. Where h depicts the amount of time between astronomical dusk and dawn, C_{Phyto} reflects the average or maximum carbon concentration (mg m^{-3}) due to phytoplankton in the upper 50 m of the water column and 0.8 is the assumed assimilation efficiency. Accordingly, zooplankton ingestion rates, e.g. copepod ingestion rates, were calculated by the equation given in Tarling et al. 2000 for *Meganyctiphanes norvegica*:

$$\text{Equation 5: } \text{Log}_{10}G = 0,746 \times \text{log}_{10}C + 1.092$$

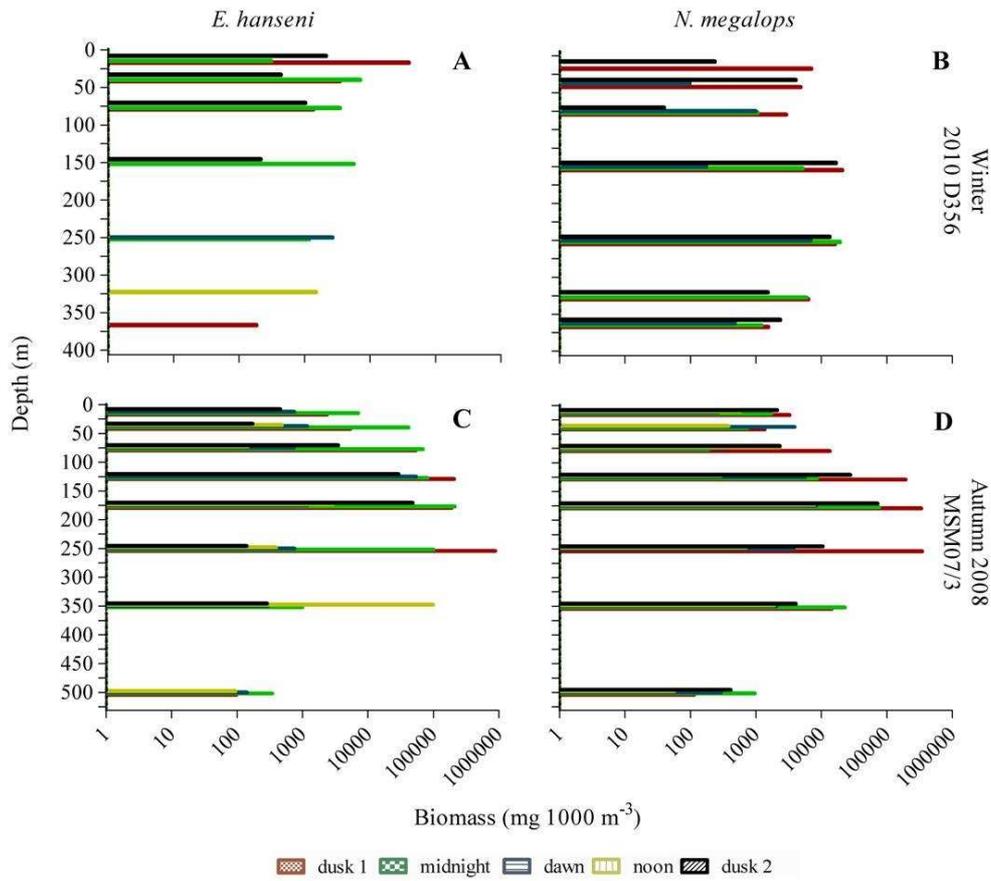
where G is the ingestion rate of copepods and C is the copepod/zooplankton biomass ($<500 \mu\text{m} - 5 \text{ mm}$) in the upper 50m of the water column or within 50 m around WMD during nighttime. The calculated time to cover the carbon demand by a carnivorous diet was then divided by 0.9, because an assimilation efficiency of 0.9 was assumed (see above). *E. hanseni* is known as a filter feeding omnivorous species whereas *N. megalops* does not have a feeding basket adapted to filter feeding (Mauchline, 1980) and *N. megalops* adults are described as carnivorous (Gurney et al., 2001, 2002). Feeding in *E. hanseni* was presumed to take place during nighttime whereas *N. megalops* fed throughout a 24h cycle, as was suggested by Barange (Barange et al., 1991). In *E. hanseni* feeding time, to cover carbon demand over 24h, was calculated using different ratios of herbivorous and carnivorous diets (100%/0%; 66%/33%; 33%/66% and 0%/100%). The calculated feeding time in both species was used as an index for “optimal” migration behaviour in terms of food availability and temperature. An overview of the presumed residence times, temperatures, migration amplitudes and swimming speeds of *E. hanseni* and *N. megalops* during the three different cruises are given in Table 3.

	time of day (night/day)	WMD (m)	migration amplitude (m)	temperature (°C)	residence time at depth (h)	migrating (h)	migrating (°C)
<i>E. hanseni</i>	night	47		15.5	9.7		
winter 2010	day	325	278	9.8	10.9	3.4	12.7
<i>E. hanseni</i>	night	158		14.3	11.7		
autumn 2008	day	326	167	10.8	10.5	1.8	12.6
<i>E. hanseni</i>	night	44		15.5	11		
summer 2004	day	249	205	11.2	10.6	2.4	11.2
<i>N. megalops</i>	night	105		13.5	9.7		
winter 2010	day	289	184	9.8	11.7	2.6	11.7
<i>N. megalops</i>	night	202		13.4	11.7		
autumn 2008	day	177	25	14.2	12	0.3	13.8
<i>N. megalops</i>	night	212		11.8	11		
summer 2004	day	249	37	11.2	12.4	0.6	11.5

↑**Table 3** Assumed residence times (h), temperatures (°C), weighted mean depth (WMD; m) and migration amplitudes (m) of *E. hanseni* and *N. megalops* used for calculation of the oxygen uptake (carbon demand) under different hydrographic conditions.

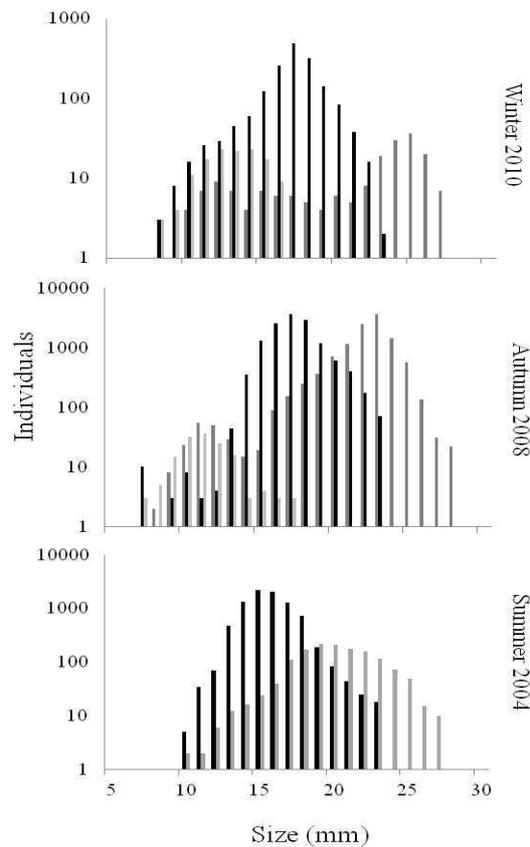
Results

During the three vertical migration studies in the NBus six euphausiid species were caught: *E. americana*; *Euphausia gibboides*; *E. hanseni*; *E. recurva*; *Nyctiphanes capensis* and *N. megalops*, where *E. hanseni* and *N. megalops* showed by far the highest biomass and abundance. An overview of the biomass at different times and depths during autumn 2008 and winter 2010 are given in Figure 4.



↑ **Figure 4** Biomass/depth plots of *E. hanseni* (A, C) and *N. megalops* (B, D) during the D356 cruise in winter 2010 (A, B) and the MSM07/3 cruise in autumn 2008 (C, D). Data were standardized to a volume of 1000m³.

Length frequency distribution for the most abundant species during each cruise is given in Figure 5.



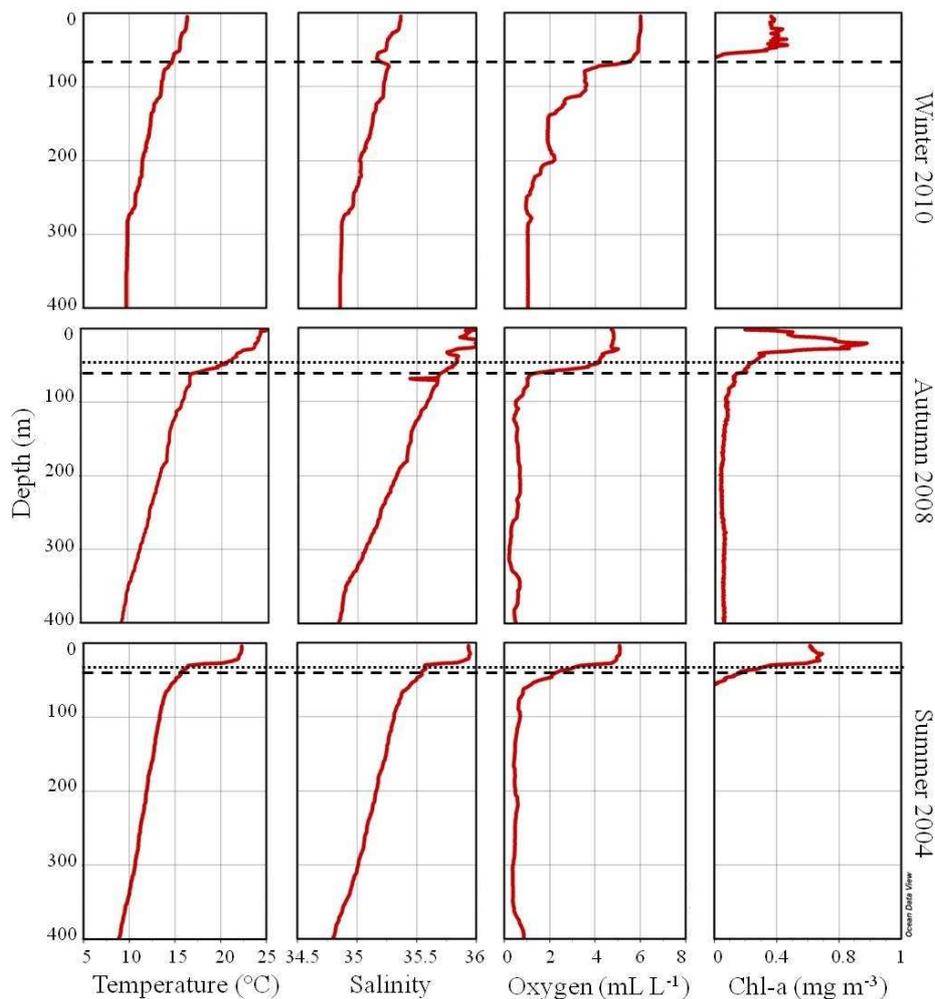
↑ **Figure 5** Length frequency distribution of *E. hanseni* (grey), *N. megalops* (black), *E. recurva* (light grey) and *E. americana* (light grey) at station T8.4a, Walvis Bay-Transect winter 2010 (top), at station T2.2, Kunene-Transect autumn 2008 (middle) and at station T2.2a, Kunene-Transect summer 2004 (bottom).

Hydrographic conditions

The sea surface temperature (SST) and salinity were highest during autumn 2008, followed by summer 2004. During both seasons the SST was well above 20°C and salinity close to 36. In contrast, during winter 2010 SST data and salinity were significantly lower, with 16.4°C and 35.57 respectively. In summer and autumn a 20-30 m thick surface layer was bordered by a strong thermocline. During autumn the thermocline ranged from 30-60 m (gradient: 0.25°C m⁻¹) where temperature decreased to 16.5°C. In summer a more intense thermocline (gradient: 0.5°C m⁻¹) prevailed between 22-33 m where temperature decreased to 16.3°C and salinity to 35.57. In winter 2010 no thermocline could be detected and the hydrographic data showed a steady decline in temperature and salinity from the surface to a depth of around 280 m. Below 280 m, salinity and temperature were constant near 34.87 and 10°C. During all seasons moderate to strong oxyclines were detected. In winter a strong oxycline was identified between 66 m and 79 m, with oxygen levels declining from 5.51 mL O₂ L⁻¹ to 3.54 mL O₂ L⁻¹

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(gradient: $0.15 \text{ mL O}_2 \text{ m}^{-1}$). Below 250 m depth the oxygen content was stable at around $1 \text{ mL O}_2 \text{ L}^{-1}$. In autumn a strong oxycline (gradient: $0.15 \text{ mL O}_2 \text{ m}^{-1}$) appeared from 50 to 70 m where the oxygen level decreased abruptly from $4 \text{ mL O}_2 \text{ L}^{-1}$ to $1 \text{ mL O}_2 \text{ L}^{-1}$. An oxygen minimum zone (OMZ) was developed between 70 and 450 m where the oxygen level was well below $1 \text{ mL O}_2 \text{ L}^{-1}$. A moderate oxycline between 22-62 m (gradient: $0.1 \text{ mL O}_2 \text{ m}^{-1}$) was detected during summer. From 66 to 400 m a linear decrease of temperature to 8.9°C and salinity to 34.80 was observed, with an almost constant oxygen level of $0.87 \text{ mL O}_2 \text{ L}^{-1}$. Oxygen depletion was most serious between 300-350 m where oxygen content below $0.4 \text{ mL O}_2 \text{ L}^{-1}$ was noted. Detailed descriptions of the different physical parameters during winter 2010, autumn 2008 and summer 2004 are given in the corresponding cruise reports (Buchholz et al. (2010), Ekau et al. (2004) and Ekau et al. (2008)).



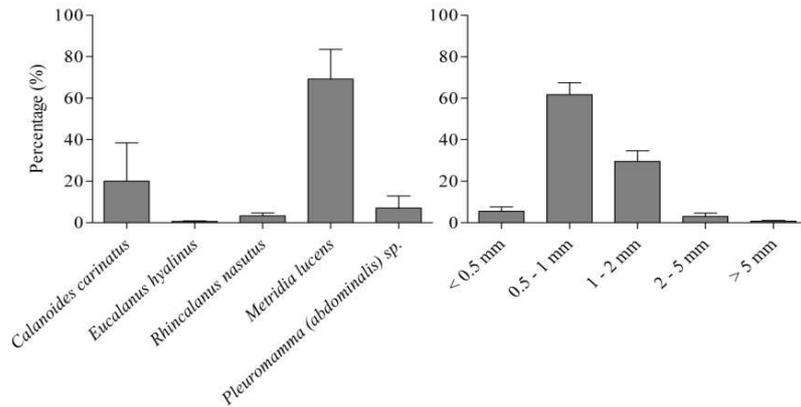
↑ **Figure 6** Hydrographic conditions (temperature ($^\circ\text{C}$), salinity, oxygen content ($\text{mL O}_2 \text{ L}^{-1}$) and Chl_a (mg m^{-3})) at the 36h station T8.4a, Walvis Bay-Transect winter 2010 (top), at station T2.2, Kunene-Transect autumn 2008 (middle) and at station T2.2a, Kunene-Transect summer 2004 (bottom). Broken line indicates position of the oxycline. Dotted line indicates position of the thermocline.

Trophic conditions

During winter the phytoplankton community in the upper 20m at station T8.4a (Walvis Bay-Transect) was dominated by dinoflagellates with a wet weight of 117.9 mg m^{-3} corresponding to 15.5 mg C m^{-3} and diatoms showed a wet weight of 33.9 mg m^{-3} or 1.8 mg C m^{-3} (N. Wasmund, IOW Warnemünde, Germany, pers. comm.). CTD data showed the highest Chl_a concentration between 20-48 m with a maximum of $0.46 \text{ mg Chl}_a \text{ m}^{-3}$ at 44 m depth. In autumn highest Chl_a and Phycoerythrin concentrations were found at around 20 m depth (CTD data; Fig. 6), with a Chl_a maximum of 0.98 mg m^{-3} at 24 m. Chl_a concentrations above 0.2 were found till 60 m depth. In summer highest Chl_a concentrations were observed between the surface and 38m where the Chl_a content was always above 0.2 mg m^{-3} , with a peak at 15 m showing a Chl_a content of 0.69 mg m^{-3} . Chl_a concentrations above 0.1 mg m^{-3} were observed down to 46 m depth.

Highest copepod abundance (73%) in winter 2010 was found close to the surface between 0-50 m during nighttime. However, approx. 10% of the whole copepod community was found between 250-300 m. During the day most of the copepods (81%) were found at depths between 250-350 m (Fig. 10). The copepod community consisted of $94.5 \pm 0.5\%$ Calanoidea and $5.5 \pm 0.5\%$ Cyclopoida (Harpacticoida were not considered). The size classes between 0.5-2 mm made up to 91% of the copepods investigated both during day and night (Fig. 7). The most abundant copepod species at all depths was *Metridia lucens* ($69.5 \pm 14.5\%$; Fig. 7), followed by *Calanoides carinatus* ($20.0 \pm 18.5\%$) and *Pleuromamma* sp. ($7.0 \pm 5.9\%$). In autumn the highest zooplankton biomass occurred at approx. 300 m during daytime (65 mg ww m^{-3}) and 500 m during nighttime (159 mg ww m^{-3} , B. Martin, Hamburg, pers. comm., Fig. 10). No zooplankton sampling was done in parallel to the 24-hour station in summer 2004.

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↑ **Figure 7** Copepod species abundance (left) and size class abundance (right) at the 36h station T8.4a, Walvis Bay-Transsect winter 2010.

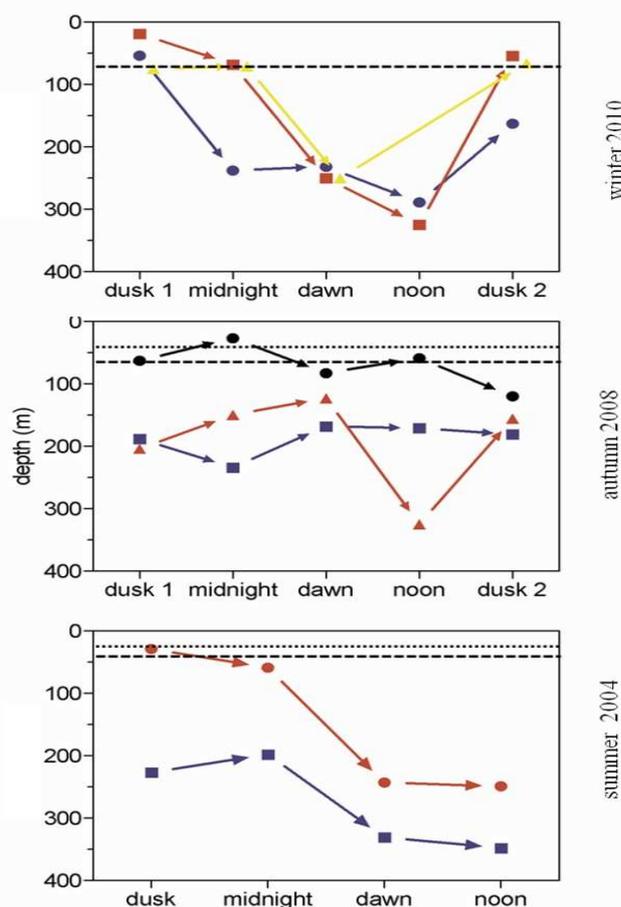
Species' vertical distribution

In winter *E. hanseni* displayed a “normal” migration pattern with an ascent at dusk and a descent at dawn. During daytime the animals resided in the oxygen depleted waters around 325 m ($<1 \text{ mL O}_2 \text{ L}^{-1}$). At night *E. hanseni* migrated out of the OMZ entering the upper layers of the water column ($\text{WMD}_{\text{midnight}} = 68 \text{ m}$). *E. hanseni* showed a migration amplitude of 306 m. A similar pattern was observed in *N. megalops* and *E. recurva* with deeper residence depths during dusk ($\text{WMD}_{\text{dusk1}} = 54 \text{ m}$ and 75 m respectively) and shallower residence depth during day in *N. megalops* ($\text{WMD}_{\text{noon}} = 289 \text{ m}$) compared to *E. hanseni*. *N. megalops* stayed in the OMZ throughout the day but at dusk (1) the animals were out of the OMZ. *N. megalops* showed a migration amplitude of up to 235 m. *E. recurva* stayed in or close to the oxycline during nighttime ($\text{WMD}_{\text{midnight}} = 71 \text{ m}$) but entered the OMZ during dawn ($\text{WMD}_{\text{dawn}} = 250 \text{ m}$; Fig. 8), suggesting pronounced vertical migration in this small species (Fig. 8).

In autumn the WMD data of *E. hanseni* showed a migration pattern with an ascent starting at dusk ($\text{WMD}_{\text{dusk1}} = 204 \text{ m}$) and a descent beginning at dawn ($\text{WMD}_{\text{dawn}} = 123 \text{ m}$) with persistence in deep waters during daytime ($\text{WMD}_{\text{noon}} = 325 \text{ m}$) and a migration amplitude of up to 200 m (Fig. 8). The animals stayed in the OMZ throughout 24h and migrated into severely oxygen depleted waters ($<0.3 \text{ mL O}_2 \text{ L}^{-1}$) during daylight. In *N. megalops* vertical migration, with a “reversed” migration pattern, was observed. Small differences of approx. 60 m between day ($\text{WMD}_{\text{noon}} = 171 \text{ m}$) and night ($\text{WMD}_{\text{midnight}} = 234 \text{ m}$) was noted in vertical distributions. *N. megalops*, like *E. hanseni*, did not leave the OMZ at all and both species avoided the thermocline. Vertical migration was evident in *E. americana* with a migration amplitude of 56 m from midnight ($\text{WMD}_{\text{midnight}} = 27 \text{ m}$) to dawn ($\text{WMD}_{\text{dawn}} = 83 \text{ m}$). At dusk 2

($WMD_{dawn} = 63$ m), contrary to dusk 1 ($WMD_{dusk1} = 120$ m), the animals entered deeper waters below 100m and therefore crossed the oxy- and thermocline (Fig. 8).

In summer *E. hanseni* occurred between 0 and 400m depth. Their vertical migration pattern showed a descent from midnight to dawn with persistence in deep waters during daytime ($WMD_{noon} = 249$ m) and a migration amplitude of up to 214 m. *E. hanseni* appeared to stay in the OMZ throughout the day and did not migrate above the oxy- and thermocline ($WMD_{dusk} = 29$ m, Fig. 8). Vertical migration behaviour in *N. megalops* showed an ascent starting at dusk ($WMD_{dusk} = 227$ m) with the shallowest vertical distribution around midnight ($WMD_{midnight} = 198$ m) and deep residence during daytime ($WMD_{noon} = 348$ m). Accordingly, the migration amplitude was 150 m (Fig. 8). Most part of the population stayed in the OMZ throughout the day and did not cross the thermocline. Animals were found between 0 and 600 m depth (Fig. 10).



↑ **Figure 8** WMD of *E. hanseni* (red line), *N. megalops* (blue line), *E. recurva* (yellow line) and *E. americana* (black line) at the 36h station T8.4a, Walvis Bay-Transect winter 2010 (top), at station T2.2, Kunene-Transect autumn 2008 (middle) and at station T2.2a, Kunene-Transect summer 2004 (bottom). Broken line indicates position of the oxycline. Dotted line indicates position of the thermocline.

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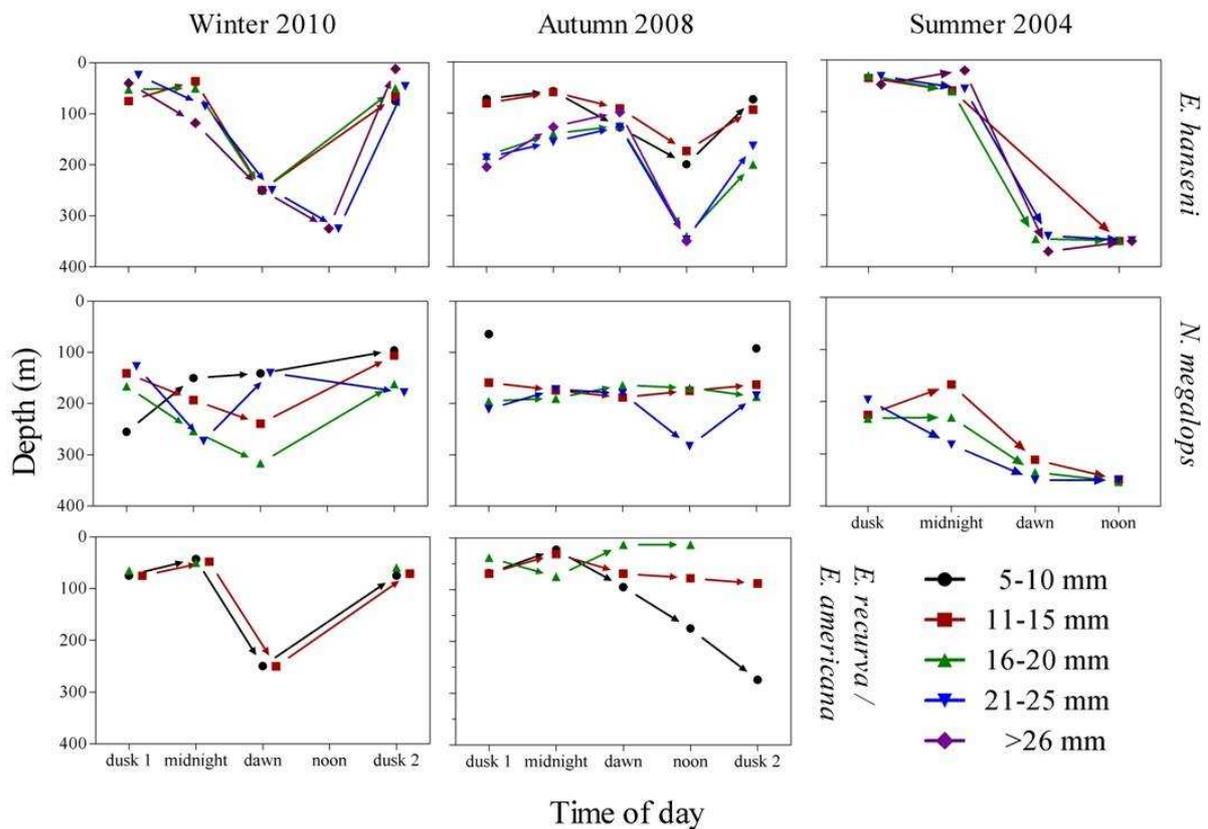
Size class distribution

Due to the 2 mm mesh size of the net our datasets did not provide enough data for the residence depth of smaller size classes < 5 mm during daytime. However, in *E. hanseni* the migration amplitude of size classes 21-25 mm and >26 mm was 310 ± 3 m in winter. Krill of all size classes, except those at 5-10 mm and 11-15 mm, entered the upper 50 m of the water column during dusk 1 or 2 and migrated close to the surface (WMD= 15 ± 2 m). However, both size classes reached their shallowest distribution at midnight (WMD= 37 ± 1 m). All sizes classes, except 5-10 mm (no data), were found at 250 m depth at dawn and the size class 11-15 and 16-20 mm may have entered deeper water layers during the day since these classes were not found at noon. WMD of size classes 21-25 mm and >26 mm was 325 m (Fig. 9). In autumn the migration amplitude of size classes 5-10 mm and 11-15 mm of *E. hanseni* with 129 ± 14 m was rather small. In contrast, the larger size classes showed a migration amplitude of 229 ± 16 m (Fig. 9). The latter migrated deeper down to 350 m (WMD) and some animals were also caught at a depth of 500 m. Additionally, the bigger size classes reached their shallowest distribution at dawn whereas the two size classes 5-10 mm and 11-15 mm arrived there at midnight. In summer the biggest size class (>26 mm) migrated into shallower waters during the night and into deeper waters at dawn compared to the other size classes (11-25 mm). Therefore its vertical migration range was slightly bigger, 350 m compared to 317 ± 2 m (Fig. 9).

In winter *N. megalops`* smallest size class (5-10 mm) reached its shallowest distribution at dusk 2 (141 m) whereas the bigger animals reached this point already at dusk (50 ± 5 m). In general, smaller animals tended to have smaller migration amplitudes than bigger ones, from 159m in size class 5-10 mm up to 263 m in size class 16-20 mm. The 11-20 mm size class showed a shallow residence at night (dusk: 50 ± 5 m) and deep residence by day (dawn: 278 ± 39 m). After reaching their shallowest distribution at dusk 1 (53 m) the 21-25 mm size class descended to 270 m depth and then ascended again up to 140 m during dawn (Fig. 9). In autumn the size class of 11-20 mm in *N. megalops* performed only weak diel vertical migration (30 ± 1 m), whereas animals between 21-25 mm showed a migration amplitude of 111 m. We cannot provide data on the persistence of the 5-10 mm size class during daytime because it was caught only during dusk. Overall, larger *N. megalops* migrated deeper than smaller ones, but only animals between 11-25 mm were found at a depth of 500 m in autumn 2008. In summer all three size classes investigated showed similar depth distributions during dusk, dawn and noon, but a clear separation was observed at midnight, where smaller animals

were found around 167 m and bigger animals around 282 m (Fig. 9). The migration amplitude in all size classes was 153 ± 24 m.

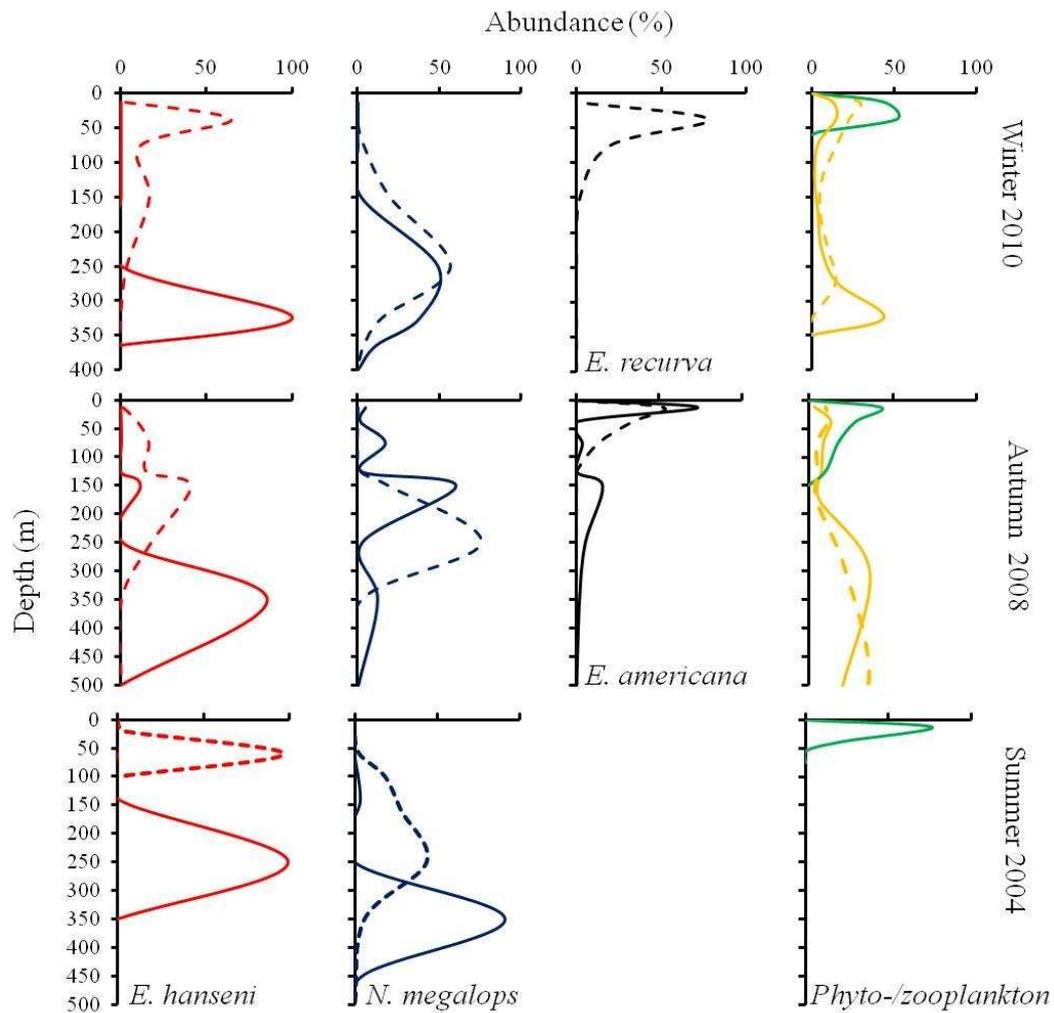
In *E. americana* the smallest size class (5-10 mm) conducted the farthest migration down to 274 m in winter 2010. However, no migration pattern was found for this size class. Animals of 11-15 mm size showed a small migration amplitude of 60 ± 3 m. A “reversed” pattern was noted in animals between 16-20 mm. They resided in the upper water layers (13 ± 0 m) during dawn and noon. During dusk and midnight the animals were located deeper at 38 and 75 m respectively. No differences in the vertical distribution between size classes were observed in *E. recurva* (Fig. 9).



↑ **Figure 9** WMD of different size classes of *E. hanseni* (top), *N. megalops* (middle), *E. recurva* (bottom, left) and *E. americana* (bottom, right) at the 36h station T8.4a, Walvis Bay-Transect winter 2010 (left), at station T2.2, Kunene-Transect autumn 2008 (middle) and at station T2.2a, Kunene-Transect summer 2004 (right).

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During all seasons *E. hanseni* showed significant differences between day and night vertical distributions. During the night the animals appeared to have spread more than during the day (Fig. 10). No differences in the daytime versus nighttime vertical distribution of *N. megalops* were found. During winter 2010 and summer 2004, a clear vertical separation between *E. hanseni* and *N. megalops* during night appeared, where *E. hanseni* stayed in the upper water layers, whereas *N. megalops* resided in deeper waters (Figure 10). In contrast, the vertical distributions of *E. hanseni* and *N. megalops* during autumn 2008 partly overlapped during nighttime (Fig. 10). During the day, vertical segregation between these species was evident during all seasons but less marked than during the night. In winter 2010, the vertical distribution of *E. hanseni* and *E. recurva* largely overlapped during night. Both species occupied the upper layers of the water column (25-50 m), whereas *N. megalops* persisted between 50–380 m (Figure 10). In autumn 2008, there was a clear vertical separation of *E. americana*, *E. hanseni* and *N. megalops* during the day (Fig. 10). *E. americana* occupied the upper layers of the water column (0-50 m), *N. megalops* persisted between 125–250 m and *E. hanseni* spent the day at a depth between 250–500 m. During night the vertical separation was blurred. Vertical distribution of *N. megalops* and *E. hanseni* partly overlapped, but *E. hanseni* showed its highest abundance around 150 m whereas *N. megalops* occurred mainly at a depth of 250 m. *E. americana* still persisted in the upper water layers (0-75 m).



↑ **Figure 10** Day (solid line) and night (broken line) distribution of *E. hanseni* (red line), *N. megalops* (blue line), *E. americana* (black line), *E. recurva* (black line), phytoplankton (green line) and zooplankton (orange line) at the 36h station T8.4a, Walvis Bay-Transect winter 2010 (top), at station T2.2, Kunene-Transect autumn 2008 (middle) and at station T2.2a, Kunene-Transect summer 2004 (bottom).

Energy demand and diel vertical migration behaviour

Oxygen consumption and carbon demands over 24h of a standardized *E. hanseni* (100 mg ww) and standardized *N. megalops* (50 mg ww) over 24h are given in Table 4. Filtration rates in *E. hanseni* were assessed at $0.9 - 1.1 \text{ L h}^{-1}$ (1 L h^{-1}), assuming phytoplankton feeding to meet 50% of the daily metabolic requirements and $1.3 - 1.6 \text{ L h}^{-1}$ (1.45 L h^{-1}), assuming phytoplankton feeding to meet 75%.

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Table 4 Calculated carbon demands (mg C d^{-1}), carbon demand due to migration (mg C d^{-1}), assumed food ratios and calculated feeding time (h) depending on the average and maximum Chl_a concentration in the upper 50m of the water column, assuming a filtration rate of 1.45 L h^{-1} of *E. hanseni* and *N. megalops* during the 36h studies in summer 2004, autumn 2008 and winter 2010.

	carbon demand (mg C d^{-1})	carbon demand for migrating (mg C d^{-1})	food ratio (phytoplankton/ zooplankton)	calculated feeding time min-max (h)
<i>E. hanseni</i> winter	0.44	0.13 (30%)	100/0	9.3-11.4
			66/33	10.3-11.6
			33/66	11.3-11.9
			0/100	12.5
<i>E. hanseni</i> autumn	0.4	0.07 (18%)	0/100	9 (day)
			0/100	19.9 (night)
<i>E. hanseni</i> summer	0.44	0.1 (23%)	100/0	6.2-9.8
			66/33	9.0-11.4
			33/66	11.7-12.0
			0/100	14.7
<i>N. megalops</i> winter	0.15	0.01 (7%)	0/100	8.5 (day)
			0/100	4 (night)
<i>N. megalops</i> autumn	0.19	0.005 (3%)	0/100	10 (day)
			0/100	16.3 (night)

The daily ratio of carbon losses due to vertical migration in *E. hanseni* varied between 4.0% body C d^{-1} during autumn and 4.4% body C d^{-1} during summer and winter. In *N. megalops* the daily ratio of carbon losses due to vertical “migration” was 2.9% body C d^{-1} during winter, 3.0 % body C d^{-1} during summer and 3.6 % body C d^{-1} during autumn. Due to the differences in the migration amplitudes observed, the percentage of migration of the daily carbon demand increased. Accordingly, the percentage of migration of the daily carbon demand in *E. hanseni* was 18% during autumn, 23% during summer and 30% during winter. In *N. megalops* this fraction was 3% during autumns, 7% during summer and 8% during winter (Table 4). During all seasons both species were able to cover their daily minimum food requirements within 24h. In *E. hanseni* the calculated feeding time, to cover its carbon demand, varied between 6.2h during summer ($F= 1.45 \text{ L h}^{-1}$; 100% phytoplankton) and 16.6h during winter ($F= 1 \text{ L h}^{-1}$; 100% phytoplankton). Although the vertical distribution of *E. hanseni* during autumn prevented feeding on living phytoplankton, it would have been able to cover its carbon demand by daytime feeding within 9h, exclusively feeding on copepods. However, 19.9h were necessary assuming nighttime feeding. *N. megalops* needed to feed between $6.2\text{h}\pm 2.1$ during winter and $12.8\text{h}\pm 3.2$ during autumn. If *E. hanseni* would have shown a migration behaviour like that during winter and summer, entering the upper water layers, assuming hydrographic and trophic conditions like those observed during autumn, its carbon demand

would have increased to 0.53 mg C d^{-1} (23% higher compared to winter+autumn), corresponding to $5.3\% \text{ body C d}^{-1}$. The estimated feeding time for such a scenario would vary between 5.3h ($F= 1.45 \text{ L h}^{-1}$; 100% phytoplankton) and 14.6h ($F= 1 \text{ L h}^{-1}$; 100% phytoplankton). The same scenario in *N. megalops* showed a 47% higher carbon demand compared to wintertime with a daily ratio of $4.2\% \text{ body C d}^{-1}$. Estimated feeding time would be $7.4\text{h}\pm 2.9$.

Discussion

Each euphausiid species occupy different vertical strata as a result of diel vertical migration in the northern Benguela upwelling system (Barange, 1990). In the current study the vertical migration behaviour of four euphausiid species, *E. hanseni*, *N. megalops*, *E. americana* and *E. recurva* and their susceptibility to being constrained by water temperatures, oxygen and food availability, was compared. The results will be discussed in the following sequence: (1) species-specific diel vertical migration, (2) vertical segregation among species, (3) DVM and hydrographic conditions, (4) DVM and trophic environment, and (5) DVM energy demand.

(1) Species-specific diel vertical migration

Animals do diel vertical migration to exploit the food rich upper water layers during the night and to decrease mortality from visual predators during the day. However, pronounced migration (DVM) leads to higher energy consumption, due to exposure to higher temperatures, low oxygen concentrations and increased swimming speeds during upward and downward migration (Torres and Childress, 1983; Lampert, 1989). In contrast, persistence in the cold deep during daytime may lead to low growth and reproduction rates (Lampert, 1989). Vertical migration in *E. americana* was not pronounced here, in contrast to James (1987) who reported that this species do extense DVM (190-300 m per day). During night maximum abundance was observed in the top 25m. Since *E. americana* may avoid nets (James, 1987) the abundance estimated in the upper layers during the day may be underestimated. In the current study most of the population stayed in the upper 150 m of the water column both during day and night and only a small part was found between 150 and 350 m by day. Accordingly, we suggest *E. americana* to be a weak-migrating species in the NBus.

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Differences in vertical distribution between dusk and dawn were distinct in *E. recurva* suggesting pronounced diel vertical migration. This contradicts Griffiths (1979) who found only little migration in *E. recurva*. However, Griffiths noted that most of the animals caught were in reproductive state, with females carrying spermatophores and most of the males having spermatophores in their ejaculatory duct. Sex dependent differences in the migration amplitude, with females migrating closer to the surface and using the energy supplement for reproduction, was also shown for *M. norvegica* (Tarling, 2003). Accordingly, the differences between Griffiths (1979) and our study could be explained by divergent reproductive status which was not analysed here.

In *E. hanseni* a clear day/night difference was observed during all cruises, indicative of intensive migration with a migration amplitude of up to 306m. Regular DVM was observed where animals ascended to the upper layers during nighttime and descended to deeper layers during the day. This is in accordance with Barange (1990). WMD plots suggested some degree of midnight sinking (cf Tarling et al., 1999). In winter 2010 *E. hanseni* reached its shallowest distribution at dusk and deepest distribution at noon. During autumn 2008 the observed DVM pattern of *E. hanseni* was distinctly different in that the animals did not migrate into the upper water layers and reached their shallowest distribution already at dawn. *E. hanseni* distributed below the thermocline, thus avoiding higher water temperatures in the uppermost water layers, may lead to lower oxygen (carbon) demand over 24h. However, in this case the omnivorous species *E. hanseni* was not able to feed, or only to a small extent, on living phytoplankton, as the phytoplankton community (Chl_a content above 0.1 mg m^{-3}) was restricted to the upper 80 m. Accordingly, a mismatch between adequate food conditions and a higher energy expenditure, due to elevated water temperatures in the upper water layers, was assumed (see below). However, other factors like high predation pressure should be taken into account. Differences in the migration behaviour between different size classes were not obvious. The migration amplitude of size classes 5-15 mm were less pronounced compared to the size class 16- >26 mm. This may have reflected the ability of larger animals to swim faster and more efficiently. Additionally, smaller animals may attain higher survival rates when avoiding larger individuals, i.e. as “predator” avoidance, since cannibalism was observed in several euphausiid species (Buchholz, 2003 and pers. obs.). Different swimming performance would explain why smaller animals reached their shallowest distribution at midnight, whereas bigger individuals already arrived there at dusk. However, prey-size spectra differ with euphausiid size (Barange et al., 1991). Accordingly, vertical segregation between different

krill species, as a matter of competition avoidance and optimized foraging, may also play a role between different size classes.

Vertical migration in *N. megalops* is yet unclear (Wiebe et al., 1982; Roe et al., 1984; Barange, 1990) but its migration amplitude is in any case smaller than in *E. hanseni* (Barange et al., 1991). These findings are in accordance with our study, where day/night differences in *N. megalops* were less marked than in *E. hanseni* and their migration amplitude was small. Although Wiebe et al. (1982) argued that day/night differences were due to net avoidance in *N. megalops* and Roe et al. (1984) suggested that these differences resulted either from net avoidance, patchiness, vertical migration or a combination of all three, we suggest a regular migration pattern with persistence in the upper layers during the night and persistence in deeper layers during the day, however, normally without crossing the thermocline. During summer 2004 *N. megalops* migrated within a layer of approx. 60 m, whereas during autumn 2008 the animals showed enhanced vertical migration of up to 150 m. In winter 2010, a migration amplitude of 235 m was observed. As stated by Mauchline (Mauchline, 1980) and Barange (Barange, 1990) the upper limit of its vertical distribution is related to the position of the thermocline. The hydrographic conditions during winter 2010, without a clear thermocline, may have supported more pronounced vertical migration with animals entering the upper 20 m of the water column. In general, *N. megalops* was distributed over the water column between approx. 50–400 m during the night, whereas *E. hanseni* was restricted to better defined strata. Size-dependent differences in DVM were obvious, where small animals (5-10 mm) stayed above the rest of the population or even showed a reversed migration pattern. Again, larger animals showed more pronounced vertical migration. Accordingly, different swimming performance could explain why small animals showed less pronounced migration amplitudes compared to larger ones. Furthermore, different size-dependent prey-size spectra (Barange et al., 1991) and possible dietary shifts from omnivory to carnivory with an increase in size could lead to different strata occupied, depending on the vertical distribution of varying prey sizes.

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(2) Vertical segregation among species

Barange (1990) showed that the euphausiid community in the northern Benguela upwelling system, regardless of migrating or non-migrating species, is segregated in space during night. Water column structure and reproductive strategies of particular species were responsible for this pattern. Vertical space partitioning was suggested to be a strategy of sharing highly productive areas, like the northern Benguela shelf/slope region, thus avoiding intraspecific competition (Barange et al., 1991). Vertical separation between *E. hanseni* and *N. megalops* was evident in this study, but it appeared not only during nighttime, as suggested by Barange (1990), but also during daytime hours. This different finding could be caused by that Barange (1990) only sampled the upper 200 m and did not provide daytime distribution data for most of the species investigated. Interspecific vertical segregation occurred also between *E. americana*, *E. hanseni* and *N. megalops* during day and night, but vertical distribution largely overlapped between *E. hanseni* and *E. recurva*, at least during nighttime. *E. hanseni* (Barange et al., 1991) and *E. recurva* (Suh and Choi, 1998) feed omnivorously and therefore, competition for food sources is likely. However, *E. recurva* is a small species (max. length in this study was 17 mm, average 12.7 mm) whereas *E. hanseni* is comparably large (max. length 27 mm, average 20 mm). Assuming size-dependent prey-size spectra, this competition may be diminished and co-occurrence in the food rich top layers may not result in food limitation in these species. As *E. hanseni* descended to deeper layers by day and the position of *E. recurva* during daytime is still unknown, food competition at least during the day may not take place. Furthermore, as different feeding patterns, and thus diel periodicity in feeding activity, could not be excluded, competition for food resources may have been further dampened.

(3) DVM and hydrographic conditions

Abiotic factors like temperature and dissolved oxygen concentration are known to influence the vertical distribution of euphausiids (Buchholz et al., 1995; Antezana, 2009; Escribano et al., 2009). A comparison of the hydrographic conditions found at the sampling stations during the cruises in summer, autumn and winter are given in Table 5. During autumn and summer the water column at the Kunene-Transect was covered by a warm surface layer (24.7°C and 22.3°C). This area lies within the Angola-Benguela frontal zone and is often influenced by a warm water intrusion from the (tropical) Angola dome. In contrast, SST at the Walvis Bay-Transect during wintertime was only 16.4°C. Here, water temperatures in summer are higher,

but temperature profiles of the upper water layers are mainly determined by irradiance, air temperature and upwelling intensity.

↓ **Table 5** Comparison of hydrographic and trophic conditions during winter 2010, autumn 2008 and summer 2004 (nd – not detectable; nda – no data available).

	winter 2010	autumn 2008	summer 2004
temperature			
SST (°C)	16.4	24.7	22.3
at 400m depth (°C)	9.8*	9.3	8.9
depth of thermocline (m)	nd	30 - 60	22 - 33
thermocline (°C m ⁻¹)	nd	0.25	0.50
oxygen level			
min (mL O ₂ L ⁻¹)	0.98	0.21	0.37
max (mL O ₂ L ⁻¹)	6.01	4.80	5.10
depth of oxycline (m)	66 - 79	50 - 70	22 - 62
oxycline (mL O ₂ L ⁻¹ m ⁻¹)	0.15	0.15	0.10
phytoplankton			
min (0-50m; Chl_a mg m ⁻³)	0.32	0.19	0.10
max (0-50m; Chl_a mg m ⁻³)	0.46	0.98	0.69
average (0-50m; Chl_a mg m ⁻³)	0.38±0.03	0.52±0.25	0.45±0.24
zooplankton			
min (0-500m; mg dw m ⁻³)	1.2**	1.4	nda
max (0-500m; mg dw m ⁻³)	31.6**	39.8	nda
average (0-500m; mg dw m ⁻³)	8.0±7.6**	10.6±10.5	nda
euphausiid species			
<i>E. americana</i>	+	+	+
<i>E. gibboides</i>	-	+	+
<i>E. hanseni</i>	+	+	+
<i>E. recurva</i>	+	-	-
<i>N. capensis</i>	-	+	-
<i>N. megalops</i>	+	+	+

* Bottom depth was 390m. Therefore, temperature at 350m depth was used.

** Bottom depth was 390m. Therefore, data are given for 0-300m depth.

According to the vertical distribution found in *E. hanseni*, this species may have entered the uppermost water layers at the Kunene-Transect in summer 2004. However, its WMD suggested that most of the population stayed close to or within the thermocline. These

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findings were in accordance with Barange (1990) who found that *E. hanseni* remained above and within the thermocline during nighttime. The need to cross the thermocline, as stated by Ohman (1990), represents major energy expenditure and thus carries a fitness cost and not a benefit. Accordingly, we presume that crossing the thermocline and entering warm waters above the thermocline may take place only for short (feeding) excursions or for reproductively-active females (Tarling et al. 1999, Tarling, 2003). During winter 2010, when no thermocline was detectable, *E. hanseni* entered the upper 20m of the water column. Here, in contrast, *N. megalops* avoided the thermocline and its shallowest distribution during winter 2010 was up to 50 m (Fig. 8 & 9), where water temperatures were comparatively low and without a thermocline. Its “normal” vertical distribution ranged between 150–350 m depth, where temperature was below 15°C. A high concentration of euphausiids below the thermocline, as reported by Barange (1990), was not observed during our studies. Differences in the vertical distribution between the two species may be explained by their different thermal adaptations with *E. hanseni* showing adaptation to temperatures between 10°C and 20°C, as indicated by a low Q_{10} of 1.5, and *N. megalops*'s adaptation to lower water temperatures with a Q_{10} of 1.9 between 5°C and 10°C (Werner et al., in press).

Irrespective of the small vertical migration amplitude, *E. americana* crossed the thermocline twice during nighttime and remained in or close to the thermocline during the day. Since *E. recurva* was caught only during winter in 2010 when no thermocline was found, vertical distribution of this species in adjustment to the thermocline could not be shown.

Oxygen levels below 1 mL O₂ L⁻¹ are common in the northern Benguela upwelling system. *E. americana*, *E. hanseni*, *E. recurva* and *N. megalops* entered the OMZ but only *E. hanseni* and *N. megalops* spent most of the time in the OMZ, irrespective of the low oxygen level (Table 5). This may have indicated that krill was divided into different ecological groups using or mainly avoiding the OMZ, as shown for copepods in the NBus (Auel and Verheye, 2007). *E. hanseni* and *N. megalops* left the OMZ during winter 2010, but did not cross the oxycline during autumn 2008. In summer 2004 *N. megalops* stayed in the OMZ throughout 24h whereas *E. hanseni* was found in the oxycline but not above it. Staying in oxygen depleted waters may be a common behaviour to avoid predation, e.g. from oxygen-dependent fish and was described for other euphausiids like *Euphausia mucronata* (Escribano et al., 2009).

(4) DVM and trophic environment

N. megalops is known as an omnivorous species, with a high degree of carnivory. No clear diurnal pattern in the feeding activity was found in this species but higher numbers of copepod fragments were found in the late afternoon/early evening in its gut (Barange et al., 1991; Gurney et al., 2002). Chlorophyll pigments in the stomach of this species were of secondary origin (Gurney et al., 2002). Accordingly, gut fluorescence, as an index for in situ grazing rates, reflected the pigment content of its prey items (Pakhomov and Perissinotto, 1996). However, due to the fact that *N. megalops* did not enter the phytoplankton rich layers and their feeding appendages lack a real filter basket (Mauchline, 1980), *N. megalops* adults are considered carnivores (Gurney et al., 2001). *E. hanseni* is known as an omnivore that opportunistically feeds on copepods, dinoflagellates, and tintinnids (Barange et al., 1991). Gut fullness and number of copepods consumed, suggested nighttime feeding in this species (Barange et al., 1991). During the vertical migration study in winter 2010 the vertical distribution of *E. hanseni* largely overlapped with the highest abundance of zooplankton in general and copepods in particular, both during day and night. Vertical distribution of *N. megalops* coincided with maximum copepod distribution during the day but did not during the night. However, during autumn 2008, *E. hanseni* persisted at approximately the same depth as the main concentration of zooplankton by day, but again no overlap was observed during nighttime. By night, *N. megalops* showed no accordance with the zooplankton distribution and also during the day only a small proportion was found at the same depth as the maximum zooplankton abundance. During winter in 2010 the maximum prey concentration may have regulated the vertical distribution of both krill species. *E. hanseni* followed its prey by day and night whereas *N. megalops* stayed at a specific depth and may have exploited the migrating copepod community, as suggested by Barange et al. (1991). In 2008 both species did not enter the upper water layers and compliance with the vertical distribution of prey items was low. However, zooplankton abundance between 200–500 m depth was very high and persistence in the vicinity of prey maxima may have been sufficient to cover their daily carbon demands. Accordingly, the necessity to migrate was only poor. The vertical distribution of euphausiids and the vertical distribution of its prey were investigated by Sameoto et al. (1987) who suggested that prey concentration may be more important than temperature in determining the vertical positioning of krill. In contrast, a re-evaluation of field data on DVM in *Daphnia* by Loose and Dawidowicz (1994) supported the assumption that vertical temperature gradients are more important than food gradients. Gibbons (1993) suggested that *Euphausia lucens* in the southern Benguela reacts to food concentrations in two

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different ways. He stated that the vertical distribution of food determines the depth of *E. lucens* during nighttime and the amount of food affects the extension of the DVM response. For *Meganyctiphanes norvegica* in the Danish Läsö-Deep, Buchholz et al. (1995) showed that the upper temperature limit excluded this species from rich food sources (copepods and phytoplankton) near the surface. However, with abundant food, other constraints, like water column structure, may act as controlling factors (Barange, 1990). Nakagawa et al. (2001, 2002) showed that *Euphausia pacifica* may switch their feeding preferences from phytoplankton to zooplankton during DVM. Accordingly, *E. hanseni* may be able to cover its carbon demand in the deep, if food conditions were appropriate and water column structure may have prevented immigration into the upper water layers. As a consequence, vertical migration may have been suspended for an unknown number of diurnal cycles.

(5) DVM energy demand

Torres and Childress (Torres and Childress, 1983) demonstrated that DVM is energetically expensive. Staying in the cold deep during the day is associated with low food concentration, low growth, fecundity rates and prolongation of the developmental time of eggs (Lampert, 1989).

The influence of temperature on the metabolism of both species differed significantly (Werner et al., in press). Accordingly, variations in the DVM behaviour, thus facing different temperatures over a diel cycle, lead to divergent carbon demands. In addition, a protein-oriented metabolism in *E. hanseni* and a lipid-oriented metabolism in *N. megalops*, with deviating RQs, accounted for an approx. 35% higher carbon demand, thus minimum food requirements, in *E. hanseni* than in *N. megalops*. The proportion needed for swimming up and down on the total daily carbon demand was $24\pm 6\%$ in *E. hanseni* and $6\pm 2\%$ in *N. megalops*. Thus, 'swimming costs' were 300% higher in *E. hanseni* than in *N. megalops*. During winter 2010, with lowest water temperatures, both species showed the least carbon demand over 24h. During autumn 2008, with highest water temperatures, both species avoided the upper water layers, and both results are indicative of temperature as a controlling and limiting factor. Despite great behavioural and temperature variations, the carbon demands calculated for both species showed only small differences between summer 2004, autumn 2008 and winter 2010, with $4.2\%\pm 0.2$ body C d⁻¹ in *E. hanseni* and $3.2\%\pm 0.4$ body C d⁻¹ in *N. megalops*, indicative of a species-specific energy (carbon) expenditure. Food concentrations during the different

seasons were high enough to cover their daily carbon demands within a reasonable amount of time. However, during autumn 2008 food concentrations for *E. hanseni* during nighttime were comparably low and feeding time would have been too long. If *E. hanseni* were able to switch their nighttime feeding activity from phytoplankton near the surface to daytime feeding of copepods in deeper layers, as shown for *E. lucens* (Stuart and Pillar, 1990) and *E. pacifica* (Nakagawa et al., 2002), feeding time would be comparably short. If *E. hanseni* had migrated into the warm upper water layers during autumn 2008, its carbon demand would have been 23% higher. However, Chl_a concentrations in the upper 50m were approx. 37% higher, counterbalancing the higher carbon demand due to higher water temperatures (Table 5). If we use the estimated feeding times as indices for favourable migration behaviour in terms of food availability and temperature, we should expect that *E. hanseni* migrated into the food rich upper layers during each season and should preferentially have fed on phytoplankton. These assumptions contradict our observations and may be explained by the different calorific content between metazoan food sources and phytoplankton and the suggested species-specific energy (carbon) expenditure. An individual feeding carnivorously may need to ingest less material than one feeding on phytoplankton. Additionally, metazoan material may be digested more readily and numbers on the carbon ratio based on weight may be fairly underestimated (Gurney et al., 2002). However, favourable food conditions in the deep may prevent animals from migrating into upper food rich water layers. Nakagawa et al. (2003) have shown that *E. pacifica* can gain energy in deeper layers during the day when the ambient food concentration is suitable. This behaviour may change if individuals of different physiological states require higher water temperature, e.g. egg bearing females. Day and night distributions of *E. hanseni* indicated that only part of the population was vertically migrating. Accordingly, differences in the DVM behaviour of individuals may be an active behavioural response to internal physiological processes and external intraspecific stimuli, as suggested for *M. norvegica* by Tarling et al. (1999). Energy budgets for *N. megalops* indicated that persistence in the deep lead to low carbon demands which can easily be satisfied by exploiting the migrating zooplankton community while staying constantly in the deep.

The data provided by this study should help to further understand the driving forces in the variable DVM patterns observed and will be implemented in a numeric modeling approach as a next step. Such a DVM-model will be integrated to broader scale modeling of ecological processes and trophic interactions and their roles in biogeochemical cycles aiming at assessing status and impact of climate variability in the Northern Benguela upwelling ecosystem. In general, the energetic considerations for both species showed that temperature

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acted as a controlling factor but could not be used alone to explain the vertical positioning and DVM behaviour of krill species in the NBus. Food abundance influenced the DVM behaviour of krill species, but the vertical distribution of krill did not always coincide with highest food concentrations. However, trade-offs between food abundance and temperature constraints suggested that different migration behaviours were energetically feasible. Therefore, other factors like predation pressure should be taken into account. In the highly productive poly-pulsed NBus, where food abundance is seldom a limiting factor over longer periods, vertical distribution may result primarily from competition and predator avoidance. In other (mono-pulsed) systems, with sometimes limiting food sources, trade-offs between temperature and food may result in different behaviours. The current study did not consider sex-dependent differences, predator abundance, or net avoidance during the day as a common capability in large euphausiids and this may have biased observations to a certain extent. Accordingly, the observed differences may be influenced by yet other factors. However, the approach evaluating different proximate factors and calculating their energetic trade-offs may be considered as a suited tool to enhance understanding of the simultaneous effects of all these factors on the DVM behaviour of zooplankton.

Conclusion and perspective

The main results of this study showed seasonal differences in the DVM behaviour of *E. hanseni* and *N. megalops*, due to different water temperatures, oxygen-depletion, food availability and energetic trade-offs. Despite behavioural differences between seasons, *E. hanseni* is a pronounced long range vertical migrant, regularly crossing the thermocline and retreating again to the oxygen minimum zone whereas *N. megalops* is a weak migrant, avoiding the thermocline and persisting in the OMZ throughout 24h. Predator and competition avoidance were ultimate cues which may determine the DVM behaviour of euphausiids in the NBus. The relative change of light intensity (day/night differences) was the principal proximate cue controlling the timing of upward and downward migration. Temperature profiles, position and intensity of the OMZ and food availability were further proximate factors which influenced the behaviour.

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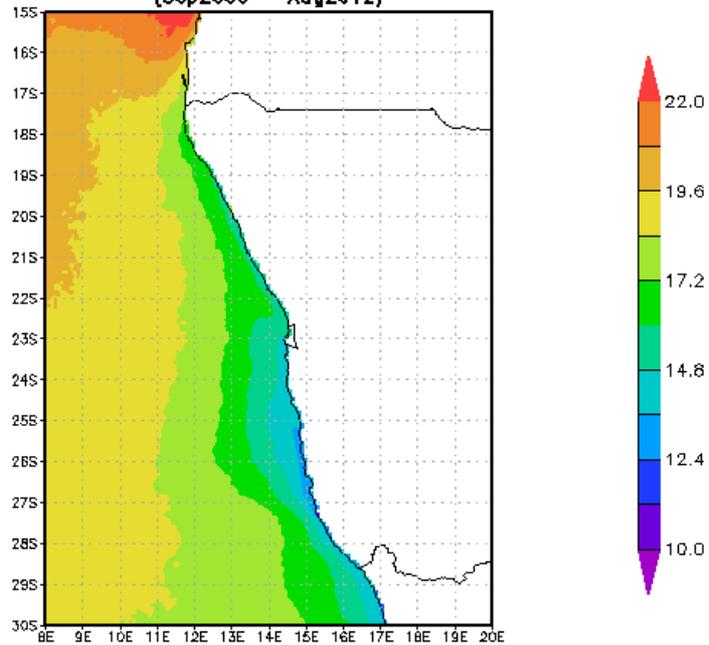
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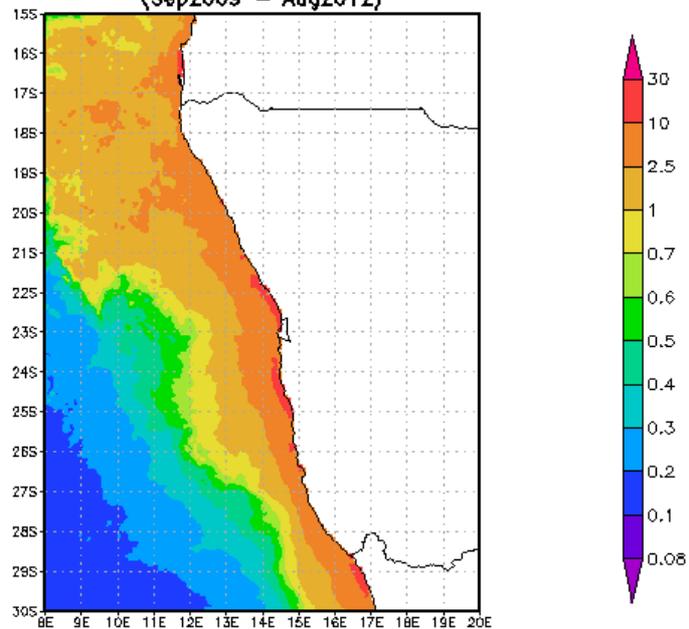
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MAMO_SST4_9km.CR Sea Surface Temperature (4 micron night) 9km [C]
(Sep2009 - Aug2012)



MAMO_CHLO_9km.CR Chlorophyll a concentration [mg/m**3]
(Sep2009 - Aug2012)



Sea surface temperature (SST, °C) and Chl_a concentration (mg m⁻³) in the NBC between September 2009 and August 2012 (Visualizations were produced with the Giovanni online data system, developed and maintained by the NASA GES DISC).

Regional and seasonal differences in physiological performance of *Euphausia hanseni* in the northern Benguela ecosystem, influenced by upwelling

Thorsten Werner and Friedrich Buchholz

Abstract

Variability in upwelling events can lead to periods of constrained food availability in the northern Benguela upwelling system (nBus), thereby affecting the physiological state and metabolic activity of euphausiids. Most research attention has so far been paid to seasonal effects and thus little is known about short time-scale effects. Accordingly, metabolic activity, as expressed through respiration and excretion rates, and the physiological state, through reproductive effort and moult activity, in *Euphausia hanseni* were scored in relation to upwelling pulses, e.g. food availability. Upwelling pulses appear to control moult and reproduction in *E. hanseni*. Additionally, higher respiration rates were found for females in higher sexual developmental stages. Moult stage did not affect oxygen consumption rates however. Overall, short time-scale events had a greater impact on metabolic rates than differences between seasons. Thus, physiological states of *E. hanseni* may be useful indicators of the current hydrographic and trophic conditions in the nBus.

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Introduction

One of the four major Eastern Boundary upwelling systems, the Northern Benguela upwelling system (nBus) is located off the west-coast of Namibia. It is bordered in the north by the Angola-Benguela front (~17°S) and in the south by the strong upwelling cell at Lüderitz (26°S). The nBus is characterized by perennial upwelling with a maximum during austral winter/spring and a minimum during austral summer/autumn. Influenced by several atmospheric and oceanographic processes the nBus is a complex and highly variable ecosystem (Shannon and Nelson 1996). Further wind-driven coastal upwelling makes this area one of the most productive ecosystems in the world's oceans. Fluctuations in upwelling intensity correlate with variations in the magnitude and direction of winds. Timing and duration of upwelling events influence the physical and biological properties of coastal seas, including the population biology of krill (Dorman et al. 2005). Upwelling events favour phytoplankton growth through nutrient input and thereby support omnivorous species, like *Euphausia hanseni* the dominant krill species in the nBus, with convenient food supply. Consequently, *E. hanseni* may show physiological adaptations to these poly-pulsed, upwelling-mediated, plankton blooms. The adjustment of metabolic rates in krill to (seasonal) differences in abiotic, e.g. temperature, oxygen and biotic, e.g. food availability, factors was the target of several studies (e.g. Buchholz and Saborowski 2000; Saborowski et al. 2002; Kim et al. 2010; Meyer et al. 2009 and 2010). However, the effects of short time scale changes in the physical and biological environment on euphausiid physiology are largely unknown (cf. Dorman et al. 2005).

Different physiological processes, such as growth and reproduction, are influenced by food availability. Accordingly, euphausiids adapt their reproductive cycles to local feeding conditions (Tarling and Cuzin-Roudy 2003); both egg production and length of the reproductive period are controlled by temperature and trophic conditions (Cuzin-Roudy and Buchholz 1999). Furthermore, the recruitment success of krill species depends both on adequate condition of the females prior to spawning and favourable trophic conditions during larval development (Tarling and Cuzin-Roudy 2003).

Growth in euphausiids is controlled mainly by temperature and food supply (Huntley and Boyd 1984) and moulting accelerates both respiration and excretion rates (Ikeda and Mitchell 1982). Furthermore, seasonal changes in food availability can alter the chemical composition of zooplankton, which in turn affects respiration and excretion rates (Conover and Corner 1968). Spring plankton blooms enhance metabolic rates in *Meganyctiphanes norvegica*,

where otherwise no respiratory adaptations occur throughout the rest of the year, under stable food conditions. Thus, during the short productive season in the Ligurian Sea, growth and reproduction are maximized by *M. norvegica* (Buchholz and Saborowski 2000). In *Euphausia pacifica* and *Thysanoessa inspinata* from the Oyashio region (North Pacific Ocean, Japan), excretion rates and O:N ratios under constant food supply suggested preferential use of dietary proteins for growth and reproduction (Kim et al. 2010). Thus, the spatial and temporal availability of food often constrains the physiological state of zooplankton. In the nBus, the plankton biomass and distribution is mainly determined by short time-scale upwelling events rather than by seasonality and thereby displays a high degree of variability. To better understand this variability in *E. hanseni*, further study on the impact of upwelling pulses on the physiology of krill species is crucial. Such investigations are also necessary, to better predict the effect that krill physiological state has on higher trophic levels and the food web structure in the nBus.

In the present paper regional differences in metabolic activity and physiological states, moult activity and reproductive state, of *E. hanseni* were examined in relation to upwelling pulses and food availability. Seasonal variability in these parameters between maximum and minimum upwelling intensities (winter and late summer), were also investigated and are discussed under a broader scope in relation to other species such as *Euphausia superba* and *M. norvegica*.

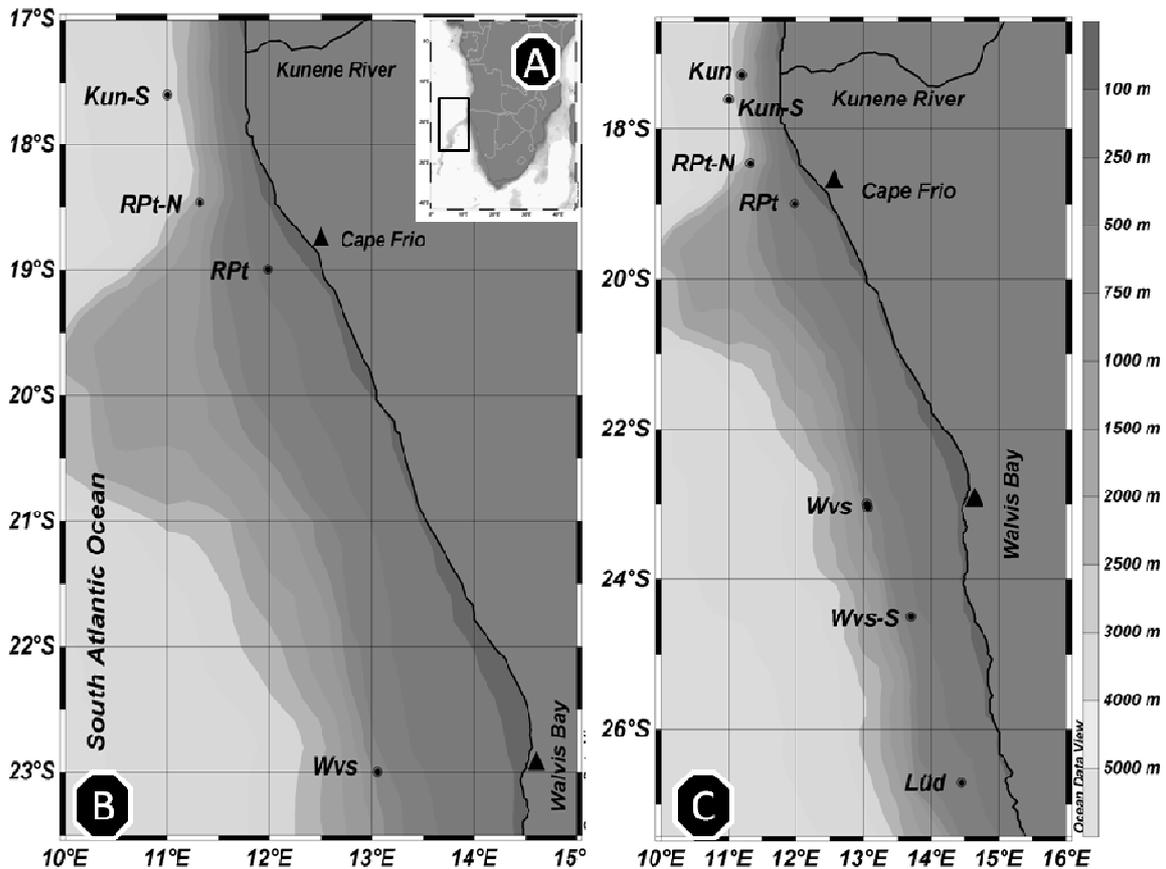
Material and Methods

Field sampling

Euphausiids were collected on board the RV *Discovery* in winter 2010 (10.09.-13.10.2010, cruise D356) and on board the RV *Maria S. Merian* in late austral summer/autumn 2011 (30.01.-07.03.2011, cruise MSM17/3). Specimens were collected between Lüderitz (26.6° S) and Kunene (17.25° S) in the northern Benguela upwelling system off Namibia. Krill were caught at various depths at different stations during nighttime with a 1-m² MOCNESS (Multiple Opening and Closing Net with Environmental Sensing System, Wiebe et al. 1985). A large mesh size (2000 µm) and a large soft closed cod-end bucket were used to reduce stress on the experimental animals. Sampling for the regional comparison of *E. hanseni* was performed at four stations between Walvis Bay (22°57`S) and Cape Frio (18°45`S; Fig. 1(B)) during winter 2010. Sampling for the seasonal comparison was performed at three additional

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stations and one repeat (Wvs), between Lüderitz and Kunene during late summer 2011 (Fig. 1(C)). The animals for metabolic measurements were randomly chosen and transferred to aerated plastic aquaria filled with filtered seawater. The leftover animals of each haul, or a representative part respectively, were used to assess the physiological state of *E. hanseni* in the field.



↑ **Figure 1** Location of the northern Benguela upwelling system in the Southwest Atlantic Ocean (inset A). The station positions sampled for regional comparison during winter 2010 (B) and the station positions sampled for seasonal comparison during winter 2010 and late summer 2011 (C). Circles denote locations of sample stations, triangles geographic landmarks. Given are the names of each sampled transect and the corresponding stations (Lüd – Lüderitz Cell; Kun – Kunene; RPt – Rocky-Point; Wvs – Walvis Bay). Letters indicate positions of the stations relative to transect (S – South; N – North).

Metabolic measurements

Respiration measurements were conducted with a closed respirometry system with Oxygen-Microsensors (PreSens, Germany) and a 4-channel micro-fiber optic oxygen transmitter (Oxy-4-micro, PreSens, Germany) on board the research vessels. Specially designed small tube-shaped chambers (volume 20 mL) were used as respiration chambers optimized for krill (cf. Werner et al. 2012). Respiration measurements were conducted in a temperature-controlled room at four different temperatures (5°C, 10°C, 15°C and 20°C), which reflect the temperature range experienced by *E. hansenii* during both seasons. *E. hansenii* was first acclimatized for approx. 12h at 8-10°C and all experiments were conducted within 24h after catch in order to minimize confounding effects due to starvation (K. Huenerlage and F. Buchholz pers. comm.). Animals were acclimated to the experimental temperatures in the respiration chamber for 1 to 4h and the oxygen uptake (M_{O_2}) was monitored over a duration of 3 to 6h in the dark. Each temperature treatment was run in triplicate with an additional empty chamber to serve as the control. The respiration chambers were placed in a water bath, which was controlled by a lab cooler ($\pm 0.5^\circ\text{C}$; Julabo F25, Germany). Filtered seawater (0.2 μm Acropak™ 1000 Capsule, Pall Filtersystems GmbH, Germany) was used, in order to minimize bacterial oxygen consumption. Since the metabolic rate of animals is known to vary both interspecifically and intraspecifically with body mass, respiration rates were standardized to units of comparison of $M_{O_2} \text{ h}^{-1} \text{ g}_{\text{ww}}^{-1}$. Animals were sexed, measured for length and weighed, and their moult stage and sexual developmental stage (SDS) determined before deep-freezing at -80°C for further analysis. Regional comparisons of metabolic rates were conducted only at 10°C . Measurement of respiration rates involved the analysis of 2ml water subsamples taken from the respiration chambers after each experiment and immediately deep-frozen in liquid nitrogen and stored at -80°C . These subsamples were later analyzed for ammonia ($\text{NH}_4\text{-N}$), the major form of dissolved nitrogen excreted by marine zooplankton (E_{NH_4}), using the phenol-hypochlorid method following Solorzano (1969). The O:N ratio was calculated by simple division between M_{O_2} and E_{NH_4} in order to determine the dominantly metabolized substrate (Mayzaud and Conover 1988).

Moult stages and sexual developmental stages

The moult stage (MS) of each *E. hansenii* specimen was determined following Buchholz (1982 and 1991), derived from Drach's method. One outer uropod was gently cut-off and

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placed on a micro slide with a drop of cooled sea water and inspected under a Zeiss microscope (100x-400x magnification). The moult cycle is divided into moult stages grouped into two phases: the postmoult period consists of MS A, B and C and the premoult period consists of MS D₀, D₁, D₂ and D₃.

Sexual developmental stages (SDSs) of female *E. hanseni* were defined following a modified staging system by Cuzin-Roudy (1993) which orders each stage according to the development of the ovaries. Overall there are 5 different SDS, with SDSs 1 and 2 being characterized by ovaries still in development and differentiation. SDS 3 is defined by the occurrence of vitellogenesis and at SDSs 4 and higher the ovaries have reached their full extension and start to mature. The SDSs were determined in live female krill, using a Zeiss microscope (100x-400x magnification) to examine the extension and structure of the ovary. The sex ratio (f/m) in a swarm was also assessed from all the individuals caught at each station.

Statistical treatment of data

Results are presented as means \pm standard deviations (SD). Either one-way ANOVAs or t-tests were performed according to the number of groups compared. Tukey's multiple comparison post-tests were also performed following ANOVAs. Data were tested for normality using the Kolmogorov-Smirnov and/or D'Agostino and Pearson omnibus normality test. Where required, data were normalized using log-transformation. If normalization was not possible, a Kruskal-Wallis test with Dunn's post test was applied. F-tests were used to compare fits (e.g. the impact of temperature on the respiration rates between seasons). Tests for correlations between parameters following Gaussian distributions were performed with Pearson's correlation calculations. Spearman correlations were conducted if the data did not show a Gaussian distribution. The significance level was set at $P < 0.05$, except where noted.

Results

Hydrographic and trophic conditions

A description of the environmental dynamics in the nBus is given by Shannon 1985; Mohrholz et al. 2001; Monteiro et al. 2008 and Hutchings et al. 2009. The hydrographic conditions during the sampling periods are described in Buchholz et al. (2010; report of cruise

D356) and Lahajnar et al. (2011; report of cruise MSM17/3). An overview of the hydrographic and trophic conditions, derived from ferrybox-measurements, at the different sampling stations is given in Table 1. Sampling stations, RPt, Kun-S and Wvs, were located in upwelling regions, as indicated by cold upper water layers bearing low salinities, whereas RPt-N was located in a non-upwelling region as indicated by considerably warmer and more saline surface water. In the following, the stations RPt, Kun-S, Wvs and RPt-N will be referred as Up_1, Up_2, Up_3 and No_1 to facilitate differentiation between upwelling and non-upwelling situations.

↓ **Table 1** Hydrographic (SST (°C), salinity) and trophic conditions (Chl_a ($\mu\text{g L}^{-1}$), Phycoerythrin levels (cells mL^{-1})) at the sampling stations during winter 2010. Data were derived from ferrybox-measurements (5-10m water depth) conducted over the whole sampling procedure. Given are the means \pm SD.

Winter 2010	temperature (°C)	salinity	Chlorophyll_a ($\mu\text{g L}^{-1}$)	Phycoerythrin (cells mL^{-1})	upwelling
Up_1	15.2 \pm 0.07	35.25	7.5 \pm 1.0	3598 \pm 25	yes
Up_2	15.8 \pm 0.06	35.35	16.6 \pm 0.4	8825 \pm 105	yes
Up_3	15.8 \pm 0.03	35.24	9.8 \pm 1.0	3859 \pm 38	yes
No_1	17.9 \pm 0.02	35.91	10.8 \pm 0.4	2119 \pm 30	no

Favourable trophic conditions were found at Up_2, with high Chlorophyll_a concentrations ($16.6 \pm 0.4 \mu\text{g L}^{-1}$) and high Phycoerythrin levels ($8825 \pm 105 \text{ cells mL}^{-1}$). In contrast, the trophic conditions at No_1, Up_1 and Up_3 were comparably poorer (Table 1). Seasonal differences in the SST between summer 2010 and winter 2011 were apparent, however, the trophic conditions during both seasons were relatively similar (Fig. 2).

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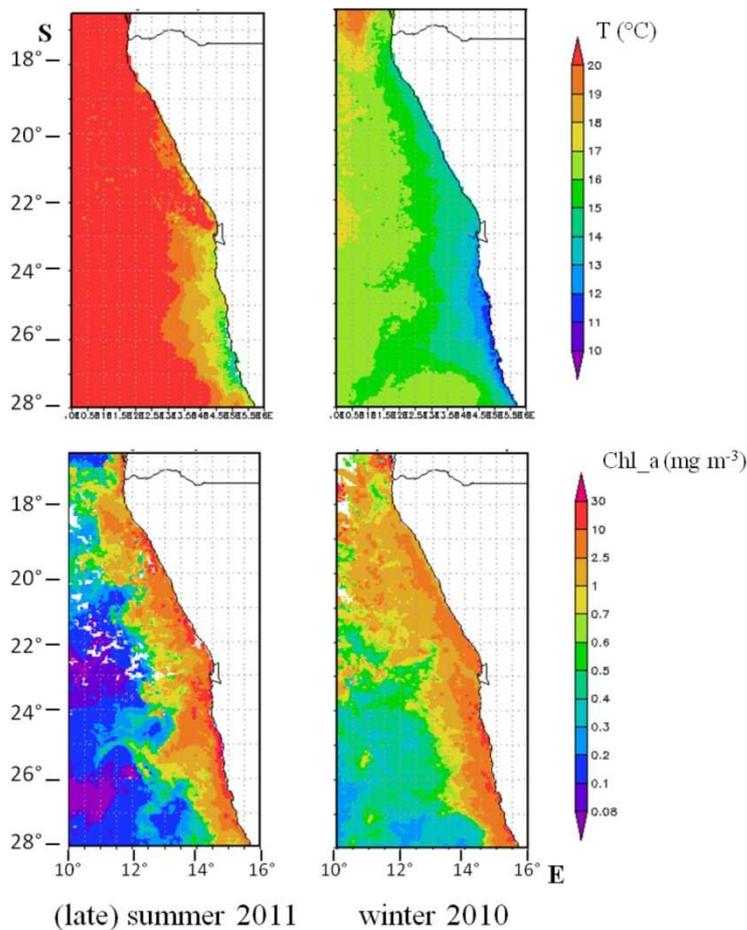


Figure 2

Sea surface temperatures (SST, °C) and trophic environment (Chl_a, mg m⁻³) during the sampling period, late summer 2011 (February) and winter 2010 (September/October).

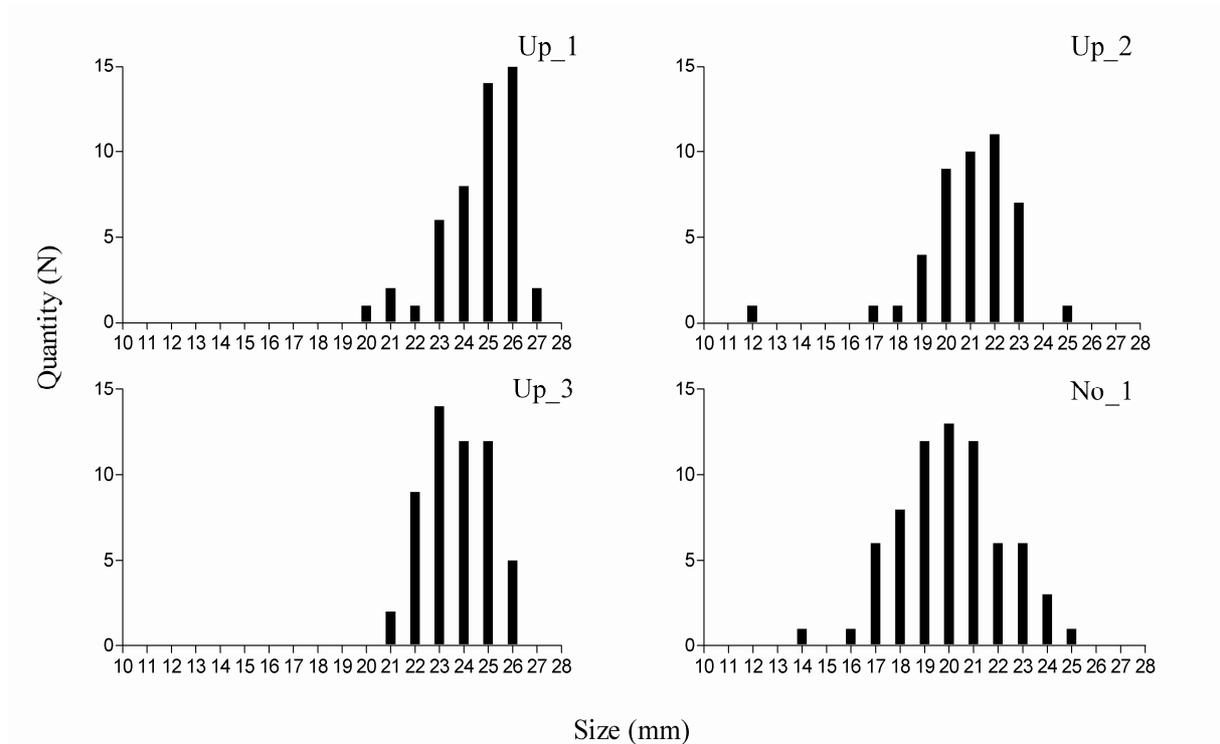
During summer 2011 the region between 17°S and 28°S was largely covered by warm upper water layers (<20°C). In contrast, during winter 2010, temperatures of the upper water layers ranged between 15°C and 17°C. The Chlorophyll_a content over the shelf, derived from satellite imagery, was high (1-10 mg m⁻³) during both seasons. However, more offshore the trophic conditions, derived from Chl_a concentrations, were more favourable during winter 2010 compared to the situation during summer 2011 (Fig. 2).

Regional comparison

Size, sex and weight

E. hanseni sampled at the four stations, used to compare upwelling from non-upwelling regions, diverged significantly in total length, weight and sex ratios. The mean krill length at No_1 (20.0 ± 2.1 mm, N=69) was significantly smaller than those at Up_1 (24.7 ± 1.5 mm, N=49) and Up_3 (24.7 ± 1.3 mm, N=54). Furthermore, the mean krill length size at Up_2

(20.9 ± 2.1 mm, $N=45$) was significantly shorter than that from Up_1 and Up_3 (Kruskal-Wallis test with Dunn's post-test, $H = 138.2$, $P < 0.0001$, Fig. 3).



↑ **Figure 3** Size frequencies of *Euphausia hanseni* at the sampled stations during winter 2010.

Accordingly, the mean weight of the krill was lowest at No_1 (53.9 ± 9.1 mg, $N = 10$) followed by Up_2 (65.5 ± 10.2 mg, $N = 10$). Heaviest mean weights were recorded at Up_1 (111.7 ± 4.7 mg, $N = 10$) and Up_3 (110.7 ± 20.1 mg, $N = 10$). The sex ratio (female/male) was highest at Up_1 (4.4) followed by the Up_3 (3.9). The sex ratios at Up_2 (1.5) and No_1 (1.2) were considerably lower. Specimens taken for determination of metabolic parameters (respiration and excretion rates) were randomly chosen out of the pool of specimens from each station. However, the size frequencies and the sex ratios reflected the in situ ratios at the sampling stations (unpaired t-tests with Welch's correction, $P > 0.05$), except at Up_1 where the sex ratio in the animals investigated was more male-biased compared to the situation in the field.

Respiration, excretion and O:N ratio

No significant differences could be detected between females and males in terms of their respiration rates (unpaired t-test, $t_{40}=0.6745$, $P > 0.05$), excretion rates ($t=1.605$, $df=36$, $P > 0.05$) and O:N ratios ($t=1.176$, $df=36$, $P > 0.05$). Thus, data from both sexes were pooled for

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further analyses. Respiration rates of *E. hanseni* individuals at No_1 were significantly lower compared to the other stations (ANOVA with Tukey's post-test, $F_{3,40}=12.04$, $P<0.0001$). Mean excretion rates were significantly greater at Up_3 compared to Up_1 (ANOVA with Tukey's post-test, $F_{3,37}=3.633$, $P=0.0224$). Calculated O:N ratios ranged from 9-13 at No_1, from 14-53 at Up_1, from 10-36 at Up_2 and from 8-27 at Up_3. Significant differences in the O:N ratio were found between No_1 and Up_1+Up_2 and between Up_1 and Up_3 (ANOVA with Tukey's post-test, $F_{3,37}=7.119$, $P=0.0008$). An overview of the metabolic activity of *E. hanseni* at the different sampling stations is given in Table 2.

↓ **Table 2.** Metabolic state (respiration rates (M_{O_2} ; $\mu\text{mol O}_2 \text{ h}^{-1} \text{ g}_{\text{ww}}^{-1}$), ammonia excretion rates (E_{NH_4} ; $\mu\text{mol NH}_4 \text{ h}^{-1} \text{ g}_{\text{ww}}^{-1}$) and O:N ratios (by atoms)) of *Euphausia hanseni* at different stations during winter 2010. The size (mm), fresh weight (mg) and sex ratio (f/m) are also presented. Values are given as range and means \pm SD in brackets. Different letters indicate significant differences between stations (ANOVA with Tukey's post test). (n=8-14)

	Size (mm)	Fresh weight (mg)	Sex ratio (f/m)	M_{O_2} ($\mu\text{mol O}_2 \text{ h}^{-1} \text{ g}_{\text{ww}}^{-1}$)	E_{NH_4} ($\mu\text{mol NH}_4 \text{ h}^{-1} \text{ g}_{\text{ww}}^{-1}$)	O:N (by atoms)
Up_1	23-27 (24.8 \pm 1.3) ^a	95.0-136.8 (111.7 \pm 14.7) ^a	1.5	8.6-18.1 (12.1 \pm 2.6) ^a	0.33-1.93 (1.0 \pm 0.5) ^b	14-53 (29 \pm 13) ^a
Up_2	19-22 (20.5 \pm 1.1) ^b	54.9-87.6 (65.5 \pm 10.2) ^b	0.6	9.1-16.9 (13.0 \pm 3.1) ^a	0.86-1.84 (1.1 \pm 0.4) ^{a,b}	10-36 (26 \pm 12) ^{a,c}
Up_3	23-27 (25.0 \pm 1.4) ^a	78.1-142.8 (110.7 \pm 20.1) ^a	3.7	8.4-20.2 (12.5 \pm 3.3) ^a	0.96-2.5 (1.6 \pm 0.5) ^a	8-27 (17 \pm 6) ^{b,c}
No_1	19-21 (19.8 \pm 0.8) ^b	43.0-73.0 (53.9 \pm 9.1) ^b	1.5	5.0-9.1 (6.5 \pm 1.4) ^b	1.02-1.49 (1.2 \pm 0.2) ^{a,b}	9-16 (11 \pm 2) ^b

Moult stages and sexual developmental stages (SDSs)

Moult activities in the *E. hanseni* populations was quantified as the proportion of individuals within an active moult stage (A,B, D₃ and the ecdysis stage) at time of sampling. In an average krill population, this number is approx. 15% (Buchholz and Buchholz 2010). At No_1, 59% of the animals sampled (N=51), were in postmoult stages and 41% in premoult stages. 14% of the animals investigated were in an active moult stage indicative of a “normal” moult activity for this population.

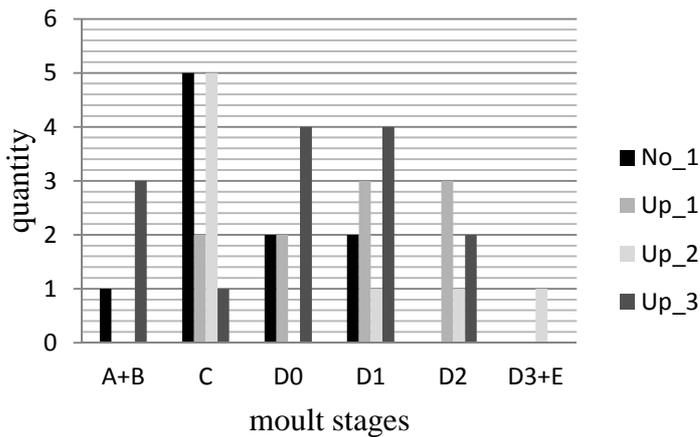


Figure 4

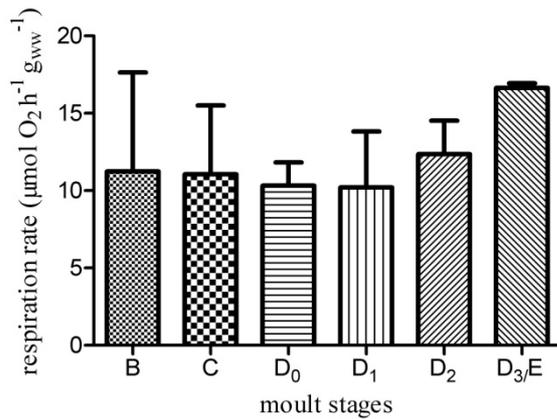
Moult stages of *Euphausia hanseni* individuals used for respiration measurements at the sampling stations during winter 2010, according to the staging system described in Buchholz (1982).

At Up_1 (N=49) only 27% of the population was in postmoult stages and 73% in premoult stages. No animals were found in an active moult stage. Up_2 (N=44) showed 45% of the population in postmoult and 55% in premoult stages. 27% of the population was found in active moult stages. Moult activity at Up_3 (N=40) was 10%, with 45% in postmoult and 55% in premoult stages. The MSs of the animals used for respiration measurements are shown in Figure 4.

Irrespective of regional differences, most of the animals investigated, were either in MS C (31%) or in MS D₂ (14%). However, moult stages did not affect the metabolic activity of *E. hanseni* (ANOVA, $F_{5,42}=1.198$, $P>0.05$; Fig. 5) in both sexes. The sexual developmental stage of the females at No_1 was significantly lower compared to the other stations (ANOVA with Tukey's post-test, $F_{8,42}=5.90$, $P<0.0001$). Respiration rates regressed against SDS (Fig. 6) showed a non-linear relationship ($df=43$, $r^2=0.42$) expressed by:

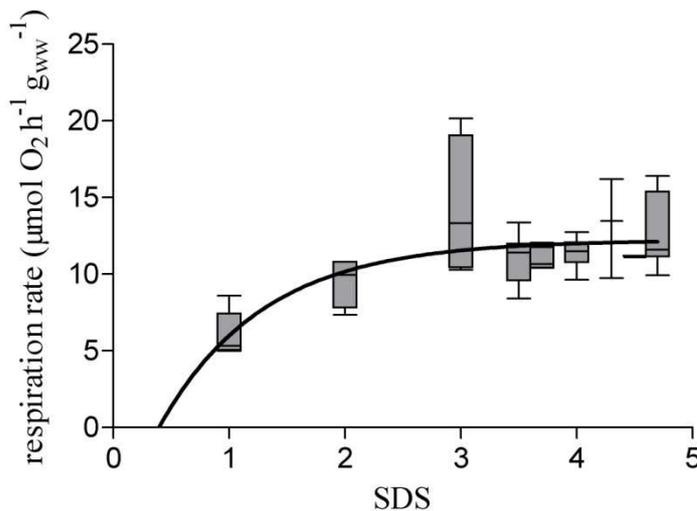
$$\text{one-phase association: } y = -6.761 + (12.22 + 6.761) * (1 - \exp(-1.114 * x))$$

The oxygen uptake was lowest at SDS 1, increased until SDS 3 and then plateaued. The high variability associated with oxygen uptake at SDS3 should be noted (Fig. 6).



◀ **Figure 5**
Moult stages of *Euphausia hanseni* and corresponding respiration rates (µmol O₂ h⁻¹ g_{ww}⁻¹) at 10°C.

Significant differences were found between SDS 1 and SDS 3+ (ANOVA with Tukey`s post-test, $F_{8,37}=4.968$, $P<0.0001$). A significant and positive correlation was detected between SDS and oxygen uptake in *E. hanseni* individuals (Spearman correlation, $r_s=0.74$, $N=46$, $P=0.023$). Correlation coefficients (r) between respiration rates, excretion rates, O:N ratios and SDS and MS in *E. hanseni* are shown in Table. 3. Since most of the animals showing an SDS of 1 or 2 were from No_1, animals sampled at other stations (not shown in this publication) were included in these analyses in order to exclude possible changes in the oxygen consumption rates due to starvation effects.



◀ **Figure 6**
Box and whisker plot of the sexual developmental stages (SDS) of *Euphausia hanseni* females and corresponding respiration rates (µmol O₂ h⁻¹ g_{ww}⁻¹) at 10°C fitted with a non-linear regression (Black line).

	Correlation coefficient (r)			
	NP	M _{O2}	E _{NH4}	O:N
SDS	26	0.542	0.129 ^{NS}	0.324 ^{NS}
MS	43	0.275 ^{NS}	0.073 ^{NS}	0.090 ^{NS}

■ **Table 3**

Correlation coefficients (r) between metabolic rates (respiration (M_{O2}; μmol h⁻¹ g_{ww}⁻¹) and excretion (E_{NH4}; μmol NH₄ h⁻¹ g_{ww}⁻¹)) and physiological states (sexual developmental stage (SDS) and moult stages (MS)) in *Euphausia hanseni* during winter 2010. NP- number of data pairs used for correlation analyses; NS – not significant

Seasonal comparison

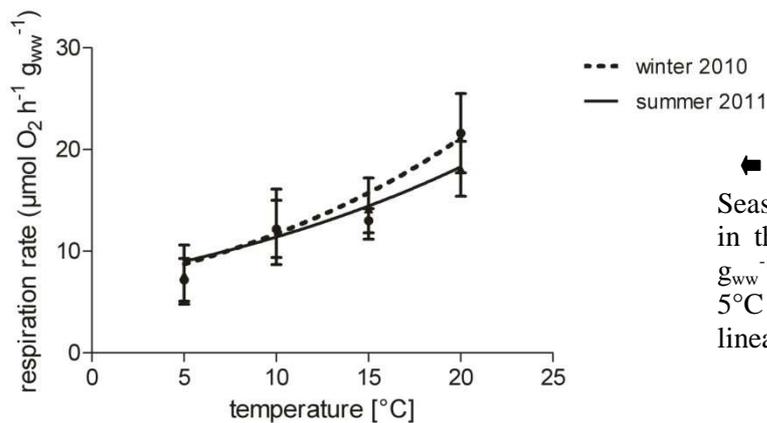
Respiration rates did not differ significantly between the sampling stations during late summer 2011. During winter 2010 one station (No_1) showed significantly lower respiration rates and was not included in the following analyses to avoid biasing the results by different physiological states possibly affecting respiration rates.

Temperature (5°C-20°C) had the same impact on oxygen uptake in *E. hanseni* individuals during winter 2010 and late summer 2011. No significant differences were detected between the slopes of the non-linear regressions describing the temperature/metabolism relationship (F-test, F_{1,113}=1.792, P>0.05; Fig. 7). The one exception was oxygen uptake at 20°C, which was significantly higher during winter 2010 compared to summer 2011 (unpaired t-test, t₁₉=2.411, P=0.0262). The relationship between temperature and respiration rates can be expressed by:

$$Y=10^{(0.8131+0.02558x)} \quad \text{- (late) summer (N=55) (Werner et al. 2012)}$$

$$Y=10^{(0.8519+0.02053x)} \quad \text{- winter (N=62)}$$

Ammonia excretion rates showed no significant differences between winter and summer, and no differences were detected in the O:N ratios between seasons either.

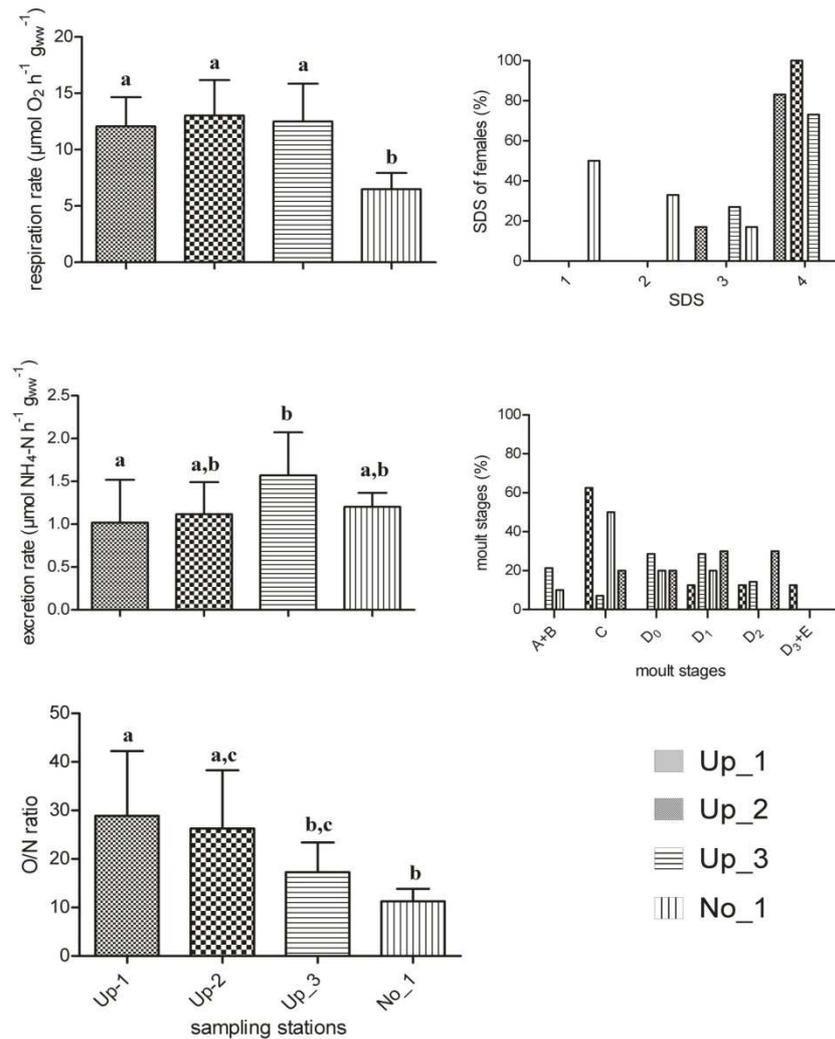


■ **Figure 7**

Seasonal differences (winter/summer) in the respiration rates ($\mu\text{mol O}_2 \text{ h}^{-1} \text{ g}_{\text{ww}}^{-1}$) of *Euphausia hanseni* between 5°C and 20°C , presented by non-linear regressions.

Discussion

Regional differences in the physiological state of *E. hanseni* were apparent during winter 2010. The swarms diverged in size, sex ratio, SDS, moult activity, oxygen uptake, excretion rates and O:N ratios (Fig. 8). Upwelling vs. non-upwelling situations, as characterized by water temperature and salinity, may have influenced the physiological state of *E. hanseni* due to divergent trophic conditions. According to Werner et al. (2012) changes in the physiological state of krill in the nBus should be related to upwelling intensity rather than to seasonality alone. The lack of seasonal differences in respiration and excretion rates for *E. hanseni* as found by the current study, further supports this notion. Besides temperature and oxygen availability, the trophic environment is the most variable factor in the nBus and may significantly influence the life histories of krill populations living there. However, trophic conditions are capable of masking thermal effects (Saborowski et al. 2002), especially when food availability is scarce or temperatures are stable. Respiration rates in *M. norvegica* were mainly affected by temperature when favourable feeding conditions prevailed throughout the year. In contrast, this species increased its oxygen uptake during the period of increased primary production in areas with oligotrophic conditions (Saborowski et al. 2002). During winter 2010 the trophic environment at Up_1 differed significantly from the other stations. Since it was only possible to distinguish between upwelling and non-upwelling situations, and no conclusions about the upwelling intensity and the `age` of the upwelled waters could be drawn, differences in the feeding conditions prior to the sampling remain unclear. Furthermore, the actual Chl_a content obtained by ferrybox data and satellite images could also have been biased by e.g. high grazing activities.



↑ **Figure 8** Physiological states of *Euphausia hanseni* individuals at different sampling stations respectively upwelling conditions during winter 2010. Given are the means \pm standard deviations for the respiration rates (M_{O_2} ; $\mu\text{mol O}_2 \text{ h}^{-1} \text{ g}_{\text{ww}}^{-1}$), excretion rates (E_{NH_4} ; $\mu\text{mol NH}_4 \text{ h}^{-1} \text{ g}_{\text{ww}}^{-1}$), O:N ratios by atoms (left side). Sexual developmental stages of the females (%) and the different moult stages (%) at the sampling stations are presented (right side). Groups were tested by one-way ANOVA. Different letters indicate significant differences between stations (ANOVA with Tukey's Multiple Comparison Test).

Since several physiological processes such as growth and reproduction are slow, the influence of the trophic environment is difficult to assess as the initiating pulse may have occurred several weeks before prior to sampling. However, *E. hanseni* can integrate trophic conditions into physiological processes (e.g. growth) over a relatively short period of time (~1-2 weeks). Furthermore, *E. hanseni* is described as an omnivorous species feeding on phytoplankton, copepods, dinoflagellates and tintinnids (Barange et al. 1991) and dietary shifts may occur when the ambient food concentration of a prey falls below a certain threshold as suggested by

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Stuart and Pillar (1990) for *Euphausia lucens* in the southern Benguela Current. Accordingly, phytoplankton levels alone may be insufficient to describe the food availability for *E. hanseni*. Repeated sampling at a fixed station over a longer time period including phases of upwelling and non-upwelling and continuous recording of the trophic environment (both phyto- and zooplankton) would help to clarify these timings. However, regional differences in upwelling activity and different physiological parameters were apparent and are discussed in the following sections.

Regional comparison

The most obvious differences in the physiological state of *E. hanseni* were evident in the respiration rates, O:N ratios, moult and reproductive activity of *E. hanseni*. At the stations where upwelling occurred, krill swarms showed comparable respiration rates ($12.1-13.0 \mu\text{mol O}_2 \text{ h}^{-1} \text{ g}_{\text{ww}}^{-1}$) and either high moult activities and/or a high SDS of females. At Up_1 no animals were in an active moult stage, however, this may be explained by the high SDSs apparent in the females here. It is likely that these animals interrupted their moult cycle and were close to a spawning event, which was further supported by multiple females showing an orange hue indicating imminent spawning. In contrast, the animals under non-upwelling conditions had significantly lower respiration rates, lower O:N ratios and showed 'normal' moult activity and low SDS. Studies on *E. superba* indicated that food availability affects moult frequency (Buchholz and Buchholz 1989) and that increasing temperatures decrease the intermoult period (Buchholz 2003). Since favourable trophic conditions promote synchronized moult activities (Buchholz et al. 1996) and reproduction (Tarling and Cuzin-Roudy 2003), a high moult or reproductive activity may be considered indicative of favourable food conditions prior to sampling.

Respiration, excretion and O:N ratio

Metabolic activity in zooplankton as a response to phytoplankton blooms have been extensively studied in copepods (Conover and Corner, 1968; Mayzaud and Conover 1988). Here, both oxygen consumption and ammonia excretion during the spring phytoplankton bloom were accelerated and the O:N ratio at least in herbivorous copepods lowered. In

contrast, during phytoplankton blooms the respiration rates in *E. superba* were accelerated but the excretion rates were depressed, resulting in higher O:N ratios (Ikeda and Kirkwood 1989).

The oxygen uptake of *E. hanseni* at No_1 under non-upwelling conditions was only half of the rates compared with the “upwelling stations”. The low metabolic activity may reflect a depression of several physiological processes under unfavourable, non-upwelling, trophic conditions (see below). Contrary to this theory, recent experiments have shown that starvation for 7 days can result in a pronounced decrease in oxygen consumption by 40-70% in *E. hanseni* (K. Huenerlage and F. Buchholz pers. comm.). However, the data received from the ferrybox measurements and satellite images showed that trophic conditions at No_1 were favourable, and dissection of specimens revealed partly filled stomachs. Therefore, a starvation effect seems unlikely. Furthermore, the actual trophic conditions did not vary significantly between No_1, Up_2 and Up_3, suggesting no starvation effect in *E. hanseni* at any station. Accordingly, the observed differences in the respiration rates, under upwelling and non-upwelling conditions, should be a result of other physiological processes, such as growth and reproduction. However, stable isotope analyses for No_1 and Up_1 indicated that *E. hanseni* at No_1 was nearly one trophic level below the animals at Up_1 (pers. obs.), suggesting different degrees of herbivory versus carnivory. These stable isotope results corresponded with the O:N ratios calculated for each station; the lowest O:N ratios were determined for No_1 (and Up_3) which is suggestive of a different quality and quantity of available food (Mayzaud and Conover 1988). Ammonia excretion rates at Up_1 and Up_2 were slightly lower compared with the two other stations, while their O:N ratios were higher. This is in agreement with observations in *E. pacifica*, *T. inspinata* (Kim et al. 2010) and *E. superba* (Ikeda and Kirkwood 1989) during a phytoplankton bloom. Lowered ammonia excretion rates and elevated O:N ratios were suggested to indicate reduced importance of protein as a metabolic substrate during the phytoplankton bloom and preferential utilization of dietary protein for somatic growth and/or reproduction rather than metabolism (Kim et al. 2010). In particular, this may be true for species with low lipid contents such as *E. hanseni*.

Sexual developmental stages (SDS)

Our results show that the SDS of *E. hanseni* females positively influenced respiration rates (Fig. 6) and that moult stage likely did not affect the oxygen uptake (Fig. 5). Thus, the observed differences in the metabolic activity between regions may be mainly due to

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differential reproductive effort. Most of the females at No_1 were in a SDS of 1 or 2, thus in stages where ovaries are still developing and differentiating. Ovaries of females from the other stations were largely fully developed (SDS 3 and higher) and some even displayed an orange hue, an indicator of egg maturation (C. Buchholz, AWI-Bremerhaven, Germany, pers. comm.). High frequencies of SDS 3+ may be an indication for suitable trophic conditions. Additionally, the oxygen uptake for both sexes at No_1 was significantly lower compared with the other stations sampled. This may suggest a reduction of metabolic rates corresponding to low reproductive effort, within the whole swarm. Furthermore, respiration rates displayed a high degree of variability in SDS 3 (vitellogenesis). The low oxygen uptake in some SDS 3 females may indicate a “waiting for favourable conditions”, whereas the high rates observed in other SDS 3 females may point to a “kick-off” in the spawning cycle. Both do not reject the scenario of a “resting” stage in the reproductive cycle, where krill females with nearly fully developed oocytes “wait for favourable conditions”, followed by the initiation of maturation and spawning if conditions allow. This “sit and wait for favourable conditions” tactic is most likely an adaptation to periodic short time-scale upwelling events, which could boost reproductive success in *E. hanseni*. In other krill species, such as *M. norvegica*, multiple egg production cycles during the reproductive season is followed by a longer period of sexual rest (Cuzin-Roudy and Buchholz 1999). However, in various krill species reproductive scheduling coincides with periods of optimal feeding conditions (Cuzin-Roudy and Buchholz 1999). Thus, a “resting” stage where females bear fully developed oocytes yet wait to spawn may allow them to quickly respond to improving trophic conditions and could evolve in poly-pulsed systems with perennial upwelling events. Ideally, vitellogenesis should only occur when trophic conditions are optimal so as to promote strong non-feeding stages of larvae, due to the optimal development and condition of eggs (Tarling and Cuzin-Roudy 2003). The quality and quantity of food influences growth rates in feeding-stages of krill larvae and accordingly, the onset of upwelling would be a critical period for this developmental stage (Siegel 2000).

These results are in agreement with observations in other krill species. In the southern Benguela Current, *E. lucens* spawned before the onset of upwelling which caused the spring phytoplankton bloom (Pillar and Stuart 1988) and thereby ensured that the larvae hatched during a period of high food supply. Dorman et al. (2005) showed that short time-scale fluctuations in Chl_a concentrations due to changes in the upwelling state positively affect the amount of eggs spawned by *E. pacifica*. Spawning intensities for *E. pacifica* were highest during upwelling events due to high food supply (Smiles and Pearcy 1971; Brinton 1976).

The subsequent zooplankton bloom may be of further importance, for omnivorous species like *E. lucens* (Siegel 2000) and *E. hanseni*. Upwelling events often induce phytoplankton blooms in the nBus and also correlate with high SDSs in *E. hanseni* as similarly observed in *E. lucens* and *E. pacifica*. However, prolonged upwelling-favourable winds decreased the reproductive effort and abundance of *E. pacifica* both on short-time and interannual scales (Dorman et al. 2005). Accordingly, there may be an optimal upwelling intensity which favours primary and secondary production without advection of plankton far off-shore (Dorman et al. 2005) which supports reproduction in euphausiids. Further analyses are needed to detail the ovarian development cycle in *E. hanseni* females as it is related to upwelling and food availability and to clarify whether this species shows a multiple or continuous breeding behaviour (Siegel 2000).

Moult activity

An interruption of the diel vertical migration possibly due to energy-demanding processes during moult was described for *M. norvegica* by Buchholz and Saborowski (2000). In *E. hanseni* no differences between the postmoult and premoult phases or between active and non-active moult phases in *E. hanseni* in terms of respiration rates were detected. This held true even after all females were excluded from the analyses, to avoid biasing the results with different SDSs. We therefore assume that accelerated metabolic activities may not be required for moulting in *E. hanseni*. If moulting were highly energy-demanding, then under continued starvation, a distinct mismatch between energy demand and expenditure should result. It is known however, that some euphausiid species continue moulting even under unfavourable trophic conditions and may even shrink in size (Buchholz 2003).

Two krill used in the experiments, actively shed their carapaces during the respiration measurements and showed comparably high metabolic rates (16.7 ± 0.4 ; N=2), indicative of an energy-intensive process. This may suggest divergent energy demands during different phases of the moult process. Most of the animals investigated were in a MS C or D₂. Under unfavourable conditions (e.g. starvation) or at the onset of sexual maturation the time span of these moult stages can be prolonged (Buchholz 1982). Accordingly, stages C and D₂ are viewed as “waiting stages” in the moulting process, analogous to SDS 3 in female reproductive development, and can be a “starting point” for moulting when conditions become suitable. Buchholz (2003) reviewed that the intermoult period (IMP) of *E. superba* decreases with an increase in water temperature and that this species is capable to fast and flexible adjust its IMP and its size increment at moult (INC) to the prevailing feeding

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conditions. Accordingly, moulting is influenced by several factors, including water temperature and food supply which in turn can relate to oxygen uptake and demand. Thus, these other factors may have interfered with the detection of differential energy requirements between moult phases.

Seasonal comparison

Investigations on seasonal differences in the metabolic activity of *M. norvegica* showed that food availability and temperature are the most relevant parameters affecting respiration rate. In areas where the trophic environment provides suitable feeding conditions throughout the year (Kattegat) the metabolic rate in *M. norvegica* was mainly affected by the thermal regime. In contrast, in the oligotrophic Ligurian Sea strong metabolic enhancement occurs when the short winter-spring plankton blooms take place (Saborowski et al. 2002). In the Northern Benguela, perennial upwelling provides appropriate food conditions throughout the year. On the other hand upwelling intensity and food availability are higher in winter/spring than in summer/autumn. However, no differences in the metabolic activity in *E. hanseni* between summer and winter could be detected. Accordingly, changes in the trophic conditions may be more pronounced between two upwelling events than over a seasonal scale. Additionally, the comparably constant metabolic rates between seasons in *E. hanseni* coincided with the *M. norvegica* from the Kattegat region investigated by Buchholz and Saborowski (2000). Higher respiration rates at an experimental temperature of 20°C were found for winter 2010 krill compared to those from summer 2011. This may indicate an adaptation to the lower water temperatures in the upper layers during wintertime. During winter 2010, the average SST for the nBus was approx. 15.7°C whereas in late summer 2011 average SST was > 20°C (Fig. 2). During summer, an intrusion of warm, saline tropical waters from the Angolan Dome is a common phenomenon (Boyd et al., 1987). Thus, *E. hanseni* an adept vertical migrator (Barange 1990) may have adapted to the elevated summer water temperatures by reducing their metabolism. During wintertime such a reduction may not be necessary, since the krill rarely encounter water temperatures > 16°C.

Adaptations of metabolic activity to (seasonal) variation in trophic conditions in E. hanseni, M. norvegica and E. superba – a species comparison

Euphausiids are distributed ubiquitously worldwide ranging from tropical waters to high latitude polar regions. They inhabit various ecosystems with marked differences in (seasonal and regional) trophic conditions. The comparably well studied species, *M. norvegica* shows a wide distribution range from boreal to sub-tropical North Atlantic waters and the adjacent seas, whereas *E. superba* is restricted to the Antarctic ring ocean. *E. hanseni* inhabits coastal Atlantic waters at the margin of the African continent at both sides of the equator (Mauchline and Fisher 1969). The seasonal changes in food availability therefore differ greatly between *M. norvegica*, *E. superba* and *E. hanseni* and this is reflected in their metabolic and physiological adaptations. *M. norvegica* encounters different trophic regimes both regionally as well as seasonally. In contrast, *E. superba* meets large seasonal differences in food availability (favourable in austral summer, poor in austral winter) though minor or no regional differences. Food availability for *E. hanseni* is largely controlled by perennial upwelling events and thus experiences both regional and seasonal fluctuations. However, all three species are omnivorous, reflecting their capability to adapt to highly variable trophic conditions in their environments (cf. Buchholz and Saborowski 2000). The seasonal adjustment of metabolic rates in relation to food availability in these krill species from very different climatic zones are discussed in the following.

In *M. norvegica*, metabolic adaptations as measured through respiration and excretion rates were recorded in response to the variable trophic conditions prevailing in the Mediterranean Sea, where a short productive season is followed by long oligotrophic conditions. In contrast, krill from areas with comparably constant food supply, such as the Kattegat and the Clyde Sea, displayed no seasonal adjustment of respiration and excretion rates (Saborowski et al. 2002). In the case of the Kattegat and Clyde Sea populations, temperature predominantly affected metabolic rates in a positive manner. Enhanced somatic growth and reproductive activity and development correlated with favourable trophic conditions during the period(s) of increased food availability (Saborowski et al. 2002). In general, *M. norvegica* preferentially displays long-term adaptations to prevailing environmental conditions rather than short-term adjustments of metabolic rate.

In *E. superba*, respiration rates may be reduced by up to 60% during autumn and winter, the periods of limited food supply (Torres et al. 1994; Atkinson et al. 2002; Meyer et al. 2010). This reduced metabolic activity was also accompanied by reduced feeding and growth and are

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considered adaptations to austral winter conditions in Antarctic waters. Reduced metabolic activity and the accumulation of large lipid reserves allows *E. superba* to survive periods without an autotrophic food supply in the water column (Meyer et al. 2010).

Metabolic activity, growth and reproductive effort in *E. hanseni* are adapted to the short-time scale changes in trophic conditions due to upwelling events in the nbus throughout the year. Accordingly, all three species are well adapted to the highly variable trophic conditions and temperature regimes in their environment/ecosystem and their physiological performance directly reflects the prevailing conditions. Different environmental conditions between each species may have lead *E. hanseni*, *E. superba* and *M. norvegica* along divergent evolutionary paths: *E. hanseni* reduces metabolic activity under food limitation and enhances physiological performance when food conditions improve though only in response to short-term and small-scale events. *E. superba* shows a drastic seasonal reduction of metabolic rate correlated with low food availability. *M. norvegica* shows enhanced metabolism during the spring phytoplankton bloom in areas with seasonal food shortage. Krill species are able to quickly respond to favourable environmental conditions and efficiently convert energy surplus into growth and reproduction (Buchholz and Saborowski 2000). Accordingly, euphausiids may serve as useful indicators of short-term or small scale changes (e.g. food availability) in environmental conditions as well as long-term and large-scale differences (seasonal and climate related changes).

In summary, regional differences in the physiological state of *E. hanseni* were more apparent than seasonal ones. These differences were mainly caused by varying upwelling situations, which induced sudden changes in the trophic environment and controlled moult and spawning in *E. hanseni*. In turn, the physiological state of *E. hanseni* and euphausiids in general may be used as an indicator for the current hydrographic and trophic conditions in the nBus and other ecosystems.

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Euphausia hanseni feeding on fish larvae
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Trophic position of euphausiid species of the northern Benguela upwelling system determined by stable isotopes - a regional and seasonal comparison

Thorsten Werner and Friedrich Buchholz

Abstract

The relative trophic position of six different euphausiid species of the northern Benguela upwelling ecosystem (nBus) were investigated to determine their role in the northern Benguela food web and to assess their dietary preferences. This eastern boundary upwelling ecosystem is characterized by perennial upwelling and highly variable environmental conditions. Accordingly, stable nitrogen ($^{15}\text{N}/^{14}\text{N}$) and stable carbon ($^{13}\text{C}/^{12}\text{C}$) isotope ratios were used to allow investigations of fluctuating trophic positions of krill species. High variations in the stable isotope ratios indicated regionally divergent roles in the food web and shifts in the dietary preferences and therefore, support the concept of opportunistic omnivorous feeding in euphausiids. Stable isotope analyses showed that the trophic level (TL) of *T. monacantha* was highest, followed by a group consistent of *N. megalops* and *N. capensis*. Species of the genus *Euphausia* were closely grouped, showing lowest trophic positions. The $\delta^{15}\text{N}$ -indicated trophic positions of krill species in the northern Benguela ranged from 5.1‰ in *E. hanseni* to 11.9‰ in *N. megalops* corresponding to a TL of 2.1 and 4.1 respectively. However, seasonal differences were less pronounced than regional ones. The fluctuating availability of phytoplankton, due to upwelling events and competition avoidance between krill species may be the reason for the TL changes observed in the omnivorous krill species of the nBus.

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Introduction

All organisms are embedded in food webs, since all organisms consume and except top-predators, are themselves consumed. The pathways of matter and energy in an ecosystem can be identified by trophic links (Paine 1988). Accordingly, a major challenge in understanding natural ecosystem processes is the investigation of trophic relationships. Recently, empirical and theoretical studies have shown that (resource) variability in space and time and the ability to quickly respond to such variations by organismal behaviour are essential to the maintenance and stability of food webs (McCann and Rooney 2009). The northern Benguela upwelling system (nBus) located at the west coast off Namibia is a typical coastal upwelling system with high plankton and moderate to high fish biomass. It is characterized by perennial upwelling and cool productive waters which occur in a narrow band from Cape Agulhas (~34°S) to Cape Frio (17°S; Hutchings et al. 2009). Despite perennial upwelling, phytoplankton biomass appears to peak in late winter till spring (Fig. 2). However, the availability of resources in consequence of upwelling pulses in such a system is highly variable. Accordingly, different zooplankton species should have developed adaptations to this fluctuating food composition and dynamic food availability. In particular euphausiids, which are mostly described as opportunistic omnivores and form a pivotal component of the mesozooplankton community and can dominate the zooplankton biomass in the nBus (Barange et al. 1991). Euphausiids are an important food source for top predators such as fish (Macpherson and Roel 1987, Roel and Macpherson 1988) and they facilitate the energy flow between primary production and higher trophic levels (Ekau et al. 2009). Accordingly, they may have a strong impact on lower and higher trophic levels (TL). In this regard, estimates of the trophic position of different euphausiid species are crucial to evaluate the trophic structure and dynamics of this plankton community (*Euphausiacea*) in the nBus and to assess their relevance in the northern Benguela food web.

During the last years the trophic relationships between very distinct groups of consumers were the target of several studies. In this regard stable isotope techniques have been frequently used (e.g., Zanden and Rasmussen 1999, Post et al. 2002). Nitrogen isotope ratios ($\delta^{15}\text{N}$) become enriched at successive trophic levels (Zanden and Rasmussen 1999) and Carbon isotopes ($\delta^{13}\text{C}$) indicate pathways of feeding and carbon flow (Hecky and Hesslein 1995). Accordingly, stable isotope analyses provide information about time-integrated food assimilation. However, determination of the trophic level of a species is usually done only for one season or averaged over seasons and sites. Accordingly, information of possible regional

and seasonal fluctuations in the TL of a species is rare. According to Vinagre et al. (2012) the TL of organisms in highly dynamic environments is not static and fluctuates in space and time, this may be particularly true for the nBus where upwelling and non-upwelling events, respectively phases of high and low productivity, appear over small scales both temporally as well as regionally. In such a highly variable ecosystem short-term events like upwelling pulses, and ensuing phytoplankton blooms, may have a greater impact on the trophic structures and dynamics than large (time-) scale processes.

Eight different krill species were observed in the nBus and most of them were considered opportunistic omnivores (Mauchline 1980; Stuart and Pillar 1990). They feed, as shown for *Euphausia lucens* in the Southern Benguela, on different prey size spectra (Stuart and Pillar 1990) and some of them may be capable of switching from herbivory to carnivory according to the ambient conditions like phytoplankton and copepod densities (Gibbons et al. 1991a). Accordingly, euphausiids may have different regional and seasonal TL in this ecosystem depending on the food environment and thus on upwelling activity. The most dominant euphausiids *Euphausia hanseni* and *Nematoscelis megalops* in the nBus consume similar size ranges of copepods but appear to partition copepod prey on the basis of size wherever the distribution of both species overlaps (Barange et al. 1991). Accordingly, the same species may show different TL at different locations during the same season. Furthermore, most euphausiids in the nBus show variable diel vertical migration patterns, thus encountering different food environments over space and time (Werner and Buchholz, *subm.*). However, the structure of the mandibles and the thoracopods determines foraging capacity (Mauchline 1967). In general, euphausiids with thoracopods of nearly uniform length, e.g. the genus *Euphausia*, are well suited for filter-feeding on phytoplankton. Whereas elongated thoracopods, e.g. *N. megalops*, are indicative of a predominant carnivorous diet (Mauchline and Fisher 1969).

In the present study the trophic positions of six different krill species were studied under regional and seasonal aspects. Accordingly, possible regional differences in the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ fractionation in different euphausiids of the nBus were examined. Furthermore, the same transect was re-sampled during each season to differentiate between spatial and temporal effects in *E. hanseni* and *N. megalops*. This study assessed the TL of the krill species in the nBus and tried to make assumptions about possible shifts in their dietary preferences.

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Material and Methods

Field sampling

Krill was caught with a 1m²-MOCNESS (Multiple Opening and Closing Net and Environmental Sensor System, Wiebe et al. 1985) with a large mesh size of 2000µm and a soft cloth cod-end. Animals were caught during three cruises in summer 2009 with the RV *Africana* (01.12.-17.12.2009), in winter 2010 with the RSS *Discovery* (10.09.-13.10.2010) and in late summer/autumn 2011 with the RV *Maria S. Merian* (30.01.-07.03.2011). Nets were hauled obliquely with a towing speed of 2 knots. Sampling was done at 9 different stations in the nBus during summer/autumn 2011 for regional comparisons. Furthermore, stations at the Walvis Bay transect (23°S) and the RockyPoint transect (19°S) were sampled during summer 2009, winter 2010 and autumn 2011 for seasonal comparisons in *E. hanseni*. For seasonal contrast in *N. megalops* the RockyPoint transect was sampled during each season. However, the species *Euphausia gibboides* was caught solely at one station close to the Angola dome (10°S, 8°E). Only adult animals were used for stable isotope analyses. An overview of the different sampling stations, except *E. gibboides*, is given in Figure 1. The hydrographic (SeaSurfaceTemperature (SST), °C) and trophic conditions (Chl_a, mg m⁻³) during each sampling period were identified using MODIS satellite images from the Giovanni online data system, NASA, US (<http://gdata1.sci.gsfc.nasa.gov>; Fig. 2).

Sample preparation

After catch the animals were sorted and deep-frozen at -80°C. In the home laboratory the 2nd and 3rd abdominal segments were severed, the cuticle removed and the muscle weighed on a microbalance. Afterwards the muscle tissue was lyophilized for 24h using an ALPHA 1-4 LSC freeze-drier (Christ GmbH, Germany), ground with a tapered glass pestle and then used for stable isotope analyses. The muscle tissue was chosen because of its relative high turnover rates and low lipid content, providing information about the response to phytoplankton blooms and seasonal food shortage (e.g. Schmidt et al. 2003), without biasing the results of the δ¹³C (e.g. Hobson and Welch 1992). Furthermore, samples from different jelly-fish (*Aequorea spp.* and *Chryosora hysocella*), salp and dinoflagellate species, collected during summer 2009 and winter 2010, were freeze-dried and used as comparative specimens. Furthermore, we were provided with data for copepod species (A. Schukat, University of

Bremen) that are characteristic for the nBus, such as *Calanus carinatus*, characteristic of upwelling areas (Arashkevich et al. 1996) and *Nanocalanus minor*.

Stable isotopes analyses

The ratio of stable isotopes of nitrogen ($\delta^{15}\text{N}$) can be used to assess the trophic position of a species because ^{15}N is typically enriched by 3.4‰ relative to its diet (Hobson and Welch, 1992). The ratio of carbon isotopes ($\delta^{13}\text{C}$) can be used to estimate the ultimate source of carbon for an organism (Post 2002). The ratio of carbon isotopes is fairly constant as carbon moves through the food web (Peterson and Fry 1987). A widely applicable trophic fractionation of $\delta^{13}\text{C}$ is 0.4‰ (Post 2002).

Specimens, thus muscle tissues, were filled into small tin caps, weighed on a microbalance scale and sent to AgroIsoLab (TÜV-Rheinland, Germany) for determination of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ fractionation. The trophic level of a species was calculated using a simple one-source model (Hobson and Welch, 1992; Post 2002) according to the equation:

$$\text{TL} = \lambda + (\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{base}})/3.4\text{‰}$$

where λ is the trophic level of the organism used to estimate $\delta^{15}\text{N}_{\text{base}}$ (TL=1 in our case) and where 3.4‰ corresponds to the average enrichment in $\delta^{15}\text{N}$ per trophic level (Minagawa & Wada, 1984).

The baseline for the nBus was evaluated as described in Schukat et al. (2012, submitted). Appropriate primary consumers were not available and therefore basal resources, e.g. primary producers with a TL of 1, were used. Additionally, we were provided with data (N. Lahanar, University of Hamburg) for phytoplankton calculated by nitrate (NO_3^-) using the denitrifier method (Sigman et al. 2001, Casciotti et al. 2002) and the international recognised nitrate standard (IAEA-N3) for isotopic comparison with air N_2 (Table 1). The $\delta^{15}\text{N}$ isotope ratio (‰) is expressed by the equation:

$$\delta^{15}\text{N}_{\text{sample}} = [({}^{15}\text{N}/{}^{14}\text{N})_{\text{sample}}/({}^{15}\text{N}/{}^{14}\text{N})_{\text{reference}} - 1] \times 1000$$

where the $^{15}\text{N}/{}^{14}\text{N}$ reference is air N_2 . The calculation of $\delta^{15}\text{N}$ for phytoplankton was performed using the following equation:

$$\delta^{15}\text{N}_{\text{phytoplankton}} = \delta^{15}\text{N}_{\text{nitrate}}/\epsilon_{\text{phytoplankton}}$$

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where ε is the isotope effect (Granger et al. 2004) for nitrate assimilation by phytoplankton. The mean value (5.8 ± 3.7) from all ε values found in the literature for marine diatoms and dinoflagellates were calculated (Montoya and McCarthy 1995; Waser et al. 1998; Needoba et al. 2003; Granger et al. 2004). Since it was not possible to create a separate baseline for each station sampled during each season a baseline compiled with data from different basal resources sampled at various stations averaged over two seasons (winter/summer) was used as the reference. In view of our main interest to estimate shifts in relative trophic position and not to compare species across various ecosystems, this approach appeared reasonable.

Table 1 Carbon $\delta^{13}\text{C}$ (‰) and nitrogen $\delta^{15}\text{N}$ (‰) fractionation of different basal resources from the northern Benguela upwelling system used for estimation of the phytoplankton baseline (trophic level: 1). Given are the quantities (n) and means \pm standard deviations. (Schukat et al. 2012 (submitted)).

source	n	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
nitrate (NO_3^-)	52	-	6.4 ± 0.7
phytoplankton (calculated by	52	-	1.0 ± 0.1
mixed phytoplankton	3	-16.2 ± 0.5	1.2 ± 0.8
diatoms	3	-17.3 ± 0.0	1.9 ± 0.2
mean phytoplankton			1.4 ± 0.5

For an overall comparison and to establish a general food web model, with a focus on krill, the ratios of stable isotopes in krill ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$), irrespective of season and sampling location, were pooled and their trophic levels determined. For possible regional contrasts, stable isotope analyses in *E. hanseni* were conducted at five, in *N. megalops* at four and in *N. capensis* at three different stations during autumn 2011 (Fig. 1). Furthermore, stable isotope analyses for a seasonal comparison in *E. hanseni* and *N. megalops* were conducted at the same transects during summer, autumn and winter. Furthermore the influence of body size (fresh weight) and sex on the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ fractionation in krill was investigated during autumn 2011. Accordingly, prior to the stable isotope sample preparation sex, size and the fresh weight of each animal were determined. The size was measured between the front of the eyes to the tip of the telson using millimetre paper under a stereomicroscope.

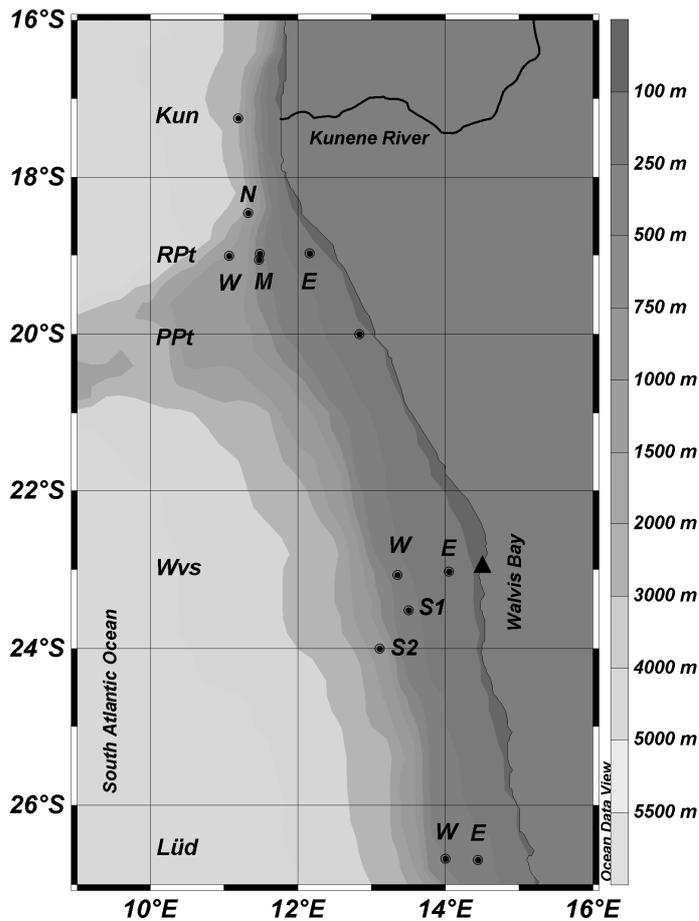


Figure 1 Sampling stations during summer 2009 (Afri09), winter 2010 (D356) and late summer/autumn 2011 (MSM17/3) in the northern Benguela upwelling system. Dots denote sampling stations, triangles denote landmarks. Given are the names of each sampled transect and the corresponding stations (Lüd – Lüderitz Cell; Kun – Kunene; PPt – Palgrave Point; RPt – Rocky-Point; Wvs – Walvis Bay). Letters indicate position of the stations relative to transects (E – East, W – West, S – South, M- Mid, N - North).

Statistical treatment of data

Results are presented as means \pm standard deviations (SD). For comparison between two groups a t-test and for three or more groups an one-way analysis of variance (ANOVA) with Tukey's multiple comparison post-test was used. Data were tested for normality using the Kolmogorov-Smirnov and/or D'Agostino and Pearson omnibus normality test. Transformation, if necessary, was done using log-transformation. If it was not possible to transform the data into a Gaussian distribution the Kruskal-Wallis test with Dunn's Multiple Comparison Test was applied. Correlation analyses between two variables were done using the Pearson's correlation. Generally, the significance level was set at $p < 0.05$.

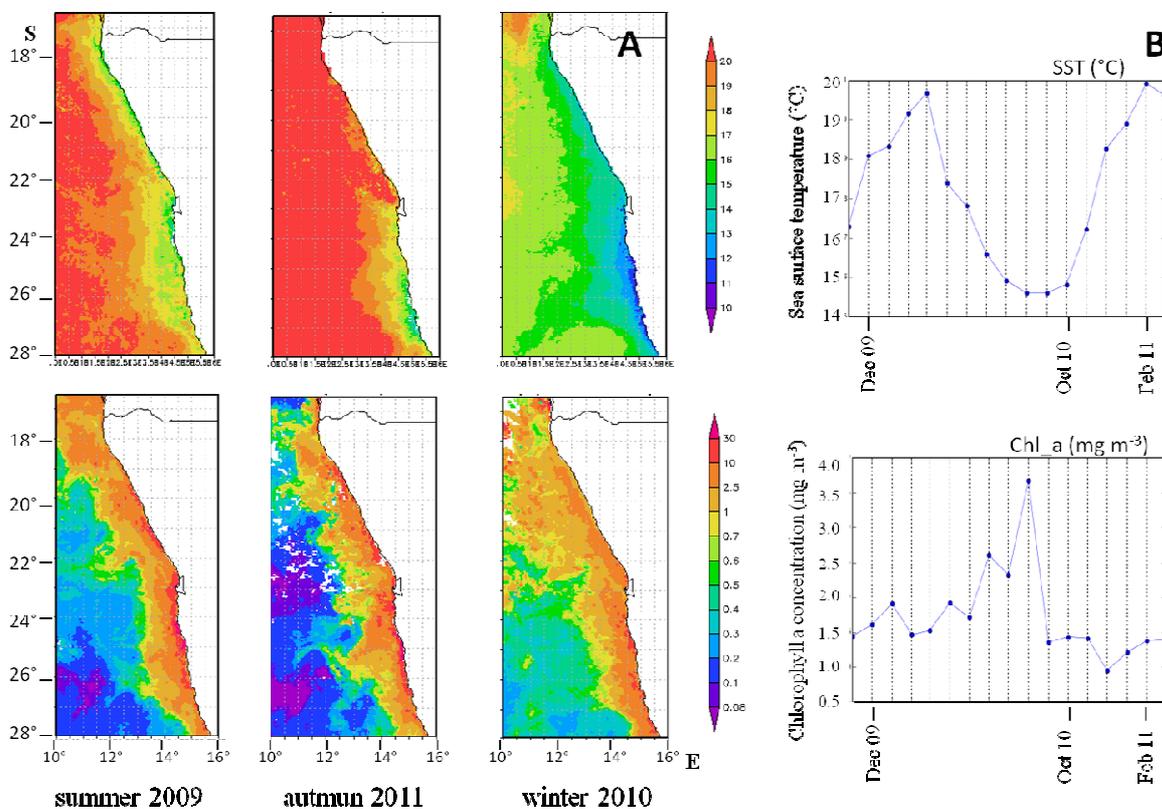
Results

Hydrographic and trophic conditions

The sea surface temperature (SST) during December 2009 (summer) varied between approximately 15-20°C. Cooler water masses occurred close to the coast, water temperatures were elevated more northward and more offshore. The area-averaged SST was 19.1°C during

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December 2009. During February 2011 (autumn) the SST ranged between 15°C and 25°C with most of the area showing a SST over 20°C. During this season an intrusion of warm, saline tropical waters is a common phenomenon (Boyd et al. 1987). The area-average value was close to 21°C. During September and October 2010 (winter) the SST fluctuated between 11°C and 20°C. The area-averaged SST was at 15.7°C. More detailed information is provided by the cruise reports (Ekau et al. 2009; Buchholz et al. 2010 and Lahajnar et al. 2011). Trophic conditions (Chl_a) near the coast were comparably good irrespective of season. However, farther off-shore the food environment became increasingly unfavourable (Fig. 2a). An overview of the SST and Chl_a content over the course of the sampling periods is given in Fig. 2b.



↑ **Figure 2a** Sea surface temperatures (°C, at the top) and Chl_a concentration (mg m⁻³, at the bottom) during summer 2009, autumn 2011 and winter 2010 in the northern Benguela upwelling system, derived from satellite images. ↑ **Figure 2b** SST and Chl_a content over the course of the sampling periods between November 2009 and March 2011.

*Stable Isotopes**Influence of size and gender*

Several studies have shown that the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ fractionation, e.g. the trophic level, may increase with increasing size of the animals tested (Gurney et al. 2001). Furthermore, sex specific differences were found in some species. Tests for possible size (weight) or gender-based variation were done in *E. hanseni*, *N. capensis* and *N. megalops* collected during autumn 2011 and for all other krill species over all seasons and sites. Instead of body size we used the fresh weight (fw) of the animals because the weight varied by a factor of 4 (e.g. 49-193 mg fw in *E. hanseni*) whereas the size variations were comparably low (e.g. 19-28 mm in *E. hanseni*).

Overall no correlation between body size, fresh weight, and stable isotope fractionation was found in the krill species tested. In *E. hanseni* there was a trend that bigger (heavier) animals had a higher $\delta^{15}\text{N}$ ratio, when one animal that showed an extreme $\delta^{15}\text{N}$ value was excluded ($r^2=0.15$, $p=0.031$). Pearson's correlation between fresh weight and $\delta^{13}\text{C}$ values was significant ($r^2=0.21$, $p=0.009$). No differences between males and females during autumn 2011 were found (unpaired t-test, $df=25$, $p=0.8657$). In *N. megalops* no correlation between isotope fractionations and fresh weight ($p>0.05$) was observed. Sex dependent differences were not significant (unpaired t-test, $df=22$, $p>0.05$). Likewise, *N. capensis* and other krill species showed neither significant correlations nor differences between sexes (males/females). Accordingly all data, irrespective of weight (size) or gender, were pooled.

Differences among euphausiid species

The results of the ANOVAs for nitrogen and carbon isotopes in euphausiids indicated that the means differed significantly between species ($F=24.42$, $df=138$, $P<0.0001$ for nitrogen, $F=6.281$, $df=138$, $P<0.0001$ for carbon). Two discrete groups could be identified. One group consisted of *N. megalops* and *N. capensis* (TL above 3) and the other one consisted of *E. gibboides*, *E. hanseni* and *E. lucens* (TL below 3). The highest value was observed in *T. monacantha* (10.8 ± 0.0) filling a TL close to 4 (Fig. 3). Salps occupied a TL of 2 whereas the two jelly-fish species showed highest $\delta^{15}\text{N}$ values of all animals tested (TL around 4). The $\delta^{15}\text{N}$ fractionation in dinoflagellates was, as supposed, slightly enriched (2.8 ± 0.2) compared to the values for diatoms and mixed phytoplankton.

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The ratio of ^{13}C isotopes in euphausiids was lowest in *E. gibboides* (-20.8 ± 0.4) and *T. monacantha* (-20.8 ± 0.0) and highest in *E. hanseni* (-18.8 ± 1.6) and *N. megalops* (-18.7 ± 1.0). In the other organisms tested, the ratio of carbon isotopes (^{13}C) ranged between -22.7 ± 0.8 in *N. minor* and -17.2 ± 0.2 in dinoflagellates. An overview of the $\delta^{15}\text{N}$ and ^{13}C fractionation given as mean \pm SD, the coefficient of variance (CV) in percentage and the range of the isotope values is given in Table 2.

↓ **Table 2** Overview of the ratios of nitrogen (^{15}N) and carbon isotopes (^{13}C) (mean \pm SD), the coefficient of variation (CV) in percent and the ranges of isotope values in euphausiids and other organisms of the northern Benguela upwelling ecosystem.

species	$\delta^{15}\text{N}$			$\delta^{13}\text{C}$			n
	Mean \pm SD	CV (%)	range	Mean \pm SD	CV (%)	range	
<i>Aequorea spp.</i>	13.1 \pm 0.3	2,1	0,4	-19.1 \pm 1.2	6,3	1,7	2
<i>Calanus carinatus</i> (females)*	8.1 \pm 0.1	1,2	0,2	-17.8 \pm 0.1	0,6	0,2	3
<i>Chryosora hysocella</i>	10.7 \pm 0.0	0,0	0,0	-19.6 \pm 0.5	2,5	0,7	2
<i>Euphausia gibboides</i>	7.5 \pm 0.4	5,1	1,1	-20.8 \pm 0.4	1,9	1,0	6
<i>Euphausia hanseni</i>	7.5 \pm 1.0	11,8	5,2	-18.8 \pm 1.6	8,7	7,0	74
<i>Euphausia lucens</i>	7.1 \pm 0.6	8,5	1,6	-19.2 \pm 1.3	6,7	2,9	5
<i>Nanocalanus minor</i> *	4.8 \pm 0.4	8,3	0,7	-22.7 \pm 0.8	3,5	1,4	3
<i>Nematoscelis megalops</i>	9.4 \pm 1.3	10,5	5,0	-18.7 \pm 1.0	5,3	4,6	38
<i>Nyctiphanes capensis</i>	9.0 \pm 0.8	8,9	3,1	-20.3 \pm 1.5	7,5	4,5	16
salps (not identified)	5.2 \pm 0.6	11,0	0,8	-21.6 \pm 1.3	5,9	1,8	3
<i>T. monacantha</i>	10.9	-	-	-20.8	-	-	1

* Data taken from Schukat et al. (2012, submitted)

E. hanseni, *N. megalops* and salps showed highest CV in the $\delta^{15}\text{N}$ ratios, followed by a group consisting of *E. lucens* and *N. capensis*. Lowest values were observed in both jelly-fish species. The range of nitrogen isotopes was greatest in *E. hanseni* (5.2‰) and *N. megalops* (5.0‰), followed by *N. capensis* (3.1‰). The observed ranges of $\delta^{15}\text{N}$ ratios in all other species were comparable low (0-1.6‰). However, the ratio of nitrogen isotopes (^{15}N) in euphausiids over all seasons and stations varied between 5.1 in *E. hanseni* at station RPt-N (winter 2010) and 11.9 in *N. megalops* at station Wvs-S2 (autumn 2011). The ratio of carbon isotopes (^{13}C) varied between -23.0 in *E. hanseni* at station RPt-M (summer 2009) and -16.0 in *E. hanseni* at station Wvs-W (autumn 2011).

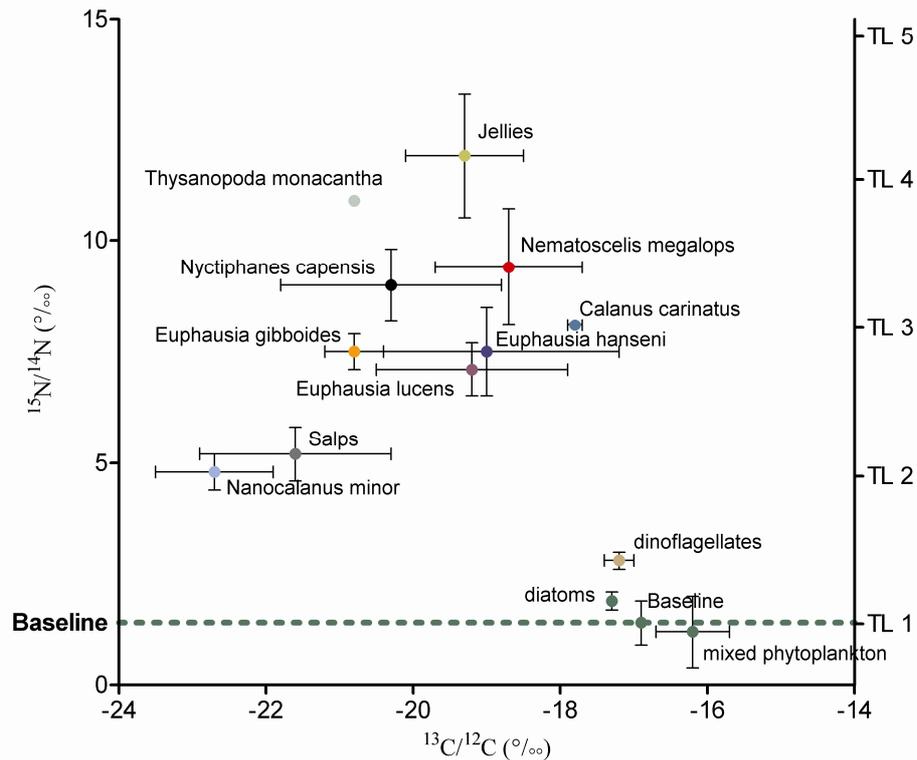


Figure 3 Nitrogen ($\delta^{15}\text{N}/\delta^{14}\text{N}$ (‰)) and carbon fractionation ($\delta^{13}\text{C}/\delta^{12}\text{C}$ (‰)) of different euphausiid species and other organisms (dinoflagellates, copepods*, salps and jellyfish) of the northern Benguela system, averaged over sites and seasons. Given are the means \pm SD (indicated by error bars). The right y-axis denotes the estimated trophic level (TL). * Data taken from Schukat et al. (2012, submitted)

Regional comparison

Regional differences (Fig. 4) in the ratio of nitrogen isotopes in *E. hanseni* during autumn 2011 were significant between station Lüd-E and station RPt-M and between station Wvs-S1 and station RPt-M (ANOVA, $F=1.13$, $df=30$, $p=0.018$). The calculated TL ranged from 2.6 at station RPt-M to 3.1 at station Lüd-W and Wvs-S1 (Table 3). The $\delta^{13}\text{C}$ fractionation diverged significantly between station Kun and station Wvs-W, between station Kun and station RPt-M and between station Lüd-E and station RPt-M and station Wvs-W (ANOVA, $F=11.64$, $df=30$, $p<0.0001$). Since there may be an influence of the body size (fresh weight) on the nitrogen isotope fractionation in *E. hanseni* and the correlation between weight and the carbon isotope fractionation was significant, we tested for significant divergent weight spectra at the stations. The animals at the two stations south of Walvis Bay (Lüd-E and Wvs-S1) were significantly heavier (ANOVA, $F=15.56$, $df=33$, $p<0.0001$) than animals at the stations north of Walvis Bay (Kun, RPt-M).

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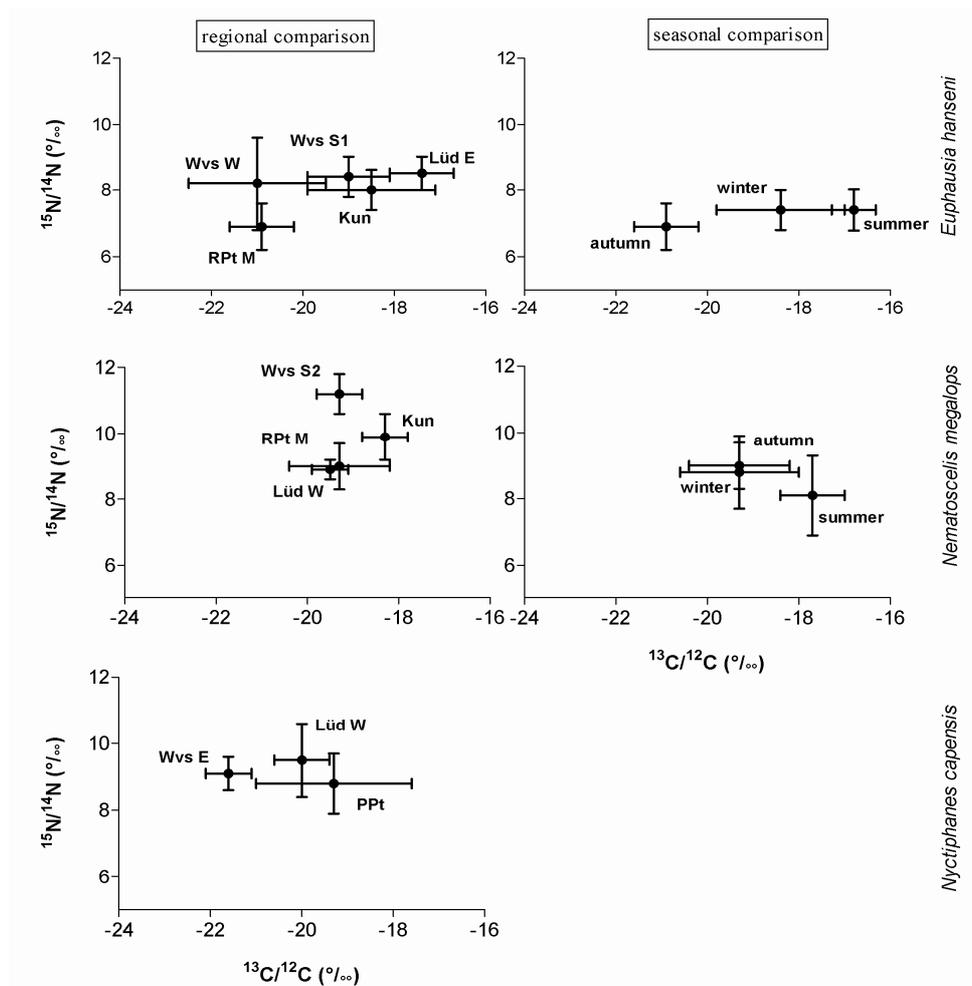
Table 3 An overview of the calculated trophic levels of *E. hanseni*, *N. megalops* and *N. capensis* at the sampling stations during autumn 2011.

trophic level	<i>Lüid</i>	<i>Lüid</i>	<i>Wvs</i>	<i>Wvs</i>	<i>Wvs</i>	<i>Wvs</i>	<i>PPt</i>	<i>RPt</i>	<i>Kun</i>
	-W	-E	-S1	-S2	-W	-E		-M	
<i>Euphausia hanseni</i>	-	3.1	3.1	-	3.0	-	-	2.6	2.9
<i>Nematoscelis megalops</i>	3.2	-	-	3.9	-	-	-	3.2	3.5
<i>Nyctiphanes capensis</i>	3.4	-	-	-	-	3.3	3.2	-	-

In *N. megalops* the results of the $\delta^{15}\text{N}$ isotopes at station Wvs-S2 were significantly different from those at station Lüid-W, Kun and station RPt-M (ANOVA; $F=11.83$, $df=25$, $p<0.0001$). Accordingly, the TL varied between 3.2 (station Lüid-W and RPt-M) and 3.9 (station Wvs-S2). The ratio of carbon isotopes was statistically different between Kun and the stations Wvs-S2 and RPt-M (ANOVA, $F=4,949$, $df=25$, $p=0.0089$). Stable isotope analyses for *N. capensis* showed no significant differences in the $\delta^{15}\text{N}$ ratio between stations (TL=3.2-3.4). However, $\delta^{13}\text{C}$ fractionation differed significantly between station PPt and station Wvs-E (ANOVA, $F=5.919$, $df=14$, $p=0.0163$).

Seasonal comparison

The stable isotope analyses for the seasonal contrast of *E. hanseni* at the Walvis Bay and RockyPoint-Transect showed no significant differences in the $\delta^{15}\text{N}$ fractionation between seasons (ANOVA, $F=1.13$, $df=22$, $p>0.05$). However, differences in the $\delta^{13}\text{C}$ ratios between seasons were highly significant (ANOVA, $F=1.13$, $df=22$, $p<0.0001$). Stable isotope analyses in *E. hanseni* revealed significant differences between the two transects investigated (unpaired t-test, $df=33$, $p=0.011$). At the RockyPoint-Transect the ratio of carbon isotopes in *N. megalops* diverged significantly between summer 2009 and winter 2010 (ANOVA, $F=4.471$, $df=17$, $p=0.0299$). No differences were found in the $\delta^{15}\text{N}$ fractionation (Fig. 4). No comparison between the RockyPoint-Transect and the Walvis Bay-Transect between seasons could be done for *N. megalops*, as insufficient specimens were caught for sensible comparisons.



↑ **Figure 4** Nitrogen ($\delta^{15}\text{N}/^{14}\text{N}$ (‰)) and carbon fractionation ($\delta^{13}\text{C}/^{12}\text{C}$ (‰)) of *Euphausia hanseni*, *Nematoscelis megalops* and *Nyctiphanes capensis*. Left side: regional comparison between stations during autumn 2011. Right side: seasonal comparison (summer/winter/autumn) at the RockyPoint-Transect during 2009, 2010 and 2011. Given are the means \pm SD (indicated by error bars). (Lüd – Lüderitz; Kun – Kunene; PPt – Palgrave Point; RPt – Rocky-Point; Wvs – Walvis Bay;). Letters indicate position of the stations relative to the transects (E – East, W – West; S – South; M- Mid).

In general, the ratio of nitrogen isotopes ($\delta^{15}\text{N}$) in *E. hanseni*, averaged over all stations, was significantly lower during winter 2010 (7.0 ± 0.9 ; $n=28$) compared to autumn 2011 (8.0 ± 0.9 ; $n=31$; ANOVA, $F=8.866$, $df=73$, $p=0.0004$). The ratio of carbon isotopes (^{13}C) in *E. hanseni* was significantly lower during summer 2009 (-17.3 ± 0.7 ; $n=15$) compared to winter 2010 (19.1 ± 1.4 ; $n=28$) and autumn 2011 (19.3 ± 1.8 ; $n=31$, ANOVA, $F=10.11$, $df=73$, $p=0.0001$) (Fig.4). However, the weight of the animals tested were significantly different between winter 2010 and autumn 2011 (ANOVA, $F=4.687$, $df=66$, $p=0.0108$). In *N. megalops* the ratio of nitrogen isotopes was lowest during summer 2009 (8.1 ± 1.2 ; $n=6$) and significantly different (ANOVA, $F=8.737$, $df=37$, $p=0.0008$) from the values during autumn 2011 (9.9 ± 1.0 ; $n=26$).

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The ratio of carbon isotopes differed significantly (ANOVA, $F=5.364$, $df=37$, $p=0.0094$) between summer 2009 (-17.7 ± 0.7 ; $n=6$) and the two other seasons (-19.3 ± 1.3 winter 2010; -18.8 ± 0.8 autumn 2011; Fig. 4). The weight of the animal was significantly different during summer 2009 compared to the other seasons (ANOVA, $F=4.488$, $df=36$, $p=0.019$). An overview of the length and weight distribution, the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ fractionation and estimated seasonal TL for *E. hanseni* and *N. megalops* is given in Table 4.

Table 4 Size (mm), fresh weight (mg), sex ratios (male/female in %), $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ fractionations and the assessed TL of *E. hanseni* and *N. megalops* during summer 2009, autumn 2011 and winter 2010.

	<i>E. hanseni</i>			<i>N. megalops</i>		
	summer 2009	autumn 2011	winter 2010	summer 2009	autumn 2011	winter 2010
size (mm)	23.7±1.2	22.9±2.6	21.1±2.5	26.0±6.5	40.4±14.7	47.3±16.9
fresh weight (mg)	89.1±15.3	106.6±39.6	78.8±25.7	14.5±1.2	17.0±2.0	17.6±2.1
sex ratio (m/f in %)	67/33	73/27	21/79	75/25	30/70	40/60
$\delta^{15}\text{N}$	7.6±0.7	8.0±0.9	7.0±0.9	8.1±1.2	9.9±1.0	8.8±1.1
$\delta^{13}\text{C}$	-17.3±0.7	-19.3±1.8	-19.1±1.4	-17.7±0.7	-18.8±0.8	-19.3±1.3
n	19	34	6	6	26	6
TL	2.8	2.9	2.6	3.0	3.5	3.2

Discussion

The concentration of food items in the environment, i.e. food availability, triggers feeding in krill (Barange et al. 1991). Accordingly, possible differences in trophic position and feeding behaviour in krill were suspected on a regional as well as on a seasonal scale. This study showed fluctuating $\delta^{15}\text{N}$ -indicated trophic positions in euphausiids of the nBus and supports the idea of a dietary segregation between different euphausiid species. The observed differences in the TL of krill in the nBus were of the same order of magnitude as reported for other (omnivores) organisms in the Tagus estuary in Portugal (Vinagre et al. 2012), both highly variable ecosystems.

Lower food web structure

Generally, the lower food web structure showed that small copepods (*N. minor*, Schukat et al. 2012, submitted) and salps occupied the same TL (TL=2), being considered primary consumers. Krill species and larger copepods like *C. carinatus* (Schukat et al. 2012, submitted) filled a TL around 3, secondary consumers, but were separated in two distinct groups. The species within the genus *Euphausia*, *E. gibboides*, *E. hanseni* and *E. lucens*, showed a TL mainly below 3 whereas the two species *N. capensis* and *N. megalops* occupied

higher levels above 3. Jellies and *T. monacantha* already filled a trophic position around 4. These findings coincide with previous studies about feeding preferences and feeding morphology of euphausiids, where krill is described as truly omnivorous, although different genera tend towards either carnivory or herbivory (Roger 1973). Most of the species within the genus *Euphausia* are considered as non-hunting opportunistic omnivores (Mauchline 1967), which are preferential herbivores (e.g. Barange et al. 1991; Stuart and Pillar 1990). In contrast, *N. megalops* is described as a carnivorous species (Barange et al. 1991; Gurney et al. 2001). Mouthparts of the genus *Euphausia* are generally more setose than those of the genus *Nematoscelis* and therefore adapted to filter feeding (Mauchline and Fisher, 1969). Here, the size of a food particle which can be ingested is determined by the space between the setae (Suh and Choi, 1998). In *N. megalops* the second pair of thoracic limbs is elongated and the eyes are divided into two lobes (Mauchline 1980). Both characteristics are supposed to represent adaptations to carnivorous feeding (hunting). Remarkable is the relatively high TL in *N. capensis*, since this comparably small species (adults 12-21mm; Barange and Stuart 1991) is lacking elongated thoracopods and is described as an omnivorous species (Stuart 1986, Stuart and Pillar 1990). The thoracic limbs in *Nyctiphanes* species are all heavily setose and are therefore suggested as effective filter feeders (Mauchline 1967), with a high degree of herbivory. However, trophic links over the shelf and more inshore, where *N. capensis* mainly occurs, may be different from the slope situation and from more oceanic waters (e.g. Post 2002). This may be indicated by high numbers of heterotrophic dinoflagellates over the shelf compared to the shelf break and more off-shore (K. Bohata, Hamburg, pers. comm.), which may have elevated the TL in *N. capensis*. At the Walvis Bay-Transect protozoa were more abundant inshore than off-shore and naupliars showed a high abundance already over the shelf (Schwinghammer 2011). Thus, micro-plankton may play an important role for the filter-feeding species *N. capensis* in the nBus and affect its trophic position. Furthermore feeding on different copepod species may result in different TL between euphausiid species, depending on the food sources, respectively the TL, of its prey (e.g. *C. carinatus* vs *N. minor*). The comparably high SD in the $\delta^{15}\text{N}$ ratios in *E. hanseni*, *N. megalops* and *N. capensis* may be indicative of their opportunistic feeding behaviour, using different food sources resulting in different TL. The calculated CV further support this assumption showing that the nitrogen isotopes varied strikingly in salps, *E. hanseni* and *N. megalops* followed by *E. lucens*, *N. minor* and *N. capensis*. Diet shifts, which occur over shorter time scales than one generation, are frequently observed in arthropods and may potentially have a major influence on community dynamics (Kondoh 2003). Accordingly, the $\delta^{13}\text{C}$ fractionation in all krill species

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tested, except *E. gibboides*, indicated a vast variety of food items ingested. Ranging from dinoflagellates (-17.2 ± 0.2) to copepods like *N. minor* (-22.7 ± 0.8). These findings coincide with stomach content analyses by Barange et al. (1991) who have found copepods, dinoflagellates and tintinnids to be the most common food items in the stomachs of *E. hanseni* and *N. megalops*. The range of the $\delta^{15}\text{N}$ fractionation was 5.2‰ in *E. hanseni* and 5.0‰ in *N. megalops*, indicative of dietary shifts and regionally different roles of these species in the northern Benguela food web. In *N. capensis* the range was less pronounced (3.1‰). However, the range was close to one TL (3.4‰), also indicative of dietary shifts and possible different roles in the nBus.

Seasonal differences

Seasonal differences in the TL of different krill species were not evident. The observed differences were mainly caused by regional variation. However, in the nBus short-term upwelling events may have a greater impact on the phytoplankton/zooplankton availability, accordingly the feeding behaviour of krill, than seasonal differences. Therefore spatial differences may blur possible seasonal effects. However, considering the fluctuating Chl_a level over the course of one year, differences in phytoplankton availability were apparent. Accordingly, we suggest different TL during phases of high (winter) and low upwelling intensities (summer). Low phytoplankton levels may lead to a higher degree of carnivory in krill species, as shown for *E. lucens* in the southern Benguela (Gibbons et al. 1991). However, stable isotope analyses provide insights into long-term food assimilation, where the time-scale of incorporation is dependent on the turnover rates of the prey or tissues used (Schmidt et al. 2003). Accordingly, the seasonal course of the phytoplankton biomass should be taken into account. However, the assessed TL of *E. hanseni* was lowest during winter 2010 (TL=2.6) and almost the same during summer 2009 (TL=2.8) and autumn 2011 (TL=2.9), supporting our assumption of seasonal differences in the TL of *E. hanseni*. Before and during the sampling periods in summer 2009 and autumn 2011 the Chl_a content was comparably low, suggesting a higher TL in *E. hanseni*. During autumn 2011 the SST was very high and this may have prevented the animals from entering the upper water layers during diel vertical migration due to energetic constraints (Werner and Buchholz 2012 (submitted)). This would have excluded this species from the phytoplankton rich upper water layers and force them to feed on the zooplankton community in deeper waters, accordingly further increasing its TL. Nonetheless, during winter 2010, phytoplankton blooms may have initiated an increase in other zooplankton species, like primary consumers such as small copepods, and thereby could

have raised the trophic position of *E. hanseni*. However, the observed distinctions were rather small, compared to regional differences, underlying the high spatial variability in this system. In *N. megalops* the TL was highest during autumn 2011 and lowest during summer 2009. Phytoplankton availability was comparably poor during both seasons. The disparities found, may be explained by different zooplankton species ingested, since *N. megalops* is described as a carnivorous species exploiting the migrating zooplankton community (Barange et al. 1991, Werner and Buchholz 2012 (submitted)). As *N. megalops* may have fed on other smaller herbivorous or omnivorous species, a drop in its TL may also result from the high amount of phytoplankton used by its prey.

Regional differences

Regional differences in the TL of the krill species were apparent. The nBus is a highly variable system with periodic upwelling events which may lead to different food environments at the different sampling stations, thus causing different isotope signatures. During the cruise in February 2011 the TL in *E. hanseni* varied between 2.6 and 3.1. Accordingly, the observed disparities were greater in space than in time (2.6-2.9). Since there was a tendency in *E. hanseni* that bigger animals have higher $\delta^{15}\text{N}$ ratios, different size structures of the populations sampled, could have influenced the TL in this species. However, it is unlikely that body weight alone would account for such marked differences in TL within a species. These disparities in $\delta^{15}\text{N}$ -indicated trophic positions suggested dietary shifts from a more omnivorous to a more herbivorous diet or vice versa. Favourable food conditions due to upwelling events may have favoured a higher degree of autotrophic food items ingested, or represented a higher degree of herbivory in its prey. At the two stations where both species occurred the TL of *E. hanseni* was comparably low (station RPt-M and Kun), giving evidence for possible dietary shifts due to competition avoidance. The trophic overlap with *N. megalops* may have forced *E. hanseni* to feed on smaller copepods. Accordingly, smaller copepods, like *N. minor*, may show lower TL compared to larger ones, thus transferring their lower $\delta^{15}\text{N}$ to its predators. Remarkable were the separation between the stations south of Walvis Bay (TL=3.1±0.0) compared with the stations north of Walvis Bay (TL=2.8±0.2). Diatoms dominated north of Walvis Bay whereas dinoflagellates prevailed south of Walvis Bay (N. Wasmund, IOW Rostock, pers. comm.). Accordingly, the TL south of Walvis Bay was higher, assuming prey selection on the basis of abundance. However, this also may have had an effect on the baseline and therefore may have biased the trophic levels calculated.

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Nevertheless, animals from stations south of Walvis Bay were significantly larger than north of Walvis Bay, so that different TLs on the basis of size could not generally be excluded.

In *N. megalops* regional differences in the $\delta^{15}\text{N}$ fractionation were also greater than seasonal ones, indicative of regionally diverse roles in the food web of the nBus. The variability in the $\delta^{13}\text{C}$ ratio was less pronounced compared to *E. hanseni* suggesting more qualified food sources. In contrast, the observed regional differences in *N. capensis* were not significant. Here, the TL changed little between regions but the carbon (food) sources, indicated by the $\delta^{13}\text{C}$ fractionation, were significantly different. Accordingly, *N. capensis* was capable of utilizing different food sources but its trophic position was comparably stable.

Segregation between krill species

The results of this study further support the idea of (dietary) segregation between dominant krill species in the nBus and a strong structure of the zooplankton community (Barange et al. 1991). *E. hanseni*, *N. megalops* and *N. capensis* are the three predominant krill species in the nBus, based on their abundance and biomass (Barange 1990). Accordingly, these species may have a strong impact on the food web structure and dynamics and may show a high degree of competition for resources. However, they are separated in space (e.g. *N. megalops* and *N. capensis* (pers. obs.)) or occupy different TL (e.g. *E. hanseni* and *N. megalops*). According to Barange et al. (1991) the two latter species partition food resources on the bases of size, when overlapping in time or space (depth). *E. hanseni* passively consumed smaller copepods compared to an active selection of larger copepods in *N. megalops*. In contrast, *N. capensis* and *N. megalops* in fact occupied the same TL but are separated in space with *N. capensis* living on the shelf and *N. megalops* being more associated with oceanic waters (Barange 1990, Werner and Buchholz in prep.). In the nBus, the preferred habitat of *N. capensis* is close inshore, whereas in other regions, like the southern Benguela, it is an inhabitant of the broad shelf. This may be a consequence of the large numbers of *E. hanseni* dominating the shelf region in this area (Barange and Stuart 1991), owing to competition avoidance between *E. hanseni* and *N. capensis*. *N. capensis* mainly dwell in the shallow coastal waters (<200m) whereas *E. hanseni* adults mainly occur over the shelf break (200-1000m; Barange and Stuart 1991). The particular larger sizes in *N. capensis* together with the “unusual” residence far inshore together with high micro-plankton abundance, may have led to the unexpected high trophic position of *N. capensis*. Furthermore *E. gibboides* and *E. hanseni* had the same TL, but *E. gibboides* is a more oceanic species. *E. lucens* mainly occurs south of the Lüderitz

upwelling cell whereas *E. hanseni* was encountered north of Lüderitz, all showing approx. the same TL. However, it should be considered that *E. gibboides* was caught north of the Angola-Benguela frontal zone and accordingly, the estimated TL of *E. gibboides* may be biased by the use of a baseline conducted with data solely from the nBus. According to Barange (1990) vertical space partitioning may be a strategy of sharing highly productive areas, thus avoiding intraspecific competition. Considering our results this may be also true for dietary segregations between species.

In summary krill species in the nBus were separated by their trophic position, with the genus *Euphausia* occupying lower and the species *N. capensis*, *N. megalops* and *T. monacantha* higher TLs. The species were either separated by their trophic positions or by their distribution patterns. Concerning different other aspects, like divergent diel vertical migration behaviour (Werner and Buchholz 2012 (submitted)) and different thermal constraints (Werner et al. 2012 (in press)) the euphausiid community in the nBus appeared to be strongly structured. In the nBus regional differences in the trophic position of krill species were more pronounced than seasonal ones. Accordingly, upwelling events may directly, through food availability, or indirectly, through the trophic position of their prey, influence the TL of krill species in the nBus. The findings support those in the literature and show that krill species are omnivorous but show species-specific preferences in their dietary emphasis.

Acknowledgements

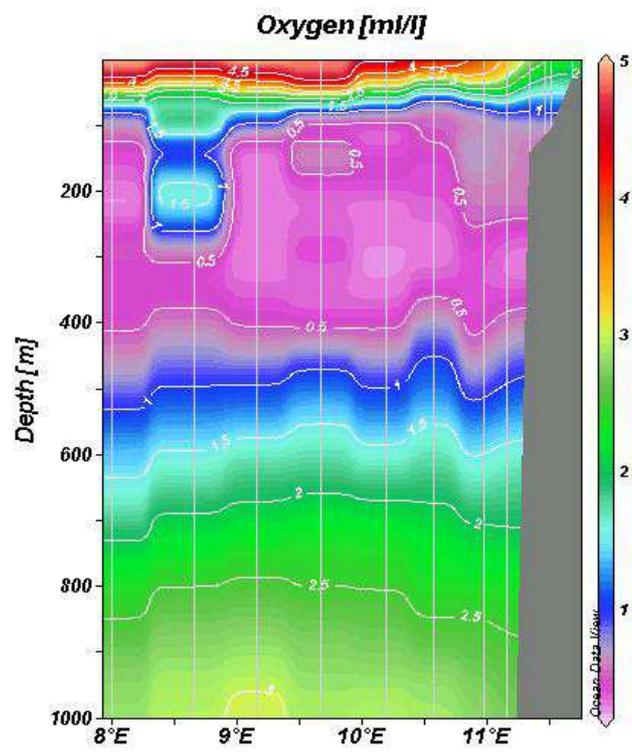
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Oxygen Minimum Layer

First results on the anaerobic capacity of *Euphausia hanseni* in the Northern Benguela Current

Thorsten Werner

Abstract

The euphausiid *E. hanseni* is a strong vertical migrator from the northern Benguela Current that encounters low oxygen levels in its environment throughout the year. It feeds in oxygenated surface waters during night and inhabits the deeper oxygen minimum zone during day. Many organisms respond to low oxygen levels by switching from aerobic to anaerobic metabolism, or even reduce their metabolism. *E. hanseni* showed a high regulatory ability with a regulation index of $RI=0.7\pm 0.2$ and was able to maintain aerobic metabolism down to $17\pm 6\%$ oxygen saturation (lowest value 6%). No suppression of metabolic rates was observed but temperature significantly shifted the critical oxygen pressure (P_C) from $13\pm 2\%$ oxygen saturation at 12°C to $19\pm 4\%$ at 20°C during summer. In winter no effect of temperature was determined ($17\pm 7\%$). Low P_C and high regulatory ability may allow *E. hanseni* the active utilization of their habitat (OMZ) and facilitate vertical migration which is in accordance with other euphausiid species from areas affected by low oxygen levels.

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Introduction

In the Northern Benguela Current (NBC), pronounced oxygen minimum zones (OMZ, $< 2 \text{ mL O}_2 \text{ L}^{-1}$) prevail between 50m to 500m depth throughout the year. Accordingly, most zooplankton species in this region had to evolve metabolic adaptations to withstand low oxygen levels or else to avoid the OMZ, thus being restricted mainly to the upper 50m of the water column. Accordingly, hypoxia affects the horizontal and vertical distribution of organisms and leads to physiological changes which in turn influence their reproductive success, life-cycle performance and growth capacity (Ekau et al. 2010). However, the biomass of mesozooplankton in the northern Benguela is severely reduced within the OMZ (Verheye et al. 2005; Auel and Verheye, 2007) and the OMZ interferes with vertical migrating mesozooplankton such as krill (Auel and Verheye, 2007). Although most euphausiids avoid the core of the OMZ below $0.1 \text{ mL O}_2 \text{ L}^{-1}$ (Sameoto, 1986; Sameoto et al., 1987; Saltzman and Wishner, 1997) two of three euphausiid species that dominate the northern Benguela upwelling ecosystem in terms of abundance and biomass, *Euphausia hanseni* and *Nematoscelis megalops*, regularly migrate through or stay within the OMZ throughout 24h (Chapter 3). Accordingly, they should have developed species-specific tolerances to hypoxic conditions, as shown for *Euphausia mucronata* in the Humboldt Current system by Gonzales and Quinones (2002). A high hypoxia tolerance allowed *E. mucronata* to avoid competition and reduce the risk of predation by oxygen-dependent hunters in the OMZ. However, the dynamics of these oxygen-depleted waters are poorly understood and their impacts on zooplankton communities are not well documented (Verheye and Ekau, 2005).

Especially the strong vertical migrant *E. hanseni* (Barange, 1990; Chapter 3) has to cope with fluctuating ambient thermal and oxygen conditions both during its vertical migration and between seasons. Furthermore, during the last decades an extension of oxygen-depleted waters and a decline in upwelling-favourable winds have been observed in the nBus. To investigate metabolic adaptations to low-oxygen levels and the anaerobic capacity of *E. hanseni* the concepts of oxyconformity, oxyregulation and the critical P_{O_2} (P_C) were applied (cf. Pörtner and Grieshaber, 1993). In general, the oxygen uptake (M_{O_2}) of an oxyconformer is strongly dependent of the ambient oxygen concentration whereas an oxyregulator maintains its oxygen uptake irrespective of the ambient oxygen concentration. However, in both cases below a critical P_{O_2} threshold (P_C) the oxygen uptake declines, which designates the onset of anaerobic metabolism. Accordingly, the activity of lactic dehydrogenase (LDH), an enzyme of the anaerobic metabolism, was determined. The ability of zooplankton species to live in, or

at least cross, the OMZ is obviously linked to the activity of anaerobic enzymes. In *Euphausia mucronata*, a strong vertical migrant from the Humboldt Current regularly crossing the OMZ, the specific activity of LDH was two orders of magnitude higher compared to the copepod *Calanus chilensis*, a species which avoids the OMZ (Gonzales and Quinones 2002). During severe hypoxia L-lactate, the primary end product of anaerobic metabolism in euphausiids, should accumulate and was used as a measure of anaerobic metabolism (Gäde 1983).

This study tried to assess the anaerobic capacity of the pronounced vertical migrant *E. hanseni* and determined the impact of temperature on its anaerobic scope. Furthermore, a comparison between (late) summer and winter was conducted to test for possible seasonal differences.

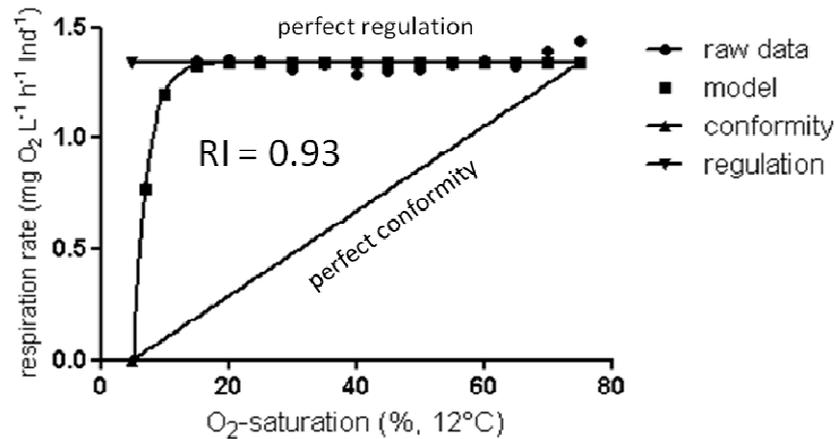
Material and Methods

Field sampling

For an exact description of the sampling gears, stations etc. please refer to Chapter 4.

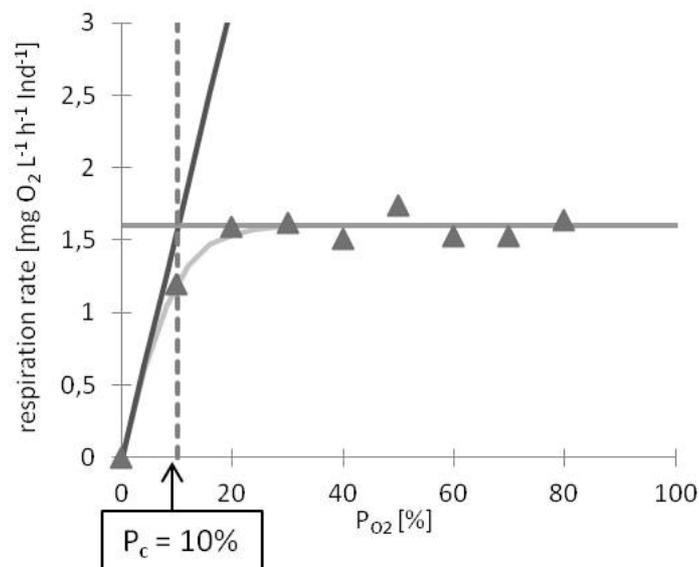
Regulation index (RI) and critical P_{O_2} (P_C)

The regulation index (RI) was determined according to the method described in Mueller and Seymour (2011). Accordingly, the oxygen uptake, determined by a closed respirometry system with Oxygen-Microsensors (PreSens, Germany - refer to Chapter 4), was plotted against the oxygen concentration and fitted with a curve (one-phase association, quadratic or linear) that showed highest R^2 . Therefore, respiration rates were calculated in 10% steps which overlapped one another by 5% (100%-90%, 95%-85%, 90-80% etc.). For a higher resolution respiration rates below 25% were calculated in 5% steps. Furthermore the first 30 min of each measurement were discarded in order to ensure a stabilization of rates. Highest respiration rates measured (start of the curve) and rates at the end of the curve (where respiration rates became 0 or at 0% oxygen saturation) were used to generate two linear regressions, visualizing extreme oxyconformity and oxyregulation. Then the area between the straight line (oxyconformity) and the curve was calculated using GraphPad Prism 5 (GraphPad Software, San Diego, CA). This area, the RI (dimension: 0-1), gives an indication of the regulation ability of the animals, where 0 corresponds to absolute conformity and 1 to absolute regulation (Mueller and Seymour (2011); Fig. 1).



↑ **Figure 1** Determination of the regulation index (RI) of *E. hanseni* at 12°C during summer 2011.

To assess the critical oxygen pressure (P_C), the oxygen level which marks the onset of anaerobic metabolism (Pörtner and Grieshaber 1993), two different methods were applied. According to Mueller and Seymour (2011) the greatest difference (GD) was calculated as the P_{O_2} at which the vertical distance between the curve and a straight line (oxyconformity) was greatest. This could be done concurrently to the determination of the RI. The second method used, fits two straight lines which intersect at a point, the P_C (Fig. 2). This method was devised by Duggleby (1984) which assumed that a two-segment line fit provides a reasonable approximation of the relationship between oxygen uptake and oxygen saturation.



↑ **Figure 2** Determination of the critical oxygen pressure (P_c) of *Euphausia hanseni* at 12°C during summer 2011 using the two-segment line approach. Triangles indicate raw data.

Biochemical analyses

Sample preparation

E. hanseni individuals from respiration measurements conducted during summer 2011 were used for the assessment of the specific activity of LDH and lactate accumulation. Respiration measurements were stopped at a specific oxygen concentration where the animals were still alive (approx. 5%, 10%, 20% and 40% O₂) and then directly frozen at -80°C using liquid nitrogen and shipped to the home laboratories in Bremerhaven.

At the laboratory the animals were rinsed with cool distilled water, carefully dried and then weighed on a microbalance (Sartorius, LA 230s, d=0.1mg). The 2nd and 3rd segment of the abdomen were severed using micro scissors on a cooling device. Afterwards, the cuticle was removed and the abdominal muscle dissected and weighed. The specimens were diluted 1:20 with cooled distilled water and grinded with a tapered glass pestle on ice. The samples were centrifuged for 15 minutes at 4°C and 13.000g using an Eppendorf centrifuge (5417R). The supernatant was used for determination of enzyme activity (LDH) and lactate accumulation. To avoid repeatedly thawing of the samples aliquots were produced and stored at -80°C for further analyses.

Specific enzyme activity

The specific activity of lactic dehydrogenase was estimated in triplicates using standard procedure according to Bergmeyer (1974). The measurements were carried out photometrically (Spectrophotometer Lambda 2, equipped with a Peltier Temperature Programmer; Perkin Elmer, England).

Metabolite accumulation

The amount of lactate was determined in duplicates using a kit provided by Biovision (Lactate Assay Kit: K607-100; Mountain View, USA). The amount of L-Alanin was determined using a kit provided by BioVision (Alanin Assay Kit: K652-100; Mountain View, USA). Both measurements were carried out with a fluorometer (Fluorscan Ascent FL, Thermo scientific).

Results

Regulation index and P_C

The regulation index in *E. hanseni* varied between 0.41 - 0.94 (0.76±0.20) at 12°C and between 0.63 - 0.89 (0.77±0.12) at 20°C during summer 2011. During winter 2010 the RI in *E. hanseni* ranged from 0.38 – 0.91 (0.63±0.18) at 10°C and from 0.44 – 0.94 (0.64±0.2) at

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20°C (Table 1). No significant differences were found between temperatures (unpaired t-test, $df=10$, $p>0.05$) or between seasons (unpaired t-test, $df=10$, $p>0.05$). Accordingly the data were pooled giving a RI of 0.70 ± 0.19 ($n=24$) in *E. hanseni*.

The critical oxygen pressure was determined using two different approaches. The GD-method provided slightly lower P_C than the intercept-method in most cases. However, only at 20°C during summer 2011 the differences, between the two methods applied, were significant (unpaired t-test, $df=10$, $p=0.023$). The P_C determined by the GD-method varied between 1.06 – 2.43 mg O₂ L⁻¹ (1.55 ± 0.44) at 12°C and 1.69 – 2.49 mg O₂ L⁻¹ (2.17 ± 0.29) at 20°C during summer 2011. In winter 2010 the P_C laid between 1.44 – 2.44 mg O₂ L⁻¹ (2.07 ± 0.35) at 10°C and 0.8 – 2.23 mg O₂ L⁻¹ (1.38 ± 0.54) at 20°C during winter 2010. The P_C calculated by the intercept-method was 0.74 – 1.48 mg O₂ L⁻¹ (1.13 ± 0.23) at 12°C and 1.25 – 2.23 mg O₂ L⁻¹ (1.68 ± 0.34) at 20°C during summer 2011. According to this, the P_C during winter 2010 varied between 1.00 – 2.33 mg O₂ L⁻¹ (1.64 ± 0.39) at 10°C and 0.53-2.85 (1.74 ± 0.82) at 20°C. In two out of six animals used for respiration measurements at 20°C during winter 2010 no P_C could be determined.

Both methods showed a significant influence of temperature on the P_C in *E. hanseni*, with $p=0.0249$ ($df=10$) for the intercept-method and $p=0.0098$ ($df=10$) for the GD-method during summer 2011, but failed to find significant differences between temperatures during winter 2010. The assessed P_C was significantly different at 10/12°C between seasons (unpaired t-test, $df=10$, $p=0.0298$). No differences between seasons at 20°C were found (unpaired t-test, $df=10$, $p>0.05$). Since the two P_C determination methods produce fairly similar results, no method could be preferred. An overview of the calculated RI and P_C is given in Table 1.

▀ **Table 1** Regulation index (RI) and calculated critical oxygen pressure (P_C in mg L^{-1} and percentage (%)) of *Euphausia hanseni* individuals at 12°C and 20°C during summer 2011 and 10°C and 20°C during winter 2010. P_C was determined using two different methods; two-segment line (intercept) and greatest differences (GD). Asterisk indicates significant differences (t-test, $\text{df}=10$, $P<0.05$) between temperatures. nd – not determinable.

<i>E. hanseni</i> summer 2011	RI (12°C)	P_C (intercept) (12°C)	P_C (GD) (12°C)	RI (20°C)	P_C (intercept) (20°C)	P_C (GD) (20°C)
animal 1	0.93	1.37 (13%)	1.06 (10%)	0.83	1.69 (19%)	1.25 (14%)
animal 2	0.94	1.27 (12%)	1.06 (10%)	0.54	2.05 (23%)	1.43 (16%)
animal 3	0.41	1.59 (18%)	1.16 (11%)	0.89	2.49 (28%)	2.23 (25%)
animal 4	0.93	1.06 (10%)	0.74 (7%)	0.63	2.23 (25%)	1.60 (18%)
animal 5	0.74	1.69 (16%)	1.27 (12%)	0.86	2.14 (24%)	1.69 (19%)
animal 6	0.62	2.43 (23%)	1.48 (14%)	0.80	2.41 (27%)	1.87 (21%)
M	0.76	1.55 (15%)*	1.13 (13%)*	0.74	2.17 (24%)*	1.68 (19%)*
SD	0.20	0.44 (4%)	0.23 (2%)	0.16	0.29 (3%)	0.34 (4%)
N	6	6	6	6	6	6
winter 2010	RI (10°C)	$P_{\text{O}_2 \text{ crit}}$ (intercept) (10°C)	$P_{\text{O}_2 \text{ crit}}$ (GD) (10°C)	RI (20°C)	$P_{\text{O}_2 \text{ krit}}$ (intercept) (20°C)	$P_{\text{O}_2 \text{ krit}}$ (GD) (20°C)
animal 1	0.52	2.44 (22%)	1.77 (16%)	0.47	2.23 (25%)	1.87 (21%)
animal 2	0.84	1.77 (16%)	1.55 (14%)	0.48	nd	nd
animal 3	0.91	1.44 (13%)	1.00 (9%)	0.62	1.07 (12%)	2.85 (32%)
animal 4	0.58	2.33 (21%)	1.66 (15%)	0.86	nd	nd
animal 5	0.56	2.22 (20%)	2.33 (21%)	0.94	0.80 (9%)	0.53 (6%)
animal 6	0.38	2.22 (20%)	1.55 (14%)	0.44	1.43 (16%)	1.69 (19%)
M	0.63	2.07 (19%)	1.64 (15%)	0.64	1.38 (15%)	1.74 (20%)
SD	0.18	0.35 (3%)	0.39 (4%)	0.20	0.54 (6%)	0.82 (9%)
N	6	6	6	6	4	4

Specific activity of LDH and lactate accumulation

The specific activity of LDH in *E. hanseni* individuals varied between $48.6 - 181.4 \text{ U min}^{-1} \text{ g}_{\text{ww}}^{-1}$ ($109 \pm 39 \text{ U min}^{-1} \text{ g}_{\text{ww}}^{-1}$, $n=14$). No significant differences were found between the treatments for specific LDH activity and the oxygen concentration at the end of the respiration experiments respectively (ANOVA, $p>0.05$, $\text{df}=13$, $F=0.5267$). Lactate values remained very low and no differences were found in relation to the oxygen concentrations.

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Discussion

Recently it was shown that several euphausiids species exhibit diel vertical migrations into oxygen minimum, tolerate hypoxic conditions and did not even avoid the core of the OMZs (Escribano et al. 2009, Antezana 2009). Studies concerning the regulatory ability of euphausiid species from other regions without an OMZ showed that e.g. *Euphausia superba* and *Thysanoessa macrura* (Torres et al. 1994) and *Meganyctiphanes norvegica* (van der Thillart et al. 1999) were able to regulate its O₂ uptake rate only down to approximately 25-30% saturation and below high mortality rates occur (conversion factors given by Ekau et al. 2010 were used to transform and compare units). As stated by Torres et al. (1994) low P_C values are not a general characteristic of mesopelagic crustaceans and low P_C values in OMZ species are specific adaptations to low oxygen levels in the water column. The mysid *Gnathophausia ingens* from the Pacific migrates into the OMZ and could regulate its oxygen uptake down to 4% oxygen saturation (Childress 1971). The RI of *E. hanseni* during both seasons was similar and *E. hanseni* showed a high regulation potential down to maximal 6% oxygen saturation (17±6%). No metabolic suppression was observed and temperature (10/12°C – 20°C) did not affect the ability of *E. hanseni* to regulate its oxygen uptake. Accordingly, this strong vertical migrator was able to keep a constant oxygen uptake irrespective of the ambient oxygen levels over a broad range of oxygen saturation. As it was not possible to include the whole experimental range between 100% oxygen saturation down to 0%, due to technical constraints, such as a stabilization of rates at the beginning of each experiment, the RI may be even higher. A high degree of regulation provides the animals with sufficient oxygen supply to maintain a high metabolic activity, irrespective of the ambient oxygen levels. The observed high swimming rates during the DVM (Chapter 3) may be reflected by this oxygen-independent metabolic activity. The high regulation potential is an adaptation to an environment with pronounced OMZs, especially in animals that regularly encounter distinct changes in oxygen availability. However, at least during summer 2011, there was a significant difference between the calculated P_C at 12°C and 20°C suggesting that higher water temperatures raised the oxygen level at which the anaerobic metabolism is initiated and therefore, negatively impacted the anaerobic capacity of *E. hanseni*. Higher metabolic rates at higher water temperatures (Chapter 1) may explain such shifts. A temperature induced shift in the P_C values was also observed in other euphausiid species, like *M. norvegica*. Here, the P_C varied between 38%-52% (8-11 kPa) at 15°C and 19%-29% (4-6 kPa) at 7°C (Strömberg and Spicer 2000). In the crab *Carcinus maenas* the P_C increased by 12% when the temperature increased from 10°C to 18°C (Taylor et al. 1977). The theory of

interactions between different factors, both biotic and abiotic, on the physiological performance of an organism was outlined by Pörtner (2010). An elevated P_C at higher water temperatures may severely constrain distribution patterns and abundance of species in view of the ongoing anthropogenic climate change: Higher P_C s at higher water temperatures may force the animals to leave or avoid areas, with low oxygen levels and concurrent elevated temperatures, earlier than OMZ with low temperatures. Accordingly, the expansion of low oxygen water masses together with increasing water temperatures (Stramma et al. 2008) could affect the physiology and behaviour of *E. hanseni* and cause distribution shifts and negatively affect its abundance in the nBus. Furthermore, the food web may be influenced and ecosystem structure and functioning may alter if the abundance of *E. hanseni* changes. However, temperature only affected the P_C during summer but not during winter 2010. To understand if this difference was a result of different thermal adaptations to different water temperatures between seasons (Chapter 5), further studies are needed. However, a high RI and a low P_C reflected the capability of *E. hanseni* to withstand low oxygen conditions. This was further supported by the observation that animals during respiration measurements were able to survive down to an oxygen level of $0.15 \text{ mg O}_2 \text{ L}^{-1}$. Sameoto et al. (1987) demonstrated that euphausiids in the eastern tropical Pacific avoided oxygen concentrations below $0.1 \text{ mL O}_2 \text{ L}^{-1}$ ($\sim 0.14 \text{ mg O}_2 \text{ L}^{-1}$).

This study neither found a correlation between the specific activity of LDH in *E. hanseni* and the ambient oxygen level nor a sign for lactate accumulation under hypoxia, which is in contrast to Schneider and Seibel (2010). It is suggested that either the chosen time span was too short to accumulate considerable amounts of lactate or *E. hanseni* may be able to quickly metabolize lactate due to oxidation or the formation of glycogen. Since the determined P_C was low in this species, an increase in LDH activity and an accumulation of lactate above approximately 20% oxygen saturation would not be expected. Accordingly, future experiments should prolong the time span for incubations and/or respiration experiments and investigate oxygen saturation levels well below 20%.

However, the results of this study coincided with observations from other ecosystems like the Eastern Equatorial Pacific and the Humboldt Current where euphausiids enter the OMZ, whereas the vertical extent of most other zooplankton species is limited by hypoxic conditions (Antezana 2002 and 2009). According to Verheye et al. (2005) and Auel and Verheye (2007) this is also the case in the NBC. Furthermore, this adaptation to hypoxic waters seems to be a competitive advantage since krill species that are able to migrate into the hypoxic zone show

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exceptionally high regional abundances, e.g. *E. mucronata* in the Humboldt Current (Antezana 2002) and *E. hanseni* in the NBC (Olivar and Barrange (1990), pers. obs.).

In summary, *E. hanseni* is well adapted to low oxygen levels by function of its high degree of regulation potential and low P_C , both facilitate its pronounced vertical migration behaviour and allow for regularly entering and/or crossing the OMZ.

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Conclusions

In the course of these conclusions I will describe physiological and behavioural adaptations of krill species to environmental conditions, demonstrate possible affects of environmental changes on krill species, and how these changes may affect the northern Benguela ecosystem. Moreover, I will discuss the potential of euphausiids as useful indicators of short-term and long-term changes in their environment and reveal their suitability as model organisms for a comparison of nutritionally mono-pulsed and poly-pulsed systems in coastal waters.

The Benguela upwelling system, an area of high commercial interest, has been subject to several studies concerning exploitable marine resources like various fish species (Mas-Riera et al. 1990, van der Lingen et al. 2006), rock lobster (Pollock and Shannon 1987, van der Lingen et al. 2006) and other important marine resources like Cape fur seals (Mecenero et al. 2006, Kirkman et al. 2007) and Cape gannets (Gremillet et al. 2004, Pichegru et al. 2007). The observed changes in e.g. distribution patterns and recruitment success of species and the drastic biological changes in the Northern Benguela Current (NBC) during the last four decades, including regime shifts, were related to environmental conditions like water temperature anomalies (Cole 2002, Daskalov et al. 2003), upwelling intensities (Olivar 1990) and anthropogenic disturbances (Boyer et al. 2001). However, all of the mentioned species are relatively large organisms of mid to high trophic levels. As stated by van der Lingen et al. (2006) there is a necessity to understand and quantify the processes, which control resource and ecosystem variability, particularly on lower trophic levels. As an important trophic link in the northern Benguela food web, and food source for many high trophic level species such as fish, birds, seals and whales (Pillar et al. 1992), this thesis assessed the impact of temperature, low oxygen levels and the fluctuating trophic environment on the physiology and behaviour of the dominant euphausiid species. Earlier studies on krill from the NBC focused on systematic records and general distribution patterns (Shannon and Pillar 1986), followed by information about biomass, community structure and production. This information helped to evaluate krill trophodynamics and their contribution to energy fluxes (Pillar et al. 1992). However, no data about physiological adaptations are yet available and the driving forces behind the observed distributional and behavioural patterns were largely not taken into account. With respect to the observed physical and biological alterations which occurred during the last four decades and the ongoing climate change, it is important to evaluate adaptations of euphausiid species to these environmental parameters. In turn, to predict

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possible changes in abundance and biomass of these species, which may affect food web structure and ecosystem functioning, it is crucial to understand the influence of these parameters on krill behaviour and physiology. Particularly, because the impact of climate change may result in a stronger biological response in upwelling systems in the Atlantic compared to the Pacific (Lachkar and Gruber 2012).

All studied parameters, both the highly stratified water column in terms of temperature and dissolved oxygen concentration and the highly fluctuating, upwelling mediated, trophic environment had largely influenced behavioural patterns and/or physiological performance of the krill species tested. The adaptive capacity of krill species to these parameters and an assessment of possible consequences for the distribution and abundance of euphausiids in the northern Benguela upwelling system (nBus) will be discussed in the following paragraph.

Physiological and behavioural adaptations of euphausiids to the fluctuating environmental conditions in the nBus – temperature, food and oxygen availability

During the research cruises between 2009 and 2012 the euphausiid community of the Benguela upwelling system consisted of nine krill species, with *N. capensis*, *E. hanseni*, *N. megalops*, *E. recurva*, *E. americana* and *Thysanopoda monacantha* (Ortmann 1893) ordered by abundance. The species *E. lucens* was solely found in the southern Benguela. The strong upwelling cell at Lüderitz (26°S) seemed to act as a physical barrier for euphausiids because the species composition north and south of this upwelling cell differed significantly. In the NBC abundance and biomass of euphausiids were highest in the northern part of this area between RockyPoint (19°S) and the Kunene River (17°S), irrespective of seasonal and interannual variability (Fig. 8). The three dominant krill species in terms of abundance and biomass were segregated horizontally (Fig. 8). While *N. capensis* lives on the shelf, *E. hanseni* is strongly associated with the shelf-break and *N. megalops* inhabits more oceanic water masses. Even though the horizontal distribution of *E. hanseni* and *N. megalops* partially overlaps, they avoid competition by segregating vertically (Chapter 2).

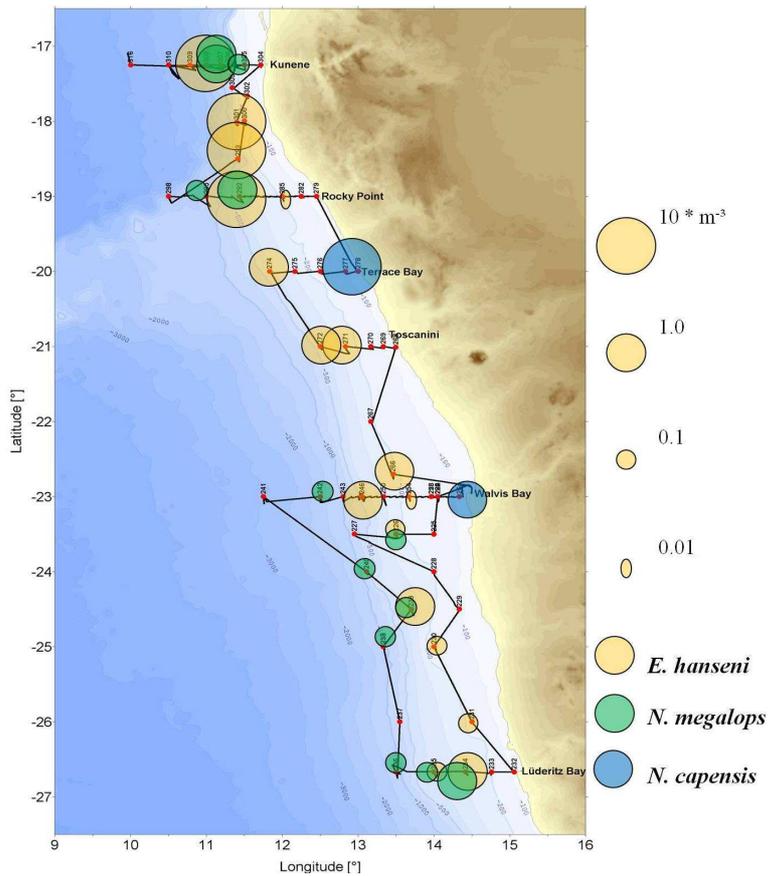
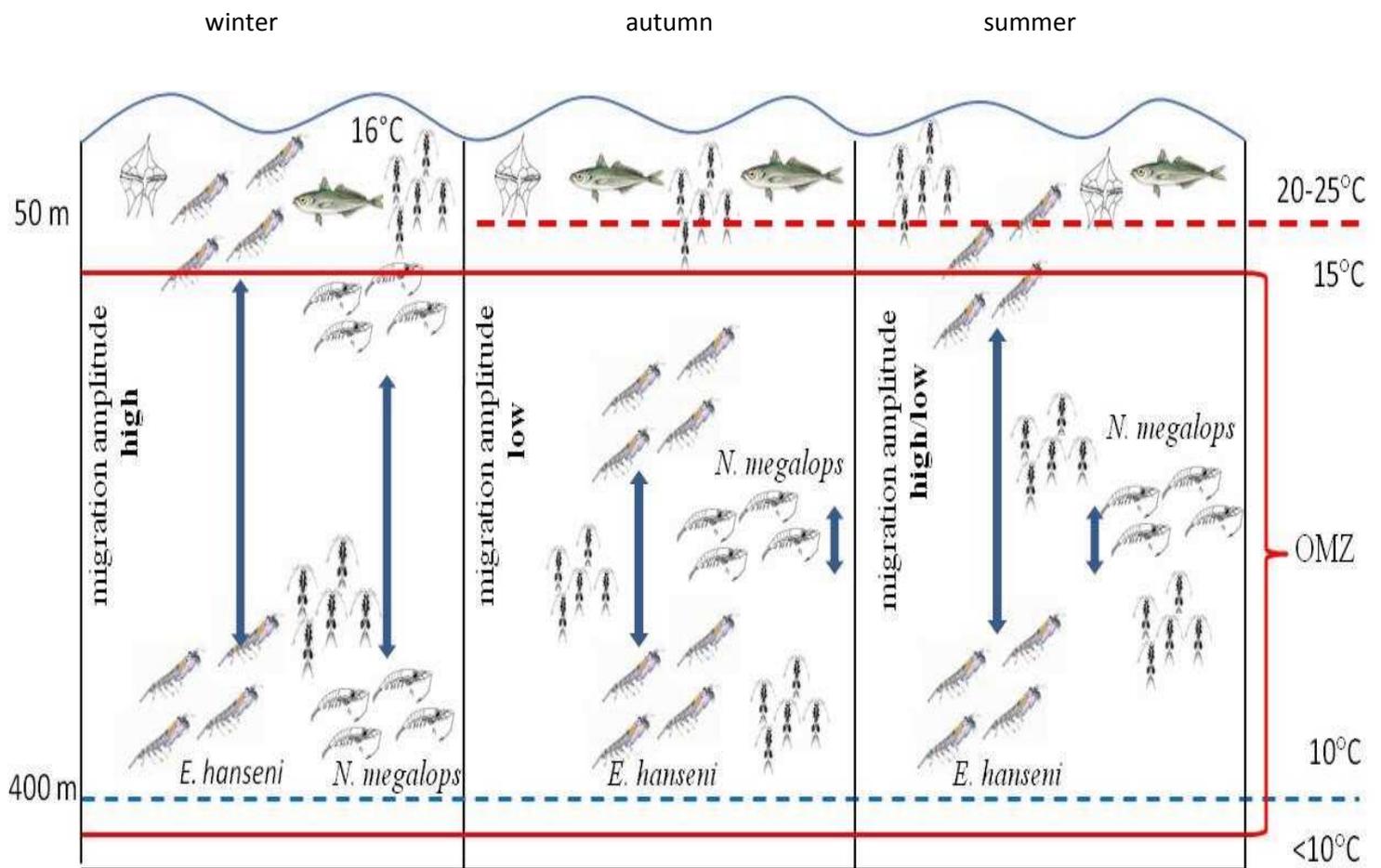


Figure 8
Horizontal distribution and abundance (Ind m^{-3}) of *E. hanseni*, *N. megalops* and *N. capensis* during a cruise with the RV *Discovery* in winter 2010. (F. Buchholz and T. Werner, unpubl. data)

Effects of temperature - One of the main driving forces causing alterations in species abundance and distribution is the warming of the world oceans (Perry et al. 2005). According to Pörtner (2001 and 2002) the effects of temperature result from physiological changes at molecular, cellular and organismal levels and the ultimate effect of a temperature increase on the ecosystem is based on species-specific responses to temperature changes. In conjunction with climate variability the underlying thermal physiology of animals plays a key role in understanding the effect of increasing water temperatures on marine ecosystems (Pörtner 2010). Accordingly, the species-specific thermal constraints were investigated (Chapter 1) in two dominant euphausiid species of the nBus, namely *N. megalops* and *E. hanseni*, and their vertical migration behaviour was followed according to seasonal differences in the temperature profile of the water column (Chapter 2). Accordingly, both species are well adapted to the prevailing water temperatures in the NBC. However, species-specific differences were apparent. The oxygen demand and NH_4-N excretion rates increased with rising water temperatures in both species. *E. hanseni* showed thermal adaptations to temperatures between $10^{\circ}C$ and $20^{\circ}C$ whereas in *N. megalops* temperatures above $15^{\circ}C$ pointed to metabolic constraints. Moreover, both species differed in their vertical position and

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diel vertical migration behaviour. *N. megalops* avoided the thermocline during its ascent and descent, which is in accordance with its thermal constraints, whereas *E. hanseni* crossed it regularly (Fig. 9).



↑ **Figure 9** A schematic model of the diel vertical migration behaviour of *Euphausia hanseni* and *Nematoscelis megalops* under different environmental conditions. Blue arrows picture the migration range; red lines indicate the lower and upper limit of the OMZ and the dotted red line describes the position of the thermocline.

During autumn and summer SST were well above 20°C and strong thermoclines were detected that influenced the DVM behaviour of krill. In contrast, during winter no thermocline was observed and SST was around 16°C. Food availability further influenced DVM behaviour of euphausiids (for details please refer to Chapter 2).

 - Dinoflagellates,  - Copepods, food sources for krill;  - Horse mackerels, a predator of krill

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In this regard, crossing the thermocline would result in detrimentally high oxygen demand. Energetic considerations showed that *E. hanseni* had a comparably high carbon demand and as a consequence, there may be a need to fulfill it via vertical migration. In contrast, calculated Q_{10} values for *E. hanseni* suggested that DVM into deeper layers allow this species to save energy. Differences in the carbon/energy demand of both species were a result of different (1) metabolic rates, (2) predominantly metabolized substrates, (3) migration amplitudes, thus swimming efforts, and (4) thermal adaptations. Assessed different metabolic rates, thus respiration rates, accounted for slightly higher carbon demands in *E. hanseni* compared to *N. megalops* between 5-15°C ($12 \pm 16\%$ of the daily carbon demand). Lipid-orientated metabolism in *N. megalops* and protein-orientated metabolism in *E. hanseni* increased the differences in daily carbon demands up to $52 \pm 21\%$ between species. Moreover, different migration amplitudes, thus “swimming costs”, accounted for $24 \pm 6\%$ of the daily carbon demand in *E. hanseni* and only $6 \pm 2\%$ in *N. megalops*. Observed differences in DVM behaviour due to dissimilar thermal constraints further influenced the carbon demand of species. However, despite behavioural and temperature variations, calculations of the daily carbon demand indicated a species-specific energy expenditure (Chapter 2) with a 31% higher carbon demand in *E. hanseni* compared to *N. megalops*. The latter species is largely carnivorous (Chapter 4) and is suggested to exploit the migrating zooplankton community as a food source (Chapter 2). It is most likely that thermal constraints and calculated energy budgets reflected adaptations to a life in deep layers with low temperatures, low oxygen levels, comparably low daily carbon demands, and convenient food supply (Chapter 1+2). In contrast, the pronounced migrant *E. hanseni* regularly crossed the thermocline and entered the upper water layers to feed on high concentrations of plankton (Chapter 2). In this species thermal constraints and determined carbon demands are indicative of a highly active species, using deeper layers to save energy and hide from predators and entering upper layers to exploit the food rich layers and use higher water temperatures for growth and reproduction. Since the horizontal distribution of both species (pers. obs.) and their dietary preferences partially overlap (Barange et al. 1991) a vertical segregation between *N. megalops* and *E. hanseni*, reflected by their different thermal adaptations, carbon demands and vertical position, promote competition avoidance in the NBC euphausiid community. Accordingly, temperature affected the oxygen uptake in euphausiids and species-specific thermal adaptations (Chapter 1) were reflected by different behavioural patterns, which in turn resulted in vertical segregation of the euphausiid community (Chapter 2).

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Dissolved oxygen concentration - The impact of oxygen minimum zones (OMZ) on the distribution of pelagic animals has been known for decades (Longhurst 1967) and oxygen availability influences the vertical distribution of euphausiids (Buchholz et al. 1995; Antezana 2009; Escribano et al. 2009). However, the relevance of the OMZ for the physiology, behaviour and the life cycles of marine animals has only recently come into focus (Ekau et al. 2010, Seibel 2011). Hypoxia is known to impact life-cycle performance, growth, reproduction and susceptibility to diseases (Ekau et al. 2010) and ambient hypoxia narrows the thermal window of a species and possibly lowers its performance optima (Pörtner and Peck 2010). Furthermore, a decline in oxygen saturation has been reported for most of the world oceans (Stramma et al. 2010). Accordingly, the anaerobic capacity (Chapter 5) and the vertical migration behaviour in relation to oxygen minimum zones (Chapter 2) were subject of this thesis. Adaptations of animals to low dissolved oxygen concentrations are driven by strong selective pressure to maintain aerobic metabolism (Seibel 2011). The use of the oxygen minimum layers as a refugium for oxygen-dependend predators was evident in *N. megalops* and *E. hanseni*. Some euphausiid species regularly entered, in the case of *E. hanseni*, or spend most of their entire adult life span, *N. megalops*, in the OMZ (Fig. 9). Others, e.g. *Euphausia recurva*, only temporarily faced low oxygen levels, and *Euphausia americana* only briefly entered the oxycline. These observations suggested that the euphausiid community is divided into two ecological groups. However, no euphausiid species in the NBC appeared to avoid the OMZ at all. The capability to withstand very low oxygen levels in species spending considerable times in the OMZ is reflected by their high regulatory capability and low critical P_{O_2} , as shown for *E. hanseni* (Chapter 5). These adaptations permit *E. hanseni* the active utilization of their habitat, as shown for *E. mucronata* in the Humboldt Current by Antezana (2002) and may explain the observed high swimming speeds during their DVM (Chapter 2). To extract the required oxygen for aerobic metabolism from the low oxygen waters within the OMZ is a challenging task and species living in this area should have developed several effective adaptations, like a greater quantity of respiratory pigments, a higher oxygen affinity or morphometric adaptations like a larger gill:cephalothorax ratio (Antezana 2002). In *N. megalops* the overall low oxygen demand, compared to *E. hanseni*, is suggested to be an adaptation to low ambient oxygen levels which allow this species to survive chronic hypoxic situations. However, to determine the anaerobic capacity of this species further analyses are needed, which failed due to technical constraints during this thesis. If different behavioural adaptations are generally reflected by different anaerobic capacities, and/or species-specific thermal constraints, as shown for *E. hanseni* and *N. megalops*, needs further evaluation. In *E.*

hanseni an increase of water temperatures led to a higher oxygen level at which the anaerobic metabolism was initiated (P_C). This phenomenon was described for several other (zooplankton) species and euphausiids (Chapter 5). A warming of the world oceans will negatively influence the anaerobic capacity of *E. hanseni* and this is most likely to affect its vertical and horizontal distribution and consequently compress its vertical habitat.

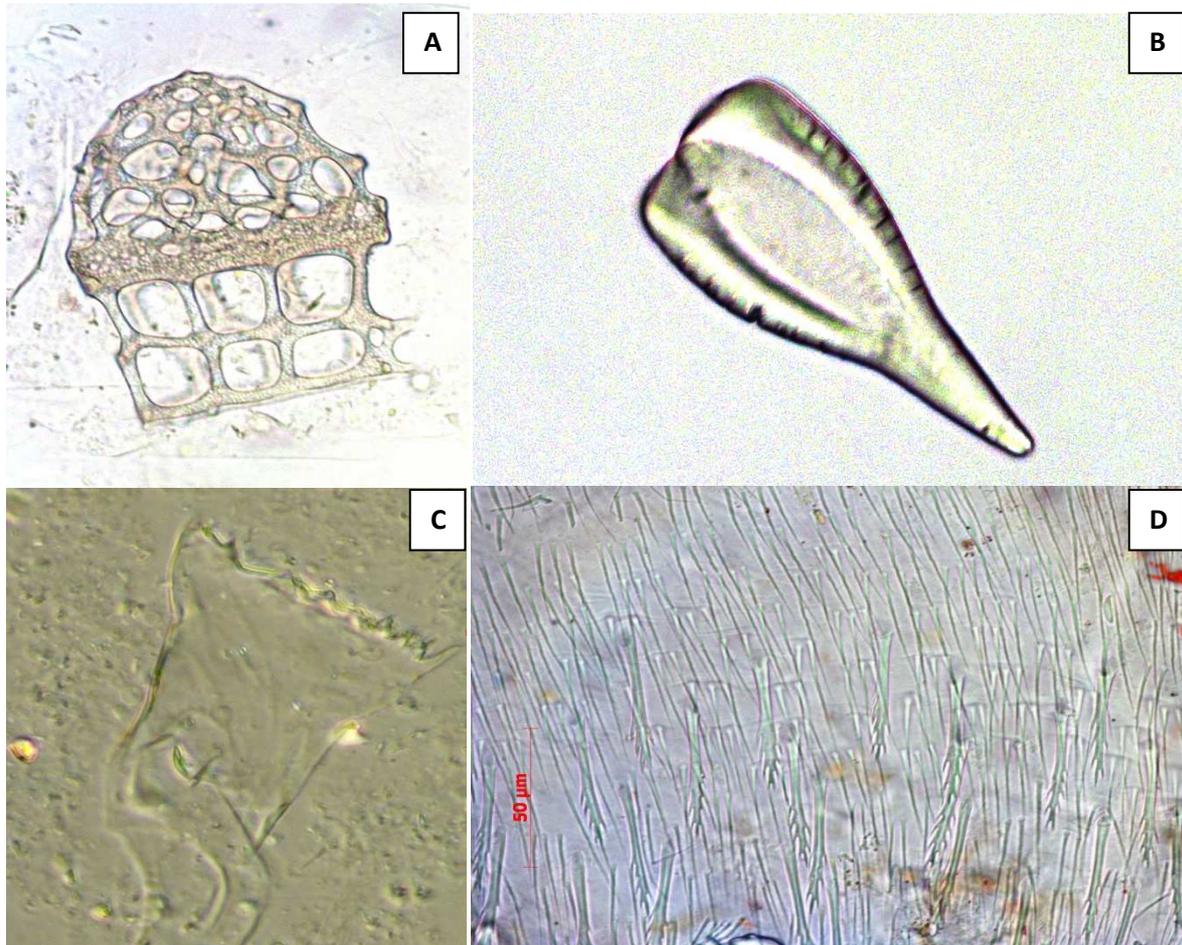
Trophic environment - The quality and quantity of the available food is known to have various effects on physiological performance. Favourable feeding conditions are known to enhance growth (Buchholz 2003) and to support a higher reproductive effort (Cuzin-Roudy and Buchholz 1999). The trophic environment influences the vertical (Gibbons et al. 1991) and horizontal (Quetin et al. 1996) distribution patterns of organisms and euphausiids in particular. Accordingly, the DVM behaviour in relation to the quality and quantity of food sources in the water column was observed (Chapter 2), the physiological performance of *E. hanseni* was related to upwelling events that determine primary production in the NBC (Chapter 3) and the trophic positions of different krill species were assessed (Chapter 4). It was shown that the trophic level of krill species in the nBus is not static in space and time but rather dependent on the regionally, and to a lesser extent, seasonally different trophic conditions. Furthermore, the variable trophic position indicated that euphausiids are omnivores (Fig. 10) which may switch their dietary emphases depending on the quantity and quality of food, which coincide with a previous study on *Euphausia lucens* (Hansen 1905) from the southern Benguela Current (Stuart and Pillar 1990). In consequence, the role of euphausiids in the nBus food web may differ between regions, seasons and upwelling conditions. The estimated trophic position indicated that euphausiids in the NBC are considered primary and secondary consumers but can also occupy the same level as jellyfish. The varying trophic positions of euphausiids and subsequent alterations in energy fluxes will be implemented in an ECOPATH/ECOSIM model approach, to describe the trophic interactions and energy fluxes in the pelagic food web. A comparison with ECOPATH models from the 1980s and 90s (Jarre-Teichmann et al. 1998., Shannon and Jarre-Teichmann 1999) will help to describe possible changes between the situation at the end of the 20th century and the situation to date. This thesis demonstrated that food abundance alters migration behaviour and that the species *E. hanseni* showed distinct physiological adaptations to the nutritionally poly-pulsed system of the NBC. During their vertical migrations, krill species did not necessarily migrate in conjunction with their prey, and the vertical position did not obligatory coincide with food maxima. However, growth and reproduction were

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controlled by upwelling events which may produce a synchronization of moult within the whole swarm (Buchholz et al. 1996) and promote suitable trophic conditions for the optimal conditions of eggs and larvae. Under non-upwelling conditions, the reproductive effort and metabolic activities were reduced whereas upwelling conditions fueled these processes. It is most likely, that upwelling pulses (food abundance) affect the physiology state, behaviour and distribution patterns of *E. hanseni* in the nBus and that these adaptations in this species are linked to small-scale processes and not to seasonal differences. Metabolic rates, thus oxygen demand, were elevated under upwelling conditions mainly due to higher reproductive efforts. It should be considered that physiological and behavioural adaptations to small-scale processes may have blurred a clear seasonal signal. Seasonality in the NBC is weak compared to temperate or polar regions, but changes in physical characteristics like upwelling-inducing winds, SST and hydrodynamics are significant. Variability is mainly influenced by the occurrence and expansion of warm, low-oxygen tropical waters from the Angolan dome into the NBC. Barange and Stuart (1991) showed that the maximum body size in *E. hanseni* and *N. capensis* coincide with the maximum intensity of upwelling and maximum densities with seasonal warming. However, continuous breeding of *E. hanseni* and *N. capensis* in this system (Barange and Stuart 1991), indicate favourable feeding conditions throughout the year, further supporting the concept of a more pronounced impact of small-scale processes compared to seasonal ones. The data shown in this thesis suggest that seasonal adjustment of metabolic rates (Meyer et al. 2010) or seasonal adaptations of growth and reproduction to times of food surplus (Buchholz 2003), as shown for *E. superba* and *M. norvegica*, did not take place to the same extent in euphausiids in the nBus. In fact, the hypothesized “resting stages” in the reproductive cycle of *E. hanseni*, with females showing nearly fully developed oocytes (Chapter 3), and the occurrence of ripe *E. hanseni* females throughout the year (Barange and Stuart 1991, pers. obs.) indicate that (1) the trophic conditions are favourable enough to allow energy expenditure for maturation irrespective of season all over the year and (2) that reproduction in *E. hanseni* is adapted to periodic short time-scale upwelling events, which could boost its reproductive success.

In summary, euphausiids in the NBC are adapted to short-term alterations in their environment, thus upwelling pulses. Seasonal adaptations may occur, as shown for maximum body sizes and maximum densities in *E. hanseni* and *N. capensis* that correlate with seasonal maxima in upwelling intensity and seasonal warming (Barange and Stuart 1991). However, the data shown in this thesis give strong evidence that short-term changes have a predominant impact on the physiology and behaviour of euphausiids, and that seasonal adjustments are less

intense. The physiology and behaviour of krill species clearly reflect the prevailing ambient conditions in the nBus, in terms of temperature and food availability.



↑ **Figure 10** Photos taken from the dissected stomach of *E. hanseni* under 200-320x magnification. **A** – a tintinnid; **B** – a crystal cone; **C**- copepod mandible; **D** – spines at the inner structure of the stomach

Simultaneous effects of multiple environmental factors - the species level

The physiological state and behavioural patterns of krill species in the NBC, which may affect their vertical and horizontal distribution and lead to changes in abundance and biomass of euphausiids, is not a result of a single factor but is most likely a result of several different factors, like temperature, oxygen levels, food availability and possibly others (e.g. light). All these observed environmental factors forced both physiological and behavioural changes in euphausiid species of the northern Benguela upwelling ecosystem. Anyhow, as these results give basic indications of the isolated effect of an environmental factor, future investigations should integrate the concurrent effects of these parameters (cf. Pörtner 2010). For example,

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the results of this thesis suggest that the simultaneous effect of rising water temperatures and an expansion and intensification of hypoxic events (Stramma et al. 2008) may be critical for the species *E. hanseni*. Since its anaerobic capacity is negatively influenced by rising water temperatures, the predicted changes may force this species to reduce the time spent in the OMZ or to avoid the core of the OMZ and accordingly, to narrow its vertical migration amplitude, respectively compress their habitat into a narrow surface layer (Chapter 2+5). These physiologically induced behavioural changes are most likely to have several, mainly negative, effects on *E. hanseni* individuals, such as:

- A higher predation risk, due to inhabitation of the upper layers during daytime and avoidance of the OMZ as a refugium for highly mobile, oxygen-demanding predators, like squids and several fish species (Chapter 2, Seibel 2011)
- A higher daily carbon demand, due to higher water temperatures in the upper water layers (Chapter 1)
- Altered retention mechanisms, due to ontogenetic shifts in the vertical migration patterns (Barange and Pillar 1992)
- Intensified competition with other zooplankton and krill species, due to overlapping vertical distribution patterns and exploitation of the same prey items (Barange et al. 1991, Chapter 2+4)
- Affecting schooling behaviour, due to trade-offs between oxygen availability and predator avoidance (Brierly and Cox 2010)

Furthermore, a restricted vertical distribution would exclude this species from its contributions to the vertical flux of carbon and this will affect carbon cycling in the NBC. Elevated water temperatures may as well cause behavioural differences due to thermal constraints in *N. megalops*, which may prevent this species from ascending during the night. As this species is well adapted to a life in cold and oxygen poor waters, a warming of water temperatures would result in higher carbon demands or even force this species to migrate deeper. To meet these requirements *N. megalops* may need to actively hunt its prey and further increase its carbon demand, due to a higher activity. As this species preys on the migrating zooplankton community, also the decoupling of predator-prey interactions should be considered. However, since several aspects of its physiology and behaviour are still uncertain and seem to be highly variable, predictions about consequences of environmental

changes are speculative. However, rising water temperatures may positively affect recruitment success and growth in krill species and accordingly, enhances productivity of the species. Furthermore, different life stages may show divergent adaptive strategies and therefore, they may either be supported or weakened. Predictions about distribution and abundance changes, thus stock alterations, are a challenging task because of the complex interactions of physical and biological factors (Flores et al. 2012).

Effects of multiple environmental factors - the ecosystem level

Changes in the abundance and distribution of predominant euphausiids of the NBC will control both low and high trophic levels in a multi-directional way. This multi-directional link was suggested as a mechanism by which species serve as constitutive components of the Benguela food webs, due to interactions with and effects on other components (Cury and Shannon 2004). Environmental induced changes in krill distribution and abundance can alter distribution, abundance and composition of other pelagic communities and therefore induce significant alterations of the current state. Krill do not merely act as a passive conduit of environmental perturbations, but bias both higher and lower trophic levels through their own species-specific dynamics.

A warming of the NBC and intensification of hypoxic events may force euphausiids, like *E. hanseni*, to compress their habitats into a narrow surface layer. From a **top-down point of view**, changes in species vertical distribution are most likely to alter predation pressure on phytoplankton and smaller zooplankton species, mainly copepods. An enhanced carbon demand, due to higher water temperatures, and a prolongation of the time-span spent in the phytoplankton rich water layers may increase the amount of phytoplankton ingested by *E. hanseni*. As a consequence, phytoplankton may be utilized more efficiently and this will decrease detritus loads and improve the dissolved oxygen concentration in the NBC. Moreover, *E. hanseni* may switch from one preferentially ingested copepod species to another, because of different ambient trophic conditions in deeper waters compared to the upper layers (Chapter 2+4). Due to these dietary shifts the energy flux in the food web may change, possibly affecting food web dynamics and the food web structure at a whole. Higher water temperatures may support higher growth rates and increase the reproductive effort, supporting high abundances of krill. However, krill species were excluded from the downward shunt of organic material, thus affecting the carbon cycle in the nBus. Changes in

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distribution patterns of euphausiids in the NBC are suggested to significantly impact lower trophic levels and to lead to alterations in the food web structure of the nBus.

On the other hand, from a **bottom-up point of view**, *E. hanseni* staying in the upper water layers throughout 24 h will allow predators, like fish, jellyfish, birds and seals to prey upon this krill species very easily and this may decrease the abundance of krill. At first, predators may benefit from the new food source, but then a decline in *E. hanseni*, will negatively affect predators and may force them to feed on other prey. Model simulations for the southern Benguela showed that shifts between sardines and anchovies may be produced by the availability of meso-zooplankton prey of proper sizes caused by alterations in environmental conditions (Shannon et al. 2004). Changes in the abundance and species composition of euphausiids in the upper water layers may cause shifts at higher trophic levels, affecting the food web and ecosystem structure. Furthermore, a reduction of krill will lead to more phytoplankton unutilized and increase detritus loads and therefore, enhance low oxygen situations. This will have negative effects on the vertical distribution range of krill. Moreover, a reduction of dissolved oxygen in the water column may negatively influence the recruitment success of small pelagics, since their early life stages are known to be sensitive to low oxygen concentrations (Breitburg 2002). It could be demonstrated that warming of water temperatures and concurrent expansion of hypoxia may narrow the vertical distribution range of *E. hanseni* and that the effects on the NBC ecosystem can be direct, such as higher grazing rates, or indirect, such as an intensification of hypoxia events.

Euphausiids of the northern Benguela upwelling system – implications for krill species as indicators of short-term and long-term changes in their environment, with emphasis on euphausiids as model organisms for a comparison of nutritionally mono-pulsed and poly-pulsed systems in coastal waters

As stated by Mangel and Nicol (2000), euphausiid species are ideal model organisms for studying the interaction between environmental and organismal variability. Studying krill consolidate different scientific fields, like ecology, biochemistry, physiology, evolution and oceanography (Mangel and Nicol 2000). Krill species are distributed ubiquitously and are central to pelagic marine food webs (Siegel 2000). Thus, gained knowledge of a certain species may be extrapolated to and compared with other krill species (Buchholz 2003).

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Euphausiids feed on a variety of phyto- and zooplankton taxa including detritus and serve as an important food source for many high trophic species. Some euphausiids provided proof to be quite good experimental animals (Nicol 2000) and can be maintained in captivity quite easily for a reasonable amount of time (pers. obs.). Their comparably large size allows studying their physiology and biochemistry even on the level of individual organs (Mangel and Nicol 2000) and their physiological and behavioural plasticity allows them to quickly adapt to changes in their environment (Buchholz 2003), and in turn their physiological performance should enable scientists to draw conclusions about the prevailing conditions. For example, Buchholz (2003) pointed out, that growth in Antarctic krill (*E. superba*) and Northern krill (*M. norvegica*) is very flexible and that these species adjust their size increment at moult (INC) and their intermoult period (IMP) to the trophic situation. Growth was further modified by temperature where the IMP decreased with increasing temperatures. Due to their different thermal constraints, krill are associated with different water masses and changes in hydrodynamics should be noticed e.g. on the community level. For example, the occurrence of three additional euphausiid species of Atlantic origin in the high Arctic Kongsfjorden indicated an increasing influence of warmer Atlantic waters in this area, whereas an unaltered community composition was a sign for persisting cold conditions in other Arctic areas (Buchholz et al. 2010). Buchholz et al. (2010) concluded from their data that the reproductive activity of krill may give an indication of further warming and shift in food web composition. In 2011, first observation of *T. raschii* spawning in the high Arctic Kongsfjorden (West Spitzbergen) gave signs for shifts in environmental temperatures and food web composition (Buchholz et al. 2012, see attachment). However, the impact of climate variability on marine resources is difficult to assess, since some species may benefit from the ongoing changes while others will not (cf. Atkinson et al. 2004, Ekau et al. 2010). It remains unclear whether the ongoing changes depart from typical climate variability and natural large scale variations have to be considered (Chavez et al. 2003). Anyhow, recent studies, e.g. Field et al. (2006), implied that the anthropogenic 20th-century warming had passed natural variability in ecosystem structure and functioning.

Nutritionally poly-pulsed systems like the NBC (Hutchings et al. 2006) differ from mono-pulsed systems (Knotz 2006) like the North Sea. In mono-pulsed systems one (mono) phytoplankton bloom is followed by a time span of relatively poor trophic conditions. Consequently, most physiological and ecological processes are linked to this “moment of food surplus”. The influence of the production regime, thus phytoplankton blooms on ecological and physiological adaptations of zooplankton to nutritionally mono-pulsed systems have been

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intensively studied. In the case of euphausiids living in these mono-pulsed systems, like *E. superba* and *M. norvegica*, growth and reproduction are linked to this phytoplankton bloom (Buchholz 2003, Cuzin-Roudy 1993). A drastic seasonal reduction of metabolic rates, correlated with low food availability, was observed in *E. superba* and contrastingly, in *M. norvegica*, higher metabolic rates during the spring phytoplankton bloom in areas with seasonal food shortage were observed. Despite regional and species-specific differences it is most likely that krill species are well adapted to this (spring) phytoplankton bloom and growth, reproduction and behaviour are linked to this short time food surplus. Krill species may therefore serve as model organisms for the impact of nutritionally mono-pulsed systems on zooplankton in coastal waters.

In upwelling areas, like the NBC, the physical and biological properties are mainly determined by the timing and duration of upwelling events that are further controlled by upwelling-favoured winds (Dorman et al. 2005), which in turn are influenced by climate change. Phases of high primary production are linked to upwelling pulses which prevail throughout the year and may support the adult animals with sufficient food supply. However, during austral summer upwelling intensities in the NBC are minimal (Hagen et al. 2001). Planktonic species like euphausiids are passively advected with water masses leading to variable environmental conditions and coming across with areas less favourable for living (Verheye and Ekau 2005). This study demonstrated that, using the results of physiological performance of *E. hanseni* (Chapter 3), adaptations took place to periodic phases of food surplus. *E. hanseni* reduces metabolic activity under food limitation (Huenerlage and Buchholz 2012) and enhances physiological performance when food conditions improve though only in response to short-term and small-scale events. Since seasonal differences, thus phases of minimal and maximal upwelling intensities, in the physiological state are less pronounced than regional ones, thus areas under upwelling and non-upwelling conditions, this notion is further supported. Krill species may change their dietary emphases according to the prevailing food items influenced by upwelling conditions (Chapter 4) and adapt their DVM behaviour to the amount of food and temperature profiles of the water column (Chapter 2). Euphausiid physiology and behaviour reflect the ambient trophic environment and due to their capability to quickly react to changes in their environment and further supported by the typically short krill food chain, euphausiids may be useful indicators for adaptations to and life history strategies in nutritionally poly-pulsed systems in coastal waters.

In conclusion, the physiological state of krill and their flexible physiological capacity, irrespective of their habitat, reflects environmental trophic conditions and moreover, euphausiids change their behaviour according to the prevailing trophic situation. A consolidated view of all these aspects indicates that euphausiids are useful indicators of short-term and long-term changes in their environment and can be used as model organisms for a comparison between mono- and poly-pulsed systems, as well as global adaptations and life history strategies of invertebrates.

Perspectives

The main focus of this thesis is to gain first insights into physiological and behavioural adaptations of dominant euphausiid species to the highly fluctuating environmental conditions in the NBC. Research results provide a comprehensive data set on the influence of environmental conditions on both the physiology and the behaviour of krill. However, several new questions and aspects arose during the course of the work which need further experimental studies.

The highly variable and dynamic system of the NBC and the complex interactions of physical and biological factors hamper the investigations of adaptations in krill to 'in situ conditions' and may blur possible seasonal effects. This emphasizes the importance of a detailed and thorough sampling strategy at a fixed station, including upwelling and down-welling events, as well as seasonal repeats. Since this thesis had not been feasible without support of other GENUS sub-projects, which provided data about the trophic environment as well as the hydrographic conditions during the sampling periods, incorporation of several environmental parameters is highly relevant.

The results of this study further indicate that the estimation of combined effects of different parameters, such as temperature and dissolved oxygen levels, on krill behaviour and physiology are crucial. This will allow us to build up a more detailed picture of the mechanisms which determine the variability in distribution and abundance of euphausiids. Further experiments should also focus on boundary layers, such as the benthic boundary layer, oxy- and thermoclines or filament structures, as these structures may strongly affect euphausiids within the zooplankton community.

Conclusions and perspectives

Krill species in the NBC are omnivores and regionally occupy different trophic levels. To study the effect of the trophic environment on krill and as well to elucidate adaptations to this factor micro-, phyto- and zooplankton should be implemented. This study shows that a description of the trophic environment only by using Chl_a data may not be useful for omnivorous euphausiids.

The results of this thesis further indicate that krill are adapted to short-term changes in environmental conditions, such as upwelling-induced phytoplankton blooms. Perennial upwelling may provide sufficient food throughout the year. Nevertheless, if krill species in this area require building up energy stores to overcome periods of food shortage needs further research.

Investigations of the anaerobic capacity of krill species should be intensified. All krill species interacted to a certain degree with the OMZ. Determination of their anaerobic scopes will help predicting possible alterations in future climate scenarios. In this regard, on the basis of past experience, new experiments should focus on oxygen levels well below 20% oxygen saturation; at least on species regularly inhabiting the OMZ. Assessment of the combined effect of temperature and low oxygen levels is reasonable. Furthermore, investigations of other adaptations to a life under low oxygen concentrations, such as gill size, quantity of respiratory pigments and their oxygen affinity will help to draw a more detailed picture of krill living in the OMZ.

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Personal contributions to national and international conferences:

Werner, T. and Buchholz, F. (2011)

Metabolism of three euphausiid species of the Northern Benguela Current under seasonal and upwelling effects.

15. Deutschsprachige Crustaceologen-Tagung, 7.-10. April 2011, Universität Regensburg 2011

Werner, T., Buchholz, C. and Buchholz, F. (2011) Performance of euphausiids of the Northern Benguela upwelling system.

BCC-Science Forum 2011, 17-19. October 2011, Swakopmund, Namibia

Werner, T., Buchholz, C. and Buchholz, F. (2011) Energy demand of *Thysanoessa inermis* in a changing environment.

Workshop “Antarctic Krill in a Changing Ocean”, 11-15. April 2011, Texel, Netherlands

Geist, S., Schukat, A. and **Werner, T.** (session chairs) (2012) Environmental changes in the pelagic – consequences and acclimatization strategies, from plankton to fish.

Youmares “Between space and seafloor – aqua vita est” 12-14. September 2012, Lübeck, Germany

Attachments

At least I would like to draw your attention on two publications concerning krill species in two fast changing ecosystems, the Arctic (I) and the Antarctic (II). During my PhD thesis I had the opportunity to participate in the sampling process, maintenance and taking pictures of eggs/larvae (I) and the discussion/development process (II) of these studies. Furthermore, I would like to advise you to have a look on the podcast of the GENUS project (III), which was shot during a research cruise with the RRS *Discovery* in 2010 by the Multimedia Kontor Hamburg.

- (I) Buchholz, F., **Werner, T.** and Buchholz, C. (2012) **First observation of krill spawning in the high Arctic Kongsfjorden, west Spitsbergen.** *Polar Biology* **35**, 1273-1279. doi: 10.1007/s00300-012-1186-3

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Abstract: In the past, two euphausiid species prevailed in the high Arctic Kongsfjorden, the arcto-boreal *Thysanoessa inermis* (Kroeyer, 1846) and *Thysanoessa raschii* (Sars, 1964). Both were considered expatriates from the Barents Sea or Norwegian Sea and non-reproductive due to low temperatures. The macro-zooplankton of the fjord has been studied as a component in an ecosystem context since 2006, including baseline investigation of distribution and functional performance of key species. In recent years, three additional krill species were regularly detected in the fjord and are the focus of an intensive long-term study. Of these species, *Thysanoessa longicaudata* (Kroeyer, 1846) and *Meganyctiphanes norvegica* (Sars, 1857) are typical for the boreal Atlantic whereas *Nematoscelis megalops* (Sars, 1883) has a broad distribution in temperate to subtropical provinces. Their occurrence in the Kongsfjorden clearly indicates increasing Atlantic influence. During the 2011 campaign, *T. raschii* was observed spawning in the field for the first time and showed development up to the naupliar stage in the laboratory. Should more evidence of reproduction be encountered in any of the five krill species in the Kongsfjorden in the future, it will be taken as an indication of a changing environment concerning temperature and food web composition.



➡ **Figure 11** A hatching nauplius larvae (Buchholz et al. 2012)

- (II) Flores, H. , Atkinson, A. , Kawagushi, S. , Krafft, B. , Milinevsky, G. , Nicol, S. , Reiss, C. , Tarling, G. A. , Werner, R. , Bravo Rebolledo, E. , Cirelli, V. , Cuzin-Roudy, J. , Fielding, S. , Groeneveld, J. , Haraldsson, M. , Lombana, A. , Marschoff, E. , Meyer, B. , Pakhomov, E. A. , Rombolá, E. , Schmidt, K. , Siegel, V. , Teschke, M. , Tonkes, H. , Toullec, J. , Trathan, P. , Tremblay, N. , Van de Putte, A. , van Franeker, J. A. and **Werner, T.** (2012) **Impact of climate change on Antarctic krill.** *Marine Ecology-Progress Series*, INTER-RESEARCH **458**, 1-19. doi: 10.3354/meps09831

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Abstract: Antarctic krill *Euphausia superba* (hereafter ‘krill’) occur in regions undergoing rapid environmental change, particularly loss of winter sea ice. During recent years, harvesting of krill has increased, possibly enhancing stress on krill and Antarctic ecosystems. Here we review the overall impact of climate change on krill and Antarctic ecosystems, discuss implications for an ecosystem-based fisheries management approach and identify critical knowledge gaps. Sea ice decline, ocean warming and other environmental stressors act in concert to modify the abundance, distribution and life cycle of krill. Although some of these changes can have positive effects on krill, their cumulative impact is most likely negative. Recruitment, driven largely by the winter survival of larval krill, is probably the population parameter most susceptible to climate change. Predicting changes to krill populations is urgent, because they will seriously impact Antarctic ecosystems. Such predictions, however, are complicated by an intense inter-annual variability in recruitment success and krill abundance. To improve the responsiveness of the ecosystem-based management approach adopted by the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR), critical knowledge gaps need to be filled. In addition to a better understanding of the factors influencing recruitment, management will require a better understanding of the resilience and the genetic plasticity of krill life stages, and a quantitative understanding of under-ice and benthic habitat use. Current precautionary management measures of CCAMLR should be maintained until a better understanding of these processes has been achieved.

- (III) <http://www.podcampus.de/channels/93>

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Eidesstattliche Erklärung

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Eidesstattliche Versicherung

Hiermit erkläre ich an Eides statt, dass ich die vorliegende Dissertationsschrift selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe.

(Thorsten Werner)

(Ort, Datum)