Trophic interactions and energetics of key components in the Benguela Current Ecosystem: The role of calanoid copepods and pelagic decapods

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Abbreviations

act	active
AMR	adjusted metabolic rate
CHOL	cholesterol
C5	copepodite stage 5
dia	diapause
DM	dry mass
DVM	diel vertical migration
EB	energy budget
ETS	electron transport system
FAME	fatty acid methyl ester
FFA	free fatty acids
MOCNESS	Multiple Opening and Closing Net with Environmental Sensing System
OMZ	oxygen minimum zone
OVM	ontogenetic vertical migration
PL	phospholipid
TL	total lipid
WE	wax ester
TAG	triacylglycerol
TFA	total fatty acids
TFAlc	total fatty alcohols
WM	wet mass

Summary

The Benguela Current is one of the major coastal upwelling systems in the world. It has changed severely in species abundance, community composition and food web dynamics during the last decades induced by environmental changes and the impact of unsustainable fisheries. While zooplankton diversity and abundance generally increased, biomass of small pelagic fish decreased. Decapods and especially copepods represent key components of the northern Benguela food web since the 1970s and play an important trophic role interlinking lower with higher trophic levels. Moreover, they enhance the vertical flux of organic matter and dissolved inorganic carbon from the euphotic zone to deeper water layers through their diel and ontogenetic vertical migrations. This study clarifies trophodynamics and energetics of dominant calanoid copepods and pelagic decapods and investigates their life strategies in terms of species-specific adaptations to upwelling conditions.

Lipid storage patterns as well as metabolic rates revealed dormant stages in the two copepod species *Calanoides carinatus* and *Rhincalanus nasutus*. The dormant stages were characterised by high wax ester levels (>80% of total lipids) and a strongly reduced metabolism in both species. However, the results indicated that *C. carinatus* and *R. nasutus* follow different life strategies: *C. carinatus* enters the extreme form of dormancy (diapause) during non-upwelling as part of its ontogenetic vertical migration, while *R. nasutus* migrates into the oxygen minimum zone in times of low food supply and enters a rather moderate and more flexible dormancy, probably quiescence.

The two biomarker approaches (e.g. fatty acids and stable isotopes) revealed a complex pattern of trophic positions for a variety of copepod species, but also highlighted the dietary importance of diatoms and dinoflagellates. Two copepod species, *C. carinatus* and *Nannocalanus minor*, occupied the lowest trophic level (predominantly herbivorous) corresponding to high amounts of fatty acid markers for diatoms (e.g. 16:1(n-7)) and dinoflagellates (e.g. 18:4(n-3)). These two copepod species represent the classical link between primary production and higher trophic levels. All other copepods belonged to secondary or even tertiary (e.g. some deep-sea copepods) consumers.

In contrast to the complex trophic interactions of calanoid copepods, pelagic decapod species represented a more homogeneous group regarding their carnivorous feeding habits, with indices for feeding on calanid copepods.

Respiration rates revealed different energy demands of copepod and decapod species. Based on these results an energy budget approach was conducted to estimate community consumption rates. The results indicated that *C. carinatus* is an important primary consumer in the northern Benguela system, consuming up to 30% of daily primary production. In contrast, the other herbivorous copepod *N. minor* has little grazing impact. Furthermore, pelagic decapods exerted a considerable predation impact on calanid copepods, consuming 2-13% of standing stock.

In conclusion, the interdisciplinary approach of this study provided new and important information on trophodynamics and energetics of various copepod and decapod species. These data are essential for the development of realistic carbon budgets and food-web models of the northern Benguela upwelling region and coastal upwelling systems in general.

Zusammenfassung

Der Benguela Strom zählt zu den wichtigsten Küstenauftriebsgebieten der Welt. In den letzten Jahrzehnten hat sich dieses Ökosystem durch Veränderungen der Umwelt und den Einfluss einer nicht nachhaltigen Fischerei in Hinblick auf die Abundanz und Zusammensetzung von Arten sowie den Prozessen im Nahrungsnetz stark gewandelt. Während die Artenvielfalt und das Vorkommen des Zooplanktons generell angestiegen sind, ist die Biomasse kleiner pelagischer Fische stark gesunken. Dekapoden und insbesondere Copepoden stellen seit den 1970-er Jahren wichtige Komponenten im Nahrungsnetz des nördlichen Benguela Stroms dar, und spielen eine wichtige trophische Rolle, indem sie niedrige mit höheren trophischen Ebenen verbinden. Des Weiteren verstärken sie durch ihre täglichen oder ontogenetischen Vertikalwanderungen den vertikalen Fluss von organischem Material und gelöstem anorganischen Kohlenstoff von der euphotischen Zone in tiefere Wasserschichten. Diese Studie erläutert trophische Interaktionen und energetische Prozesse dominanter calanoider Copepoden- und pelagischer Dekapodenarten und untersucht ihre Lebensstrategien in Bezug auf artspezifische Anpassungen an Auftriebsbedingungen.

Lipidspeicherungsmuster und Stoffwechselraten zeigten Ruhestadien in zwei Copepoden Arten (*Calanoides carinatus* und *Rhincalanus nasutus*) auf, die in nahrungsknappen Zeiten während geringem Auftrieb eingegangen werden. Charakteristisch für diese Ruhestadien sind hohe Wachsestergehalte (>80% des Gesamtlipidgehalts) und ein stark reduzierter Stoffwechsel. Die Ergebnisse deuteten jedoch darauf hin, dass *C. carinatus* und *R. nasutus* unterschiedliche Lebensstrategien verfolgen: *C. carinatus* begibt sich während auftriebsschwachen Perioden als Phase seiner ontogenetischen Vertikalwanderung in eine extreme Form des Ruhezustands (Diapause), während *R. nasutus* in Zeiten von geringem Nahrungsangebot in die Sauerstoffminimumszone wandert und dort einen eher moderaten und flexibleren Ruhezustand, möglicherweise die Quieszenz, einnimmt.

Die Biomarkeransätze (Fettsäuren und stabile Isotope) enthüllten komplexe trophische Interaktionen für eine Vielfalt von Copepoden, stellten aber außerdem die Wichtigkeit von Diatomeen und Dinoflagellaten in der Ernährung von Copepoden heraus. Die beiden Copepodenarten *C. carinatus* und *Nannocalanus minor* besetzten die unterste trophische Ebene (überwiegend herbivor), einhergehend mit hohen Anteilen von Fettsäuremarkern für Diatomeen (z.B 16:1(n-7)) und Dinoflagellaten (z.B. 18:4(n-3)). Diese beiden Copepodenarten stellen demnach die klassische Verbindung zwischen Primärproduktion und höheren trophischen Ebenen dar. Alle anderen Copepodenarten zählten zu Sekundär- bzw. Tertiärkonsumenten (z.B. einige Tiefsee Copepoden). Im Gegensatz zu den komplexen trophischen Interaktionen von calanoiden Copepoden, stellten pelagische Dekapoden eine homogenere Gruppe hinsichtlich ihrer carnivoren Ernährungsweise dar. Calanide Copepoden scheinen einen wichtigen Anteil in ihrer Ernährung zu spielen.

Sauerstoffverbrauchsraten ergaben unterschiedliche Energieansprüche für Copepoden- und Dekapodenarten. Basierend auf diesen Ergebnissen wurden Energiebilanzen aufgestellt, um Verbrauchsraten von Copepoden- und Dekapodengemeinschaften abzuschätzen. Es konnte gezeigt werden, dass *C. carinatus* ein wichtiger Primärkonsument im nördlichen Benguela Strom ist und bis zu 30% der täglichen Primärproduktion verbrauchen kann. Im Gegensatz dazu hat der ebenfalls herbivore Copepode *N. minor* einen geringen Wegfraßeinfluss. Pelagische Dekapoden zeigten einen erheblichen Prädationsdruck auf calanide Copepoden, indem sie zwischen 2-13% deren Bestandes konsumieren können.

Zusammengefasst liefert der interdisziplinäre Ansatz dieser Studie neue und wichtige Informationen über trophische Interaktionen und energetische Prozesse diverser Copepoden- und Dekapodenarten. Diese Daten sind essentiell für die Entwicklung von realistischen Kohlenstoffbudgets und Nahrungsnetzmodellen des nördlichen Benguela Auftriebsgebiet und von Küstenauftriebsregionen im Allgemeinen.

OUTLINE OF PUBLICATIONS

The following overview outlines the four publications included in this thesis and my contributions to the respective chapters. The overall objectives of this study were derived from the GENUS project (Geochemistry and Ecology of the Namibian Upwelling System) and the general concept was developed by Prof. D. W. Hagen and PD Dr. H. Auel (subproject 6). Sampling and on board measurements were conducted during three cruises with the *FRS Africana* (2009), *RRS Discovery* (2010) and *RV Maria S. Merian* (2011). Further analyses were operated between 2009 and 2012 in the laboratories of the Marine Zoology at the University of Bremen and in the laboratories of the Institute for Hydrobiology and Fisheries Science (IHF) in Hamburg.

CHAPTER I

Schukat A, Auel H, Teuber L, Hagen W

Complex trophic interactions of calanoid copepods in the Benguela upwelling system

The idea and concept of this manuscript was compiled by myself with assistance from H. Auel and W. Hagen. Field work was done by all authors and I performed the biochemical analyses. The manuscript was written by myself with scientific and editorial advice by all co-authors.
The manuscript was submitted (July) to Journal of Sea Research and is under review since September 2012.

CHAPTER II

Schukat A, Teuber L, Hagen W, Auel H

Energetics and carbon budgets of dominant calanoid copepods in the northern Benguela upwelling system

I developed the concept of the manuscript with advice of H. Auel. I shared sampling and experimental work on board with all co-authors and did the analytical work in the home labs by myself. I wrote the manuscript. All co-authors provided ideas and scientific advice.
The manuscript was submitted (August) to Journal of Experimental Marine Biology and Ecology and is in revision since October 2012.

CHAPTER III

Bode M, Schukat A, Auel H, Hagen W

Predicting metabolic rates of calanoid copepods

I shared field work with M. Bode as well as the development of experimental design and performance and gave scientific advice by the preparation of the manuscript.
The manuscript was submitted (September) to Journal of Experimental Marine Biology and Ecology.

CHAPTER IV

Schukat A, Bode M, Auel H, Carballo R, Martin B, Koppelmann R, Hagen W

On the distribution, ecophysiology and carbon consumption of pelagic decapods in the northern Benguela upwelling system

I developed the concept for this manuscript with H. Auel and shared field work with M. Bode, Bettina Martin, Rolf Koppelmann and H. Auel. I did the experimental work on board and shared analytical work with M. Bode and R. Carballo. Based on the results I wrote the manuscript with advice of the co-authors.
The manuscript was submitted (October) to Deep-Sea Research.

1 SCIENTIFIC BACKGROUND AND OBJECTIVES

1.1 The Benguela Current coastal upwelling system

Eastern boundary currents are characterised by coastal upwelling of cold nutrient-rich water, which provides optimal environmental conditions for phytoplankton growth and thus supports high biomass of zooplankton, fish, seabirds and marine mammals (Hardman-Mountford et al. 2003, Shannon & O'Toole 2003). Furthermore, coastal upwelling systems play an important role in the oceans' carbon cycling (Vargas & Gonzalez 2004). They occupy only 1% of the ocean area but generate 11% of new primary production (Chávez & Toggweiler 1995).

The south-west coast of Africa is strongly affected by the Benguela Current, one of the major coastal upwelling systems in the world, influencing coastal environments of western South Africa, Namibia and southern Angola (Boyer et al. 2000). It stretches from east of the Cape of Good Hope in the south northwards into Angolan waters (from ca. 37°S to 16˚S), and encompasses the entire marine environment of Namibia (Fig. 1). The Benguela Current is characterised by a predominately equatorward flow and high levels of Ekman-driven coastal upwelling (Boyer & Hampton 2001, Shannon & O'Toole 2003). Its distinctive bathymetry, hydrography, chemistry and tropho-dynamics combined make it one of the most productive oceanic region in the world, together with the Humboldt Current (Chávez & Toggweiler 1995, Carr 2002, Shillington et al. 2006), with an annual primary productivity ranging from 400 to 900 g C m^{-2} yr^{-1} (Brown et al. 1991, Carr 2002, Heileman & O'Toole 2008, Monteiro 2010). However, fish catches in the Benguela Current do not reflect these high primary production rates; less than 1 million tons of small pelagic fish were recorded for the Benguela Current in 2006, whereas the Humboldt as well as the Canary Current yielded much higher fish landings with 7.5 and 1.7 million tons, respectively (www.seaaroundus.org).

The Benguela upwelling system shares many of the generic characteristics of other eastern boundary currents, but it has the unique feature that it is bounded by warm water currents on both, the northern and the southern end, i.e. the tropical Angola

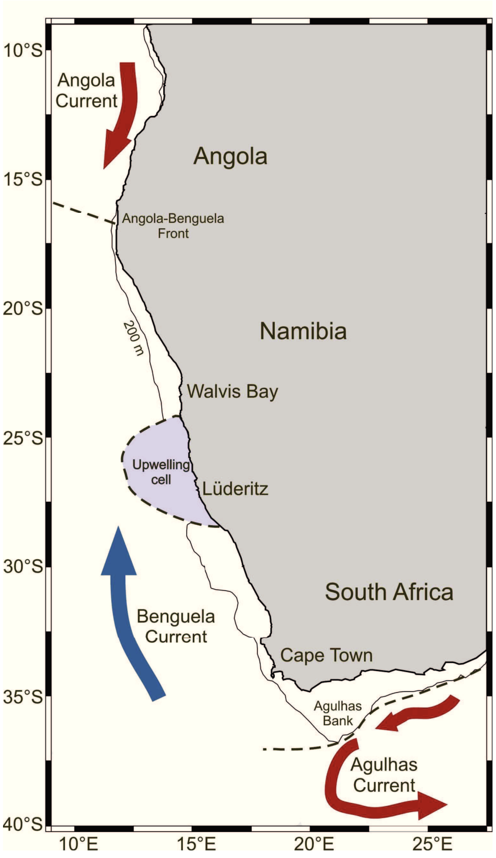

Figure 1: The Benguela upwelling system. Red arrows indicate warm currents, blue cold (modified after Shannon 2006).

Current in the north and the Agulhas Current system in the south (Fig. 1) (Shannon & O'Toole 2003, Shillington et al. 2006). Water masses of different origins are prevalent in the Benguela upwelling region. Tropical and subtropical waters occur at the surface, whereas the thermocline is characterised by South Atlantic and tropical Atlantic Central Water. Antarctic Intermediate Water occurs below the thermocline in average depths of 700-800 m. A poleward flowing undercurrent prevails along the shelf break at depths of 200-300 m as compensation current to the northward flowing Benguela Current (Nelson & Hutchings 1983, Shannon & Nelson 1996). Coastal upwelling of cool nutrient-rich South Atlantic Central Water is induced by strong south-easterly trade winds (Hardman-Mountford et al. 2003) and occurs in separate upwelling cells along the coast (Nelson & Hutchings 1983, Shannon & Nelson 1996). Upwelling intensity fluctuates throughout the year in five- to ten-day cycles between active upwelling and relaxation. Highest upwelling intensity occurs in austral winter and spring (Branch et al. 1987). The only structure within the Benguela system with intense perennial upwelling, is the Lüderitz upwelling cell at 26-28°S (Fig. 1) (Boyer & Hampton 2001, Duncombe Rae 2005). This cell is the most intense upwelling zone found in any upwelling regime. It separates the Benguela region in a northern and southern part (Shannon & Jarre-Teichmann 1999, Boyer et al. 2000, Shillington et al. 2006). In the northern Benguela system, active upwelling cells have also been identified around 18°S near Cape Frio, 20°S and 23°S (Shannon & Nelson 1996, Hardman-Mountford et al. 2003). A narrow shelf strip of only 40 km width, as in the vicinity of Cape Frio, is a characteristic topographic feature for major upwelling cells in the northern Benguela system (Nelson & Hutchings 1983, Hardman-Mountford et al. 2003).

The Angola-Benguela Front (ABF) is another important structure associated with the Benguela upwelling system (Fig. 1). This convergence zone occurs, where the cool equatorward flowing Benguela Current meets the warm, highly saline poleward flowing Angola Current (Shannon et al. 1987). The ABF is a permanent feature at the surface (noticeable to a depth of ~200 m) and is maintained throughout the year within a narrow band of latitudes, characteristically between 14°S and 17°S (Shannon et al. 1987, Hutchings et al. 2009). Approximately every 10 years, the ABF can reach as far as 25°S caused by enhanced southward intrusions of warm, highly saline waters originating from the Angola Basin (Boyer et al. 2000) referred to as Benguela Niños. However, these events are less frequent and less intense than the El Niños in the Pacific Humboldt region (Shannon et al. 1987).

Oxygen minimum zones (OMZ), which occur at intermediate depths, are also a major feature of the Benguela region. On the shelf of the northern Benguela Current water masses with low oxygen levels result from complex interactions of several processes. These include the influx of oxygen-depleted water from the Angola Dome via a poleward undercurrent, the influx of well-oxygenated central water from the Cape Basin via upwelling at Lüderitz and a stratification of shelf waters (Nelson & Hutchings 1983, Monteiro & van der Plaas 2006, Hutchings et al. 2009). Low oxygen concentrations of less than 1.4 ml O_2 l^{-1} are found within the OMZ at 50 to 600 m water depth (Auel & Verheye 2007, Ekau et al. 2010). Furthermore, there is an almost permanent hypoxic layer above the seafloor with occasional outbreaks of toxic hydrogen sulphide in the near-bottom waters of the northern Benguela system (Weeks et al. 2002).

The occurrence of extended OMZs and hypoxic conditions, as well as the intrusion of the Angola Current and the impact of unsustainable fisheries caused substantial changes in the Benguela Current during the past decades. Environmental changes can lead to alterations in species abundance, community composition and food web dynamics of pelagic ecosystems at all trophic levels (Kraberg et al. 2011).

1.2 Food web of the Benguela system

Classical food chains of coastal upwelling regions are regarded as short, typically consisting of only three trophic levels (e.g. primary producers via small pelagic fish to top predators), and very efficient (Fenchel 1988, Cushing 1989). This kind of food-web structure was also present in the Benguela coastal upwelling system until the 1970s. Large chain-forming diatoms dominated at the base of the food web, with high abundances and biomasses of small pelagic fish, such as sardine (*Sardinops sagax*) and anchovy (*Engraulis encrasicolus*), which are able to feed directly on diatoms (Verheye et al. 1998, Cury & Shannon 2004). High standing stocks of small pelagics represented favourable conditions for predators, such as cape carnets, cape cormorants and fur seals (Boyer & Hampton 2001). These three trophic levels represented the dominant components of a highly efficient energy flow in the Benguela Current (Fig. 2), which resulted in a very productive region in terms of fish yield until the 1970s.

Since then, severe changes were observed in the Benguela Current. In addition to large diatoms, smaller phytoplankton organisms, such as cyanobacteria and dinoflagellates became more abundant at the base of the food web and the high biomass of small pelagics decreased from about 2 million tons to less than 700,000 tons as recorded in 2006 (www.seaaroundus.org). This decline was only caused to some extent by overfishing but also by environmental changes, which can induce regime shifts as well as alterations in species abundance at all trophic levels (Kraberg et al. 2011). While the standing stock of sardines recovered to a certain degree in the southern Benguela region, catches of sardines in the northern subsystem remained low (Cury & Shannon 2004). Instead, other fish species, such as the zooplanktivorous horse mackerel *(Trachurus trachurus)*, pelagic goby (*Sufflogobius bibarbatus*), hakes (*Merluccius capensis* and *Merluccius paradoxus*) and myctophids became more abundant (Cury & Shannon 2004, van der Lingen et al. 2006). Coinciding with the decline of landings of small pelagics, zooplankton abundance increased by more than two orders of magnitude from the 1950s to 1996 and copepod biomass increased about ten times during that time period (Verheye & Richardson 1998, Verheye et al. 1998). Copepods as well as euphausiids belong to the most important zooplankton components in the Benguela region; they dominate plankton biomass by more than 50% (Olivar & Barange 1990, Timonin et al. 1992). However, gelatinous zooplankton, especially the jellyfish species *Chrysaora hysoscella* and *Aequorea aequorea*, also increased in abundance (Boyer & Hampton 2001, Lynam et al. 2006). Jellyfish are significant predators of zooplankton and ichthyoplankton in other marine ecosystems and thus may influence the size of pelagic fish stocks in the Benguela region (Gibbons et al. 1992, Lynam et al. 2006). Changes in the community structure of zooplankton or pelagic fish also affect top predators (e.g. seabirds and seals) (Cury & Shannon 2004). The above mentioned alterations in the Benguela region resulted in a very complex food web with diverse trophic interactions and longer food chains (Fig. 2) making the energy flow less efficient compared to earlier times.

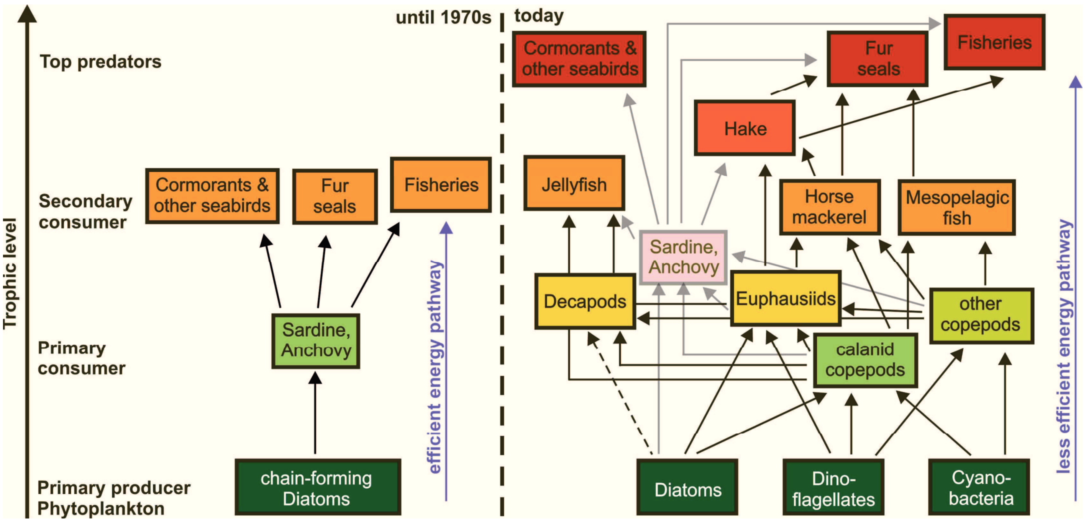

Figure 2: Conceptual models of the Benguela food web and its trophic interactions until 1970s (left side) and since then (right side). The major trophic links and energy pathways are highlighted.

The increasing zooplankton biomass underlines the now vital role of this group in the energy transfer of the Benguela upwelling system. Trophodynamics and energetics of dominant zooplankton species are crucial processes for the understanding of ecosystem structure and function. Therefore, this study focuses on two important components (copepods and decapods) of the zooplankton and micronekton community of the northern Benguela upwelling system, which are described in detail in the following.

1.3 Copepods off northern Namibia

Copepods prevail with up to 90% of mesozooplankton abundance in coastal upwelling systems and some key species represent major trophic links between primary production and higher trophic levels (Loick et al. 2005, Verheye et al. 2005). Moreover, copepods play a crucial role in the cycling of organic matter in the ocean, e.g. via moulted exoskeletons, faecal pellets, and respiration processes (Longhurst et al. 1990, Dam et al. 1995, Al-Mutairi & Landry 2001, Steinberg et al. 2008).

Annual copepod production for the northern and southern Benguela Current is higher than in the other major coastal upwelling systems; for the northern subsystem of the Benguela Current, copepod production varies between 34-45 g C m^{-2} yr^{-1} (Cushing 1971, Richardson et al. 2001), while copepod production for the southern Benguela Current is between 11-160 g C m^{-2} yr^{-1} with estimates generally higher than 50 g C m^{-2} yr^{-1} (Cushing 1971, Richardson et al. 2001, Huggett et al. 2009). Calculations of copepod production for the California, Humboldt and Canary Current were generally less than 25 g C m^{-2} yr^{-1} (Cushing 1971, Peterson et al. 2002).

The copepod community in the northern Benguela Current is perennially dominated by *Calanoides carinatus*. Other common copepods of this area are *Eucalanus hyalinus*, *Rhincalanus nasutus*, *Metridia lucens*, *Centropages brachiatus*, *Nannocalanus minor*, *Neocalanus gracilis*, *Neocalanus robustior*, *Pleuromamma borealis*, *Pleuromamma xiphias*, *Pleuromamma abdominalis* and *Aetideopsis carinata* (Timonin et al. 1992). Numerous smaller (<2 mm) copepods, such as *Acartia* spp., *Paracalanus* spp., *Clausocalanus* spp., and cyclopoid copepods are widely distributed, but their contribution to total copepod biomass is limited (Timonin et al. 1992). The copepods mentioned above differ in their feeding behaviours. Most of them are omnivorous, such as *M. lucens*, *Pleuromamma* spp. and *A. carinata,* while the calanid copepods *C. carinatus* and *N. minor* are primarily herbivorous (Loick et al. 2005).

C. carinatus (Fig. 3A) dominates the copepod biomass in the Benguela Current with up to 67% and can reach very high abundances of about 120 000 Ind. m^{-2} (Verheye 1991, Verheye et al. 2005).

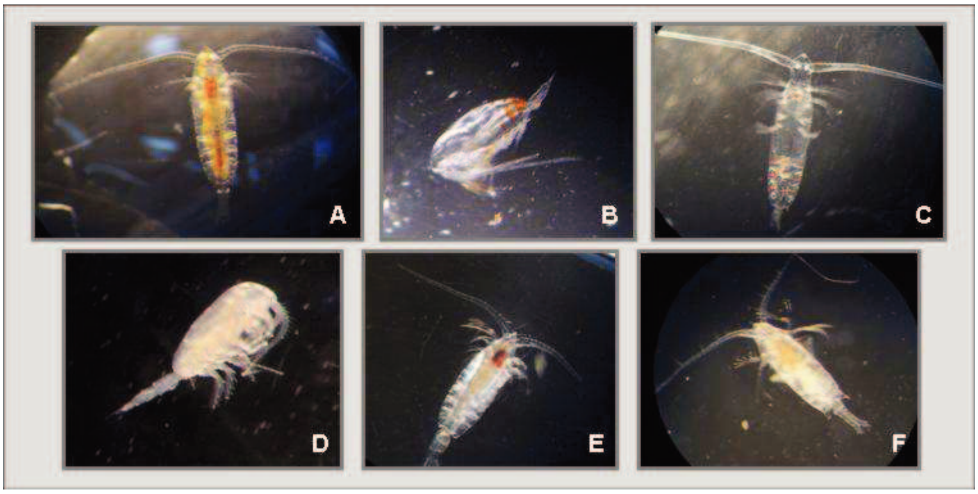

Figure 3: Calanoid copepods of the northern Benguela upwelling system. Females of A) *Calanoides carinatus*, B) *Nannocalanus minor*, C) *Eucalanus hyalinus*, D) *Metridia lucens* E) *Pleuromamma xiphias* and F) *Aetideopsis carinata*.

Due to its dominance, *C. carinatus* is the only well studied copepod species of the Benguela upwelling system with regard to its trophodynamics and metabolic activities (Kosobokova et al. 1988, Verheye et al. 1991, Arashkevich & Drits 1997, Auel et al. 2005, Verheye et al. 2005, Auel & Verheye 2007). *C. carinatus* is highly adapted to life in coastal upwelling systems. Its life strategies are characterised by lipid (wax ester) storage, ontogenetic vertical migration and diapause, to ensure its population retention within the highly seasonal coastal upwelling system (Verheye et al. 1991, Verheye et al. 2005). This species thrives in plumes of nutrient-rich, recently upwelled water close to the coast and exhibits a multi-generational life cycle typical of *Calanus* species (e.g. *C. marshallae*, *C. chilensis*) from other upwelling regions (Conover 1988, Peterson 1998). Reproduction takes place on the shelf within the upwelling region. A part of the population is transported towards the open ocean by Ekman drift and pre-adult copepodite stages C5 descend to depths below 400 m. At depth they overcome periods of food shortage in a dormant stage with extremely reduced metabolic activity and extensive lipid reserves (Auel et al. 2005, Verheye et al. 2005). They return to surface waters and moult to adults at the onset of a new upwelling event (Kosobokova et al. 1988, Verheye 1991, Verheye et al. 1991). Copepodite stage C5 of *C. carinatus* shows a bimodal vertical distribution in the northern Benguela and the ABF region. They were found concurrently in the surface layer and at depth below 200 m during substantial periods of the annual cycle (Kosobokova et al. 1988, Arashkevich & Drits 1997, Loick et al. 2005, Verheye et al. 2005). Apparently, only part of the C5 population performs vertical migrations and enters diapause.

Another common calanid copepod is the epipelagic species *Nannocalanus minor* (Fig. 3B) that occurs further offshore in generally warmer and nutrient-depleted waters. This species as well as smaller copepods such as *Pseudocalanus*, *Paracalanus*, *Clausocalanus* and *Centropages* are not known to enter a resting stage and are often present in low numbers during non-upwelling seasons. Their population size starts to increase within a month or two after an upwelling event (Peterson & Miller 1977). *Centropages* spp. and *Acartia* spp. are known to produce resting eggs, another strategy to retain individuals within the system during non-upwelling periods (Peterson 1998).

The dominant eucalanid species (e.g. *Eucalanus hyalinus* (Fig. 3C) and *Rhincalanus nasutus*) of the northern Benguela Current, show a rather sluggish life style (Flint et al. 1991). Together with their transparent body this inactivity reduces the risk of predation by tactile and visual predators (Castellani et al. 2005). Dormant stages are assumed for some eucalanid copepods, but in contrast to *C. carinatus* they enter these resting stages as adult females or as both, adult females and copepodids C5 (Ohman et al. 1998). For *R. nasutus* indications of dormancy, e.g. high wax ester levels of non-feeding, non-reproducing females, are found in mid-water layers (Ohman et al. 1998, Schnack-Schiel et al. 2008). Reduced metabolic rates were also detected in females of *Eucalanus californicus* and *R. nasutus* of the California Current, suggesting a dormant stage of these species in winter (Ohman et al. 1998). *Eucalanus* spp. and *R. nasutus* are known to inhabit OMZs in various regions, such as the Arabian Sea and the

Humboldt Current (Flint et al. 1991, Schnack-Schiel et al. 2008, Escribano et al. 2009, Hidalgo et al. 2010). These species may survive periods of food scarcity by retreating into the OMZs and reducing their metabolic rate, which may enable them to exist at such low oxygen levels (Weikert 1980). Ohman et al. (1998) described eucalanid copepods as ‘event-driven’ with flexible responses to environmental stimuli rather than to seasonal phenomena.

Species of the family Metridinidae such as *Metridia lucens* (Fig. 2d) and *Pleuromamma* spp. are strong diel vertical migrants (Peterson 1998). Generally, *M. lucens* occurs further offshore and developmental stages are not separated in their occurrence, as it is observed for *C. carinatus*. At night, when the majority migrates into upper water layers (100-200 m), the population of *M. lucens* is advected north-westward by the Benguela Current and the surface drift. During daytime, individuals of *M. lucens* inhabit water layers below 200 m and are carried south-eastward by the deep counter current to partly compensate their night drift (Timonin 1997). It is suggested that the further offshore occurrence of *M. lucens* exposes the species to a lesser degree to the variability of physical parameters as compared to *C. carinatus*.

A comparison of studies in the 1960s and 1970s (Unterüberbacher 1964, Brenning 1985) with more recent investigations (Timonin et al. 1992, Hansen et al. 2005) off Walvis Bay (23°S) indicated a change in dominance towards larger copepod species in the northern Benguela Current over the past four decades. The small copepod, *Paracalanus parvus* (<1.5 mm) has decreased markedly in abundance, whereas *R. nasutus* (up to 5.5 mm) has become one of the dominant species since the 1970s (Hansen et al. 2005). *M. lucens* (1.6-2.9 mm) also increased in dominance and at some locations it even reaches similar abundances as *C. carinatus* (2.3-2.9 mm) and it is occasionally the most abundant species in offshore areas (Hansen et al. 2005). The ability of individuals to remain in an upwelling zone, largely determines which species dominates the upwelling systems (Peterson 1998). The diversity of copepods during upwelling seasons is generally quite low, with only 4 to 5 key species accounting for ≥80-90% of the copepod biomass (Peterson 1998). Since the 1980s, *C. carinatus*, *M. lucens*, *R. nasutus* and *C. brachiatus* represent the typical dominant copepods during upwelling off Walvis Bay (Timonin et al. 1992, Hansen et al. 2005). Verheye et al. (1998) suggest that changes in copepod community structure are not only controlled by environmental forcing mechanisms (bottom-up), but also by altered predator-prey interactions (top-down) associated with the regime shifts and collapse of small pelagic fish (Verheye & Richardson 1998, Verheye 2000).

1.4 Pelagic decapods off northern Namibia

Pelagic decapods represent an abundant and important component of the macrozooplankton/micronekton communities throughout many regions of the world's oceans (Maynard et al. 1975, Hopkins et al. 1989, Flock & Hopkins 1992) and constitute a vital link between zooplankton and higher trophic levels (Omori 1974, Walters 1976). They are a zooplanktivorous group that can utilise various kinds of food including other decapods, copepods, mysids, euphausiids, chaetognaths, amphipods, ostracods and fish larvae (Omori 1974, Heffernan & Hopkins 1981, Flock & Hopkins 1992). On the other hand, they have been reported as prey of oceanic tuna and flying fishes in the open ocean and of various commercially important fish species in shelf waters (Smale 1992, Karuppasamy et al. 2006).

However, pelagic decapods and micronekton specimens in general (e.g. mysids, adult krill, cephalopods and juvenile fish) are often considered too small to be caught by most large mesh-size (~3 cm) pelagic trawls, but too mobile to be collected efficiently by conventional plankton nets (Brodeur et al. 2005). Their patchy distribution and high mobility makes unbiased sampling difficult (Pakhomov & Yamamura 2010) and thus pelagic decapods tend to be poorly quantified and their biology little understood.

In the Benguela upwelling system, 29 species of pelagic decapods have been recorded (Macpherson 1991). Their distribution in the area has been mapped from the Kunene River as the northern limit (15-16°S) down to the Cape of Good Hope in the south (Kensley 1981, Macpherson 1991, Kensley 2006). All species recorded belong to the Infraorder Caridea or the Superfamily Penaeoidea. Common caridean species are *Acanthephyra pelagica* (Fig. 4a), *Oplophorus novazeelandiae* (Fig. 4b), *Pasiphaea semispinosa* (Fig. 4c), and *Systellaspis debilis*, while *Gennadas* spp., several *Sergestes* species (e.g. *S. armatus*, *S. arcticus*, *S. corniculum*) and *Sergia robusta* (Fig. 4e) are the most common penaeoid species (Kensley 1981, Macpherson 1991, Kensley 2006).

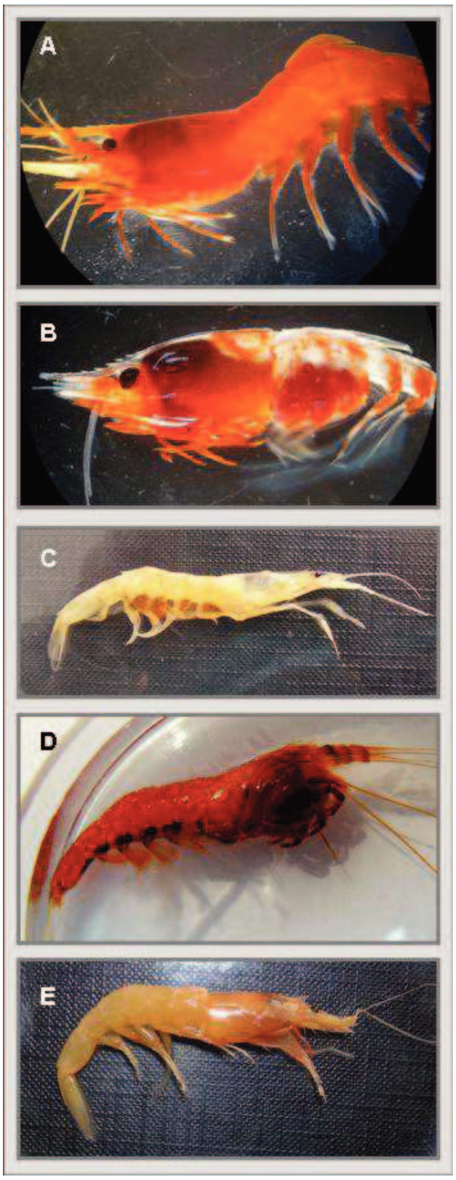

Figure 4: Pelagic decapods of the Benguela system. a) *Acanthephyra pelagica*, b) *Oplophorus novazeelandiae*, c) *Pasiphaea semispinosa*, d) *Gennadas brevirostris* and e) *Sergia robusta*

Publications of pelagic decapods for the area are mainly taxonomic in nature or expedition reports (Kensley 1971a, Kensley 1971b, Macpherson 1991, Kensley 2006). Little is published on biological aspects of pelagic decapods. Reasons for this scarcity of information may be: their assumed low occurrence and the difficulty to assess their abundance with conventional nets as well as the lack of commercial value. As a consequence, Gibbons et al. (1992) concluded that pelagic decapods play a minor trophic role in the Benguela system.

However, *P. semispinosa* is suggested to be an important component of the northern Benguela shelf assemblage (Olivar & Barange 1990, Macpherson 1991) and it is the only decapod species of the Benguela Current that has been studied in greater detail. Gibbons et al. (1994) determined high densities of up to 27 ind. m^{-2} for *P. semispinosa* in the northern Benguela Current and concluded that this species tends to form swarms. High densities of *Pasiphaea* have also been observed off Oregon and in the Ligurian Sea (Krygier & Pearcy 1981, Dagnino et al. 1985, Relini & Relini 1990). Abundance and distribution of pelagic decapods appear to be strongly correlated with the quantity and distribution of their prey (Omori 1974, Relini & Relini 1990). *P. semispinosa* shows a long-shore distribution that overlaps with areas of dense populations of euphausiids over the shelf of the Benguela Current (Pillar et al. 1992). Accordingly, euphausiids seem to be a principal food source for adult *P. semispinosa* and the potential impact of *Pasiphaea* on euphausiid populations in local areas may be high. Some pelagic decapods (e.g. *P. semispinosa*) may therefore have a more important trophic role than previously assumed by Gibbons et al (1992) for the Benguela region.

1.5 Objectives

The present study is embedded in the GENUS project (Geochemistry and Ecology of the Namibian Upwelling System), which aims to resolve and model the relationships between climate changes, biogeochemical cycles of nutrients, climate relevant gases and ecosystem structures within the Benguela upwelling system. For the modelling of possible consequences of climate change, a better understanding of trophodynamics, physiological tolerances, adaptations, energetics and constraints of key species within a certain ecosystem are required.

The focus of this study is on calanoid copepod and pelagic decapod species as two important components of the mesozooplankton and macrozooplankton/micronekton community, respectively, in the Benguela Current. This study aims to quantify trophic interactions and energetics of these species and to evaluate their life strategies in the Benguela Current. For this purpose, energy requirements, dietary preferences, trophic levels and lipid storage patterns were determined applying a variety of methods including lipid extraction, fatty acid and stable isotope analyses as well as respiration and enzyme measurements. Abundance data of copepods and decapods were additionally determined to calculate from individual ingestion rates to population carbon consumption rates. The data of the present study are essential for the development of realistic carbon budgets and food-web models of the northern Benguela upwelling region and coastal upwelling system in general. This work addresses three main objectives which are summarised below:

- **Adaptations to upwelling:** Coastal upwelling systems are highly variable in their food availability, oxygen concentrations and water temperatures. Moreover, pelagic species in these upwelling areas are exposed to an offshore transport, driven by the cross-shelf component in the Ekman layer (Verheye & Field 1992, Peterson 1998). Hence, poor feeding conditions during non-upwelling periods and the retention of their populations in the upwelling area represent major challenges for zooplankton species. The success of species in upwelling region thus depends on their ability to develop adaptive strategies to the conditions of the ecosystem. To clarify species-specific strategies of dominant calanoid copepods and pelagic decapods, lipid analyses and respiration measurements (as proxy for metabolic activity) were conducted. Lipid analyses reveal information about life-cycle adaptations due to different storage functions of lipid classes and differences in metabolic rates can be related to species-specific life-cycle characteristics (e.g. dormancy, diel vertical migration).

- **Trophodynamics:** Trophic interactions in the Benguela food web have changed during the last decades from three principal dietary pathways to a very complex food web. Zooplankton became a major component of the food web since the 1970s (Verheye & Richardson 1998). The understanding of trophic interactions of key species in ecosystems is a major concern to determine energy pathways and their efficiencies. Trophic positions and dietary preferences of key copepod and decapod species were investigated applying fatty acid biomarker and stable isotope ($\delta^{13}C$, $\delta^{15}N$) analyses to reveal their trophic role and their function in the energy transfer of the northern Benguela food web.

- **Energetics:** Metabolic demands of zooplankton organisms are of great importance to estimate their contribution to biogeochemical cycles, especially under changing environmental conditions. Copepod and decapod species play an essential role in the cycling of organic matter in the ocean. Through their diel and ontogenetic vertical migrations, they enhance the vertical flux of organic matter and dissolved inorganic carbon from the euphotic zone to deeper layers (Longhurst et al. 1990, Steinberg et al. 2008). Respiration rates as well as enzyme activities were conducted with dominant copepod and decapod species to gain information of their individual energy demands as well as community energy requirements.

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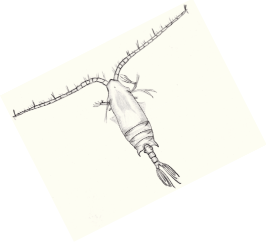
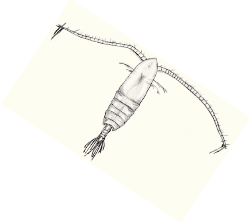
CHAPTER I





COMPLEX TROPHIC INTERACTIONS OF CALANOID COPEPODS IN THE BENGUELA UPWELLING SYSTEM

Schukat A, Auel H, Teuber L, Hagen W

under review

Journal of Sea Research

Complex trophic interactions of calanoid copepods in the Benguela upwelling system

Anna Schukat, Holger Auel, Lena Teuber and Wilhelm Hagen

ABSTRACT

Life-cycle adaptations, dietary preferences and trophic levels of calanoid copepods from the northern Benguela Current were determined via lipid classes, marker fatty acids and stable isotope analyses, respectively. Trophic levels of the copepod species were compared to other zooplankton and higher taxa. Lipid class analyses revealed that three of the dominant calanoid copepod species stored wax ester, four accumulated triacylglycerols and another three species were characterised by high phospholipid levels. The two biomarker approaches (via stable isotopes and fatty acids, resp.) revealed a complex pattern of trophic positions for the various copepod species, but also highlighted the dietary importance of diatoms and dinoflagellates. *Calanoides carinatus* and *Nannocalanus minor* occupied the lowest trophic levels (predominantly herbivorous) corresponding to high amounts of fatty acid markers for diatoms (e.g. 16:1(n-7)) and dinoflagellates (e.g. 18:4(n-3)). These two copepod species represent the classical link between primary production and higher trophic levels. All other copepods belonged to secondary or even tertiary (i.e. some deep-sea copepods) consumers. The calanoid copepod species cover the entire range of $\delta^{15}N$ ratios, as compared to $\delta^{15}N$ ratios of all non-calanoid taxa investigated, from salps to adult fish. These data emphasize that the trophic roles of calanoid copepods are far more complex than just interlinking primary producers with pelagic fish, which should also be considered in the process of developing realistic food-web models of coastal upwelling systems.

KEYWORDS

zooplankton, fatty acids, stable isotopes, biomarkers, trophic level, food web

INTRODUCTION

Calanoid copepods of highly productive coastal upwelling systems were traditionally considered as being primarily herbivorous, acting as a key link between primary producers, pelagic fish and higher trophic levels (Lowndes 1935, Anraku & Omori 1963). This view of the trophic role of calanoid copepods has changed during the last decades. Further research revealed that many calanoid copepod species are omnivorous with picoplankton, nanoplankton and microzooplankton as major components of their diet (e.g. Kleppel et al. 1996, Calbet et al. 2007, Escribano & Pérez 2010). However, the natural diets of most planktonic copepods are still not well characterised and it is difficult to clearly assign them to specific trophic levels.

A classical method to determine dietary components is gut content analysis, which provides detailed taxonomic information (Hyslop 1980). However, this method fails for soft-bodied and fragile organisms or in case of advanced digestion, and it can only provide a snap-shot impression. In contrast, trophic biomarkers (e.g. fatty acids and stable isotopes) integrate dietary signals over longer time spans of days to weeks (Graeve et al. 1994a, Gentsch et al. 2009). They represent a complementary approach to determine dietary preferences and trophic positions.

The analysis of lipids, particularly fatty acid compositions, is used for the identification of trophic relationships in marine ecosystems. Some fatty acids are characteristic of specific groups of phyto- or zooplankton and are incorporated into consumers' body tissues largely unmodified, thus retaining a dietary signature (Graeve et al. 1994b, Dalsgaard et al. 2003). Stable isotope ratios of naturally occurring nitrogen ($^{15}N/^{14}N$) and carbon ($^{13}C/^{12}C$) are useful in aquatic food web analyses for identifying trophic levels and different carbon sources (Hobson & Welch 1992, Michener & Shell 1994), considering that heavier isotopes accumulate in the animals' body tissues (Vander Zanden & Rasmussen 2001, Bode & Alvarez-Ossorio 2004). Thus, fatty acid and stable isotope biomarkers can be used to trace trophic pathways and to model the structure of food webs.

Within the northern Benguela upwelling system off Namibia, one of the four major coastal upwelling ecosystems in the world, the calanoid copepod *Calanoides carinatus* is a perennially prevailing species. Other frequently occurring copepods include *Metridia lucens*, *Centropages brachiatus*, *Nannocalanus minor*, *Aetideopsis carinata*, *Pleuromamma* spp., *Eucalanus hyalinus* and *Rhincalanus nasutus* (Hansen et al. 2005, Loick et al. 2005). Little is known about the feeding ecology and dietary spectra of these subtropical species, compared to their high-latitude congeners and even less about trophic interactions within subtropical environments. Precise knowledge about the feeding preferences of dominant copepods is necessary to understand the tropho-dynamics of the respective ecosystems.

The aim of this study was to reveal adaptive strategies of calanoid copepods to cope with the variability of upwelling systems and to identify their dietary relationships and trophic levels in this environment. Therefore, a large dataset of ten dominant calanoid copepods, as well as of other dominant zooplankton and upper trophic level taxa was assessed and trophic interactions were analysed combining the two complementary methodological approaches, fatty acids and stable isotopes.

MATERIAL AND METHODS

Sampling

Copepods

Copepods were collected in the northern Benguela upwelling system during cruises in March 2008 (RV *Maria S. Merian*) and December 2009 (FRS *Africana*) (Fig. 1). Copepod samples for lipid and stable isotope analyses were taken from the same station, net and depth, if possible. They were mainly sampled by stratified vertical multiple opening/closing net hauls (Hydro-Bios Multinet Midi, mouth opening 0.25 m^2, mesh size 200-300 µm), but additional samples were taken from a Current Underway Fish Egg Sampler (CUFES, mesh size 500 µm), a Tucker trawl (mesh size 1000 µm) and a drift net (mesh size 200 µm). Immediately after the hauls, samples were rapidly but gently sorted into species and stages and deep-frozen at -80°C for further analyses.

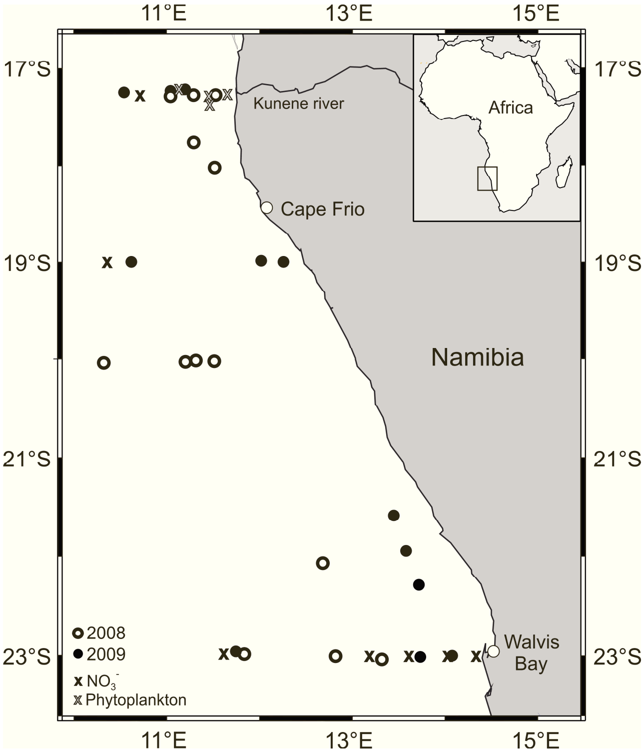


Figure 1: Study area in the northern Benguela upwelling system with stations sampled for zooplankton in March/April 2008 (open circles) and December 2009 (black dots) and for NO_3^- (2009; black crosses) and phytoplankton (2009, 2010; grey crosses) stations.

Other zooplankton and higher taxa

For the comparison of trophic levels, also other taxa of the northern Benguela ecosystem were sampled. Zooplankton (amphipods, euphausiids, decapods, chaetognaths and gelatinous zooplankton, e.g. salps) were collected from Multinet, 1 m^2-MOCNESS (Multiple Opening and Closing Net with Environmental Sensing System, 333 µm) and Tucker trawl hauls. Fish samples were taken from a demersal fish trawl and seabird feathers (storm petrel) were collected from animals landed onboard. All samples were immediately deep-frozen at -80°C.

Phytoplankton and water samples

At some stations in December 2009, extensive blooms of large phytoplankton cells occurred and were sampled by Multinet hauls in the upper 30 m. In these seston samples, microzooplankton could not be excluded. However, the vast majority of seston during these extreme bloom events consisted of large centric diatoms

(*Coscinodiscus wailesii*), smaller chain-forming diatoms (e.g. *Chaetoceros* sp.) and/or the dinoflagellate *Noctiluca*. Additionally, samples of diatoms were collected in October 2010, also by Multinet hauls in the upper 30 m. Diatoms could be separated from the rest of the seston sample based on their buoyancy. Phytoplankton samples were enriched over a 55-100 µm gauze, transferred into glass vials and deep-frozen at -80°C until analysis of stable isotopes.

Water samples were taken regularly of almost every CTD cast in 2009 from epi- to meso- and bathypelagic depth and filtered through pre-combusted GF/F filters (Whatman). The filtrate was used to measure the isotopic composition (^{15}N) of nitrate (NO_3^-) to determine the ecosystem isotopic baseline for the calculation of $\delta^{15}N$ of phytoplankton.

Lipid analysis

Body dry mass of copepods was determined after lyophilisation of the deep-frozen samples for 48 h. Identical species and stages from the same station, net and depth stratum were pooled to obtain sufficient biomass. Lipids were extracted with dichloromethane:methanol (2:1 per volume) according to Folch et al. (1957) and total lipid content was measured gravimetrically essentially after Hagen (2000).

Lipid classes were determined by high-performance (HP) TLC-scanning densitometry as described by Stübing et al. (2003). 2-3 µg lipid were applied in duplicate on HPTLC plates (silica gel 60, Merck), which were developed for 17 min in hexane:diethyl ether:acetic acid (80:20:2, v/v) for the separation of neutral lipids. The lipid bands were quantified at 550 nm wavelength and calibrated using commercial standards for each detected lipid class.

Fatty acid were converted to their methyl ester derivatives (FAME) by trans-esterification (Kattner & Fricke 1986, Peters et al. 2007) and analysed together with the fatty alcohols using a gas chromatograph (Agilent Technologies 7890A), equipped with a DB-FFAP column of 30 m length and 0.25 mm diameter and a programmable temperature vaporiser injector (Peters et al. 2007). Peaks were identified according to retention times in comparison to a fish oil and a copepod lipid standard of known composition.

Fatty acid compositions are evaluated following the trophic biomarker concept (Dalsgaard et al. 2003). 16:1(n-7), as well as 16:4(n-1) and 18:1(n-7) are used as indicators of a diatom-dominated diet. 18:4(n-3) is applied as dinoflagellate marker (Graeve et al. 1994b, Dalsgaard et al. 2003). The ratio 18:1(n-9)/18:1(n-7) was calculated to estimate the degree of carnivory versus herbivory (Auel et al. 2002, Dalsgaard et al. 2003). In addition, the fatty acids ratio of 18:1(n-9)/[16:1(n-7) + 16:4(n-1) + 18:1(n-7) + 18:4(n-3)], in the following named 18:1(n-9)/∑herb. markers, was applied as a new relative measure of carnivory.

Stable isotope analysis

Dried samples were transferred in tin capsules and stable isotope analyses were performed by Agroisolab GmbH in Jülich, Germany, using a mass spectrometer (EA NA1500 Series 2, Carlo Erba Instruments) and helium as carrier gas. Copepods were used as whole animals; for larger organisms only parts of their muscles, caudal fin or feathers were taken. Samples of small species were pooled to obtain sufficient biomass (0.5-7.0 mg) for proper analysis.

Determination of carbon and nitrogen stable isotope ratios was conducted using the standards IAEA-VPDB (IAEA-C1) and atmospheric air (IAEA-N1), respectively. Isotopic ratios are expressed as $\delta^{13}C$ and $\delta^{15}N$ in ‰, according to the equation given by Hobson et al. (2002).

In contrast to some publications (Hobson et al. 2002, Mintenbeck et al. 2008), lipids were not extracted prior to stable isotope analysis, since biomass values were generally low and we wanted to avoid any bias of $\delta^{15}N$ by lipid extraction, as discussed by Mintenbeck et al. (2008).

Creation of a $\delta^{15}N$ baseline and calculation of trophic levels

An isotopic baseline of a food web is required to estimate distinct trophic levels. We applied the following three methodological approaches to obtain a consistent and robust $\delta^{15}N$ food web-baseline for the northern Benguela Current:

i) Calculation of $\delta^{15}N$ of phytoplankton by nitrate assimilation of phytoplankton. Here, the $^{15}N/^{14}N$ ratio of nitrate (NO_3^-) from seawater samples was determined following the denitrifier method (Sigman et al. 2001, Casciotti et al. 2002) using the internationally recognised nitrate standard (IAEA-N3) for isotopic comparison with air N_2. The isotope ratio is expressed as $\delta^{15}N$ in ‰ according to the equation in Granger et al. (2004).

$\delta^{15}N$ of phytoplankton was then calculated via the following equation:

$\delta^{15}N_{phytoplankton} = \delta^{15}N_{nitrate}/\varepsilon_{phytoplankton}$

where ε is the isotope effect (Granger et al. 2004) for nitrate assimilation by phytoplankton. The $^{15}N/^{14}N$ of nitrate in the ocean typically suggests an isotope effect of 5-10‰ for nitrate assimilation (Wu et al. 1997, Sigman et al. 1999, Altabet 2001). We calculated a mean ε value (6.6 ± 3.6) from all published data available for marine diatoms (45 data points, seven species) (Montoya & McCarthy 1995, Waser et al. 1998, Needoba et al. 2003, Granger et al. 2004).

ii) Direct measurement of $\delta^{15}N$ of mixed phytoplankton and diatom sub-samples.

iii) Use of primary consumers as baseline. We defined the copepod with the lowest $\delta^{15}N$ (*Nannocalanus minor*, 4.8‰) as trophic level 2.0.

Trophic levels were then calculated according to the equation:

Trophic level = $\lambda + (\delta^{15}N_{\mathrm{consumer}} - \delta^{15}N_{\mathrm{base}})/\Delta_n$

where λ is the trophic level of the organism used to estimate $\delta^{15}N_{\mathrm{base}}$ and Δ_n is the enrichment in $\delta^{15}N$ per trophic level. Consumers generally become enriched by 3–4‰ in ^{15}N relative to their diet (Minagawa & Wada 1984, Peterson & Fry 1987, Cabana & Rasmussen 1994). We used the common average enrichment of 3.4‰ per trophic level (Peterson & Fry 1987, Hobson & Welch 1992).

The application of all three approaches produced similar results. Differences ranged between 0.1-0.3 for trophic level of a species. Calculated and measured $\delta^{15}N$ data of phytoplankton covered two main transects of the investigation area of the northern Benguela Current (Fig. 1) and represent a large data set. Therefore, we decided to use the mean value (1.36‰) of calculated and measured $\delta^{15}N$ of phytoplankton to obtain a proper baseline for this area.

Statistical analyses

Species-specific differences in biochemical composition were analysed using one-way ANOVA and a proximate post-hoc test (Tukey) within the Prism software package

(5.0). A two-tailed unpaired t-test (confidence interval 95%) was applied to test for intra-specific differences between sex, stage or sampling year using the same software package. Prior to statistical analysis, data distribution was tested for normality.

RESULTS

Dry mass and lipid composition

Centropages brachiatus, *Metridia lucens* and *Nannocalanus minor* belonged to the smallest copepods (0.05-0.07 mg dry mass (DM), Table 1) in this study. These were followed by *Calanoides carinatus*, *Aetideopsis carinata* and *Euchaeta marina* (0.11-0.20 mg DM). *Pleuromamma robusta*, *Eucalanus hyalinus*, *Rhincalanus nasutus* and *Euchirella similis* were the largest and heaviest copepods (0.30-1.05 mg DM, Table 1). Total lipid content (TL) ranged from 7% to 50% DM with lowest lipid levels in females of *E. similis* (6.7 ± 4.0% DM), followed by females of *C. carinatus* (11.3 ± 2.5% DM), *A. carinata* (2008: 12.2 ± 4.7%), *M. lucens* (12.5 ± 2.3% DM) and males of *E. hyalinus* (12.8 ± 1.6% DM) (Table 1, Fig. 2). Females of *E. hyalinus* showed a moderate lipid amount of 16.8 ± 2.2% DM similar to *E. marina* (14.7% and 18.9% DM) and *N. minor* (17.5% and 19.8% DM). Higher lipid levels occurred in females of *A. carinata* from 2009 (23.9 ± 3.2% DM) and in copepodite stages C5 of *C. brachiatus* (27.9 ± 17.0% DM). Females of *R. nasutus* and copepodids C5 of *C. carinatus* (both years) contained significantly higher lipid levels (46-50% DM) than the other copepods (ANOVA, Tukey, $p \leq 0.01$).

Copepod species also differed in lipid class compositions (Table 1, Fig. 2). High wax ester (WE) levels (51-87% TL) were found in females of *E. marina*, *R. nasutus* and copepodids C5 of *C. carinatus*. Females of *C. carinatus* had lower but highly variable WE contents (26.7 ± 17.4% TL) and a significantly higher phospholipid (PL) level compared to the copepodids C5 (ANOVA, Tukey, $p \leq 0.001$). Triacylglycerols (TAG) were the main neutral lipid class in *E. similis*, *P. robusta*, *E. hyalinus* and *A. carinata* with 37-60% TL.

Table 1: Dry mass (DM), total lipid content (TL) and lipid class compositions as well as fatty acid and fatty alcohol compositions (>4% not shown) and carnivory ratios of dominant calanoid copepods from the northern Benguela upwelling system. WE: wax esters; TAG: triacylglycerols; CHOL: cholesterol; FFA: free fatty acids; PL: phospholipids; TFA: total fatty acids; TFAlc: total fatty alcohols; ∑herb. markers: sum of 16:1(n-7), 16:4(n-1), 18:1(n-7), 18:4(n-3); n (ind): number of samples (total number of individuals).

	Calanidae				Eucalanidae			Aetideidae			Euchaetidae	Metridinidae		Centropagidae
	C. carinatus			*N. minor*	*E. hyalinus*		*R. nasutus*	*A. carinata*		*E. similis*	*E. marina*	*M. lucens*	*P. robusta*	*Centropages* sp.
	C5	C5	female	C5	male	female	female	female	female	female	female	female	female	male
month/year	March/2008	Dec/2009	Dec/2009	Dec/2009	Dec/2009	Dec/2009	Dec/2009	March/2008	Dec/2009	March/2008	March/2008	Dec/2009	Dec/2009	Dec/2009
n (ind)	6 (200)	5 (160)	3 (90)	2 (100)	3 (30)	4 (40)	5 (32)	4 (88)	3 (90)	7 (37)	2 (16)	4 (200)	3 (48)	4 (172)
Dry mass (mg ind^{-1})	0.15 ± 0.02	0.15 ± 0.01	0.11 ± 0.02	0.07/0.07	0.33 ± 0.01	0.77 ± 0.05	0.54 ± 0.08	0.19 ± 0.02	0.18 ± 0.01	1.05 ± 0.1	0.20/0.16	0.06 ± 0.01	0.30 ± 0.80	0.05 ± 0.01
Total lipid (% DM)	50.1 ± 6.7	50.2 ± 4.2	11.3 ± 2.5	17.5/19.8	12.8 ± 1.6	16.8 ± 2.2	46.2 ± 7.5	12.2 ± 4.7	23.9 ± 3.2	6.7 ± 4.0	14.7/18.9	12.5 ± 2.3	13.9 ± 1.7	27.9 ± 17.0
Lipid classes (% TL)														
WE	84.4 ± 2.7	87.4 ± 2.2	26.9 ± 17.4	-	-	-	83.3 ± 1.6	17.8 ± 7.3	12.1 ± 4.1	3.2 ± 3.4	51.3/82.4	3.3 ± 0.6	-	-
TAG	7.0 ± 2.3	6.2 ± 1.2	10.5 ± 6.9	9.3/14.3	38.9 ± 6.7	59.3 ± 4.7	1.2 ± 1.0	50.6 ± 5.4	59.7 ± 3.2	36.5 ± 10.8	4.5/-	5.0 ± 1.4	44.0 ± 0.8	-
CHOL	1.0 ± 0.2	0.2 ± 0.4	7.1 ± 3.0	11.9/11.3	9.7 ± 1.8	6.0 ± 0.7	2.0 ±0.7	4.1 ± 0.7	3.3 ± 0.8	8.2 ± 3.6	5.4/2.2	18.1 ± 2.3	14.2 ± 1.4	14.5 ± 3.4
FFA	0.7 ± 0.5	1.1 ± 0.2	5.3 ± 1.6	6.8/1.9	2.2 ± 0.7	2.8 ± 0.5	1.7 ± 1.0	2.3 ± 0.6	2.8 ± 0.5	4.3 ± 3.2	2.1/1.2	6.7 ± 2.4	3.5 ± 0.7	7.0 ± 2.1
PL	6.9 ± 0.9	5.0 ± 0.8	50.2 ± 8.4	71.9/72.4	49.1 ± 5.0	31.8 ± 4.8	11.8 ±1.4	25.2 ± 2.1	22.1 ± 2.1	47.8 ± 5.1	36.7/14.2	66.9 ± 4.7	38.3 ± 0.9	78.6 ± 3.4
Fatty acids (% TFA)														
14:0	5.9 ± 1.5	4.4 ± 0.8	3.2 ± 1.2	1.3/2.7	3.5 ± 1.1	4.0 ± 0.3	1.0 ± 0.4	4.7 ± 1.2	4.2 ± 0.8	2.0 ± 0.7	1.3/0.3	1.0 ± 0.7	3.4 ± 1.5	2.3 ± 0.7
16:0	6.8 ± 0.6	6.6 ± 1.0	22.3 ± 9.3	12.5/18.2	34.7 ± 6.1	24.1 ± 1.9	3.8 ± 0.8	20.5 ± 2.0	24.4 ± 0.2	18.0 ± 2.9	11.7/1.6	14.7 ± 1.6	21.4 ± 1.1	18.2 ± 1.1
18:0	1.4 ± 0.1	1.4 ± 0.2	5.3 ± 2.4	4.5/5.0	7.9 ± 1.8	3.8 ± 0.5	1.1 ± 0.2	2.6 ± 0.2	4.4 ± 0.4	3.7 ± 0.8	2.4/0.4	3.7 ± 0.2	3.1 ± 0.5	4.4 ± 0.3
16:1(n-7)	10.4 ± 0.6	7.9 ± 0.4	8.0 ± 2.5	1.4/2.9	6.7 ± 2.2	8.6 ± 0.3	17.9 ± 1.5	6.6 ± 0.7	5.5 ± 0.3	3.2 ± 1.5	4.5/9.5	2.0 ± 0.5	5.0 ± 2.4	1.5 ± 1.3
16:2(n-4)	1.6 ± 0.2	1.7 ± 0.1	0.8 ± 0.7	-/0.2	0.9 ± 0.4	1.8 ± 0.1	2.4 ± 0.3	0.8 ± 0.4	0.9 ± 0.0	0.3 ± 0.2	0.9/1.3	0.1 ± 0.1	0.8 ± 0.4	0.5 ± 0.3
16:4(n-1)	2.2 ± 1.1	4.4 ± 0.6	0.7 ± 0.6	-	0.5 ± 0.3	2.8 ± 0.3	3.9 ± 0.6	0.5 ± 0.2	1.1 ± 0.1	-	0.4/0.8	-	0.3 ± 0.4	0.1 ± 0.2
18:1(n-7)	0.6 ± 0.1	0.5 ± 0.1	1.3 ± 0.2	0.7/1.0	2.4 ± 0.1	3.3 ± 0.1	1.4 ± 0.4	4.2 ± 0.2	3.8 ± 0.0	2.6 ± 0.3	1.6/0.7	1.6 ± 0.0	3.0 ± 0.4	2.5 ± 0.5
18:1(n-9)	4.2 ± 0.4	3.6 ± 0.3	2.9 ± 0.3	2.8/6.4	9.0 ± 0.7	6.0 ± 0.6	33.8 ± 3.4	9.5 ± 2.4	10.0 ± 0.6	14.8 ± 5.2	20.4/44.0	2.5 ± 0.1	8.2 ± 3.4	2.1 ± 0.7
18:2(n-6)	1.3 ± 0.1	1.1 ± 0.0	1.6 ± 1.3	8.0/1.4	1.2 ± 0.3	0.8 ± 0.1	1.2 ± 0.2	1.0 ± 0.1	1.1 ± 0.1	1.2 ± 0.2	1.5/1.3	1.3 ± 0.1	1.5 ± 0.6	0.7 ± 0.2
18:4(n-3)	3.8 ± 0.3	3.3 ± 0.4	1.4 ± 0.7	9.2/0.7	0.6 ± 0.3	1.4 ± 0.1	1.6 ± 1.5	0.9 ± 0.2	1.4 ± 0.1	0.5 ± 0.4	1.7/1.8	2.0 ± 0.1	1.1 ± 0.8	0.5 ± 0.2
20:1(n-9)	13.5 ± 0.6	12.6 ± 1.1	1.2 ± 1.5	-/0.5	0.3 ± 0.0	0.4 ± 0.0	0.6 ± 0.1	4.0 ± 1.3	1.3 ± 0.3	0.7 ± 0.4	0.8/-	0.8 ± 0.1	1.4 ± 2.0	0.3 ± 0.1
20:2(n-6)	-	-	0.1 ± 0.1	7.5/-	0.3 ± 0.3	-	0.2 ± 0.2	0.2 ± 0.1	-	0.5 ± 0.3	0.2/0.2	0.4 ± 0.4	0.1 ± 0.2	-
20:5(n-3)	14.3 ± 2.5	20.8 ± 1.7	16.0 ± 3.0	12.0/15.7	12.1 ± 1.6	26.4 ± 2.0	20.0 ± 4.2	14.8 ± 1.5	21.2 ± 0.5	10.3 ± 0.9	14.6/12.1	20.5 ± 1.7	13.4 ± 7.6	16.1 ± 1.1
22:1(n-11)	16.7 ± 1.2	15.2 ± 0.3	1.8 ± 2.9	-	-	-	-	7.4 ± 2.5	2.1 ± 0.6	0.4 ± 0.2	0.7/-	-	2.5 ± 2.6	0.1 ± 0.2
22:6(n-3)	7.4 ± 1.0	5.6 ± 0.2	20.0 ± 3.3	28.7/35.1	10.7 ± 2.0	5.8 ± 0.1	3.8 ± 1.3	11.5 ± 1.0	8.4 ± 0.4	29.7 ± 6.8	28.7/18.9	35.9 ± 2.6	16.6 ± 2.4	40.3 ± 3.8
24:1(n-11)	0.5 ± 0.1	0.5 ± 0.1	2.6 ± 1.5	1.6/3.7	2.8 ± 1.0	0.9 ± 0.1	0.8 ± 0.1	2.0 ± 0.5	1.3 ± 0.1	5.8 ± 2.1	2.4/0.3	5.5 ± 0.6	5.7 ± 4.0	1.6 ± 0.3
18:1(n-9)/18:1(n-7)	6.6 ± 0.8	7.0 ± 0.8	2.3 ± 0.4	3.9/6.6	3.7 ± 0.1	1.9 ± 0.2	24.2 ± 4.1	2.3 ± 0.6	2.6 ± 0.2	5.8 ± 1.7	12.9/66.3	1.5 ± 0.0	2.7 ± 0.8	0.8 ± 0.2
18:1(n-9)/Σherb. markers	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.1	0.2/1.4	0.9 ± 0.2	0.4 ± 0.0	1.4 ± 0.2	0.8 ± 0.2	0.8 ± 0.1	2.3 ± 0.4	2.5/3.5	0.4 ± 0.0	1.0 ± 0.4	0.5 ± 0.1
Fatty alcohols (% TFAlc)														
14:0	11.7 ± 0.8	12.3 ± 1.0	10.1 ± 18.7	-	-	-	36.0 ± 3.6	16.3 ± 3.3	17.1 ± 1.8	38.1 ± 6.9	4.0/31.6	52.3 ± 7.1	-	-
16:0	16.2 ± 1.1	16.8 ± 1.2	17.8 ± 19.5	-	-	-	58.0 ± 3.0	20.6 ± 4.6	40.2 ± 15.1	61.9 ± 6.9	89.5/63.7	47.8 ± 7.1	-	-
18:0	1.7 ± 0.2	2.1 ± 0.1	1.9 ± 8.8	-	-	-	6.1 ± 0.6	-	-	-	3.2/2.3	-	-	-
20:1	19.1 ± 1.0	19.9 ± 0.6	16.1 ± 9.3	-	-	-	-	13.8 ± 2.1	13.5 ± 4.5	-	-	-	-	-
22:1	50.7 ± 1.9	48.8 ± 1.7	54.1 ± 31.2	-	-	-	-	47.2 ± 4.0	29.2 ± 10.1	-	1.6/0.9	-	-	-

Males of *E. hyalinus* had significantly lower TAG levels compared to females (t-test, p ≤0.001). PL was also a dominant lipid class in these four copepod species. Maximum PL contents (63-79% TL) were determined in *M. lucens*, *N. minor* and *C. brachiatus*, where TAG and WE occurred only in small amounts or were completely absent (i.e. *Centropages* sp.). Cholesterol (CHOL) and free fatty acids were only minor lipid classes (combined <10% TL), except for *M. lucens*, *P. robusta* and *C. brachiatus*, which exhibited higher portions of CHOL (14-18% TL).

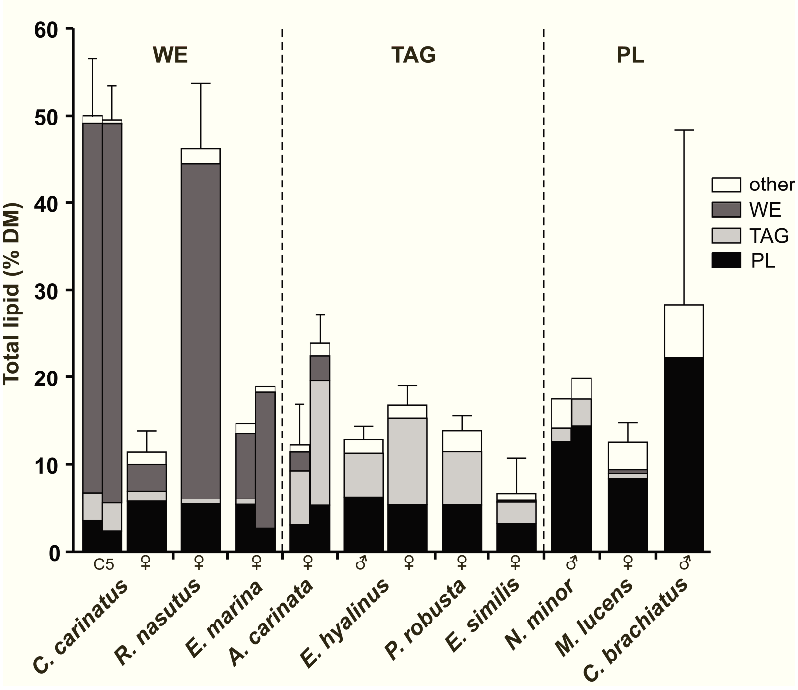


Figure 2: Total lipid content (% dry mass, DM) and percentage contribution of major lipid classes to total lipid of dominant calanoid copepods. WE: wax esters, TAG: triacylglycerols, PL: phospholipids. Staged columns with error bars represent two sampling years (2008, 2009), no error bars: only two replicates were analysed. See Table 1 for the number of samples.

Fatty acid and alcohol compositions

Fatty acid compositions of most copepod species were characterised by high amounts of typical membrane fatty acids, i.e. 16:0, 20:5(n-3) and 22:6(n-3) (Table 1). Principal fatty acids in copepodids C5 of *Calanoides carinatus* (both years) were the marker fatty acid for diatoms 16:1(n-7) (7.9-10.4% total fatty acids (TFA)) and the long-chain mono-unsaturated fatty acids typical of calanid copepods 20:1(n-9) (12.6-13.5% TFA) and 22:1(n-11) (15.2-16.7% TFA). Females of *C. carinatus* also showed elevated values of 16:1(n-7), whereas 20:1(n-9) and 22:1(n-11) occurred in low amounts <2% TFA. One of the two samples of *Nannocalanus minor* showed a high amount (9.2%) of the dinoflagellate marker 18:4(n-3).

Eucalanus hyalinus (both sexes), *Aetideopsis carinata* (both years), *Euchirella similis* and *Pleuromamma robusta* had, in addition to the membrane fatty acids, higher amounts of the carnivory marker 18:1(n-9). The diatom marker 16:1(n-7) also showed enriched values in these species (except in *E. similis*). *A. carinata* from 2008 exhibited higher amounts of 20:1(n-9) (4.0 ± 1.3% TFA) and 22:1(n-11) (7.4 ± 2.5% TFA). *Euchaeta marina* and *Rhincalanus nasutus* had significantly higher amounts of 18:1(n-9) with up to 44% TFA, as compared to all other copepods (ANOVA, Tukey, $p \leq 0.001$). Lipids of these two species also typically contained 16:1(n-7). *R. nasutus* was the only species which contained phytanic acid (~7% TFA, not listed in Table 1). *Metridia lucens* and *Centropages brachiatus* were clearly dominated by the three membrane fatty acids (Table 1).

Six of the calanoid copepod species stored wax esters in variable amounts and thus also contained fatty alcohols (Table 1). The fatty alcohol compositions of *C. carinatus* and *A. carinata* were characterised by the alcohol moieties 14:0, 16:0, 20:1 and 22:1, with the long-chain mono-unsaturated fatty acids as dominant components (together >60% and >40%, resp.). *R. nasutus*, *E. similis* and *M. lucens* had high amounts of the two shorter-chain saturated fatty alcohols 14:0 and 16:0, while 16:0 dominated with 90% in *E. marina*.

Fatty acid biomarker ratios

The ratios 18:1(n-9)/18:1(n-7) and 18:1(n-9)/∑herb. markers are used as indicators for the degree of carnivory (Table 1, Fig. 3). *Euchaeta marina* showed the highest degree of carnivory with significantly higher fatty acid ratios (ANOVA, Tukey, $p \leq 0.05$), as compared to all other copepods (except for *Euchirella similis* for 18:1(n-9)/∑herb. markers, $p = 0.13$). *Rhincalanus nasutus* and *E. similis* also exhibited enhanced values for both ratios. The two samples of *Nannocalanus minor* varied between 3.9 and 6.6 for 18:1(n-9)/18:1(n-7) and 0.2 and 1.4 for 18:1(n-9)/∑herb. markers. Intermediate ratios were found in *Aetideopsis carinata*, *Pleuromamma robusta* and males of

Eucalanus hyalinus (18:1(n-9)/18:1(n-7): 2.3-3.7, 18:1(n-9)/Σherb. markers: 0.8-1.0). Females of *E. hyalinus* exhibited low ratios, similar to *Centropages brachiatus* and *Metridia lucens* (18:1(n-9)/18:1(n-7): 0.8-1.5, 18:1(n-9)/Σherb. markers: 0.4-0.5). Deviating results with regard to the two marker ratios occurred in *Calanoides carinatus*, particularly in the copepodids C5, with higher values (2.3-7.0) for 18:1 (n-9)/18:1(n-7) and lowest values (0.2-0.3) for 18:1(n-9)/Σherb. markers.

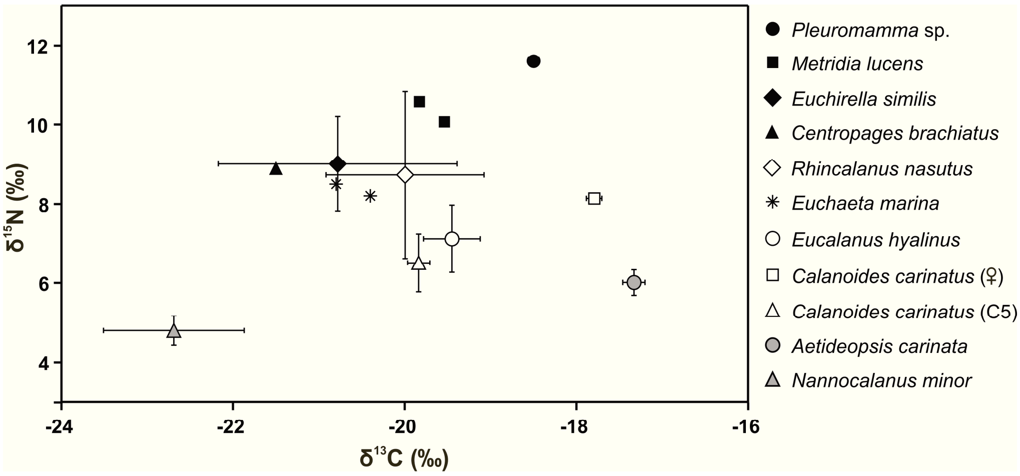


Figure 4: Stable isotope signatures of $\delta^{15}N$ and $\delta^{13}C$ for dominant calanoid copepod species. Error bars represent standard deviations. See Table 2 for the number of samples.

Stable isotope ratios and trophic levels

Stable isotope ratios of carbon and nitrogen of the ten calanoid copepod species ranged from -17.3‰ to -22.7‰ and 4.8‰ to 11.6‰, respectively (Table 2, Fig. 4). *Nannocalanus minor* had the lowest isotope ratios of all copepods ($\delta^{13}C$: -22.7‰, $\delta^{15}N$: 4.8‰). The majority of the copepods clustered around $\delta^{13}C$ values of -19.4 to -21.5‰. Higher carbon isotope ratios were found in females of *Aetideopsis carinata*, *C. carinatus* and *Pleuromamma* sp. (-17.3‰ to -18.5‰). Low $\delta^{15}N$ values of 6-7‰ occurred in *Aetideopsis carinata*, *Calanoides carinatus* (C5) and *Eucalanus hyalinus*. Higher $\delta^{15}N$ values (8-9‰) were found in females of *C. carinatus*, *Euchaeta marina*, *Euchirella similis*, *Rhincalanus nasutus* and *Centropages brachiatus*. Both species of the family Metridinidae, *Metridia lucens* and *Pleuromamma* sp., exhibited the highest $\delta^{15}N$ values (10-11‰).

Table 2: Stable isotope compositions of several components of the northern Benguela food web. Trophic levels are calculated using a δ^{15}N enrichment of 3.4‰. n = number of samples (total number of individuals).

Species	Stage	n	δ^{13}C (‰)	δ^{15}N (‰)	Trophic level
nitrate (NO_3^-)		41	-	6.3 ± 0.8	
phytoplankton (calculated by NO_3^-)		41	-	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	1
copepods (<700 m depth)					
Nannocalanus minor	f	3 (35)	-22.7 ± 0.8	4.8 ± 0.4	2.0
Aetideopsis carinata	f	4	-17.3 ± 0.1	6.0 ± 0.3	2.4
Calanoides carinatus	C5	4 (58)	-19.9 ± 0.2	6.1 ± 0.3	2.4
Calanoides carinatus	f	3 (56)	-17.8 ± 0.1	8.1 ± 0.1	3.0
Eucalanus hyalinus	f	7 (16)	-19.4 ± 0.4	6.9 ± 0.5	2.6
Euchaeta marina	f	2 (6)	-20.4/-20.8	8.2/8.5	3.1
Rhincalanus nasutus	f	5 (11)	-20.0 ± 0.9	8.7 ± 2.1	3.2
Centropages brachiatus	adult	1 (25)	-21.5	8.9	3.2
Euchirella similis	f	3	-20.8 ± 1.4	9.0 ± 1.2	3.2
Metridia lucens	f	2 (100)	-19.5/-19.8	10.1/10.6	3.7
Pleuromamma sp.	f	1 (2)	-18.5	10.9	3.8
copepods (>700 m depth)					
Paraeuchaeta gracilis	f	5 (12)	-19.2 ± 0.7	8.9 ± 0.6	3.2
Paraeuchaeta hanseni	f	1	-19.1	9.3	3.3
Gaetanus pileatus	f	2	-18.1/-18.3	9.1/9.4	3.3
Megacalanus princeps	C5	1	-18.6	10.8	3.8
Pseudochirella sp.	f	1	-20.1	11.0	3.8
Lucicutia sp.	f	1	-21.9	11.9	4.1
Gaussia sp.	m,f	3	-19.8 ± 0.6	12.8 ± 0.6	4.3
decapods					
Oplophorus novazeelandiae		2	-16.9/-16.3	5.7/7.3	2.5
Acanthephyra pelagica		2	-15.4/-15.1	6.6/7.1	2.6
Sergestes orientalis		1	-16.7	6.9	2.6
Gennadas brevirostris		3	-15.4 ± 0.3	8.0 ± 1.0	3.0
Sergestes armatus		3	-17.6 ± 0.6	8.5 ± 0.8	3.1
Pasiphaea semispinosa		8	-16.1 ± 0.4	8.7 ± 0.6	3.1
Sergia robusta		1	-15.5	8.8	3.2
salps					
Salpa fusiformis		8	-21.6 ± 0.9	6.4 ± 0.4	2.5
Pyrosoma sp.		2	-20.8/-20.4	7.8/7.9	2.9
pteropods (*Cymbulia* sp.)		5	-19.1 ± 0.6	7.3 ± 0.8	2.7
chaetognaths (*Sagitta* sp.)		5	-18.4 ± 0.7	8.8 ± 0.9	3.2
euphausiids (*Nematoscelis* sp.)		3	-18.2 ± 0.2	9.6 ± 0.7	3.4
amphipods					
Hyperia galba		1	-18.1	9.2	3.3
Themisto gaudichaudi		3	-17.8 ± 0.5	9.9 ± 0.6	3.5
jellyfish					
Chrysaora hysoscella		1	-17.8	9.3	3.3
Beroe sp.		1	-19.4	10.3	3.6
Aequorea aequorea		5	-17.5 ± 0.6	12.1 ± 1.6	4.2
fish					
anchovy (*Engraulis capensis*)	adult	2	-16.4/-16.0	7.6/7.0	2.7
Cyclothone sp.	adult	2	-19.7/-18.1	13.4/10.4	4.1
cape hake (*Merluccius capensis*)	adult	1	-17.1	14.1	4.7
snoek (*Thyrsites atun*)	adult	2	-21.4/-15.7	14.5/14.0	4.8
seabird (storm petrel)		4	-17.3 ± 0.4	13.5 ± 0.7	4.6

A comparison of stable isotope ratios and calculated trophic levels of the copepods with other taxa of the northern Benguela food web is shown in Table 2. Mean δ^{15}N values varied greatly among taxa (1.0-14.5‰). As expected, the most depleted δ^{15}N values were determined for phytoplankton and the most enriched values were found in fish and storm petrels.

The $^{14}N/^{15}N$ ratio of nitrate did not show differences between sampling depths and therefore the mean value was estimated (6.3 ± 0.8). $\delta^{15}N$ of phytoplankton calculated from nitrate was 1.0 ± 0.1. Stable isotope ratios of nitrogen of mixed phytoplankton samples varied between 0.3 and 1.8 and ranged between 1.6-2.1 for diatoms. The mean $\delta^{15}N$ value of all three approaches for phytoplankton (1.4 ± 0.5‰) was used as baseline for the northern Benguela Current (Table 2).

Calanoid copepod species from depths shallower than 700 m exhibited trophic levels of 2.0 to 3.8. *N. minor*, *A. carinata*, as well as copepodids C5 of *C. carinatus* showed low trophic levels of around 2, whereas females of *C. carinatus* occupied a higher trophic level (3.0). The highest trophic level (3.8) among epi- to mesopelagic copepods was determined for *Pleuromamma* sp. Copepod species from deeper water layers (>700 m) had on average higher trophic levels (3.2 to 4.3) than those of shallower waters.

Salps, pteropods, decapods and chaetognaths exhibited trophic levels from 2.5 to 3.2, whereas euphausiids, amphipods and jellyfish had higher trophic levels of 3.3 to 4.2. The trophic level for fish ranged from 2.7 for anchovy (*Engraulis capensis*) to 4.8 for snoek (*Thyrsites atun*). The storm petrel also occupied a high trophic level of 4.6.

DISCUSSION

Life-cycle adaptations of calanoid copepods to upwelling

Lipids may reveal information about life-cycle strategies (Hagen & Auel 2001). Due to their specific biochemical and metabolic properties, different major lipid classes, i.e. wax esters, triacylglycerols and phospholipids, serve different storage functions in zooplankton (Hagen et al. 1993, Albers et al. 1996, Lee et al. 2006). Wax esters serve as long-term metabolic reserves, especially in copepods, whereas triacylglycerols are utilised for short-term demands (Conover 1988, Båmstedt et al. 1990, Mayzaud et al. 1998). Studies of lipid compositions of zooplankton mainly focussed on high-latitude regions, since lipid accumulation and storage is more pronounced in these highly seasonal environments than in subtropical or tropical regions. Most of the polar calanoid copepods accumulate extensive lipid deposits as wax esters (Kattner & Hagen 1995). In contrast, in the tropical regions usually lipid-poor copepods with low wax ester levels occur in epipelagic waters (Lee & Hirota 1973). Data of lipid compositions of copepods from upwelling regions are scarce, except for *Calanoides carinatus* from the Benguela Current (Verheye et al. 2005). Upwelling areas are highly variable ecosystems and - although at different time scales - the periodicity of active vs. inactive upwelling is comparable to the seasonal food supply at high latitudes and may lead to similar strategies of lipid accumulation in zooplankton.

Among the ten dominant copepod species in the Benguela upwelling system covered in this study, three species stored wax esters (*Calanoides carinatus*, *Rhincalanus nasutus*, *Euchaeta marina*), four deposited triacylglycerols (*Eucalanus hyalinus*, *Aetideopsis carinata*, *Euchirella similis*, *Pleuromamma robusta*) and another three were characterised by high phospholipid levels (*Nannocalanus minor*, *Metridia lucens Centropages brachiatus*). The high wax ester levels (>80% of total lipid) of copepodite stage C5 of *C. carinatus* and females of *R. nasutus* (data herein, Sommer et al. 2002, Verheye et al. 2005, Schnack-Schiel et al. 2008) are associated with diapause or resting stages. This energy accumulation represents an adaptative strategy to buffer the variability of upwelling systems and to cope with food-limited periods (Lee et al. 2006). Both species resemble the lipid storage patterns of their Antarctic congeners (e.g., *Calanoides acutus* and *Rhincalanus gigas,* respectively) and thus exhibit essentially similar life-history traits in the Benguela upwelling system as those species in polar regions.

The wax ester deposits of *Euchaeta marina* in the present study are comparable to wax ester levels measured in the tropics (Lee & Hirota 1973). In this species, they primarily function as buoyancy aids rather than as energy stores to ensure their 'drift and wait' strategy (Auel & Hagen 2005, Laakmann et al. 2009).

Triacylglycerols are generally used by copepod species that feed year-round and usually do not rely on extensive lipid reserves (Hagen 1999). In this study *Eucalanus hyalinus*, *Aetideopsis carinata*, *Euchirella similis*, and *Pleuromamma robusta* contained low to moderate levels of triacylglycerol as main lipid class, which is in line with findings for species of the same genera in tropical and temperate regions of the ocean (Lee & Hirota 1973, Kotani 2006).

The smallest species in this study, *Nannocalanus minor*, *Metridia lucens* and *Centropages brachiatus*, all contained high phospholipid levels (>70% TL) and very low neutral lipid reserves. Studies from other regions found high levels of wax esters in *Metridia* and triacylglycerols in *Centropages* (Albers et al. 1996, Lee & Hirota 1973, Kattner 1981). Therefore, additional studies are needed to clarify the lipid storage strategies of *Nannocalanus*, *Metridia* and *Centropages* in the northern Benguela upwelling system.

Overall, the calanoid copepod species of the Benguela upwelling system have developed similar lipid storage strategies, i.e. the same principal lipid classes, as their congeners in polar, temperate and tropical regions.

Comparison of methodological approaches, fatty acids vs. stable isotopes

The present study combined fatty acid trophic biomarkers and stable isotopes as two complementary approaches to assess dietary preferences and trophic levels of different copepod species in the northern Benguela upwelling region.

We are aware that samples were derived from two cruises in March 2008 and December 2009 but interannual variability was not very pronounced. The comparison of sea surface temperatures (CTD data) of both sampling times indicated similar upwelling events and fatty acid profiles of species collected in both years did not show significant differences, indicating that both sampling times were comparable.

Both methods resulted in rather similar trophic classifications of copepods. Most copepod species had calculated trophic levels of 2.5 to 3.7 based on $\delta^{15}N$ ratios supporting a predominately omnivorous to carnivorous feeding mode. Correspondingly, relatively high indices of carnivory as derived from fatty acid trophic biomarkers suggest omnivorous to carnivorous feeding for many species. However, for some species (*Metridia lucens*, *Pleuromamma* sp., *Nannocalanus minor*) both approaches differed in their indication of the carnivory degree and trophic level. *M. lucens* and *Pleuromamma* sp. showed surprisingly high $\delta^{15}N$ values, but had low or intermediate "carnivory" fatty acid ratios. Likewise, *N. minor* with the lowest $\delta^{15}N$ value had carnivory indices above the median, based on both fatty acid ratios. The differences for *N. minor* and *Pleuromamma* may be explained by the fact that samples for fatty acids and stable isotopes were not from the same station. It is also possible that the two different methodological approaches varied in their sensitivities to changes in dietary composition, particularly with regard to component-specific turnover times and, hence, the time span over which dietary signals are integrated by different trophic biomarkers (Graeve et al. 1994a, Gentsch et al. 2009).

Deviations in the outcome of the two indices of carnivory based on fatty acid composition are mainly caused by the diatom marker 16:1(n-7), which is included in the 18:1(n-9)/∑herb. markers ratio, but neglected by the 18:1(n-9)/18:1(n-7) ratio. Particularly copepodids C5 of *C. carinatus* contained rather high amounts of 16:1(n-7), but only traces of 18:1(n-7). Therefore, they had the lowest degree of carnivory according to the 18:1(n-9)/∑herb. markers ratio, but intermediate values based on the 18:1(n-9)/18:1(n-7) ratio. We suggest that the new ratio with all herbivory markers allows for a more robust interpretation than the previously used 18:1(n-9)/18:1(n-7) ratio, because 18:1(n-7) originates from 16:1(n-7) and is often only a minor component of the total fatty acids. The combination of fatty acids and stable isotope measurements to provide complementary insights into trophic levels proved to be very useful, albeit certain inconsistencies still exist.

Trophic role of calanoid copepods in upwelling systems

Zooplankton taxa, especially copepods, play a central role in the energy flux of marine ecosystems interlinking primary producers with pelagic fish and higher trophic levels (Loick et al. 2005, Verheye et al. 2005). Zooplankton organisms are particularly sensitive to climate forcing, making them excellent indicators of climate effects (Hays et al. 2005). Due to their often non-linear responses, subtle changes at the base of the food web can propagate along the food chain and may even lead to regime shifts altering entire ecosystems.

According to our data for the northern Benguela system, only the two calanid species *Calanoides carinatus* and *Nannocalanus minor* fit into the classical scheme of herbivorous copepods interlinking primary production with higher trophic levels. Both species occupy low trophic levels as primary consumers and contain high amounts of ‘herbivory’ fatty acid biomarkers. *C. carinatus* thrives in plumes of nutrient-rich, recently upwelled water close to the coast, which are generally dominated by diatoms. Indeed, females and diapausing copepodids C5 of *C. carinatus* had accumulated high amounts of the characteristic diatom marker 16:1(n-7), emphasizing the dietary importance of diatoms for the nutrition of copepods within upwelling plumes (Smith 2001, Ceballos et al. 2006). However, the 2‰ higher $\delta^{15}N$ ratio of *C. carinatus* females compared to C5s indicates that surface-dwelling females also rely on other resources than diatoms. Protozoa could provide an additional food source (Ceballos et al. 2006).

In contrast to *C. carinatus*, the epipelagic *Nannocalanus minor* occurs further offshore in generally warmer and nutrient-depleted waters. It contained elevated levels of the dinoflagellate marker 18:4(n-3). These data together with very low $\delta^{15}N$ values suggest that autotrophic dinoflagellates are preferred by *N. minor*, consistent with a role as primary consumer. The differences in the fatty acid patterns of *C. carinatus* and *N. minor* are in line with the general zonation pattern of phytoplankton across coastal upwelling systems with diatom-dominated assemblages in recently upwelled, nutrient-rich waters nearshore and dinoflagellate communities prevailing further offshore, where silicate has already been depleted (Gibbons & Hutchings 1996, Probyn et al. 2000). Both species represent important food items for higher trophic levels such as mesopelagic fish, chaetognaths and euphausiids (Kinzer & Schulz 1985, Liang & Vega-Peréz 1995) and can be seen as important ‘classical’ links between primary production and higher trophic levels in the Benguela upwelling system.

The third copepod species in our dataset with a low trophic level of 2.4 was *Aetideopsis carinata.* This species differs in distribution pattern and life cycle from the previous two epipelagic herbivores. *A. carinata* occurred almost exclusively at mesopelagic depths above the continental rise, where dense blooms of senescent diatoms (mainly *Coscinodiscus*) sink out of the euphotic zone. Under these conditions, females of *A. carinata* were very active and showed high egg production rates (pers. obs.). Hence, they seem to feed specifically on sinking phytodetritus after extreme bloom events to fuel their reproductive processes. With this feeding behaviour, *A. carinata* may have a strong impact on the sinking particle field and may regulate the export of organic material from the upwelling shelf to greater depths and the adjacent open ocean. At times of low phytodetritus availability, *A. carinata* may switch to a carnivorous diet including younger stages of *Calanoides carinatus*. Females of *A. carinata* collected in 2008 contained elevated amounts of the fatty acids 20:1(n-9) and 22:1(n-11), which usually occur in pre-adult *C. carinatus*.

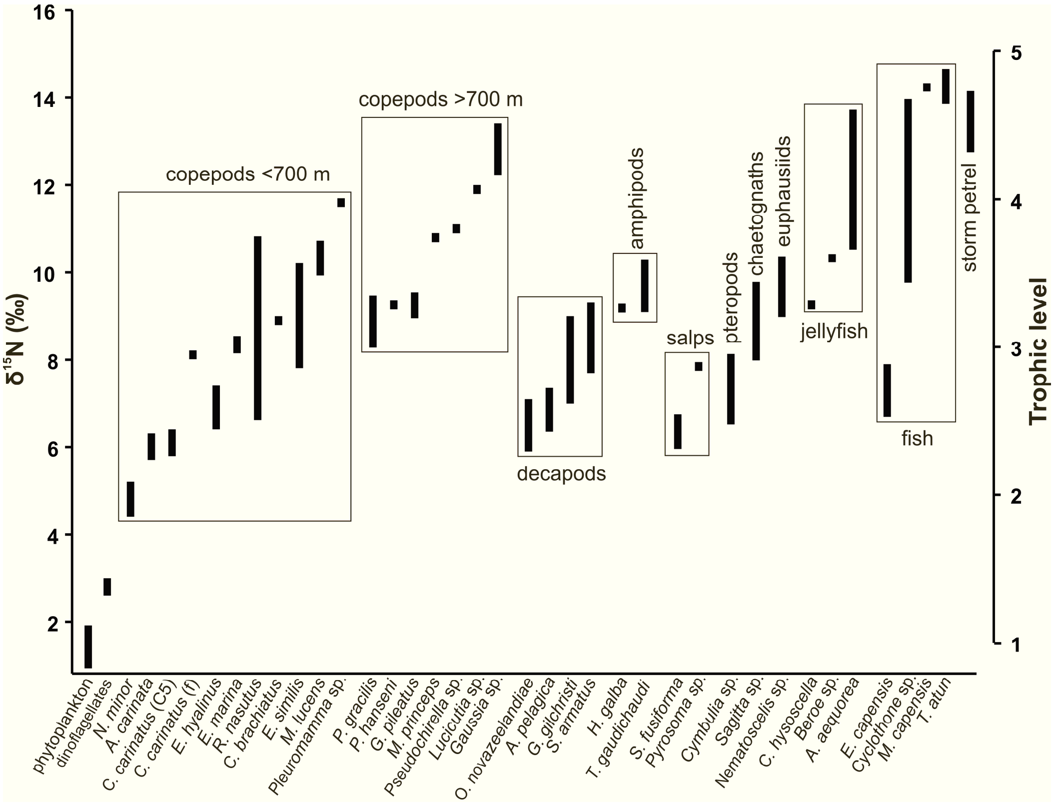


Figure 5: Ranges of $\delta^{15}N$ and their associated trophic levels for 39 plankton taxa and higher trophic levels, e.g. fish and birds, from the northern Benguela Current. Trophic levels were calculated by applying a trophic enrichment value of 3.4‰.

Several studies from different regions of the World Ocean have shown that supposedly herbivorous zooplankton taxa may rather have an omnivorous diet depending on local conditions (e.g. Gifford 1993, Vargas et al. 2006). The present study supports the suggestion that pure herbivory is rarely found in copepods, whereas omnivory is very common also among calanoid copepods of the northern Benguela upwelling system. In our investigation, seven of ten epi- to mesopelagic and all deep-sea copepod species had calculated trophic levels ≥2.5, indicating a rather omnivorous or even carnivorous feeding behaviour. Copepods often show high clearance rates or even a preference for microheterotrophs such as ciliates compared to phytoplankton (Fessenden & Cowles 1994, Zeldis et al. 2002). Motile prey may be easier to detect and/or more attractive to ingest than immobile and armoured algal cells such as diatoms. Omnivory by copepods may play an important role, establishing a link between the microbial loop and the ‘classical food’ chain, with copepods ingesting large amounts of ciliates and flagellates (Calbet & Saiz 2005).

Moreover, many copepods, like *Euchaeta* spp., are true predators, feeding on comparably large prey items such as other mesozooplankton organisms. This has been demonstrated by feeding experiments, gut content analysis, trophic biomarkers, and functional morphology of their mouth parts (Hopkins 1987, Hagen & Auel 2001, Michels & Schnack-Schiel 2005). In this study the predatory behaviour of euchaetid species is supported by the high level of carnivory, as indicated by fatty acid trophic markers and also the trophic level of 3.1 for *Euchaeta marina*.

Shifts from herbivory to omnivory may depend on food availability in the field (Vargas et al. 2006) or they may be performed to maintain reproduction and population growth year-round, even during periods, when phytoplankton is scarce (Escribano & McLaren 1999, Hidalgo & Escribano 2007). For example, *Rhincalanus* species are supposed to shift from herbivory to omnivory in unproductive periods or regions (Bathmann et al. 1993, Graeve et al. 1994b, Schnack-Schiel et al. 2008). In our study, the high trophic level and carnivory indices of *R. nasutus* suggest omni- to carnivorous feeding. At the same time, *R. nasutus* contained high amounts of diatom markers. This combination supports the assumption of a shift in dietary composition and feeding mode dependent on food availability by *Rhincalanus*.

The fact that most copepod species occupy rather high trophic positions, leads to the unusual situation that the taxonomic group of calanoid copepods in total, from the small epipelagic *N. minor* to large deep-sea species such as *Gaussia princeps*, covers nearly the whole range of δ^{15}N ratios, similar to all other higher taxa from salps to adult fish (Fig. 5). *C. carinatus* and *N. minor* share trophic positions with the herbivorous filter-feeding salp *Salpa fusiformis* at the base of the food web, whereas six of the epipelagic copepod species are truly carnivorous, similar to predatory zooplankton such as amphipods, the chaetognath *Sagitta* sp. and jellyfish, for which copepods usually serve as prey items (Liang & Vega-Pérez 1995).

This study revealed that most calanoid copepods of the northern Benguela exhibit trophic roles that are far more complex than just interlinking primary producers with pelagic fish. Although difficult to implement, this should be considered for the development of realistic food-web models. Such models often include just one box for zooplankton or a parameterisation of zooplankton by size class. Here we show that zooplankton taxa, particularly copepods, represent many different trophic guilds. Food-web models should therefore focus on dominant and ecologically important key species with their specific trophic positions to ensure an authentic description of trophic interactions in coastal upwelling ecosystems.

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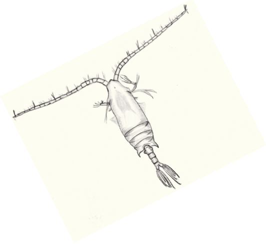
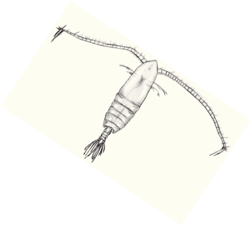
Chapter II





Energetics and carbon budgets of dominant calanoid copepods in the northern Benguela upwelling system

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Energetics and carbon budgets of dominant calanoid copepods in the northern Benguela upwelling system

Anna Schukat, Lena Teuber, Wilhelm Hagen and Holger Auel

ABSTRACT

Respiration rates of 16 calanoid copepod species from the northern Benguela upwelling system were measured on board RRS *Discovery* in September/October 2010 to determine their energy requirements and assess their significance in the carbon cycle. Mass-specific respiration rates were standardized to a mean copepod body mass and a temperature regime typical of the northern Benguela Current. These adjusted respiration rates revealed two different activity levels (active and resting) in copepodids C5 of *Calanoides carinatus* and females of *Rhincalanus nasutus*, which reduced their metabolism during dormancy by 72% and 62%, respectively. An allometric function (I_{max}) and an energy budget approach were performed to calculate ingestion rates. I_{max} overestimated the ingestion rates derived from the energy budget approach by >50%. We suggest that the energy budget approach is the more reliable approximation with a total calanoid copepod (mainly adults) consumption of 78 mg C m^{-2} d^{-1} in neritic regions and 21 mg C m^{-2} d^{-1} in oceanic regions. The two primarily herbivorous copepods *C. carinatus* and *Nannocalanus minor* contributed 83% and 6%, respectively, to total consumption of calanoid copepods. On average, *C. carinatus* removed 12% of phytoplankton biomass daily and consumed about 5% of daily primary production. In contrast, *N. minor* removed on average 3% of phytoplankton biomass and consumed approximately 1% of daily primary production. These estimates imply that *C. carinatus* is an important primary consumers in the northern Benguela system, while *N. minor* has little grazing impact. Data on energy requirements and total consumption rates of dominant calanoid copepods of this study are essential for the development of realistic carbon budgets and food-web models for the northern Benguela upwelling system.

KEYWORDS

respiration, ingestion, community consumption, grazing pressure

INTRODUCTION

Coastal upwelling systems play an important role in the oceans´ biological productivity and carbon cycling (Vargas & Gonzalez 2004). They occupy only 1% of the ocean area but generate 11% of the new primary production (Chávez & Toggweiler 1995). The Benguela current system is one of the most productive upwelling region in the world, together with the Humboldt Current (Chávez & Toggweiler 1995, Carr 2002, Shillington et al. 2006), with estimates for annual primary production ranging from 400 g C m^{-2} yr^{-1} to 900 g C m^{-2} yr^{-1} (Brown et al. 1991, Carr 2002, Heileman & O'Toole 2008, Monteiro 2010).

In coastal upwelling areas, copepods prevail with up to 90% of mesozooplankton abundance and some key species represent major trophic links between primary production and higher trophic levels (Loick et al. 2005, Verheye et al. 2005). Furthermore, copepods play a crucial role in the cycling of organic matter in the ocean, e.g. via moulted exoskeletons, faecal pellets, and respiration processes. Several studies have shown that diel as well as seasonal or ontogenetic vertical migrations of copepods contribute substantially to the downward carbon flux, as they consume organic carbon in surface waters, which they transfer to depth as gut contents to be either defecated, or assimilated into biomass, and then respired or consumed by predators (Longhurst et al. 1990, Dam et al. 1995, Al-Mutairi & Landry 2001, Steinberg et al. 2008). Quantifying the rates of phytoplankton utilization by copepods is an essential step to better understand the mechanisms that regulate phytoplankton populations and the downward flux of organic matter in marine ecosystems.

Within the northern Benguela upwelling system, the calanoid copepods *Calanoides carinatus*, *Metridia lucens*, *Nannocalanus minor*, *Aetideopsis carinata*, *Pleuromamma* spp., *Eucalanus hyalinus* and *Rhincalanus nasutus* are very common (this study, Hansen et al. 2005, Loick et al. 2005). These larger-sized pelagic copepods include herbivorous as well as omnivorous and carnivorous species and some important vertical migrants, which thus contribute to the biological carbon pump of the northern Benguela Current.

Respiration rates can be used as a direct measure of metabolism and provide information about minimum energetic demands of an animal. They represent useful estimates of its metabolic activity and serve to trace energy pathways and carbon fluxes (Ikeda 1985, Ikeda et al. 2000, Hernández-León & Ikeda 2005). Factors that influence metabolic rates include feeding mode, activity level, migration and developmental stage of an organism, but the most pronounced effects on respiration rates are related to body mass and temperature (Ikeda 1985, Ikeda et al. 2001, Hernández-León & Ikeda 2005).

In this study, respiration rates of 16 calanoid copepod species (1-7 mm size range) were determined 1) to reveal differences in respiration rates that are independent of temperature and body mass and 2) to estimate carbon consumption rates to assess the grazing pressure on phytoplankton populations and the contribution of copepods to the carbon flux in the northern Benguela system.

MATERIAL AND METHODS

Sampling of copepods

Copepods for respiration measurements were collected by RRS *Discovery* in the northern Benguela upwelling system in September/October 2010. Copepod species were sampled by different net types: vertical and oblique Multinet hauls (Hydro-Bios Multinet Midi, mesh size 200-500 µm), towed 1 m^2-MOCNESS (Multiple Opening and Closing Net with Environmental Sensing System, 333 µm), WP-2 (300 µm), Bongo net (300 and 500 µm), ring trawl (1000 µm) and a driftnet (200 µm). Immediately after the hauls, samples were sorted to species and stages (16 species; females, males and C5 copepodids). Sorting was carried out with special care to ensure that the copepods remained in good condition. Specimens were kept in temperature-controlled refrigerators for at least twelve hours before they were used in experiments.

Abundance data of copepods were derived from vertical Multinet hauls (mouth opening 0.25 m^2, mesh size 200-300 µm) at ten stations, carried out in the northern Benguela upwelling system in December 2009 (FRS *Africana*) and September/October 2010 (RRS *Discovery*). Three transects along ~17°S, 19°S and 23°S with three stations each (neritic, shelf break, oceanic) and one station at 21°S were analysed for copepod abundance. Maximum sampling depth was either close to the seafloor (neritic and shelf break stations) or 700 m (2009) and 1000 m (2010) for the oceanic stations. Calanoid copepod species and stages were identified and enumerated separately. Corresponding to the respiration experiments, adult females and males and copepodite stages C5 (in case of *Calanoides carinatus* and *Nannocalanus minor*) were included in the abundance calculations. Abundance is expressed as number of individuals per m^2, calculated from the volume of water filtered (calibrated flowmeter, Hydro-Bios) and the maximum sampling depth at each station.

Respiration measurements

Respiration rates of different copepod species were measured by optode respirometry (for details see Köster et al. 2008) with a 10-channel optode respirometer (PreSens Precision Sensing Oxy-10 Mini, Regensburg, Germany) under simulated *in situ* conditions in temperature-controlled refrigerators on board. For each experiment, seawater was UV-treated, oxygenated and filtered through 0.2 µm Whatman GF/F filter. The filtered seawater was stirred for further 10 to 20 min to avoid supersaturation.

Temperature profiles derived from the CTD sensor were used to set refrigerators to the ambient temperature at sampling depth. Experiments were run in gas-tight glass bottles (12-13 ml). For each set of experiments, two controls without animals were measured under exactly the same conditions to compensate for potential bias. The number of animals per bottle depended on the copepods' size, stage and metabolic activity. It varied between one individual for large species and ten individuals for small species or diapausing *Calanoides carinatus*. Animals were not fed during the experiments but they showed natural species-specific movements. Therefore, we consider that the metabolic activity during experiments approximated routine metabolism. Immediately after the experiments, all specimens were deep-frozen at -80°C for later dry mass determination (after lyophilisation for 48 h) in the home lab.

Carbon demand and ingestion rates

For the estimation of carbon requirements of copepod species, individual oxygen consumption rates were converted to carbon units, assuming that the expiration of 1 ml O_2 mobilises 0.44 mg of organic carbon (Williams & Carlucci 1976, Mahaut et al. 1995). Based on these results, ingestion rates were calculated according to an energy budget approach with the equation Consumption = 1.79 x Respiration (for details see Pakhomov & Perissinotto 1996, Auel & Werner 2003).

For comparison, potential maximum ingestion rates (I_{max} in day^{-1}) were calculated by an allometric function according to Moloney and Field (1989), which is based on body mass (M in pg C). For that purpose, body dry mass (mg DM) of copepods was converted to units of carbon (mg C). The carbon content of each species was measured by mass-spectrometry in association with stable isotope analysis (for details see Schukat et al. submitted). Carbon content varied from 37% to 62% of dry mass (DM) between different species and stages, mainly depending on the lipid content. For species without available carbon data, the mean value of all species (44% DM) was applied. The original function $I_{max} = 63 \times M^{-0.25}$ (valid for 20°C) was adjusted to a typical upwelling temperature regime of 15°C applying a Q_{10} of 2 (Thuesen et al. 1998, Ikeda et al. 2006). The scaling coefficient of -0.25 was exchanged by -0.24, which was derived from the slope of the regression line by plotting log temperature-adjusted respiration versus log dry mass (Fig. 1). The allometric function for 15°C reads then $I_{max} = 47.25 \times M^{-0.24}$.

To evaluate the impact of different calanoid copepod species on phytoplankton and carbon flux in the northern Benguela Current, ingestion rates of these communities were estimated, based on the median abundance of species and stages and the respective ingestion rate, derived from both, the energy budget approach and I_{max}.

Standardisation of respiration rates

To allow for physiological comparisons of respiration rates of deep- and shallow-living copepod species without the effects of ambient temperature and different individual body mass, mass-specific respiration rates were adjusted to a typical temperature of upwelling systems of 15°C using a Q_{10} of 2 (van’t Hoff equation: $R_{15°C} = R_1 {*} Q_{10}{}^{(15-}$

$^{T1)/10}$, where R_1 is the original respiration rate and T_1 the corresponding temperature) and size-adjusted. The scaling coefficient of -0.24 (Fig. 1) is used for the standardisation of body dry mass to 0.3 mg (mean dry mass of all analysed copepods) applying the allometric equation $R_{15°C,\ 0.3mg} = a \times 0.3^{-0.24}$, where a is the rate coefficient (for details see Thuesen et al. 1998, Ikeda et al. 2001, Ikeda et al. 2006).

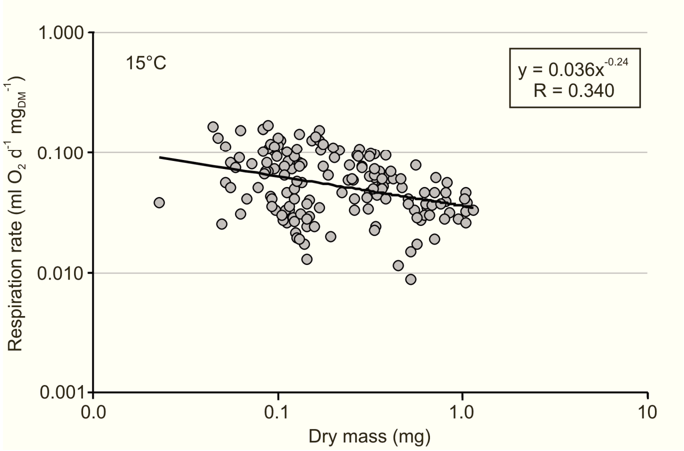

Figure 1: Relationship between body dry mass and mass-specific respiration rate standardised to 15°C for subtropical calanoid copepods.

RESULTS

Respiration and adjusted respiration rates

A total of 127 respiration measurements were conducted with 16 different calanoid copepod species (Table 1). Individual respiration rates ranged from 2.1 ± 0.7 µl O_2 d^{-1} for diapausing copepodids C5 of *Calanoides carinatus* to 42.1 ± 3.9 µl O_2 d^{-1} for females of *Euchirella splendens* (Table 1).

Regression analysis of individual and mass-specific respiration rates showed that 72% and 40% of their variances could be explained by ambient temperature and individual body mass, respectively (r = 0.85; 0.64). All copepods combined had a mean adjusted metabolic rate (AMR) of 51.8 ± 24.5 ml O_2 d^{-1} g_{DM}^{-1} (Fig. 2). Three copepod species showed pronounced differences in AMR within the same stage (Fig. 2).

Table 1: Sampling depth, experimental temperature (*in situ*), mean dry mass, mass-specific (adjusted: 15°C, 0.3 mg dry mass) and individual respiration rates and ingestion rates of 16 calanoid copepod species from the northern Benguela system . f = female, m = male, C5 = copepodite stage 5, act = active, dia = diapause, n = number of samples (total number of individuals),* not *in situ* temperature but adapted for 72 h, EB = energy budget approach, I_{max}= allometric function.

Species	Stage	n (ind.)	Depth (m)	Temp. (°C)	Dry mass µg ind^{-1}	Mass-specific Respiration ml O_2 d^{-1} g_{DM}^{-1}		Respiration µl O_2 ind.$^{-1}$ d^{-1}	µg C ind.$^{-1}$d^{-1}	Ingestion µg C ind.$^{-1}$ d^{-1}	
Calanidae						not adjusted	adjusted			EB	I_{max}
Calanoides carinatus	f	2 (8)	50-0	17	85 ± 2	102.0 ± 27.0	63.3 ± 16.5	8.7 ± 2.1	3.8 ± 0.9	6.8 ± 1.7	31.9 ± 0.5
	f	5 (10)	10-0	14	121 ± 19	88.0 ± 19.4	75.3 ± 18.7	10.9 ± 4.3	4.8 ± 1.9	8.6 ± 3.4	41.7 ± 7.0
	f	4 (15)	50-0	10*	99 ± 19	70.1 ± 13.6	75.4 ± 15.7	7.0 ± 2.1	3.1 ± 0.9	5.5 ± 1.7	35.7 ± 5.1
	m	2 (2)	50-0	14	112 ± 27	56.1 ± 31.1	46.8 ± 28.2	6.7 ± 5.0	2.9 ± 2.2	5.3 ± 3.9	39.2 ± 7.2
	C5 act	5 (12)	50-0	14	84 ± 37	100.5 ± 34.8	77.9 ± 25.3	7.7 ± 2.9	3.4 ± 1.3	6.2 ± 2.3	31.2 ± 10.6
	C5 dia	14 (121)	950-500	8	135 ± 14	15.8 ± 4.7	21.4 ± 6.5	2.1 ± 0.7	0.9 ± 0.3	-	-
Nannocalanus minor	f	5 (24)	130-0	17-18	101 ± 11	55.3 ± 21.7	34.9 ± 14.1	5.5 ± 2.0	2.4 ± 0.9	4.3 ± 1.6	36.2 ± 2.9
	m	2 (10)	50-25	18	60 ± 17	132.2 ± 45.3	71.3 ± 19.5	7.5 ± 0.4	3.3 ± 0.2	5.9 ± 0.3	24.3 ± 5.4
	C5	2(11)	130-0	18	59 ± 4	106.8 ± 8.3	58.6 ± 5.5	6.3 ± 0.9	2.8 ± 0.4	5.0 ± 0.7	24.1 ± 1.2
Neocalanus robustior	f	3 (4)	100-40	16-17	422 ± 239	57.1 ± 21.6	56.1 ± 22.5	17.9 ± 2.0	7.9 ± 0.8	14.1 ± 1.5	106.1 ± 46.7
Eucalanidae											
Eucalanus hyalinus	f	9 (9)	80-0	14	733 ± 143	31.7 ± 5.6	41.4 ± 8.4	23.2 ± 5.6	10.2 ± 2.5	18.3 ± 4.4	163.4 ± 23.9
	C5	2 (6)	130-0	17-18	375 ± 11	59.8 ± 12.3	53.9 ± 13.7	22.4 ± 4.0	9.8 ± 1.8	17.6 ± 3.1	98.5 ± 2.1
Rhincalanus nasutus	f	3 (5)	130-0	17	515 ± 59	15.0 ± 5.4	14.4 ± 5.4	7.8 ± 3.5	3.5 ±1.5	6.2 ± 2.7	125.3 ± 11.0
	f	4 (7)	30-0	14	418 ± 96	69.3 ± 15.2	81.3 ± 17.3	28.8 ± 8.6	12.7 ± 3.8	22.7 ± 6.7	106.6 ± 18.3
	f	8 (8)	110-80	13	551 ± 178	28.5 ± 10.7	36.8 ± 13.5	15.4 ± 7.0	6.8 ± 3.1	12.1 ± 5.5	130.9 ± 32.5
Pareucalanus sewelli	f	4 (10)	100-50	16-18	290 ± 55	62.5 ± 19.1	49.7 ± 18.5	20.5 ± 7.5	9.0 ± 3.3	16.2 ± 5.9	80.8 ± 11.5
Aetideidae											
Aetideopsis carinata	f	9 (28)	730-500	8	170 ± 48	33.0 ± 10.6	46.9 ± 14.4	5.5 ± 1.9	2.4 ± 0.8	4.3 ± 1.5	53.5 ± 11.5
Euchirella rostrata	f	5 (12)	130-0	17	464 ± 124	55.6 ± 15.0	52.2 ± 10.5	24.6 ± 3.2	10.8 ± 1.4	19.3 ± 2.5	115.2 ± 23.4
	f	2 (5)	30-0	14	323 ± 18	26.6 ± 7.7	28.7 ± 7.9	8.5 ± 2.0	3.7 ± 0.9	6.7 ± 1.6	87.8 ± 3.7
Euchirella similis	f	2 (2)	500-200	8	1035 ± 21	26.9 ± 2.2	59.6 ± 5.2	27.9 ± 2.9	12.3 ± 1.3	22.0 ± 2.3	213.0 ± 3.3
Euchirella splendens	f	3 (5)	130-0	16-17	1084 ± 59	38.9 ± 4.3	47.6 ± 4.4	42.1 ± 3.9	18.5 ± 1.7	33.2 ± 3.1	220.6 ± 9.2
Metridinidae											
Metridia lucens	f	2 (11)	50-0	13-14	56 ± 5	60.4 ± 8.9	43.9 ± 9.7	3.4 ± 0.8	1.5 ± 0.3	2.7 ± 0.6	23.1 ± 1.5
	f	4 (22)	110-80	13	59 ± 7	32.2 ± 9.3	25.0 ± 7.9	1.9 ± 1.0	0.8 ± 0.4	1.5 ± 0.5	24.1 ± 2.5
Pleuromamma abdominalis	f	3 (6)	30-0	14	403 ± 17	70.5 ± 17.5	80.0 ± 19.6	28.2 ± 5.9	12.4 ± 2.6	22.2 ± 4.6	103.9 ± 3.2
Pleuromamma robusta	f	3 (6)	50-0	17	317 ± 16	64.9 ± 14.0	56.5 ± 12.2	20.7 ± 5.1	9.1 ± 2.2	16.3 ± 4.0	86.7 ± 3.2
	f	7 (14)	80-20	14	273 ± 26	74.0 ± 16.5	75.6 ± 17.7	23.2 ± 6.0	10.2 ± 2.6	18.2 ± 4.7	77.3 ± 5.5
	f	3 (6)	500-200	8	360 ± 13	30.3 ± 3.1	52.0 ± 5.9	10.9 ± 1.5	4.8 ± 0.6	8.6 ± 1.2	95.4 ± 2.6
Pleuromamma xiphias	f	3 (3)	30-0	14	778 ± 76	44.7 ± 7.9	59.4 ± 10.9	34.9 ± 7.5	15.3 ± 3.3	27.5 ± 5.9	171.5 ± 11.2
Candaciidae											
Candacia bipinnata	f	1 (1)	100-25	18	315	115.9	95.2	36.5	16.1	28.7	86.3
Candacia ethiopica	f	5 (10)	130-0	17	363 ± 72	74.5 ± 11.8	68.2 ± 10.4	26.9 ± 5.5	11.8 ± 2.4	21.2 ± 4.4	95.8 ± 14.3

Active copepodids C5 of *C. carinatus* exhibited a significantly higher AMR of 77.9 ± 25.3 ml O_2 d^{-1} g_{DM}^{-1} than diapausing C5 stages with 21.4 ± 6.5 ml O_2 d^{-1} g_{DM}^{-1} (t-test, $p \leq 0.0001$, Table 1, Fig. 2). This is equivalent to a metabolic reduction of 72% in diapausing copepodids C5 at depth as compared to active C5 stages from the surface (Fig. 2). Without compensation for temperature and body mass, metabolic reduction was even higher with 83% due to lower water temperatures at depth.

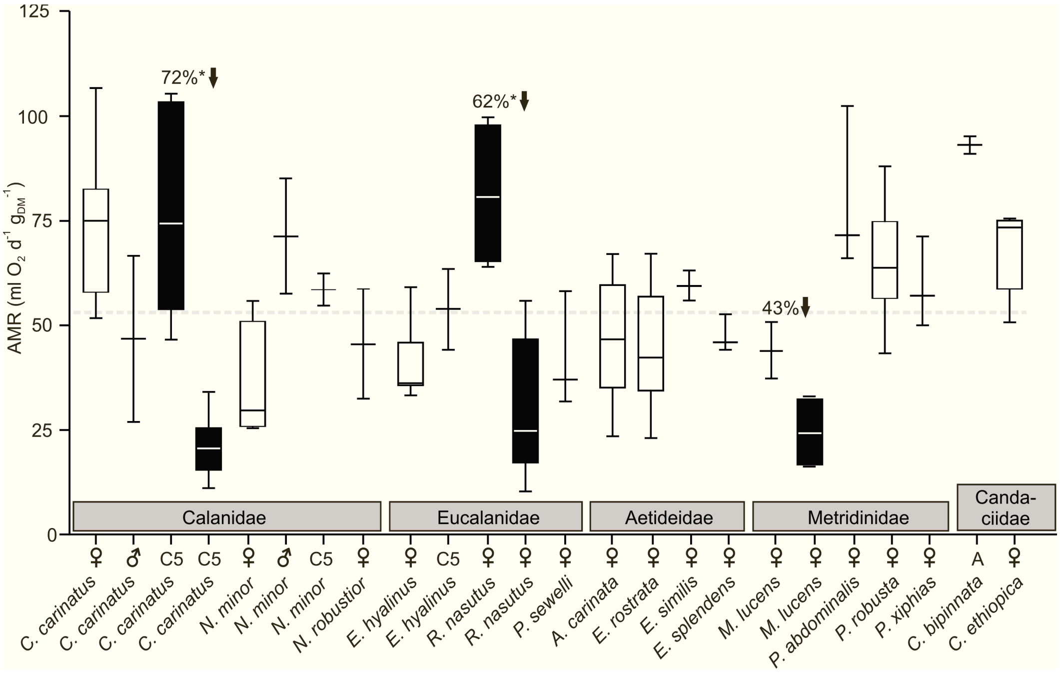

Figure 2: Adjusted metabolic rates (AMR, 15°C, 0.3 mg dry mass) of 16 calanoid copepod species from the northern Benguela system. Broken line signifies average respiration of all copepods. Black boxes indicate differences within the same stage of a species (*=significant, t-test, $p \leq 0.0001$). % values express the metabolic reduction within these stages. C5: copepodite stage 5, A: adult.

Significant intraspecific differences in metabolic activity were also found in *Rhincalanus nasutus* (t-test, $p \leq 0.0001$). Females sampled at 21°S at the surface had an average AMR of 81.3 ± 17.3 ml O_2 d^{-1} g_{DM}^{-1}, whereas females collected at 23°S in deeper water layers up to 130 m showed a 62% lower AMR of 30.7 ± 15.6 ml O_2 d^{-1} g_{DM}^{-1} (Fig. 2). Females of *Metridia lucens* exhibited a 43% lower AMR in depth of 110-80 m compared to females at the surface (t-test, $p = 0.06$).

Abundance of copepods for community carbon demands

Neritic (water depth <200 m) and oceanic (water depth >200 m) abundances of the analysed copepod species was measured to determine total carbon ingestion rates for copepod species communities (Table 2). For neritic abundances, three stations were enumerated, while seven stations were evaluated for the oceanic abundances. Abundance data were derived from two different years, but did not differ significantly between the years (station 23°S, 13°E; t-test, $p > 0.05$).

Near the coast down to a maximum depth of 200 m, *Calanoides carinatus* prevailed with a maximum abundance of 15,633 ind. m^{-2} for females, males and copepodids C5 combined. Females of *Metridia lucens* were the second most abundant group with 5,867 ind. m^{-2} in neritc waters, followed by females of *Rhincalanus nasutus* (271 ind. m^{-2}). All other copepod species showed a low abundance (<100 ind. m^{-2}) or did not occur at all at the neritic stations (Table 2).

At oceanic stations diapausing copepodids C5 of *C. carinatus* dominated with a maximum abundance of 18,839 ind. m^{-2}. Females of *Aetideopsis carinata* reached a maximum of 10,045 ind. m^{-2}, while females of *M. lucens* showed little differences between oceanic (5,060 ind. m^{-2}) and neritic abundances. Maxima of 500-1,200 ind. m^{-2} were determined for females of *R. nasutus* and *Pleuromamma robusta*, as well as for copepodids C5 and females of *Nannocalanus minor* (Table 2).

Carbon demands and ingestion rates

Individual oxygen consumption rates were converted to carbon units to express minimum food requirements for each species (Ikeda et al. 2000). Minimum individual carbon demands were determined for diapausing C5s of *Calanoides carinatus* (0.9 ± 0.3 µg C d^{-1}) and females of *Metridia lucens* (1.1 ± 0.4 µg C d^{-1}), while high individual carbon requirements >10 µg C d^{-1} were measured for females of *Eucalanus hyalinus*, *Rhincalanus nasutus*, *Euchirella* spp. and *Pleuromamma* spp., as well as for *Candacia bipinnata* and *Candacia ethiopica* (Table 1).

Ingestion rates were calculated via an energy budget approach and an allometric equation, except for diapausing copepodids C5 of *C. carinatus*, which do not feed.

Ingestion rates in µg C d^{-1} $ind.^{-1}$ are shown in Table 1 and daily ingestion rates in % d^{-1} are illustrated in Fig. 3.

Ingestion rates of all analysed copepods, as derived from an energy budget approach, averaged 10 ± 5% d^{-1}. Highest ingestion rates beyond 15% d^{-1} were determined for adults of *C. carinatus*, *Nannocalanus minor* and *C. bipinnata*. In contrast, low ingestion rates of <6% d^{-1} were measured for females of *R. nasutus*, *Euchirella similis* and *Aetideopsis carinata*. Maximum potential ingestion rates (I_{max}) averaged 54 ± 12% d^{-1} (Fig. 3). I_{max} ranged from ~40% d^{-1} in large *Euchirella* species (~1 mg DM) to 75 ± 2% d^{-1} in the small *M. lucens* (<100 µg DM) and was generally more than four times higher than the respective daily ingestion rate determined via the energy budget approach (Fig.3).

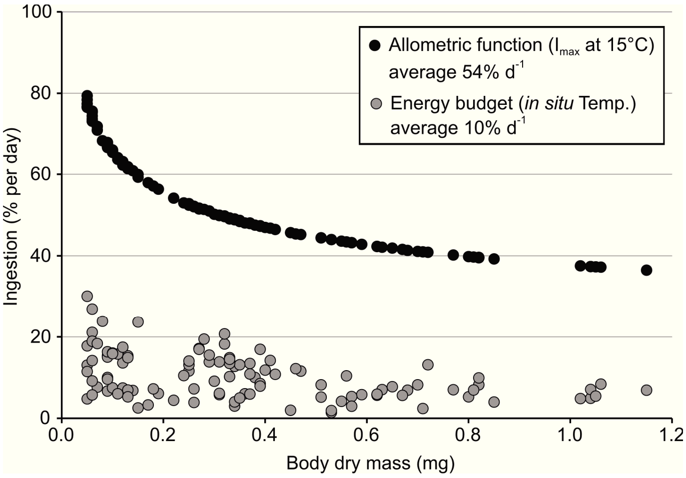

Figure 3: Results of the allometric approach (Q_{10} = 2, 15°C) and the energy budget approach (*in situ* temperature) converted to daily ingestion rates and plotted against the individual dry mass of analysed copepods. Diapausing C5 stage of *C. carinatus* is not included in this graph.

Total copepod community consumption in neritic and oceanic regions varied between the two approaches for the calculation of ingestion rates (Table 2). Applying the energy budget approach, total carbon ingestion yielded 78 mg C m^{-2} d^{-1} for neritic and 21 mg C m^{-2} d^{-1} for oceanic copepod communities. Maximum carbon ingestion calculated via I_{max} was higher with 273 mg C m^{-2} d^{-1} for neritic and 123 mg C m^{-2} d^{-1} for oceanic regions (Table 2).

Active copepodids C5 and females of *C. carinatus* contributed up to 83% to total carbon ingestion in neritic regions (energy budget, EB: 64 mg C m^{-2} d^{-1}, I_{max}: 191 mg C m^{-2} d^{-1}). *M. lucens* and *R. nasutus* females consumed much less carbon (6-52 mg C m^{-2} d^{-1}). The other copepod species did not occur at all or only in very low abundances at the neritic stations and, thus, their carbon consumption was negligible. The oceanic calanoid copepod community showed much lower total carbon consumption compared to the neritic community. Females of *A. carinata* exhibited the highest consumption, followed by *M. lucens*, *Pleuromamma robusta*, the two eucalanids *E. hyalinus* and *R. nasutus* and *N. minor* (Table 2).

Table 2: Neritic (<200 m) and oceanic (>200 m) abundances (individuals per m^2) and population demands (median abundance x ingestion rate) of 16 calanoid copepod species from the northern Benguela system. f = female, m = male, C5 = copepodite stage 5, act = active, dia = diapause, nd = not determined, EB = energy budget approach, I_{max}= allometric function.

Species	Stage	Abundance (ind. m^{-2})				Population demand			
		neritic		oceanic		neritic		oceanic	
		max.	median	max.	median	(mg C m^{-2} d^{-1})		(mg C m^{-2} d^{-1})	
Calanidae						EB	I_{max}	EB	I_{max}
C. carinatus	f	2240	1078	158	0	15.5	40.7	0	0
	m	737	114	17	0	0.9	4.5	0	0
	C5 act	12656	4808	324	25	48.1	150.0	0.3	0.8
	C5 dia	0	0	18839	3636	-	-	-	-
N. minor	f	10	0	762	92	0	0	0.7	3.2
	m	0	0	163	26	0	0	0.2	0.6
	C5	0	0	898	86	0	0	0.5	2.1
N. robustior	f	0	0	83	6	0	0	0.1	0.6
Eucalanidae									
E. hyalinus	f	16	16	383	87	0.4	2.6	2.3	14.2
	C5	nd	nd	nd	nd	nd	nd	nd	nd
R. nasutus	f	271	176	522	116	5.6	21.7	3.7	14.3
P. sewelli	f	0	0	14	0	0	0	0	0
Aetideidae									
A. carinata	f	80	0	10045	878	0	0	5.7	47.0
E. rostrata	f	nd	nd	nd	nd	nd	nd	nd	nd
E. similis	f	0	0	20	0	0	0	0	0
E. splendens	f	nd	nd	nd	nd	nd	nd	nd	nd
Metridinidae									
M. lucens	f	5867	2176	5060	1100	6.7	51.8	3.4	26.2
P. abdominalis	f	0	0	122	0	0	0	0	0
P. robusta	f	6	0	1154	169	0	0	4.1	14.1
P. xiphias	f	0	0	27	0	0	0	0	0
Candaciidae									
Candacia spp.	adult	400	18	486	0	0.5	1.6	0	0
all copepods						**78**	**273**	**21**	**123**

DISCUSSION

Physiological effects on respiration rates

Respiration rates of organisms reflect their metabolic activities and serve to trace energy pathways and carbon fluxes (Ikeda 1985, e.g. Hernández-León & Ikeda 2005). Metabolic rates in aquatic animals are influenced by physiological factors such as feeding mode, activity level (e.g. diapause), migration, body mass, and developmental stage as well as by abiotic parameters such as ambient temperature and oxygen partial pressure (Hirche 1987, Hernández-León & Ikeda 2005, Ikeda et al. 2006, Seibel 2011). However, up to 95% of variability in individual respiration rates of zooplankton are in fact due to differences in temperature and body mass (Ikeda 1985, Ikeda et al. 2001).

Regression analysis in this study revealed that 72% and 40% of variations in individual and mass-specific respiration rates, respectively, can be explained by body mass and temperature. Therefore, mass-specific respiration rates were adjusted (Thuesen et al. 1998, Ikeda et al. 2007) to a mean copepod body mass and a temperature regime typical of the northern Benguela upwelling system to compare respiration rates without the influence of these two parameters. The scaling coefficient of -0.24 determined in this study for subtropical calanoid copepods fits well in the range of scaling coefficients (-0.1 to -0.4) published for marine zooplankton, which is usually close to -0.25 (Conover 1960, Ivleva 1980, Moloney & Field 1989).

Intraspecific differences in adjusted metabolic rates (AMR) were determined for *Calanoides carinatus*, *Rhincalanus nasutus* and *Metridia lucens.* All three species exhibited deviating metabolic activities within a stage. *C. carinatus* is well known to enter diapause at depth with an extremely reduced metabolism (Arashkevich & Drits 1997, Auel et al. 2005), which is reflected in the very low AMR of copepodids C5 below 400 m. Their metabolic activity was about 72% lower than the activity of surface-dwelling C5s. Metabolic reduction in diapausing C5s of *C. carinatus* without compensation for habitat temperature and body mass is at least 10% higher (this study, Arashkevich & Drits 1997, Auel et al. 2005).

In contrast to *C. carinatus*, *R. nasutus* is assumed to enter a resting stage as adult females (Ohman et al. 1998, Schnack-Schiel et al. 2008). There are indications of dormancy, e.g. high wax ester levels of non-feeding, non-reproducing females in mid-water layers (Ohman et al. 1998, Schnack-Schiel et al. 2008). Females of *R. nasutus* found in mid-water layers in this study exhibited an adjusted metabolic reduction of 62% compared to females in surface layers, supporting the hypothesis of dormancy in deeper-dwelling females. However, metabolic depression during *R. nasutus* dormancy is less pronounced than during diapause in *C. carinatus*. Resting stages of *R. nasutus* showed an almost 1.5 times higher metabolic activity compared to diapausing C5s of *C. carinatus*.

According to Weikert (1980), *R. nasutus* survives long periods of starvation by retreating into the oxygen minimum zone, and thus reducing its metabolism. Our data strongly support this observation. Females with high metabolic activities originated from the upper 30 m, whereas females with reduced activities were mainly found between 80 m and 110 m, where the oxygen minimum zone (OMZ) was located (~70-130 m, CTD data).

M. lucens is not known to enter dormant stages as *C. carinatus* and *R. nasutus* but this species performs diel vertical migrations and has been observed to migrate trough the OMZ in the Benguela Current (Timonin 1997). The reduced respiration rates (43%) of female *M. lucens* in the OMZ compared to females above the OMZ shows the ability of *M. lucens* to regulate its metabolic rate during its vertical migrations.

Metabolic demands and grazing impact of calanoid copepods

Copepods are major components of marine pelagic ecosystems and play an essential role in the cycling of organic matter in the ocean. Therefore, understanding the metabolism of copepods is of vital importance to estimate their contribution to oceanic biogeochemical cycles.

We consider metabolic activities measured in this study as representative for routine metabolism because animals showed natural mobility and behaviour in the respiration vials but were not fed during experiments. Respiration rates of non-feeding copepods are known to result in significantly lower metabolic rates as compared to feeding animals (Ikeda 1977, Ikeda et al. 2000, Almeda et al. 2011). Thus, our metabolic rates derived from respiration measurements with filtered seawater are likely to be lower than natural metabolic rates of copepods in the field. Taking this underestimation into account, maximum respiration rates were used for further calculations (e.g. population demands).

Individual respiration rates of copepods at *in situ* temperatures ranged from 2.1 to 42.1 µl O_2 d^{-1} $ind.^{-1}$ and are in good accordance with published data for the same species (Raymont 1959, King & Packard 1975, Arashkevich & Drits 1997, Ikeda et al. 2001, Auel et al. 2005, Ikeda et al. 2007). Individual ingestion rates derived from an energy budget approach agree well with published data of copepod ingestion rates from the southern Benguela Current and the Humboldt Current (Dagg et al. 1980, Peterson 1989, Vargas & Gonzalez 2004).

For the calculation of community carbon ingestion, two independent approaches were applied; (i) respiration rates were converted to ingestion via an energy budget approach, and (ii) maximum ingestion rates (I_{max}) were estimated based on an allometric equation. I_{max} overestimated the respective data derived from the energy budget approach by more than 50%. The allometric approach approximated maximum potential ingestion rates under optimum conditions (Moloney & Field 1989, Gradinger et al. 1999). Hence, *in situ* measurements should generally reveal lower ingestion rates. We suggest that the energy budget approach is the more reliable approximation with a total consumption of 78 mg C m^{-2} d^{-1} in neritic regions and 21 mg C m^{-2} d^{-1} in oceanic regions. Thus, the following estimates of grazing pressure on the ambient phytoplankton are discussed based on these results.

Calanoides carinatus and *Nannocalanus minor* are predominantly herbivorous, whereas all other copepod species covered in this study are omnivorous to carnivorous (e.g. Loick et al. 2005, Schukat et al. submitted). Therefore, the grazing pressure upon primary production was determined for *C. carinatus* and *N. minor*. Active copepodids C5 at the surface together with adults of *C. carinatus* comprised 83% of total neritic copepod consumption. In contrast, C5s and adults of *N. minor*, which occur further offshore, accounted for only 6% of total oceanic copepod ingestion.

Phytoplankton biomass and primary production in the northern Benguela system are highly variable as consequence of local and temporal upwelling events (Estrada & Marrasé 1987, Wasmund et al. 2005, Heileman & O'Toole 2008, Barlow et al. 2009). Phytoplankton biomass was determined at 25 stations (8 neritic, 17 oceanic) during the same expedition in September/October 2010 and ranged from 24 to 10,186 mg C m^{-2} for neritic regions and 62 to 2,882 mg C m^{-2} for oceanic regions (Wasmund, unpublished data). Daily consumption of phytoplankton biomass by active copepodids C5 and adult *C. carinatus* ranged between 0.1 and 30% for different stations in the northern Benguela system, whereas C5s and adults of *N. minor* consumed 0.1 to 16% of daily phytoplankton biomass. Published data for primary production in the northern Benguela are rather scarce compared to the southern Benguela; estimates range from 0.1 to 6.7 g C m^{-2} d^{-1} for different seasons and regions (Estrada & Marrasé 1987, Wasmund et al. 2005, Barlow et al. 2009). For the onshore (<500 m water depth) area of the northern Benguela a long-term average primary production rate of 1.2 g C m^{-2} d^{-1} is calculated (Brown et al. 1991). Adults and active C5 stages of *C. carinatus* consumed 64.5 mg C m^{-2} d^{-1} in neritic regions. Using the average value of 1.2 g C m^{-2} d^{-1} for primary production, *C. carinatus* would consume 5.4% of primary production, only considering C5s, females and males.

The other herbivorous species, *N. minor* consumed less carbon with 1.4 mg C m^{-2} d^{-1} in offshore areas. No comparable data are available for primary production of offshore regions of the northern Benguela system. However, even at a low primary production rate of 0.1 g C m^{-2} d^{-1}, *N. minor* would only consume about 1% of primary production in offshore areas.

As compared to our study, Hansen et al. (2005) and Verheye et al. (2005) reported much higher abundances of *C. carinatus* of about 100-170 x 10^3 ind. m^{-2} for the northern Benguela system. These high abundances indicate that locally *C. carinatus* could consume up to 27% of daily primary production at maximum primary production levels (e.g. 6.7 g C m^{-2} d^{-1}). These results imply that *C. carinatus* is an important primary consumer in neritic waters while the impact of *N. minor* on primary production in offshore areas is clearly smaller.

Several studies have shown that migrating copepods enhance carbon flux from the euphotic zone to deeper water layers, since they consume small particulate material in surface waters and respire and produce faecal pellets at depth, or they are eaten by deeper-living carnivores (Longhurst et al. 1990, Steinberg et al. 2008, Wilson & Steinberg 2010). The two most abundant species in this study, *C. carinatus* and *M. lucens,* are either ontogenetic or diel vertical migrants and thus may contribute substantially to the export of carbon from the productive upwelling shelf to deeper layers and/or to the adjacent oligotrophic open ocean. Robust empirical data on energy requirements and carbon consumption of calanoid copepods, such as presented in this study, are fundamental for the development of realistic carbon budgets and food-web models of the northern Benguela upwelling region and coastal upwelling systems in general.

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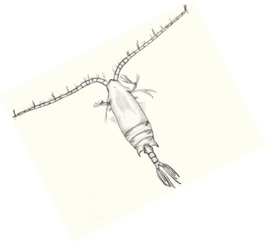
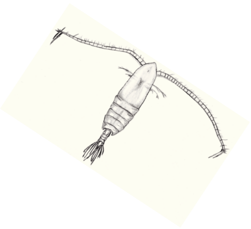
CHAPTER III





PREDICTING METABOLIC RATES OF CALANOID COPEPODS

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Predicting metabolic rates of calanoid copepods

Maya Bode, Anna Schukat, Holger Auel and Wilhelm Hagen

ABSTRACT

Respiration rates and electron transport system (ETS) activities were measured in dominant copepod species from the northern Benguela upwelling system in January-February 2011 to assess the accuracy of the ETS assay in predicting *in vivo* respiration rates. Individual respiration rates varied from 0.06 to 1.60 µL O_2 h^{-1} ind^{-1}, while ETS activities converted to oxygen consumption ranged from 0.14 to 4.46 µL O_2 h^{-1} ind^{-1}. Respiration rates were significantly correlated with ETS activities ($r^2 = 0.8$, $p = 0.0001$), with ratios between respiration and ETS activities of 0.11 to 0.76 for different species. Up to 69% and 90% of the variance of respiration rates and ETS activities, respectively, could be explained by differences in body mass and temperature. Therefore, two regression models applying respiration rates and ETS activities were developed to parameterize metabolic rates of calanoid copepods based on body mass and ambient temperature. Metabolic rates could be expressed as a function of these two easily measurable parameters, while ETS activity was much more precisely predictable due to its much lower variance. Such predictive mathematical models may contribute to assess the impact of copepods on nutrient and carbon fluxes in marine ecosystems.

KEYWORDS

respiration rate, ETS activity, allometric modelling, zooplankton, Benguela Current, coastal upwelling

INTRODUCTION

Coastal upwelling systems such as the Benguela Current are characterized by special features concerning their bathymetry, hydrography, trophic relationships and productivity. Together with the Humboldt Current, the Benguela Current is the most productive upwelling system in the world reaching an average annual primary production of 394 g C m^{-2} yr^{-1} (Heilemann & O'Toole 2008). With a proportion of 55-95%, copepods are the most abundant mesozooplankton components throughout the world's oceans (Longhurst 1985) and thus represent an important linkage between primary producers and higher trophic levels. Moreover, they play an important role within the biological carbon pump (Longhurst & Harrison 1989). Through diel and ontogenetic vertical migrations copepods enhance vertical flux of organic matter and dissolved inorganic carbon from the euphotic zone to deeper layers (Longhurst et al. 1990). Annual copepod production for the Angola-Benguela frontal region and the southern Benguela upwelling system was estimated at 39 g C m^{-2} yr^{-1} and 99 g C m^{-2} yr^{-1}, respectively (Richardson et al. 2001, Verheye et al. 2005). However, few data on copepod production exist for the northern Benguela ecosystem (Verheye et al. 2005).

Due to their key role in the cycling of organic matter, predicting copepod metabolism may be a useful tool to estimate their contribution to marine biogeochemical cycles in future, especially under changing environmental conditions. Physiological processes such as ingestion rates are difficult to assess in zooplankton, because knowledge about their natural food composition is limited and experimental approaches are very time-consuming (Ikeda 1985). In contrast, certain metabolic measurements are much more feasible and cost-efficient. On the one hand, direct measurements of oxygen consumption of copepods in a controlled system provide a robust estimate of the organisms' metabolic demand and energy expenditure (Ikeda 1985, Brown et al. 2004). On the other hand, in vivo respiration rates are physiologically closely coupled to the activity of the electron transport system (ETS). Therefore, the ETS assay was introduced as a biochemical proxy for respiration rates of zooplankton specimens (Packard 1971, Owens & King 1975). Since the response of the ETS to changes in environmental factors is much slower and often delayed by 1-3 days compared to respiration rates (Båmstedt 1980), the ETS assay circumvents additional problems such as long incubation times and capture stress to the animals (Schalk 1988). However, large intra- and interspecific variations in the ratios of respiration rates to ETS activities have been recorded, which questioned the predictive accuracy of ETS concerning population or community metabolism (King & Packard 1975, Båmstedt 1980, Ikeda et al, 2006).

Different ecological and physiological factors have been identified to influence copepod metabolism, e.g. body mass and temperature (Ikeda 1985, Ikeda et al. 2001, Ivleva 1980), depth of occurrence (Ikeda et al. 2006, 2007), oxygen concentration (Ikeda et al. 2007), regional and seasonal variability, e.g. food availability and feeding behaviour (Conover 1959, 1960), general activity, e.g. dormancy and different maturity levels (Arashkevich & Drits 1997, Pfaffenhöfer 2006) and diel vertical migrations (Pavlova 1994). Since body mass and temperature have been identified as the most important influencing factors, their combined effect on metabolic rates may be used to model and predict metabolic rates in order to assess carbon and energy flows in marine ecosystems (Ikeda 1985, Ikeda et al. 2001). In this study, respiration rates and ETS activities of dominant copepod species from the epipelagic and upper mesopelagic zones in the northern Benguela upwelling system were analyzed. Their correlation was determined to assess the precision of the ETS assay to estimate *in vivo* respiration, and thus metabolic rates of copepods. To establish generalized relationships of metabolic rates as a function of body mass and temperature, two regression models of respiration and ETS activity were developed and tested in order to provide a simplified approach for determining metabolic rates of copepods based on easily measurable parameters such as body mass and temperature.

MATERIAL AND METHODS

Sampling

Samples were collected during the GENUS (Geochemistry and Ecology of the Namibian Upwelling System) research cruise on board of RV Maria S. Merian in January and February 2011. Copepods were collected in the northern Benguela upwelling system along two cross-shelf transects at 23°00’S and 26°40’S and at various stations in between these transects (Fig. 1). Sampling was carried out using double (18 nets) and single (9 nets) Multiple Opening/Closing Net Environmental Sensing Systems (MOCNESS, 1 m^2 mouth opening, mesh sizes 333 µm) and vertical or oblique Multinet hauls with 300 to 500 µm mesh sizes (Hydrobios Multinet Midi: 5 nets, 0.25 m^2 mouth opening). Specimens were collected in discrete depth layers between 25 m and 800 m (Table 1). Sorting was carried out rapidly but gently and only apparently healthy individuals were selected for experiments. Copepod species and stages were identified under a dissecting microscope. One subsample of the individuals was used for onboard respiration measurements, while the other subsample was shock-frozen at -80°C for subsequent ETS measurements in the home laboratory.

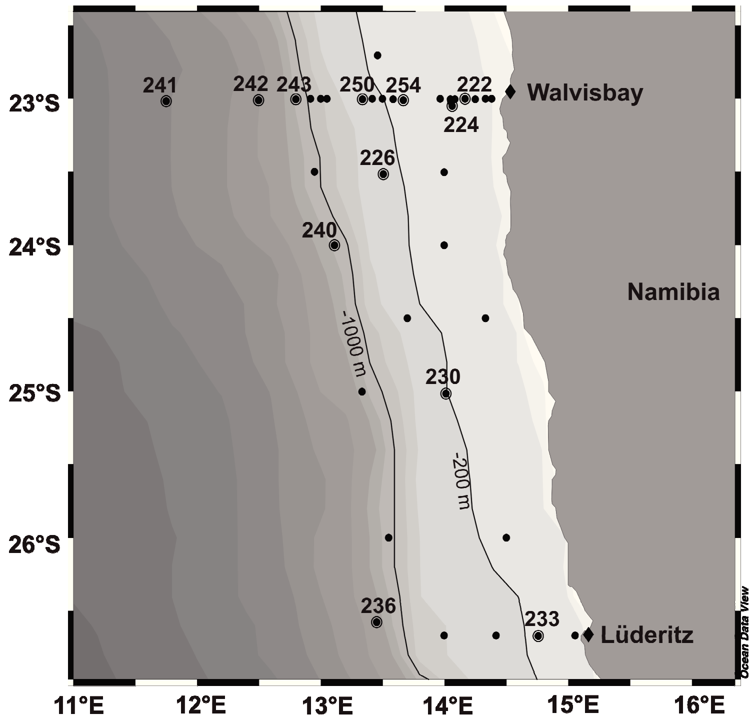

Figure 1: Station map. All stations of the first leg of the GENUS research cruise on the RV Maria S. Merian off the Namibian coast in January and February 2011 are shown. The stations where copepods were caught for this study are encircled and marked with their station number.

Respiration

Copepods were transferred into gas-tight glass bottles containing 13 ml of UV treated, 0.2 µm filtered and oxygenated seawater. For larger copepods such as adult females of

Gaetanus pileatus, *Eucalanus hyalinus*, *Rhincalanus nasutus*, *Euchirella rostrata*, *Pleuromamma xiphias*, *Pleuromamma quadrungulata* and *Pleuromamma robusta*, one individual was used per bottle. In case of smaller body size, several individuals were incubated together (2-3 individuals of females of *Calanoides carinatus*, *Candacia cheirura* and copepodids CIV of *R. nasutus*; 4-6 individuals of females of *Nannocalanus minor*, *Metridia lucens*, *Aetideus armatus* and copepodids CII/III of *R. nasutus*; 9-11 individuals of diapausing copepodids CV of *C. carinatus*). The oxygen consumption was measured using a 10-Channel Fiber-Optic Oxygen Meter (OXY-10, PreSens, Precision Sensing GmbH). An oxygen-sensitive foil was glued to the inside wall of the incubation bottles to detect the decrease in dissolved oxygen concentrations non-invasively and non-destructively by oxygen minisensors from the outside. Prior to the measurements, the oxygen meter was calibrated according to the OXY-10 instruction manual (see also Köster et al. 2008). In each experimental run, two bottles without copepods were monitored as controls to correct for unspecific microbial respiration. At least four replicates were measured for each species and stage. The bottles were connected to the sensors and kept in a water bath in a temperature-controlled refrigerator. Experiments were conducted at simulated *in situ* temperatures and at lower and higher temperatures to detect the dependency of respiration rates on temperature (*in situ* temperatures ± 5°C). The decrease in oxygen concentration in each bottle was measured every 15 s. After each experiment, copepods were frozen at -80°C for subsequent determination of dry mass (DM) using a Sartorius Microbalance (Sartorius, NCII1S, precision ± 10 µg) after lyophilization for 48 h (see also Auel et al. 2005).

Respiratory Electron Transport System Activity

ETS activities of nine copepod species were measured applying the method described by Packard (1971) and modified by Owens & King (1975). The method was adjusted to the species used in this study. The amount of polyvinylpyrrolidone (PVP) in the homogenizing buffer was reduced from 1.5 mg ml^{-1} to 0.5 mg ml^{-1}, pH 8.1. Substrate solution contained 1.3 mM NADH, 0.05 mM NADPH, and 1 mM succinate, pH 8.1, while INT solution comprised 2.5 mM 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride (INT), pH 7.7. Prior to each experiment, the wet mass (WM) of the copepod specimens was determined, so that the crude homogenate contained 1 mg wet mass per ml homogenizing buffer. The homogenate was centrifuged for 10 min at 4700 g at 0-4°C. The final reaction mixture of 5 ml was reduced to 1 ml: 600 µL substrate solution and 200 µL INT solution were incubated with 200 µL supernatant (3:1:1) in a 2 ml plastic cup, while stirred in a thermomixer (Eppendorf comfort) at respective *in situ* temperatures. No quench solution was used. Absorption was measured at 490 nm directly after a species-specific incubation time using a temperature-controlled photometer (Kontron Instruments, UVIKON 941) and distilled water as reference. In order to receive maximum ETS activities, incubation times were determined for each species: 20 min for copepodids CV of *C. carinatus*, 40 min for females of *C. carinatus*, *N. minor*, *A. armatus* and *M. lucens*, 50 min for females of *E. hyalinus*, *R. nasutus* and copepodids CIV of *R. nasutus*, 70 min for females of *Pleuromamma* spp. Each sample was measured in four replicates and corrected by a sample blank (600 µL phosphate buffer, 200 µL INT solution, 200 µL supernatant) and substrate blank (600 µL substrate solution, 200 µL INT solution, 200 µL homogenizing buffer).

Regression Model

Multiple regression technique was applied to analyze the dependency of either respiration rate ($Y = \mu L\ O_2\ h^{-1}\ ind^{-1}$, $\mu L\ O_2\ h^{-1}\ mg\ DM^{-1}$) or ETS activity ($Y = \mu L\ O_2\ h^{-1}\ ind^{-1}$, $\mu L\ O_2\ h^{-1}\ mg\ WM^{-1}$) on body mass ($X_1$ = mg DM or WM, respectively) and temperature (X_2 = °C). Allometric relationships between metabolic rate and body mass are usually expressed as $Y = a\ W^b$ or $\ln Y = \ln a + b \ln W$, where Y is the metabolic rate, W body mass, a the normalization constant and b the scaling coefficient (Hochachka & Somero 2002). The scaling coefficient is independent of temperature (Vidal & Whitledge 1982) (Fig. 3). To obtain the best fit for respiration rates all measurements at all experimental temperatures were added into the model. The following regression model was applied with a_0, a_1 and a_2 as new constants (Ikeda et al. 2001):

$$\text{Ln } Y = a_0 + a_1 \ln X_1 + a_2 X_2$$

RESULTS

Individual and mass-specific respiration rates (R) and ETS activities are presented in Table 1 along with body mass and R:ETS ratios. To exclude temperature effects from body mass effects copepods were divided into two groups, specimens collected from above 400 m (measured at 13-16°C) and from below 400 m (measured at 5-10°C) (Fig. 2).

Table 1: Individual and mass-specific respiration rates and ETS activities of dominant copepod species (adult females and copepodite stages). Incubation temperature (T) and sampling depth are presented. Dry mass is given for copepods used for respiration experiments, while wet mass is given for individuals used for ETS measurements. Data are expressed as mean values with standard deviations and number of experiments in parentheses (n). R:ETS ratios are based on respiration and ETS activities per individual. The letter behind each species identifies its location in Fig. 3.

Species	Stage	Depth [m]	T [°C]	Dry mass [mg]	Respiration rate $[\mu L\ O_2\ h^{-1}\ mg_{DM}^{-1}]$	$[\mu L\ O_2\ h^{-1}\ ind^{-1}]$
Eucalanus hyalinus (a)	f	50-25	16	0.862 ± 0.153	1.73 ± 0.54	1.60 ± 0.46 (4)
Candacia cheirura	f	100-50	15	0.112 ± 0.026	2.84 ± 0.21	0.34 ± 0.04 (3)
Aetideus armatus (b)	f	100-50	15	0.084 ± 0.004	3.52 ± 0.41	0.30 ± 0.03 (4)
Nannocalanus minor (c)	f	100-50	15	0.096 ± 0.007	2.67 ± 0.93	0.26 ± 0.10 (4)
Calanoides carinatus (d)	f	75-50	13	0.098 ± 0.007	3.26 ± 1.33	0.32 ± 0.14 (4)
Rhincalanus nasutus (e)	f	90-40	13	0.364 ± 0.110	0.81 ± 0.56	0.29 ± 0.24 (5)
Metridia lucens (f)	f	100-50	13	0.056 ± 0.009	3.14 ± 0.59	0.17 ± 0.02 (4)
Rhincalanus nasutus (g)	CIV	50-25	13	0.076 ± 0.010	1.01 ± 0.36	0.08 ± 0.02 (4)
Rhincalanus nasutus	CII/III	50-25	13	0.027 ± 0.013	2.15 ± 0.45	0.06 ± 0.03 (4)
Euchirella rostrata	f	400-200	10	0.479 ± 0.052	0.71 ± 0.13	0.34 ± 0.03 (4)
Pleuromamma robusta (h)	f	600-400	8	0.427 ± 0.152	1.51 ± 0.36	0.72 ± 0.36 (10)
Eucalanus hyalinus (i)	f	400-300	8	1.051 ± 0.192	0.37 ± 0.29	0.40 ± 0.33 (4)
Calanoides carinatus (j)	CV	600-400	8	0.125 ± 0.028	0.95 ± 0.26	0.11 ± 0.03 (8)
Gaetanus pileatus	f	600-400	7	1.351 ± 0.293	0.78 ± 0.11	1.03 ± 0.14 (3)
Pleuromamma xiphias (k)	f	600-400	7	0.644 ± 0.033	1.06 ± 0.23	0.68 ± 0.13 (8)
Pleuromamma quadrungulata (l)	f	800-600	5	0.508 ± 0.048	1.10 ± 0.14	0.55 ± 0.04 (5)

Species	Stage	Wet mass [mg]	T [°C]	ETS activity $[\mu L\ O_2\ h^{-1}\ mg_{WM}^{-1}]$	$[\mu L\ O_2\ h^{-1}\ ind^{-1}]$	R:ETS
Eucalanus hyalinus (a)	f	7.240 ± 2.244	16	0.63 ± 0.09	4.46 ± 1.183 (6)	0.36
Aetideus armatus (b)	f	0.420 ± 0.032	15	2.80 ± 0.19	1.17 ± 0.09 (6)	0.25
Nannocalanus minor (c)	f	0.454 ± 0.031	15	2.12 ± 0.10	1.12 ± 0.09 (6)	0.23
Calanoides carinatus (d)	f	0.541 ± 0.075	13	1.94 ± 0.09	1.05 ± 0.18 (5)	0.31
Rhincalanus nasutus (e)	f	2.032 ± 0.452	13	0.97 ± 0.23	1.91 ± 0.36 (9)	0.15
Metridia lucens (f)	f	0.366 ± 0.034	13	1.52 ± 0.04	0.56 ± 0.04 (3)	0.31
Rhincalanus nasutus (g)	CIV	0.648 ± 0.191	13	1.14 ± 0.14	0.72 ± 0.17 (6)	0.11
Pleuromamma robusta (h)	f	2.714 ± 0.731	8	0.71 ± 0.09	1.94 ± 0.65 (6)	0.37
Eucalanus hyalinus (i)	f	8.965 ± 1.482	8	0.32 ± 0.03	2.86 ± 0.49 (6)	0.14
Calanoides carinatus (j)	CV	0.454 ± 0.031	8	0.31 ± 0.06	0.14 ± 0.04 (6)	0.76
Pleuromamma xiphias (k)	f	4.143 ± 0.539	7	0.47 ± 0.11	1.92 ± 0.41 (5)	0.35
Pleuromamma quadrungulata (l)	f	3.824 ± 0.575	5	0.47 ± 0.05	1.778± 0.25 (6)	0.31

Between the groups in each graph, slopes of the regression curves were not significantly different ($p = 0.313$). However, Y intercepts differed significantly from each other due to generally higher metabolic rates at higher temperatures ($p < 0.0001$) (Fig. 2). As expected, individual respiration rates and individual ETS activities showed a significant positive correlation with body mass (p values < 0.0001), whereas mass-specific respiration rates and mass-specific ETS activities significantly decreased with increasing body mass (p values < 0.0001).

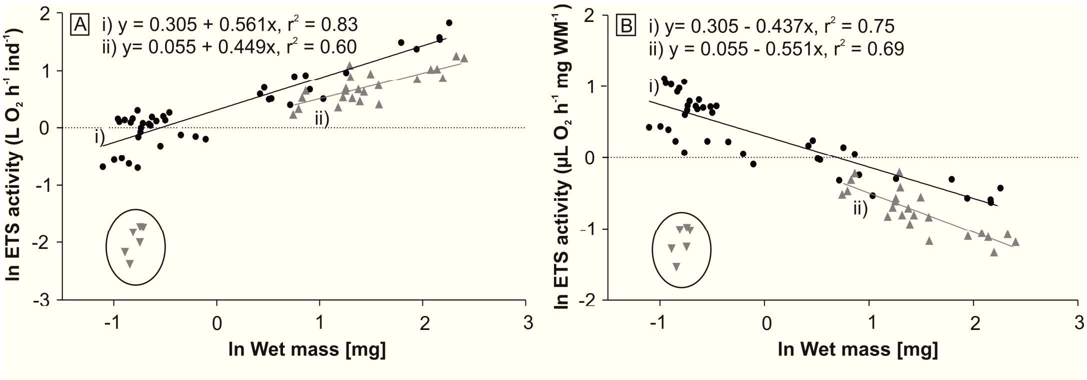

Figure 2: Relationship between a) individual and b) mass-specific ETS activity and wet mass categorized in specimens from above 400 m measured at 13-16°C (i, black dots) and from deeper than 400 m measured at 5-8°C (ii, grey triangles). The conspicuously lower ETS activities of copepodids CV of *C. carinatus* (encircled) were not included in the calculations. Respective equations of the regression lines are presented (p-values < 0.0001). In each graph slopes are not significantly different (p-values = 0.313), while intercepts differ significantly from each other (p-values < 0.0001).

In copepods from depths shallower than 400 m individual respiration rates varied from 0.06 ± 0.03 µL O_2 h^{-1} ind^{-1} in the smallest specimens, copepodids CII/III of *Rhincalanus nasutus* with 0.027 ± 0.013 mg dry mass (DM), to 1.6 ± 0.5 µL O_2 h^{-1} ind^{-1} in *Eucalanus hyalinus* (0.862 ± 0.153 mg DM), the largest copepod in this group. Copepods caught deeper than 400 m exhibited individual respiration rates from 0.11 ± 0.03 µL O_2 h^{-1} ind^{-1} in diapausing copepodids CV of *Calanoides carinatus* (0.125 ± 0.028 mg DM), the smallest specimens in this group, to 1.03 ± 0.14 µL O_2 h^{-1} ind^{-1} in the largest copepod *Gaetanus pileatus* (1.35 ± 0.29 mg DM). The lowest mass-specific respiration rate of 0.81 ± 0.56 µL O_2 h^{-1} mg DM^{-1} in copepods from depths shallower than 400 m were measured in females of *R. nasutus* (0.36 ± 0.11 mg DM), while *A.*

armatus (0.084 ± 0.004 mg DM) showed the highest mass-specific respiration rate of 3.5 ± 0.4 µL O_2 h^{-1} mg DM^{-1}. In copepods from below 400 m mass-specific respiration rates varied from 0.78 ± 0.11 µL O_2 h^{-1} mg DM^{-1} in *G. pileatus* to 1.51 ± 0.36 µL O_2 h^{-1} mg DM^{-1} in *P. robusta,* the second smallest specimens in this group after copepodids CV of *C. carinatus*.

Individual ETS activities of copepods collected between the surface and 400 m varied from 0.56 ± 0.04 µL O_2 h^{-1} ind^{-1} in *Metridia lucens* , the smallest adult copepod with 0.366 ± 0.034 mg wet mass (WM), to 4.46 ± 1.18 µL O_2 h^{-1} ind^{-1} in *E. hyalinus* (7.240 ± 2.244 mg WM). Copepods sampled below 400 m exhibited individual ETS activities between 0.14 ± 0.04 µL O_2 h^{-1} ind^{-1} in copepodids CV of *C. carinatus* (0.454 ± 0.031 mg WM) and 2.86 ± 0.49 µL O_2 h^{-1} ind^{-1} in *E. hyalinus* (8.97 ± 1.48 mg WM). Mass-specific ETS activities in copepods caught above 400 m ranged from 0.63 ± 0.09 µL O_2 h^{-1} mg DM^{-1} in *E. hyalinus* to 2.8 ± 0.2 µL O_2 h^{-1} mg DM^{-1} in *A. armatus*, while in copepods from below 400 m ETS activities varied from 0.31 ± 0.06 µL O_2 h^{-1} mg DM^{-1} in copepodids CV of *C. carinatus* to 0.71 ± 0.09 µL O_2 h^{-1} mg DM^{-1} in *P. robusta*.

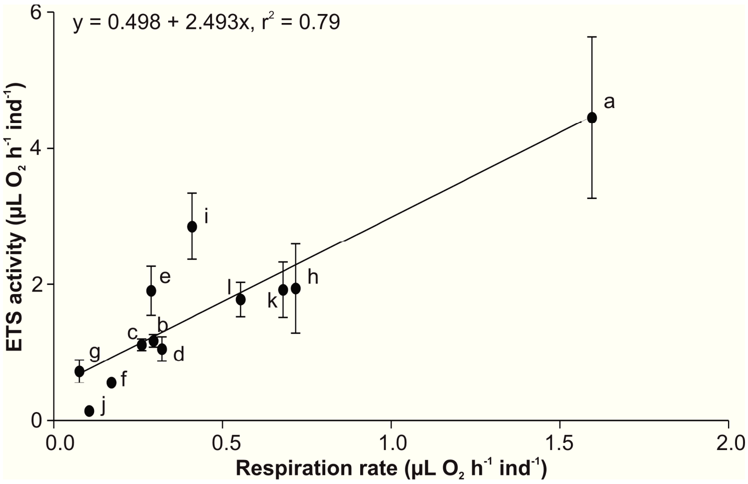

Figure 3: Relationship between individual ETS activities and individual respiration rates. Mean ETS activities with standard deviations are plotted against mean respiration rates. The letter besides each point identifies the associated species in Table 1. The equation of the regression line is shown (p-value = 0.0001).

The correlation of individual ETS activities and individual respiration rates was significantly positive (p = 0.0001) resulting in a coefficient of determination (r^2) of 0.8

(Fig. 3). ETS activities of females of *R. nasutus* and *E. hyalinus* lied conspicuously above the regression line, while they showed the lowest ratios of respiration rate to ETS activity (R:ETS) between 0.11 and 0.15 (Table 1). In contrast, ETS activity levels of diapausing copepodids CV of *C. carinatus* were located below the regression line, with the highest R:ETS ratio of 0.76 (Fig. 3). All other ratios varied from 0.23 in *Nannocalanus minor* to 0.37 in *P. robusta* (Table 1).

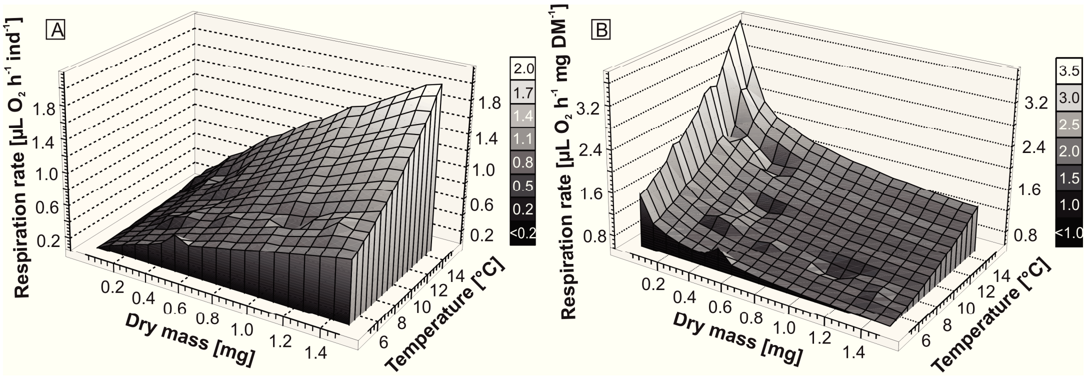

Figure 4: A) Individual and B) mass-specific ETS activities plotted against wet mass and temperature. The mean values of measured respiration rates at ambient temperatures were added to the model to demonstrate their deviation from calculated values. The most pronounced peaks belong to species of *Pleuromamma* and *G. pileatus*, while the deepest valleys represent *R. nasutus*, *E. rostrata* and deep-living *E. hyalinus*. For regression statistics see Table 2.

Results of the multiple regression analyses are listed in Table 2. Respiration rates were calculated from multiple regression equations and are illustrated in a three-dimensional plot in Fig. 4. The means of measured respiration rates at ambient temperatures were added to the model, which occur as peaks and valleys in the graph (Fig. 4). Copepodids CV of *C. carinatus* were excluded from the regression model, since diapausing animals are expected to act differently than active ones. They showed strongly reduced respiration rates and even more severely reduced ETS activities (Table 1, Fig. 2). The effect of body mass (a_1) and temperature (a_2) on metabolic rates was both significant: Regression coefficients were significantly different from zero (p values < 0.0001). 69% of the variance of individual respiration rates was explainable by dry mass and temperature ($r = 0.82$, $r^2 = 0.69$), while for mass-specific respiration rates 51% of the variance could be explained by the two parameters ($r = 0.72$, $r^2 =$

0.51). The scaling coefficient b derived from the allometric equation $Y = a\ W^{b}$ was 0.746 for individual respiration rates and -0.255 for mass-specific respiration rates. The following equations were derived, where R_i is the individual respiration rate, R_m the mass-specific respiration rate, X_1 copepod dry mass in mg and X_2 temperature in °C:

1) $\text{Ln } R_i$ (µL O_2 h^{-1} ind^{-1}) = $-0.989 + 0.746 \ln(X_1) + 0.096\ X_2$

2) $\text{Ln } R_m$ (µL O_2 h^{-1} mg DM^{-1}) = $-0.989 -0.255 \ln(X_1) + 0.096\ X_2$

Table 2: Regression statistics of metabolic rates (respiration rate (R), ETS activity (E)) on body mass (X_1) and temperature (X_2). Body mass is expressed as dry mass (DM) for respiration rates and wet mass (WM) for ETS activities. The number of specimens (N) is given. Regression coefficients a_1 and a_2 are significantly different from zero ($p < 0.0001$).

	Metabolic rate	N	Log (R, E) = $a_0 + a_1 \log X_1 + a_2 X_2$			R	R^2	p-value a_1	p-value a_2
			a_0	a_1	a_2				
Respiration:	R_i [µL O_2 h^{-1} ind^{-1}]	127	-0.989	0.746	0.096	0.82	0.69	< 0.0001	< 0.0001
	R_m [µL O_2 h^{-1} mg DM^{-1}]		-0.989	-0.255	0.096	0.72	0.51	< 0.0001	< 0.0001
ETS activity:	E_i [µL O_2 h^{-1} ind^{-1}]	64	-0.506	0.541	0.059	0.93	0.86	< 0.0001	< 0.0001
	E_m [µL O_2 h^{-1} mg WM^{-1}]		-0.506	-0.459	0.058	0.95	0.90	< 0.0001	< 0.0001

In contrast, 86% of the variance of individual ETS activities could be attributed to differences in wet mass and temperature ($r = 0.93$, $r^2 = 0.86$), while 90% of the variance of mass-specific ETS activities was explainable by these two parameters ($r = 0.95$, $r^2 = 0.90$) (Table 2). The scaling coefficient b was 0.541 for individual ETS activities and -0.459 for mass-specific ETS activities. The following equations were derived for ETS activities, where E_i is the individual ETS activity, E_m the mass-specific ETS activity, X_1 is now copepod wet mass in mg and X_2 again temperature in °C:

3) $\text{Ln } E_i$ (µL O_2 h^{-1} ind^{-1}) = $-0.506 + 0.541 \log(X_1) + 0.059\ X_2$

4) $\text{Ln } E_m$ (µL O_2 h^{-1} mg WM^{-1}) = $-0.506 – 0.459 \log(X_1) + 0.058\ X_2$

Considering the regression analyses based on body mass and temperature, females (f) and copepodids CIV of *R. nasutus*, *Euchirella rostrata* (f), *E. hyalinus* (f) from deeper than 400 m and copepodids CV of *C. carinatus* showed lower than expected respiration rates, while *A. armatus* (f), *G. pileatus* (f) and species of *Pleuromamma* (f) exhibited higher respiration rates than expected (Table 1). In contrast, with regard to ETS activities, only *A. armatus* exhibited higher than expected ETS activities. *M. lucens* (f), *R. nasutus* (CIV) and *C. carinatus* (CV) showed noticeably lower ETS activities than expected, based on the regression analyses, while the reduction of the ETS activity in *C. carinatus* (CV) was much more pronounced (Table 1, Fig. 2).

DISCUSSION

Due to their high abundance in mesozooplankton communities, copepods play a critical role in the marine carbon flux and energy flow. In order to assess the energy requirements of dominant copepods, we compared two cost-efficient complimentary approaches to measure metabolic rates. On the one hand, respiration measurements during incubation of (non-feeding) copepods in controlled systems indicate minimal food requirements of an organism and provide good estimates of its metabolic demand (Ikeda 1985). On the other hand, the electron transport system (ETS) assay represents a simple approach to measure the potential metabolic rate of copepods (Schalk 1988, Hernández-Léon & Gómez 1996). A third and even easier approach is to model metabolic rates from key parameters such as body mass and temperature. ETS activities proved to give good estimates of copepod respiration, while the prediction of ETS activities from body mass and temperature was even much more reliable than the one based on respiration rates. Thus, under certain constraints, which are discussed below, the ETS assay and mathematical modelling seem to be useful methods to assess copepods metabolic rates.

Our range of respiration rates (0.06-1.60 µL O_2 h^{-1} ind^{-1}) is in good agreement with previous studies. These data are compared in Table 3. However, comparison to other studies is sometimes difficult due to different geographical regions, temperatures and body masses or missing body mass data. 51% and 69% of the variance of mass-specific and individual respiration, respectively, could be explained by differences in body mass and temperature. Estimated scaling coefficients for mass-specific respiration rates vary between -0.1 and -0.4, while most values are close to -0.25

(Moloney & Field 1989, Thuesen et al. 1998). Scaling coefficients for individual respiration rates usually range between 0.6 and 0.9 for copepods and other marine crustaceans (Ivleva 1980, Vidal & Withledge 1982, Ikeda 1985, Ikeda et al. 2001). The scaling coefficients derived from this study lie well within these ranges. However, Ikeda (1985) and Ikeda et al. (2001) even attributed 93-96% of the variance of individual oxygen consumption rates to differences in body mass and habitat temperature applying a global model comprising tropical, subtropical, boreal, Arctic and Antarctic regions.

Table 3: Comparison of the individual respiration rates from this study with published data from different oceanic regions. Mean rates are given, if available, ± standard deviation and dry mass in parentheses (NA = North Atlantic, NP = North Pacific, ABFZ = Angola Benguela frontal zone, Pac. = Pacific, north. = northern).

			Present study	Published data			
Species	Stage	T (°C)	Respiration rate [µL O_2 h^{-1} ind^{-1}]	T (°C)	Respiration rate [µL O_2 h^{-1} ind^{-1}]	Study area of published data	References
Nannocalanus minor	f	15	0.26 ± 0.10 (97 µg)	26.9	0.21 ± 0.01 (40 µg)	Tropical Pacific Ocean	Ikeda et al. 2001
Metridia lucens	f	13	0.17 ± 0.02	15.5	0.32	North western Atlantic	Raymont 1959
Calanoides carinatus	f	13	0.32 ± 0.14	16	0.47 ± 0.32	Eastern NA	King and Packard 1975
				10	0.05 ± 0.01	Northern Benguela	Kosobokova et al. 1988
				11	0.31	Benguela Current	Arashkevich and Drits 1997
				13-15	0.59 ± 0.14		Arashkevich and Drits 1997
Calanoides carinatus	CV	8	0.11 ± 0.03	8	0.12	Benguela Current	Arashkevich and Drits 1997
		13	0.23 ± 0.11	10	0.042 ± 0.002*	Northern Benguela	Kosobokova et al. 1988
				14	0.16 ± 0.10	Northern Benguela	Kosobokova et al. 1988
Euchirella rostrata	f	10	0.34 ± 0.03 (480 µg)	3	0.56 (930 µg)	Western subarctic Pac.	Ikeda et al. 2007
Pleuromamma xiphias	f	7	0.68 ±0.13 (644 µg)	3	0.47 (563 µg)	Western subarctic Pac.	Ikeda et al. 2007
				12	0.55 (387 µg)	unknown	Ikeda et al. 2007
			[µL O_2 h^{-1} mg DM^{-1}]		[µL O_2 h^{-1} mg DM^{-1}]		
Pleuromamma robusta	f	8	1.51 ± 0.36 (427 µg)	3-5	0.93 (362 µg)	NA	Conover and Corner 1968
				8	2.26-2.80 (283 µg)	Western NA	Conover 1960
Rhincalanus nasutus	f	13	0.81 ± 0.56 (364 µg)	3-5	0.39 (529 µg)	NA	Conover and Corner 1968
				7	0.59-0.65 (826-1079 µg)	Western NA	Conover 1960
Euchirella rostrata	f	10	0.71 ± 0.13 (479 µg)	7	1.05-1.46 (820- 947 µg)	Western NA	Conover 1960
Calanoides carinatus	f	19	6.37 ± 1.84 (156 µg)	22	6.90 (118 µg)	ABFZ and north. Benguela	Auel et al. 2005
Calanoides carinatus	CV	8	0.952 ± 0.264 (125 µg)	8	0.21 ± 0.08 (156 µg)	ABFZ and north. Benguela	Auel et al. 2005
				20	5.23 (92 µg)		

ETS activities measured in this study (0.14-4.46 µL O_2 h^{-1} ind^{-1}) matched the range of 0.10-9.15 µL O_2 h^{-1} ind^{-1} reported by Ikeda et al. (2006) and are in close agreement to previous studies (Arashkevich et al. 1996, Kosobokova et al. 1988, Flint et al. 1991, Ohman et al. 1998). Although several studies about zooplankton and ETS measurements have been published, knowledge on individual activities of copepods is still limited. Within the Benguela upwelling region, ETS activities of 0.10-0.21 µL O_2 h^{-1} ind^{-1} were measured in copepodite stages CV of *C. carinatus* from 600-300 m depth (Arashkevich et al. 1996), which are in close agreement with the data of this study (0.14 ± 0.04 µL O_2 h^{-1} ind^{-1}). However, other CV stages of *C. carinatus* from 500-200 m and from the surface showed considerably higher ETS activities of 0.5 µL O_2 h^{-1} ind^{-1} and 0.61-1.32 µL O_2 h^{-1} ind^{-1}, respectively (Arashkevich et al. 1996). In contrast, significantly lower ETS activities of 0.05-0.08 µL O_2 h^{-1} ind^{-1} were determined in CV copepodids from 500-200 m (Kosobokova et al. 1988). Such differences in ETS activities may be associated with different activity levels and dormancy phases of the copepods, further discussed below. The relationship of ETS activities to body mass and ambient water temperature had a much higher fit than the one for respiration. 86% and 90%, respectively, of the variance of individual and mass-specific ETS activities could be explained by differences in temperature and body mass. The slower response of the ETS to environmental changes, in comparison to respiration rates, may lead to the lower variance, and thus a better fit, because results are not biased by short-term impacts caused by sampling and handling stress of the copepods (Schalk 1988). Apparently, a new ETS equilibrium is only established three to four days after the environmental change occurred (Båmstedt 1980).

Besides body mass and temperature, other ecological and physiological factors such as locomotory activity (Pfaffenhöfer 2006), feeding behaviour (Conover 1960) and diel vertical migration (DVM) (Pavlova 1984) have been identified to influence metabolism. Copepods respiration is dependent on intrinsic factors, e.g. its general activity and maturity level, which may cause considerable inter- and intraspecific variations (Arashkevich & Drits 1997). Species of *Metridia* and *Pleuromamma* undergo DVMs, leading to higher activity and thus higher respiration rates at night for instance in *P. xiphias* (Pavlova 1994, Timonin 1997). *Euchirella rostrata* appeared to be a weak- or non-migrant in the Mediterranean Sea (Andersen et al. 2001). It was found in the Arabian Sea within the lower oxygen minimum zone (OMZ) where oxygen concentrations fell below 1 ml L^{-1} (Madhupratap and Haridas 1990), while *M. lucens* has been observed to migrate through OMZs in the Benguela Current system (Timonin

1997, Loick et al. 2005). This potential of some species to occur in OMZs may be reflected in their physiology and biochemical composition by reducing their metabolic rates. Furthermore, omnivorous to carnivorous species such as *Gaetanus pileatus* (Thuesen et al. 1998) are expected to have higher oxygen requirements due to their more active life style (searching and catching prey) compared to herbivorous and omnivorous species (Conover 1960).

Calanoides carinatus, the key biomass species within the Benguela upwelling region, seems to be perfectly adapted to a life in the coastal upwelling system. Its life cycle is characterized by ontogenetic vertical migrations (OVM) to ensure the population´s retention within the upwelling system (Verheye et al. 1991). Reproduction takes place on the shelf within nutrient-rich plumes of recently upwelled water. A part of the population is transported towards the open ocean by Ekman drift and later descends as copepodids CV to 400 to 800 m depth, where they endure periods of food shortage in diapause. Eventually, they are brought back to the shelf by new upwelling events (Verheye et al. 1991). Deep-dwelling copepodids CV of *C. carinatus* from the Benguela Current system have reduced their respiration rates by 25-30% (Kosobokova et al. 1988) to 96% (Auel et al. 2005), respectively, in comparison to active individuals at the surface. Differences in respiration rates and ETS activities are associated with different activity levels of the copepods, as some individuals may have been at the beginning or already at the end of their resting period (see Table 3, Kosobokova et al. 1988, Arashkevich & Drits 1997, Auel et al. 2005).

ETS activities showed a significant positive correlation with respiration rates of the different copepod species, confirming the findings of King and Packard (1975) and Packard and Williams (1981). The general ratio of respiration to ETS activity (R:ETS) in zooplankton communities was given as 0.38 (Owens and King 1975), while a ratio of 0.5 was reported for *Calanus pacificus* (Bigidaire et al. 1982). Copepodids CV and females of *C. carinatus* from the Benguela Current region had ratios of 0.56-1.08 (CV stages) and 0.27 (females), respectively (Kosobokova et al. 1988), which agree well with the respective ratios of 0.75 and 0.31 calculated in this study. Since the R:ETS ratio approximates the proportion of respiratory capacity an organism is effectively using, ETS activities were expected to exceed respiration rates by at least 100% (Packard et al. 1974, Simčič & Brancelj 1997). In this study, individual ETS activities were on average even four times higher than individual respiration rates. *In vivo* respiration measurements in the laboratory most likely depict the organisms' routine or standard metabolism (Ikeda 1985), whereas the standard metabolic rate is around two to three times lower than the average daily *in situ* energy expenditure of a pelagic copepod (Brown et al. 2004). Thus, the actual metabolic rate may lie somewhere in between measured respiration rates in the laboratory and ETS activities. R:ETS ratios for copepod species in this study varied from 0.11 to 0.76, which may be explained by their different activity levels. Surface-dwelling *Rhincalanus nasutus* and deeper-living *Eucalanus hyalinus* exploited merely 10-15% of their potential metabolic rates, whereas the respiration rate of diapausing CV stages of *Calanoides carinatus* was 76% of their ETS activity. For *E. hyalinus* and *R. nasutus* a rather lethargic life style has been observed (Flint et al. 1991), which may coincide with low respiration rates and – together with their transparent body – reduce predation risk from tactile and visual predators (Castellani et al. 2005). *Eucalanus* spp. permanently inhabits OMZs in the Arabian Sea and the Humboldt Current (Flint et al. 1991, Fabian et al. 2005, Escribano et al. 2009, Hildalgo et al. 2010), while *R. nasutus* is also a well known species inhabiting OMZs in various regions (Schnack-Schiel 2008). In the Benguela Current *R. nasutus* was also found in the OMZ, which occurs between 60 and 500 m with oxygen concentrations <1.4 ml O_2 L^{-1} (Auel & Verheye 2007). They may survive periods of food paucity by retreating into the OMZ and reducing their metabolic rate, which may enable them to exist at such low oxygen conditions (Weikert 1980). Reduced metabolic rates were also detected in the California Current in females of *Eucalanus californicus* and *R. nasutus*, indicating dormancy to a certain extent in winter. Ohman et al. (1998) described them as "event-driven" species that respond to environmental stimuli rather than seasonal phenomena, which would coincide with their "normal" ETS activities but reduced respiration rates. In contrast, diapausing CV stages of *C. carinatus* showed a strong reduction of both, respiration rate and ETS activity.

ETS activities provide good estimates of the copepods' metabolic rate, since the actual *in situ* metabolic rate lies between measured respiration rates and ETS activities.

However, the copepods' life history traits (e.g. activity level, feeding and migratory behaviour and sexual maturity) have to be considered in this context for more meaningful interpretations, which holds also true for mathematical modelling. ETS measurements, combined with mathematical models based on parameters such as body mass and temperature, may simplify future measurements of metabolic rates since they seem to be applicable in various marine ecosystems. The model predicting ETS activities was much more precise than the one for respiration rates due to the much lower variance. To ensure a higher accuracy when predicting metabolic rates from ETS activities a larger data set needs to be established of a wider species and temperature range from different depth layers. Overall, the parameterization of metabolic rates offers a relatively easy and inexpensive approach to estimate energy requirements of a larger species spectrum of zooplankton, and thus seems to be a useful tool to assess their role in nutrient and carbon fluxes in marine ecosystems.

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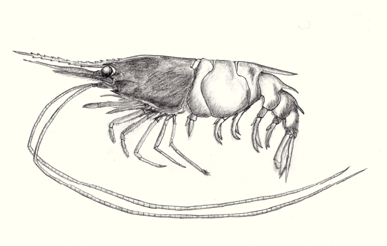
CHAPTER IV



ON THE DISTRIBUTION, ECOPHYSIOLOGY AND CARBON CONSUMPTION OF PELAGIC DECAPODS IN THE NORTHERN BENGUELA UPWELLING SYSTEM

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submitted

Deep-Sea Research

On the distribution, ecophysiology and carbon consumption of pelagic decapods in the northern Benguela upwelling system

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Rolf Koppelmann and Wilhelm Hagen

ABSTRACT

This study shows that pelagic decapods are an important ecological component of the northern Benguela upwelling system and may exert a substantial predation impact on calanid copepods. Although pelagic decapods are abundant in the macrozooplankton/micronekton community, information about their ecophysiological role is limited. Species-specific horizontal distribution limits were detected for some decapod species (e.g. *Plesionika carinata, Sergestes arcticus, Pasiphaea semispinosa*). Significant diel vertical migration patterns were determined for two caridean (*Acanthephyra pelagica*, *Oplophorus novazeelandiae*) and two penaeiodean species (*Gennadas brevirostris*, *Sergia robusta*). Biomass was variable and ranged from 23 to 2770 mg dry mass m^{-2} with highest values for *Pasiphaea semispinosa*. Fatty acids and stable isotope analyses of pelagic decapods revealed that the examined decapod species are omnivorous to carnivorous except the herbivorous to omnivorous species *Plesionika carinata*. Calanid copepods such as *Calanoides carinatus* were identified as an important prey item especially for caridean species. Community consumption rates based on respiration rates of pelagic decapods ranged from 7 mg C d^{-1} m^{-2} (23°S) to >20 mg C d^{-1} m^{-2} in the north (19°S, 17°S). Overall, pelagic decapods apparently play a prominent trophic role in the Benguela Current marine ecosystem by consuming up to 13% d^{-1} of the calanid copepod standing stock.

KEYWORDS

Benguela, Namibia, zooplankton, lipids, marker fatty acids, respiration, ETS activity, C/N isotopes, ingestion

INTRODUCTION

Pelagic decapods are an abundant and important component of the macrozooplankton/ micronekton community throughout many regions of the world's oceans (Maynard et al. 1975, Hopkins et al. 1989, Flock & Hopkins 1992). They constitute a vital link between zooplankton and upper trophic levels in pelagic ecosystems (Omori 1974, Walters 1976) and have been reported as prey of oceanic tuna and flying fish in the open ocean and of various commercially important fish species in shelf waters (Smale 1992, Karuppasamy et al. 2006).

Pelagic decapods often have widespread distribution ranges and, as typical of most mesopelagic micronekton species, they undergo vertical migrations (Omori 1974). The migrations are performed either on a seasonal or daily basis, with migrants occupying surface waters at night and descending to mesopelagic depths during the day. Thus, decapods also contribute to the vertical flux of organic matter. They accelerate the transfer of organic material to greater depth by feeding at the surface and defecating at depth. The production of fast sinking faecal pellets enhances vertical carbon flux (Robinson et al. 2010). In addition, organic carbon assimilated into decapod biomass can be consumed by predators at depth or released as dissolved inorganic carbon via respiration (Longhurst & Harrison 1988, Longhurst et al. 1990, Mincks et al. 2000, Karuppasamy et al. 2006).

The Benguela Current upwelling system is one of the major coastal upwelling ecosystems of the world. It is characterized by a predominately equatorward flow and high levels of Ekman-driven coastal upwelling (Boyer & Hampton 2001, Shannon & O'Toole 2003). Its distinctive bathymetry, hydrography, chemistry and tropho-dynamics combined make it one of the most productive ocean areas in the world, with an annual primary productivity ranging from 400 to 900 g C m^{-2} yr^{-1} (Brown et al. 1991, Carr 2002, Heileman & O'Toole 2008, Monteiro 2010).

Macpherson (1991) recorded 29 species of pelagic decapods in the Benguela upwelling system. Their distribution in the region has been mapped from the Kunene River as the northern limit (15-16°S) down to the Cape of Good Hope in the south (Kensley 1981, Macpherson 1991, Kensley 2006). Most reports for the area are mainly taxonomic in nature: Kensley (1981) provided a brief discussion on the biogeography of decapods in southern African waters; Macpherson (1991) presented data on the community structure of benthic and pelagic decapods off the coast of Namibia, and their distribution; Gibbons et al. (1994) recorded data on the distribution and abundance of one caridean species *Pasiphaea semispinosa*; and Kensley (2006) documented the species diversity and associations off South Africa. However, in comparison with surface-living species, the biology and biochemistry of meso- and bathypelagic organisms is far less understood. No data exist on the physiology of pelagic decapods and their role in the food web in the Benguela upwelling system. Therefore, this study aims to reveal their trophic position and role in the Benguela upwelling system by combining a complex set of analyses (e.g. lipid, stable isotopes, respiration and ETS measurements) for dominant pelagic decapods in addition to their distribution and abundance.

MATERIAL AND METHODS

Sampling of pelagic decapods

For the assessment of abundance and biomass, pelagic decapods were collected in the northern Benguela upwelling system off the coast of Namibia between 17°-23°S on three cruises with FRS *Africana* (December 2009), RRS *Discovery* (September/October 2010) and RV *Maria S. Merian* (February/March 2011). Three transects along 17°S, 19°S and 23°S were sampled (see Fig. 1). Decapods were collected with a 1 m^2 single (December 2009) and double (September/October 2010, February/March 2011) MOCNESS (Multiple Opening and Closing Net and Environmental Sensing System; Wiebe et al. 1985). The single MOCNESS was equipped with 9 and the double MOCNESS with 18 nets of 333 μm mesh size. The towing speed of the vessel was 2 knots and the heaving speed of the winch was 0.5 m s^{-1}. Maximum sampling depth was close to the seafloor at the shallow stations (shelf and shelf break) or down to 750 m (2009), 2600 m (2010) and 3000 m (2011) at the deeper slope and oceanic stations. Discrete depths intervals (0-25, 25-50, 50-100, 100-200, 200-400, 400-600, 600-800, 800-1000 m) were sampled at most stations. At stations deeper than 1000 m, 250 m intervals were performed to a maximum depth of 3000 m.

After the net hauls, samples were preserved in 4% buffered formaldehyde-seawater solution. In the laboratory of the Institute for Hydrobiology and Fisheries Science (IHF) at the University of Hamburg, preserved samples were sieved in size fractions of <0.5, 0.5-1, 1-2, 2-5 and >5 mm and for taxonomic analysis stored in Steedman-solution (Steedman 1976). The size class of >5 mm was used for the recording of decapods. Decapod species were identified according to Crosnier & Forest (1968, 1969), Kensley (1971a, 1971b) and Pérez Farfante & Kensley (1997) and enumerated and measured separately (total length, anterior margin of eye to end of telson). Abundance is expressed as both, numbers of individuals per m^{-2} and per m^{-3}.

Adult decapods for biochemical analyses and respiration experiments were collected alive in 2009 and 2011, respectively. Samples were taken by opportunity from different net types deployed during the cruises: MOCNESS (integrated haul), vertical and oblique Multinet hauls (Hydrobios Multinet Midi) and WP2 net.

Specimens for biochemical analyses were sorted on board, briefly rinsed with deionised water, blotted dry and individually deep-frozen at -80°C. For respiration measurements, only individuals in apparently very good condition were selected and kept in temperature-controlled refrigerators (10°C) for at least 8 h before the start of the experiments.

Vertical distribution

Day/night hauls were conducted in February/March 2011 to investigate vertical distribution patterns. Weighted mean depths (WMD) of decapods were calculated for both day and night hauls with the following equation:

$\mathrm{WMD} = \Sigma(n_i * z_i * d_i) / \Sigma(n_i * z_i)$,

where d_i is the median depth of the sample interval i (in m), z_i the depth range of the sample interval i (in m) and n_i the abundance within the sample interval i (number of individuals m^{-3}) (Andersen et al. 2001). A two-tailed unpaired t-test (confidence interval 95%, Prism software package 5.0) was applied to test for significant differences between day and night WMD to determine, if a species performed diel vertical migration. Prior to statistical analysis, data distribution was tested for normality.

Biomass

Biomass of decapods was calculated based on length/dry mass relationships for each genus. Total length of preserved and deep-frozen decapod samples was measured separately. Dry mass of decapods was determined after lyophilisation of the deep-frozen samples for 48 h and dried samples were used for biochemical analysis. To express the biomass also in mg carbon, body dry mass of decapods was converted to carbon. The carbon content of decapods was measured by mass-spectrometry in association with the analysis of stable isotopes (see next chapter) and the mean carbon value of all decapods of 36% of dry mass was applied.

Lipid and stable isotope analysis

Lipids were extracted with dichloromethane:methanol (2:1 per volume) according to Folch et al. (1957) and total lipid content measured gravimetrically after Hagen (2000). Fatty acid and fatty alcohol compositions were determined by converting the fatty acids to their methyl ester derivatives (FAME) in methanol containing 3% concentrated sulphuric acid at 80°C for 4 h (Kattner & Fricke 1986). After cooling, 4 ml of aqua bidest. were added and FAMEs were extracted with hexane (3 x 1.5 ml). Fatty acids and alcohols were analysed using an Agilent Technologies gas chromatograph (7890A), equipped with a DBFFAP column (30 m length, 0.25 mm diameter, 0.25 µm film thickness) using temperature-programming and helium as carrier gas. Peaks were identified according to retention times in comparison to a fish oil and a copepod lipid standard of known composition.

Fatty acid compositions are evaluated following the trophic biomarker concept (Dalsgaard et al. 2003). 16:1(n-7) and 18:1(n-7) are used as indicators of a diatom-dominated diet, while 20:1(n-9) is applied as calanid copepod marker (Dalsgaard et al. 2003). The fatty acid ratio 18:1(n-9)/18:1(n-7) was calculated to estimate the degree of carnivory versus herbivory (Auel et al. 2002, Dalsgaard et al. 2003). In addition, the fatty acids ratio of [18:1(n-9) + 20:1(n-9)]/[16:1(n-7) + 18:1(n-7)], in the following named carni./herb., was applied as a new relative measure of carnivory.

For stable isotope ratios of $\delta^{13}C$ and $\delta^{15}N$, dried subsamples (fraction of tissue, 0.5-7.0 mg) of decapods were transferred to tin capsules and analyses were performed by

Agroisolab GmbH in Jülich, Germany, using an elemental analyser (EA NA1500 Series 2, Carlo Erba Instruments) coupled to a mass spectrometer (ISO-CHROM; reproducibility <2 µg) with helium as carrier gas.

Determination of carbon and nitrogen stable isotope ratios was conducted using the standards IAEA-VPDB (IAEA-C1) and atmospheric air (IAEA-N1), respectively. Isotopic ratios are expressed as $\delta^{13}C$ and $\delta^{15}N$ in the unit ‰, according to the following equation:

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

where X is ^{13}C or ^{15}N of the sample and R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. In contrast to some publications (Hobson et al. 2002, Smyntek et al. 2007, Mintenbeck et al. 2008), lipids were not extracted prior to stable isotope analysis to avoid any bias of $\delta^{15}N$ by lipid extraction, as discussed by Mintenbeck et al. (2008).

Electron Transport System (ETS) activity

According to Packard (1971) and Owens & King (1975) and as described by Bode et al. (submitted), ETS activities of eight decapod species were measured with an optimised method for pelagic decapods: Polyvinylpyrrolidone (PVP) in the homogenizing buffer was reduced to 0.5 mg ml^{-1} (pH 8.1). The substrate solution comprised 1.3 mM NADH, 0.05 mM NADPH (pH 8.1), while the INT solution contained 2.5 mM 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride (INT) (pH 7.7). Wet mass (WM) of the decapod specimens was determined, so that the crude homogenate contained 3 mg WM per ml homogenizing buffer. The homogenate was centrifuged for 10 min at 4700 g at 0-4°C. The final reaction mixture was reduced to 1 ml: 600 µl substrate solution and 200 µl INT solution were incubated with 200 µl supernatant (3:1:1) while stirred in a thermomixer (Eppendorf comfort) at a chosen standard temperature of 10°C. No quench solution was used. Absorption was read at 490 nm directly after a species-specific incubation time using a temperature-controlled photometer (Kontron Instruments, UVIKON 941) and distilled water as reference. Incubation times varied from 50 min for *Plesionika carinata* to 90 min for *Sergia robusta*, depending on the highest activity. Each sample was measured four times and corrected by a sample blank (600 µl phosphate buffer, 200 µl INT solution, 200 µl supernatant) and substrate blank (600 µl substrate solution, 200 µl INT solution, 200 µl homogenizing buffer).

Respiration measurements

Respiration rates of three decapod species were measured at a chosen standard temperature of 10°C in closed-bottle experiments using Winkler titration to determine oxygen concentrations (Ikeda 2000). A total of 12 experiments was conducted with one individual each. Specimens were transferred into glass bottles (2 L) with filtered and oxygen-saturated seawater. Experiments were conducted for 18-26 h. After termination of experiments, oxygen concentration in the bottles was determined by Winkler titration (precision: 0.05 ml O_2 l^{-1}, Ikeda 2000) and compared to the oxygen concentration of animal-free controls. After the experiments, decapods were deep-frozen (-80 °C) for later dry mass (DM) determination. The long incubation times and difficulty to obtain the decapod species in very good conditions from depth, limited the number of respiration experiments.

To express respiration rates also per wet mass (WM), the following equation according to Podeswa (2012) obtained by more than 100 data points with identical species was applied: WM = DM/0.179 (r^2=98).

Estimation of daily ingestion

Based on the results of the respiration measurements and ETS activities converted to respiration rates, daily ingestion rates of eight decapod species were calculated. Therefore oxygen consumption rates were converted to carbon units according to the equation RC = R*RQ*12/22.4, where RC is the respiratory carbon equivalent, R the measured respiration rate and RQ the respiratory quotient. RQ was assumed to be 0.97 (Pakhomov et al. 1999). Afterwards an energy budget approach was applied, based on estimates of metabolic requirements and net conversion efficiency with the equation Ingestion = 1.62*RC (for details see Pakhomov et al. 1999).

RESULTS

Biomass

The biomass of pelagic decapods along the three transects in the northern Benguela system for three sampling years is shown in Fig. 1. Both northern transects, at 17°S and 19°S, showed higher biomasses of decapods (median 827 mg_{DM} m^{-2}, 298 mg C m^{-2}) than the transect at 23°S (median 188 mg_{DM} m^{-2}, 68 mg C m^{-2}) in all three sampling years. Maximum biomass of pelagic decapods (2770 mg_{DM} m^{-2}, 997 mg C m^{-2}) was determined in September/October 2010 over the continental rise at 19°S.

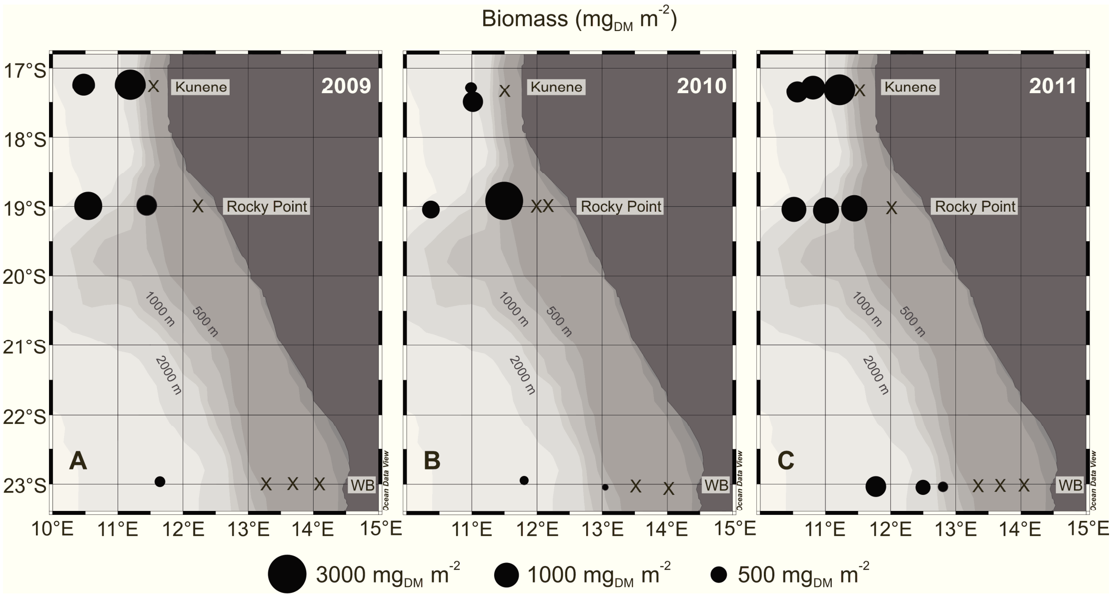

Figure 1: Biomass of pelagic decapods along three transects in the northern Benguela ecosystem for three sampling years A) December 2009, B) September/October 2010 and C) February/March 2011. Crosses indicate stations with no decapods in the net hauls. WB: Walvis Bay.

Species composition

22 decapod species could be identified in the investigation area between 23°S (Walvis Bay) and 17°S (Angola-Benguela Front) (Table1). Ten species belonged to the infraorder Caridea and 12 species belonged to the superfamily Penaeoidea. On average 90% of the decapods in the net hauls were adults (~30-80 mm total length), the other 10% were juveniles. Four species that occurred only rarely or once could not be identified and these specimens were listed as "unidentified". The family Sergestidae

was most diverse with ten species, followed by the family Acanthephyridae with four species, Pandalidae with three species, Oplophoridae and Aristeidae with two species each and Pasiphaeidae with one species (Table 1).

Table 1: Species list with abundance (mean (median), ind. 10^{-1} m^{-2}) integrated over the whole sampling depth (usually 1000 m to surface; for exceptions see table) and main depth distribution of all pelagic decapods found in the northern Benguela system during three years. Maximum abundances in individuals per 1000 m^{-3} are given for the main depth distribution. Species are listed in order of their dominance in three sampling years.

Species	Family	2009 (ind. 10^{-1} m^{-2})	2010 (ind. 10^{-1} m^{-2})	2011 (ind. 10^{-1} m^{-2})	Depth (m)	max. abund. (ind. $1000m^{-3}$)
Pasiphaea semispinosa	Pasiphaeidae, Caridea	20 (0)	73 (8)	23 (0)	100-400	864.0
Sergia robusta	Sergestidae, Penaeoidea	25 (25)	3 (2)	71 (28)	400-600	57.5
Oplophorus novazeelandiae	Oplophoridae, Caridea	25 (7)	8 (4)	43 (14)	200-600	47.8
Gennadas brevirostris	Aristeidae, Penaeoidea	4 (3)	7 (7)	14 (12)	400-800	8.9
Acanthephyra pelagica	Acanthephyridae, Caridea	13 (3)	4 (2)	7 (8)	100-600	8.3
Plesionika carinata	Pandalidae, Caridea	3 (0)	1 (0) /4	12 (1)	200-400	7.5
Sergestidae (not identified)	Sergestidae, Penaeoidea	<0.5 (0)	3 (4)	12 (3)		
Sergestes arcticus	Sergestidae, Penaeoidea	-	-	10 (0)	600-800	22.2
Sergestes armatus	Sergestidae, Penaeoidea	4 (0)	1 (0)	4 (2)	50-600	20.4
Systellaspis debilis	Oplophoridae, Caridea	1 (0)	2 (4)	2 (0)	100-600	10.2
Unidentified	?	2 (0)	1 (0)	1 (0)		
Sergestes corniculum	Sergestidae, Penaeoidea	1 (0)	-	3 (0)	100-400	10.7
Sergestes sargassi	Sergestidae, Penaeoidea	-	-	3 (0)	50-600	15.4
Sergia regalis	Sergestidae, Penaeoidea	-	<0.5 (0)	1 (0)	200-400	3.6
Sytlopandalus richardi	Pandalidae, Caridea	1 (0)	-	-	400-600	0.7
Plesionika rossignoli	Pandalidae, Caridea	-	1 (0)	-	200-250	8.3
Sergia laminatus	Sergestidae, Penaeoidea	-	-	1 (0)	280-2500	1.2
Sergia potens	Sergestidae, Penaeoidea	-	-	1 (0)	50-2500	5.1
Acanthephyra quadrispinosa	Acanthephyridae, Caridea	-	1 (0)	-	100-200	2.7
Gennadas gilchristi	Aristeidae, Penaeoidea	-	-	<0.5 (0)	500-650	1.5
Sergestes grandis	Sergestidae, Penaeoidea	-	<0.5 (0)	-	400-600	1.2
Sergestes orientalis	Sergestidae, Penaeoidea	<0.5 (0)	-	-	100-200	0.8
Notostomus sp.	Acanthephyridae, Caridea	-	<0.5 (0)	<0.5 (0)	200-600	1.5
Hymenodora sp.	Acanthephyridae, Caridea	-	<0.5 (0)	-	1500-1750	0.9

Pasiphaea semispinosa, *Sergia robusta* and *Oplophorus novazeelandiae* were the most dominant decapod species during the three sampling periods with average abundances ranging from 3-73 ind. 10^{-1} m^{-2} (Table 1) and together accounting for >60% of all decapods in the net hauls. *Gennadas brevirostris, Acanthephyra pelagica*, *Plesionika carinata*, *Sergestes armatus* and *Systellaspis debilis* occurred also each year but were less dominant. *Sergestes arcticus* occurred only in 2011 with an average abundance of 10 ind. 10^{-1} m^{-2}. The other decapod species occurred only infrequently (Table 1).

Horizontal distribution and abundance

All decapods were distributed along the continental rise and in oceanic waters; no animals were found on stations in neritic waters or along the shore during the three cruises (Fig. 1). Species-specific horizontal distribution was determined for six species. *Plesionika carinata* occurred exclusively at the 17°S transect with abundances of 0.2-1.5 ind. m^{-2} (Fig. 2) mainly at depths of 200-400 m (Table 1), whereas *Sergestes arcticus* occurred at the southernmost transect at 23°S only in 2011, with abundances of 0.2-4.4 ind. m^{-2} at a main depth stratum of 400-800 m (Table 1, Fig. 2). *Pasiphaea semispinosa* was only sampled at the continental rise stations not deeper than 400 m (Table 1), showing an average abundance of 11 ind. m^{-2} with maxima (43 ind. m^{-2}) at 19°S in September/October 2010 (Fig. 2). In contrast, *Acanthephyra pelagica*, *Gennadas brevirostis* and *Sergestes armatus* were collected at the oceanic stations in a wider depth range of 50-800 m (Table 1), showing similar abundances of 0.2-2.5, 0.2-1.8 and 0.2-1.4 ind. m^{-2}, respectively (Fig. 2). *Oplophorus novazeelandiae* and *Sergia robusta* occurred at almost every

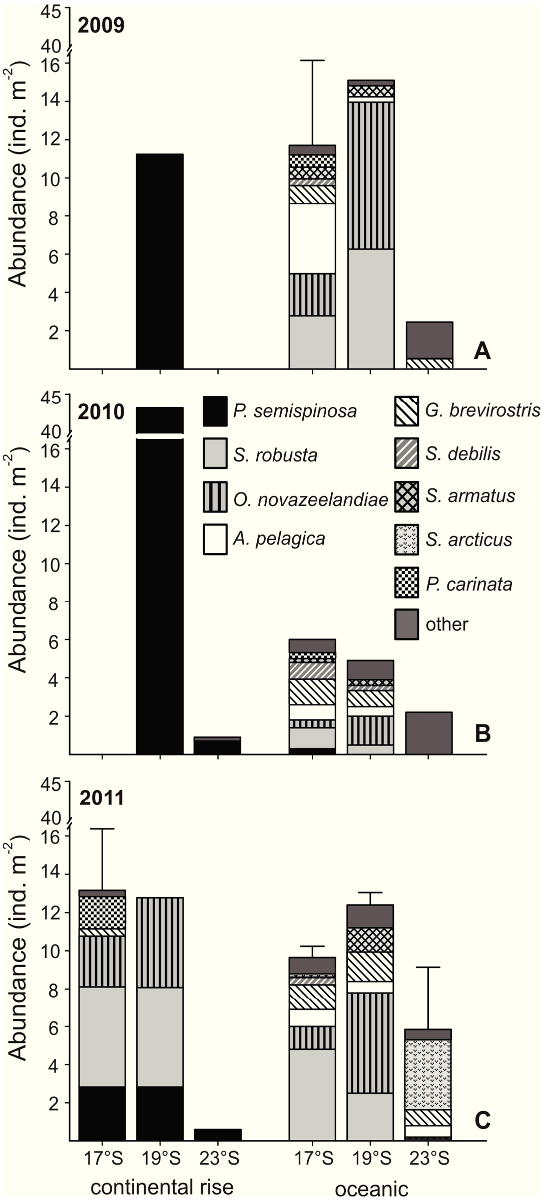

Figure 2: Abundance and species composition of pelagic decapods over the continental rise and at oceanic stations in A) December 2009, B) September/October 2010 and C) February/March 2011. In case of more than one station, mean value ± standard deviation is shown.

station at a main depth of 400-600 m with abundances of 0.2-7.8 and 0.2-6.4 ind. m^{-2}, respectively. The two congeners *Sergestes corniculum* and *Sergestes sargassi* also occurred in similar abundances of 0.2-1.1 and 1.1-2.3 ind. m^{-2} in 2011.

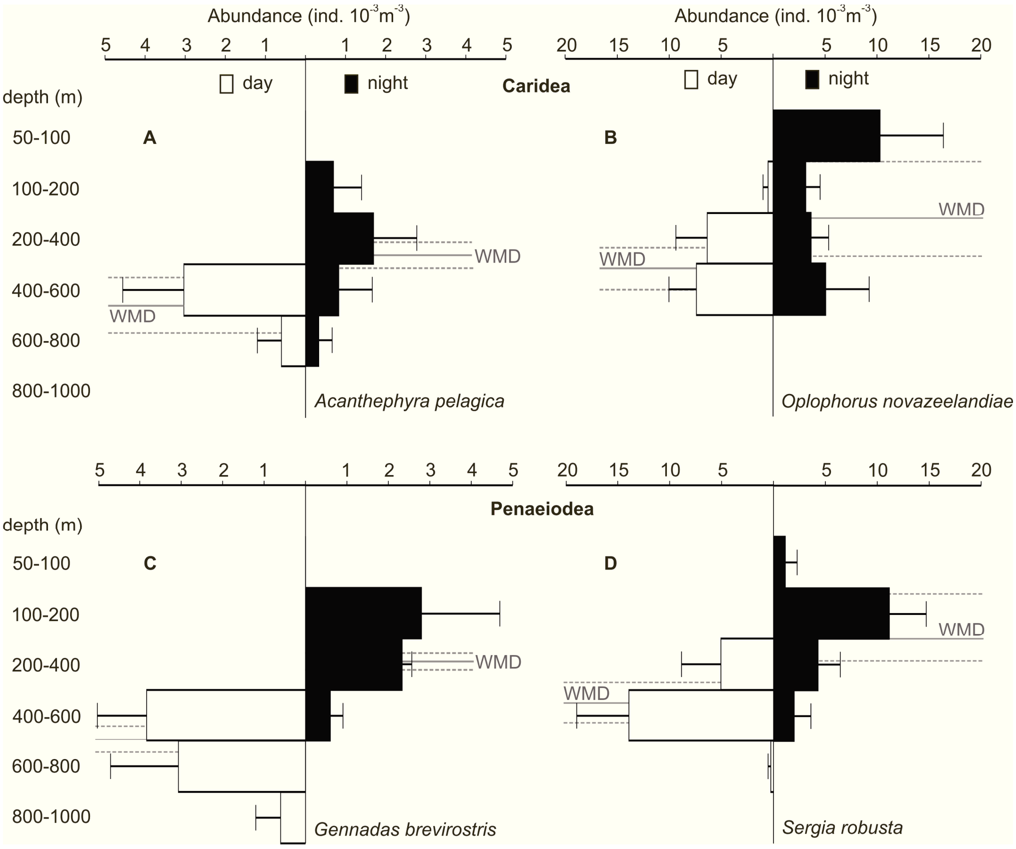

Figure 3: Diel vertical migration pattern and abundance depth profiles of four pelagic decapod species (A-D) off the Namibian coast. Daytime (white) and nighttime (black) weighted mean depths (WMD) are shown as solid lines, with dotted lines indicating standard errors (n=3).

Vertical distribution

Day/night hauls were conducted in February/March 2011 to study vertical migration patterns. Diel vertical migrations were determined for two caridean and two penaeiodean species (Fig. 3). The other species occurred in insufficient numbers in the day/night hauls to test for vertical migration activities.

For all four species significantly different weighted mean depths (WMD) were determined for the day and night period (t-test, $p<0.05$). The majority of *Acanthephyra pelagica* specimens occurred at a depth of 400-600 m (WMD: 567 ± 115

m) at day, whereas at night individuals were more widely distributed, and the majority occurred in shallower waters (WMD: 359 ± 52 m). Individuals of *Oplophorus novazeelandiae* mainly occurred at depths of 200-600 m during daytime (WMD: 415 ± 86 m), whereas at nighttime the majority of *O. novazeelandiae* was found between 100 and 50 m, although some individuals also occurred down to 400-600 m (WMD: 225 ± 124 m). *Gennadas brevirostris* exhibited the most pronounced diel vertical migration. Individuals were found from 400-1000 m (WMD: 599 ± 107 m) at daytime, whereas at night specimens migrated to shallower depths of 100-600 m (WMD: 288 ± 30 m). During the day *Sergia robusta* was encountered at depths of 200-600 m (WMD: 446 ± 84 m) and at nighttime it migrated into shallower waters between 50 and 600 m (WMD: 203 ± 92 m).

Total lipid content, fatty acids and alcohols

All species exhibited a moderate lipid content of 11-19% of dry mass (DM), except for *Oplophorus novazeelandiae*, which accumulated higher lipid stores of 31% DM (Table 2). *Acanthephyra pelagica* and *Sergia robusta* had the highest wax ester levels with more than 30% of total lipids (TL). Lower amounts of wax esters (15-24% TL) were determined for the *Sergestes* species. The two caridean species, *O. novazeelandiae* and *Plesionika carinata*, contained only low amounts of wax ester <10%. The fatty acid compositions of all decapod species were dominated by four fatty acids: the long-chain polyunsaturated fatty acids (PUFA) 20:5(n-3) and 22:6(n-3), the monounsaturated fatty acid (MUFA) 18:1(n-9) and the saturated fatty acid (SFA) 16:0 (Table 2). In addition, *Acanthphyra pelagica* and *Gennadas brevirostris* showed elevated amounts of 20:1(n-9). Three of the caridean species and four of the penaeiodean species contained fatty alcohols. Fatty alcohol compositions were dominated by the 22:1 moiety, exceeding 50% of total fatty alcohols (TFAlc) in the caridean species, whereas fatty alcohols of penaeiodean species were clearly dominated by 16:0 (>50 % TFAlc). The alcohols 14:0, 18:0 and 20:1 were also present in smaller amounts in the decapods (Table 2).

Table 2: Dry mass (DM), lipid content (TL) and fatty acid and alcohol composition (in % of total fatty acids or fatty alcohols, resp.) as well as wax ester content (in % TL) of caridean and penaeiodean decapods from the northern Benguela upwelling system.

Caridea	***Acanthephyra pelagica***	***Oplophorus novazeelandiae***	***Pasiphaea semispinosa***	***Plesionika carinata***	***Stylopandalus richardi***
No. samples	3	3	8	1	1
Dry mass	114.2 ± 22.0	138.0 ± 27.3	62.3 ± 13.1	49.7	110.5
TL (% DM)	15.4 ± 4.9	30.6 ± 8.0	11.3 ± 3.5	14.0	10.9
Fatty acids					
14:0	0.9 ± 0.3	2.2 ± 0.6	1.3 ± 0.6	3.6	2.4
16:0	11.2 ± 0.3	17.1 ± 2.8	15.9 ± 0.7	20.1	18.0
18:0	2.0 ± 0.1	2.7 ± 0.5	5.0 ± 0.7	6.4	4.0
16:1(n-7)	3.8 ± 0.2	4.5 ± 0.4	3.7 ± 0.7	7.5	2.5
18:1(n-7)	4.6 ± 0.2	2.8 ± 0.4	4.3 ± 0.4	5.1	3.3
18:1(n-9)	26.3 ± 6.6	32.4 ± 1.9	20.4 ± 1.9	16.5	18.0
18:2(n-6)	1.0 ± 0.2	1.1 ± 0.1	1.1 ± 0.1	0.6	1.6
20:1(n-9)	7.1 ± 1.1	3.0 ± 0.1	2.9 ± 0.6	2.7	1.9
20:4(n-6)	1.3 ± 0.1	1.2 ± 0.1	1.3 ± 0.3	0.7	2.4
20:5(n-3)	11.4 ± 1.1	10.8 ± 1.3	16.4 ± 1.7	12.4	12.6
22:6(n-3)	10.3 ± 1.8	11.0 ± 1.6	13.9 ± 1.4	11.0	22.1
18:1(n-9)/18:1(n-7)	5.7 ± 1.3	11.9 ± 2.4	4.8 ± 0.4	3.2	5.5
carni./herb.	4.0 ± 0.5	4.9 ± 0.6	2.9 ± 0.2	1.5	3.5
Fatty alcohols					
14:0	8.0 ± 1.1	8.4 ± 0.8	-	9.6	-
16:0	16.8 ± 4.2	10.9 ± 3.6	-	10.6	-
18:0	1.9 ± 0.6	-	-	-	-
20:1	15.2 ± 3.0	18.9 ± 4.1	-	14.7	-
22:1	53.7 ± 12.7	61.8 ± 4.6	-	65.1	-
Wax ester (% TL)	39.5 ± 11.8	8.4 ± 2.6	-	5.9	-
Penaeiodea	***Gennadas brevirostris***	***Sergestes armatus***	***Sergestes orientalis***	***Sergestes* spp.**	***Sergia robusta***
No. samples	4	3	1	15	2
Dry mass	135.4 ± 38.2	53.8 ± 22.4	77.5	95.9 ± 34.5	134.5 ± 32.6
TL (% DM)	19.4 ± 2.8	14.2 ± 4.9	18.2	15.6 ± 3.2	19.3 ± 1.8
Fatty acids					
14:0	1.3 ± 0.3	1.2 ± 0.2	2.4	2.0 ± 0.6	2.2 ± 0.0
16:0	17.1 ± 1.6	19.4 ± 1.4	17.4	15.9 ± 1.0	13.6 ± 0.4
18:0	3.3 ± 0.7	3.7 ± 0.3	2.7	3.0 ± 0.2	2.2 ± 0.4
16:1(n-7)	3.0 ± 0.6	4.6 ± 0.8	6.4	7.3 ± 1.1	9.8 ± 0.7
18:1(n-7)	1.9 ± 0.3	2.9 ± 0.3	3.0	3.2 ± 0.6	3.6 ± 0.1
18:1(n-9)	39.0 ± 2.8	17.9 ± 0.6	18.7	19.4 ± 2.9	24.1 ± 0.7
18:2(n-6)	0.6 ± 0.2	1.5 ± 0.1	1.3	1.5 ± 0.1	1.3 ± 0.1
20:1(n-9)	7.0 ± 0.8	3.0 ± 1.0	3.6	2.6 ± 0.5	3.5 ± 0.1
20:4(n-6)	0.8 ± 0.1	1.1 ± 0.1	0.7	1.1 ± 0.3	1.0 ± 0.1
20:5(n-3)	5.8 ± 0.6	13.5 ± 1.0	13.5	13.1 ± 1.9	10.5 ± 0.2
22:6(n-3)	10.1 ± 0.6	14.9 ± 2.2	11.6	12.9 ± 2.1	12.4 ± 0.7
18:1(n-9)/18:1(n-7)	20.6 ± 3.9	6.2 ± 0.4	6.3	6.2 ± 0.5	6.8 ± 0.0
carni./herb.	9.6 ± 2.1	2.8 ± 0.3	2.4	2.1 ± 0.3	2.1 ± 0.1
Fatty alcohols					
14:0	-	9.2 ± 1.1	11.6	9.2 ± 1.8	10.6 ± 1.3
16:0	-	60.2 ± 7.6	54.6	64.6 ± 5.0	59.2 ± 0.1
18:0	-	3.6 ± 0.3	3.8	5.9 ± 0.5	5.3 ± 0.4
20:1	-	5.9 ± 2.3	6.5	6.6 ± 1.8	9.0 ± 0.4
22:1	-	20.3 ± 6.8	20.7	10.9 ± 2.9	12.5 ± 2.4
Wax ester (% TL)	-	14.6 ± 7.8	23.9	18.6 ± 2.8	33.3 ± 1.2

The fatty acid ratios ranged from 3.2 to 11.9 (18:1(n-9)/18:1(n-7)) and 1.5-4.9 (carni./herb.) for the caridean species with highest ratios for *O. novazeelandiae* as compared to the other carideans. For the penaeiodeans, 18:1(n-9)/18:1(n-7) varied between 6.2 and 20.6 and ranged from 2.1 to 9.6 for carni/herb. (Table 2). All

Sergestidae species exhibited similar ratios, whereas *G. brevirostris* showed the highest ratios of all decapods.

Stable isotopes

Stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) of pelagic decapods from the northern Benguela system were closely clustered (Fig. 4). $\delta^{13}C$ values ranged from -17.6‰ (*Sergestes armatus*) to -15.2‰ (*Acanthephyra pelagica*), while $\delta^{15}N$ ratios comprised values of 6.1‰ for *Plesionika carinata* to 8.8‰ for *Sergia robusta* (Fig. 4). Caridean and penaeiodean decapods showed no significant differences in their food source or trophic level, as indicated by their similar mean values for $\delta^{13}C$ (-16.1 vs. -16.3) and $\delta\ ^{15}N$ (7.8 vs. 7.8), respectively.

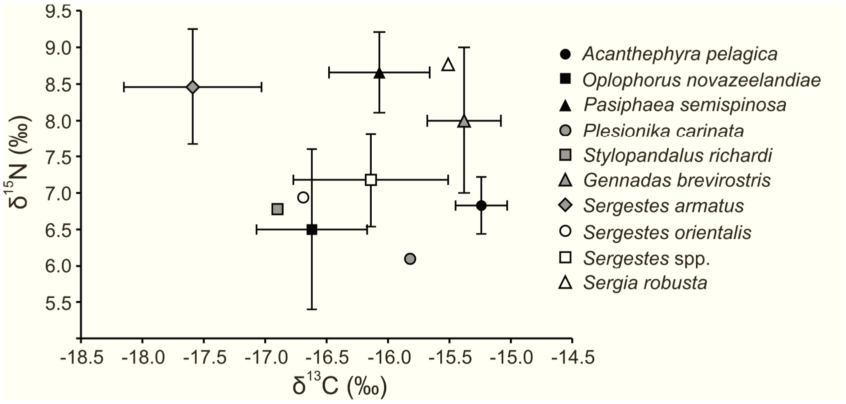

Figure 4: Stable isotope signatures of $\delta^{13}C$ and $\delta^{15}N$ for ten pelagic decapod species from the northern Benguela Current system. Error bars represent standard deviations.

ETS activity and respiration rates

The measurement of the Electron Transport System (ETS) activity was used as a proxy for oxygen consumption. ETS activities of eight decapod species were determined and ranged for the six caridean species from 0.23 ± 0.06 ml O_2 h^{-1} g_{WM}^{-1} (*Acanthephyra pelagica*) to 0.68 ± 0.13 ml O_2 h^{-1} g_{WM}^{-1} (*Oplophorus novazeelandiae*) (Fig. 5a). The two penaeiodean species had ETS activities of 0.62 ± 0.15 ml O_2 h^{-1} g_{WM}^{-1} (*Gennadas brevirostris*) and 0.24 ± 0.09 ml O_2 h^{-1} g_{WM}^{-1} (*Sergia robusta*) (Fig. 5a).

Respiration experiments were conducted for only three species in 12 experiments. Individual respiration rates of *Systellaspis debilis* (n = 2), *O. novazeelandiae* (n = 6) and *S. robusta* (n = 4) ranged from 0.09-0.12 ml O_2 h^{-1} ind.$^{-1}$, while mass-specific respiration varied between 0.27-1.48 ml O_2 h^{-1} g_{DM}^{-1} (Fig. 5b).

In general, individual ETS activities were two to three times higher than individual respiration rates. The correlation between ETS activities and respiration rates is shown in Fig. 5b. ETS activity (per g wet mass) showed a significant positive relationship with the measured respiration rates (per g dry mass) with a derived slope equation of $y = 0.58x - 0.07$ ($r^2=0.88$, $p<0.0001$, Fig. 5b). If respiration is expressed per wet mass, the slope equation reads $y = 3.24x - 0.07$ ($r^2=0.87$, $p<0.0001$). The equations allow the conversion of ETS activities into respiration rates or vice versa for pelagic decapods.

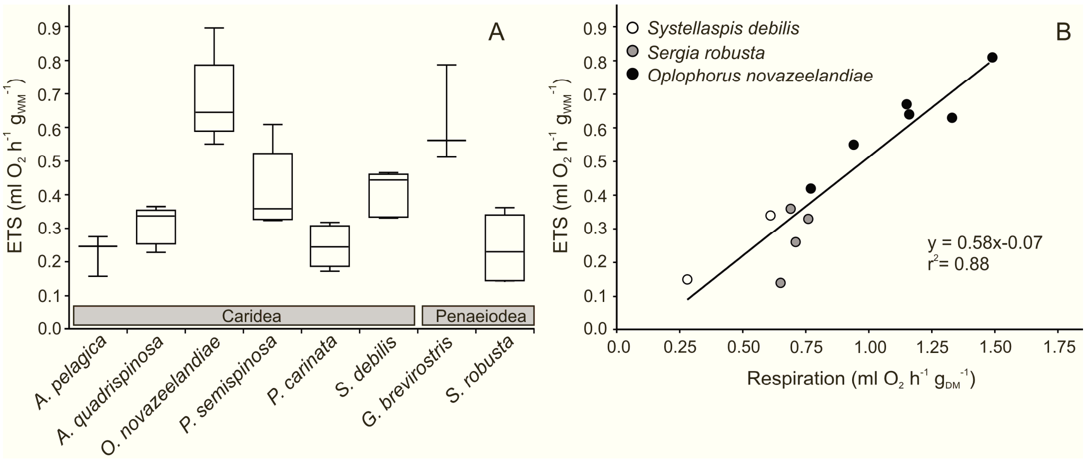

Figure 5: A) ETS activities of eight pelagic decapod species from the northern Benguela upwelling system and B) correlation between ETS activity and respiration rate for three decapod species.

Ingestion rates

Ingestion rates, derived from an energy budget approach based on metabolic requirements, were calculated for eight decapod species. Ingestion rates were lowest in *Pasiphaea semispinosa* with 0.9 mg C ind.$^{-1}$ d^{-1} and highest in *Plesionika carinata* with 3.4 mg C ind.$^{-1}$ d^{-1}. Using the average ingestion rate of all decapods (2.3 mg C d^{-1}), a decapod community consumption of 21.6 mg C d^{-1} m^{-2} at the 17°S transect and 25.8 mg C d^{-1} m^{-2} at the 19°S transect was calculated. Total consumption by decapods at the southernmost transect (23°S) was about three times lower with 6.8 mg C d^{-1} m^{-2}.

DISCUSSION

In many regions of the world's oceans, pelagic decapods are important components of oceanic macrozooplankton/micronekton communities and can constitute a vital link between zooplankton and higher trophic levels in pelagic ecosystems (Walters 1976, Hopkins et al. 1989, Flock & Hopkins 1992).

Generally, the abundance of decapods is underestimated by conventional plankton nets because of their patchy distribution and ability to avoid nets (Omori 1974, Pakhomov & Yamamura 2010). To evaluate the efficiency of different sampling gears for micronekton, a *Micronekton Sampling Gear Intercalibration Experiment* was established in 2002 under the direction of the PICES Biological Oceanography Committee. Six different micronekton gears were tested in experimental approaches, and the Matsuda-Oozeki-Hu Trawl (MOHT) turned out to be the most reliable and cost-effective micronekton gear (Yamamura & Yasuma 2010). The 1 m^2 MOCNESS used in the present study caught on average ~3 times less micronekton than the MOHT (Yamamura & Yasuma 2010). Consequently abundance/biomass data presented in this study should be considered underestimations and may have to be multiplied by a factor of 3.

Earlier investigations reported 29 pelagic decapod species in the northern Benguela system from Lüderitz (26°S) to the Kunene river mouth (Macpherson 1991). In this study 26 species were found in a smaller area of the northern Benguela Current from Walvis Bay (23°S) to the Kunene River (17°S). *Pasiphaea semispinosa*, *Sergia robusta* and *Oplophorus novazeelandiae* were the most dominant decapod species, together accounting for >60% of all decapods in the net hauls. Comparable data for pelagic decapods are scarce, especially for the northern Benguela system. Kensley (2006) listed decapods in order of their presence at 57 stations in the southern Benguela Current: *Sergestes armatus* was most widespread and occurred at 52% of the stations, followed by *Sergia prehensilis* (48%) and *Systellaspis debilis* (40%).

Species-specific horizontal distribution limits were found for some decapods in this study. *Plesionika carinata* exclusively occurred at the 17°S transect, whereas *Sergestes arcticus* only appeared at the southernmost transect at 23°S. The distribution limit for *S. arcticus* is in agreement with the results of Macpherson (1991). However, he reported a wide distribution range for *P. carinata* between 17°S and 29°S. Macpherson (1991) classified/grouped pelagic decapods of the Benguela system in an 'inshore association' over bottom depth between 50 m and 1000 m and an 'offshore association' with bottom depth exceeding 1000 m. The inshore community is closely coupled to the most active upwelling areas and consisted of three species: *P. semispinosa*, *Processa namibiensis* and *Merhippolyte ancistrota*. All other pelagic decapods are assigned to the offshore community. In the present study, *P. semispinosa* was found exclusively at continental rise stations and it was the only species at these stations in 2009 and 2010. This distribution pattern agrees well with the classification by Macpherson (1991). However, the diversity of the continental rise stations increased with up to six decapod species in February/March 2011. Species, which were considered having an offshore distribution by Macpherson (1991), also occurred at the continental rise stations in 2011 (e.g. *Sergia robusta*, *Oplophorus novazeelandiae* and *Plesionika carinata*).

Pelagic decapods are encountered at various depth layers ranging from the surface to at least 4,000-6,000 m. Most decapod species are strong diel vertical migrants (Omori 1974, Heffernan & Hopkins 1981, Flock & Hopkins 1992, Mincks et al. 2000) and some of the sergestid species showed a bimodal distribution, especially at night. Immatures are centred near the surface and mature stages prefer deeper waters (Flock & Hopkins 1992). Significant diel vertical migrations could be confirmed for two caridean and two penaeiodean species in this study. *Acanthephyra pelagica* and *O. novazeelandiae*, as well as *Gennadas brevirostris* and *S. robusta* ascended to shallower depths at night and they migrated to deeper water layers during the day.

Our abundance data of pelagic decapods in the northern Benguela system (~10 ind. m^{-2}, upper 1,000 m) are more than twice as high as in other regions. At subtropical latitudes in the North Atlantic (Azores), a total decapod abundance of 1.2-3.7 ind. m^{-2} (upper 1,200 m) was calculated (rectangular midwater trawl, RMT) (Domanski 1986). Similar densities (3.3-3.9 ind. m^{-2}, upper 1,000 m) are estimated for four decapod taxa in the northwestern Arabian Sea (10 m^2 MOCNESS) (Mincks et al. 2000). Standing stocks of sergestid shrimps off Hawaii (upper 1,200 m) (Walters 1976) and in the eastern Gulf of Mexico (upper 1,000 m) (Flock & Hopkins 1992) are 0.9 and 1.14 ind. m^{-2} (both Tucker trawls), respectively. In the present study, sergestid species showed a fourfold higher abundance. Apparently, upwelling regions such as the Benguela system with their high zooplankton stocks compared to non-upwelling areas or oligotrophic waters also support higher abundances of decapods. Total biomass of pelagic decapods ranged from 0.1 to 2.8 g_{DM} m^{-2} in the present study. Maximum concentrations consisted of only one species, *P. semispinosa*, indicating swarming behaviour. Previous estimates of *P. semispinosa* biomass were much lower with 6-40 mg_{DM} m^{-2} (Gibbons et al. 1994). Considering the net efficiency of the 1 m^2 MOCNESS, biomass values may at least increase to 0.3-8.3 g_{DM} m^{-2} equivalent to 0.1-3.0 g C m^{-2}. Hence, they are in a similar range as compared to estimates for euphausiids and mesozooplankton for this region (1.0-6.2 g_{DM} m^{-2} and 0.4-1.3 g C m^{-2}, resp.) (Pillar et al. 1992, Verheye et al. 1992).

Generally, decapods are described as a zooplanktivorous group that can utilise various kinds of food including other decapods, copepods, mysids, euphausiids, fish larvae, chaetognaths, amphipods and ostracods (Omori 1974, Heffernan & Hopkins 1981, Flock & Hopkins 1992). All these food items were revealed by gut content analyses. Some authors claim that net feeding by decapods should be a major concern (Omori 1974). Biomarker-based approaches such as fatty acids and stable isotopes have in common that information on dietary composition and/or trophic level is integrated over a time scale of several weeks to months. Thus, these methods are not affected by cod-end feeding and may supplement conventional stomach and/or gut-content analyses, which provide data for substantially shorter time scales.

The carnivorous feeding behaviour of decapods is supported by the higher fatty acid ratios in this study. 18:1(n-9)/18:1(n-7) was generally higher than 5, whereas values for the newly chosen ratio including the calanid copepod biomarker 20:1(n-9) and the diatom marker 16:1(n-7) were higher (1.5-9.6) than in copepods (0.2-2.5) (Schukat et al. submitted-a). The highest ratios were determined for *Oplophorus novazeelandiae* and *Gennadas brevirostris*, respectively, indicating that these two species may have a higher degree of carnivory than the other decapods.

The long-chain monounsaturated fatty acids and alcohols 20:1(n-9) and 22:1(n-11) are typical components of wax esters synthesised *de novo* by herbivorous calanid (family: Calanidae) copepods (Hopkins et al. 1993 and references therein). Thus, high amounts of these components in carnivores, imply feeding on calanid copepods. *Acanthephyra pelagica* and *G. brevirostris* contained elevated amounts of the fatty acid 20:1(n-9), and the wax esters of all caridean decapods were clearly dominated by the alcohols 20:1 and 22:1, indicating predation on calanid copepods such as *Calanoides carinatus*. Indeed, specimens of *A. pelagica* and *G. brevirostris* for fatty acids analyses were sampled at stations, at which diapausing copepodite stages C5 of *C. carinatus* occurred at the same depth range in higher abundances of 100 ind. m^{-3} (Schukat et al. submitted-b). Accordingly, *C. carinatus* is likely an important food source for decapods in the Benguela Current.

The high ratios of stable nitrogen isotopes of *P. semispinosa*, *G. brevirostris*, *Sergestes armatus* and *Sergia robusta* correspond to trophic levels of 3.0-3.2 for the northern Benguela Current (Schukat et al. submitted-a) and support the hypothesis of a carnivorous feeding behaviour based on fatty acid biomarkers. However, a surprisingly low $\delta^{15}N$ (6.1) corresponding to a trophic level of 2.1 (Schukat et al. submitted-a) were measured for *Plesionika carinata*. This species additionally exhibited lowest carni./herb. ratio as compared to all other decapods. Hence, it seems that *Plesionika carinata* follows another feeding strategy with a rather herbivorous to omnivorous feeding. However, these data should be treated with caution because only one sample for both, fatty acids and stable isotopes, was available.

Respiration rates of three decapod species were measured in this study and ranged from 0.09-0.12 ml O_2 h^{-1} ind.$^{-1}$, while mass-specific respiration varied between 0.27-1.48 ml O_2 h^{-1} g_{DM}^{-1} and are in agreement with published data for the same genera (Teal 1971, Childress 1975). The long incubation times (up to 26 h) and difficulties to obtain decapods in good conditions after capture, limited the number of respiration experiments. To circumvent such problems, the electron transport system (ETS) assay is used as an alternative measure of potential metabolic rates to approximate respiration (Packard et al. 1971, Owens & King 1975).

ETS activities showed a significantly positive correlation (r^2=0.88) with respiration rates and turned out to be a useful alternative approach to measure respiration rates of decapods applying the correlation determined in the present study. However, the correlation of ETS activity and respiration rates is based on only 12 data points and further data are required for a robust relation of both methods. Nevertheless, a first estimation of ingestion rates of decapod communities in the northern Benguela Current based on metabolic requirements measured by respiration rates and ETS activity was conducted. For the northern transects at 17°S and 19°S, a community consumption of all decapods of 22 mg C d^{-1} m^{-2} and 26 mg C d^{-1} m^{-2}, respectively, was estimated. The total consumption by pelagic decapods along the southernmost transect (23°S) was lower with 7 mg C d^{-1} m^{-2}. A potential consumption by decapods of 2-13% d^{-1} of the standing stock of the calanid copepod *Calanoides carinatus* could be estimated for the northern transects (17°S, 19°S), using the oceanic abundance data for *C. carinatus* calculated by Schukat et al. (submitted-b). Considering the sampling net efficiency, this consumption by pelagic decapods probably is much higher.

In conclusion, pelagic decapods are an important component of the northern Benguela upwelling system, reaching similar biomass (*P. semispinosa*) as euphausiids and mesozooplankton and exerting a considerable predation impact on calanid copepods. Furthermore, some decapods frequently fall prey to key fish species of the region, such as cape horse mackerel and hake (Andronov 1985, Roel & Macpherson 1988). Thus, more attention should be paid to pelagic decapods. They apparently play a more prominent role in the food web and energy transfer of the Benguela Current ecosystem than previously assumed.

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3 SYNOPTIC DISCUSSION

This thesis focuses on trophodynamics and energetics of dominant calanoid copepods and pelagic decapods in the northern Benguela upwelling system. Three research expeditions in the Benguela Current yielded a comprehensive set of biochemical and metabolic data for more than 20 species of copepods and decapods.

Lipid contents, fatty acid patterns, stable isotope signatures and metabolic rates were determined to elucidate life-cycle adaptations (section 3.1) and to discuss the feeding ecology and trophic positions based on trophic biomarker comparisons (section 3.2). In addition, metabolic and carbon consumption rates are provided on an individual and a population basis level to estimate carbon budgets and to assess the potential contribution of copepods and decapods to the energy flow in the northern Benguela system (section 3.3).

3.1 Life-cycle adaptations of copepods and decapods to upwelling conditions

Upwelling regions are highly variable ecosystems with regard to their food availability, oxygen concentrations and water temperatures. Furthermore, pelagic species in coastal upwelling areas are exposed to an offshore transport, driven by the cross-shelf component in the Ekman layer (Verheye & Field 1992, Peterson 1998, Escribano et al. 2001). The upper water layers in upwelling regions can drift offshore at rates of several km day^{-1} (Weisberg et al. 2000). This advection can severely impact the population dynamics and ontogenetic distribution of epipelagic species by transporting individuals away from the productive coastal upwelling zone. Hence, poor feeding conditions in between upwelling events and the retention of their populations in the upwelling area represent major challenges for zooplankton species to thrive in coastal upwelling regions. The key species have developed sophisticated ecophysiological and behavioural adaptations to cope with these dynamic challenges of upwelling systems. The maintenance of coastal species within upwelling areas is associated with active vertical migration between water layers moving in opposite directions (Peterson et al. 1979, Verheye & Field 1992).

Several copepod and decapod species undergo intense diel vertical migrations (DVMs): individuals usually occupy deeper layers during daytime and ascend into near-surface waters at night in order to feed (Timonin 1997, Mincks et al. 2000). Among the copepods of the Benguela Current, *Metridia lucens* and *Pleuromamma* spp. as well as decapods of the families Acanthephyridae, Oplophoridae and Sergestidae are strong diel vertical migrants (Chapter IV, Timonin 1997). These species migrate about 300 m (copepods) or more than 500 m (decapods) during the daily cycle. This migration from the surface layer to mesopelagic depths at dawn allows the species to avoid the offshore Ekman transport. The more oceanic decapods, e.g. *Acanthephyra pelagica*, *Gennadas brevirostris* and *Sergestes armatus* (Chapter IV), are less influenced by upwelling circulation patterns than neritic species.

Besides the DVMs, also ontogenetic vertical migrations (OVMs) are common in some zooplankton species. High-latitude herbivorous copepods of the genus *Calanus* and *Calanoides* perform seasonal OVMs associated with a resting stage (Schnack-Schiel 2001). They generally accumulate extensive lipid deposits as wax esters (up to 90% of total lipids) during copepodite stages C4 and/or C5 and overwinter at depth in a diapausing mode (Sargent & Falk-Petersen 1988, Schnack-Schiel & Hagen 1995, Hagen & Auel 2001). Dominant *Calanus* species, e.g. *C. marshallae* and *C. chilensis*, of the California and Humboldt upwelling region as well as *Calanoides carinatus*, the prevailing copepod in upwelling areas off NW- and SW-Africa, exhibit similar life history traits. They also perform OVMs in combination with diapause to overcome times of low food supply during non-upwelling periods (Peterson 1998, Verheye et al. 2005).

C. carinatus is a key component of the Benguela system and inhabits the entire coastal zone of the Benguela upwelling area (Timonin 1997). Reproductive processes from hatching via nauplii to early copepodite stages mainly occur in upwelling waters of the shelf zones (Fig. 5). Younger stages and females of *C. carinatus* inhabiting surface waters intensively utilise the phytoplankton bloom (mainly diatoms) in the productive shelf water. They are transported offshore and northward within the upwelling plume by the Ekman drift and the Benguela Current, respectively (Timonin 1997). During this movement the population gradually matures. When productivity of the plume decreases following nutrient depletion and mixing with surrounding oligotrophic waters over the continental rise and further offshore, copepodite stages C5 of *C. carinatus* descend to deeper water layers below 400 m and enter diapause. These diapausing copepodids C5 are characterised by high lipid and wax ester levels and an extremely reduced metabolic activity (chapter I, chapter II, Auel et al. 2005, Verheye et al. 2005). Compared to surface-dwelling copepodids C5, metabolic reduction during diapause reaches between 80-96% (Kosobokova et al. 1988, Arashkevich & Drits 1997, Auel et al. 2005). In the present study, metabolic rates were standardised to a typical temperature of upwelling systems and mass-adjusted (chapter II), because they are strongly influenced by these two parameters (Ikeda et al. 2001). Even without the effect of temperature and body mass, metabolic rates of diapausing C5 were strongly reduced by 72% as compared to copepodids C5 at the surface (chapter II).

The extremely reduced metabolism and large amounts of wax esters enable *C. carinatus* to survive long periods of about 4-6 months without feeding (Kosobokova et al. 1988, Timonin et al. 1992, Verheye et al. 2005). The diapausing copepodids C5 remain in arrested development and growth until the onset of a new upwelling event. Meanwhile, they are transported southward by the deep counter current and the bottom transverse circulation moves them towards the shore, where they ascend with the upwelling water to the surface and moult into adults (Verheye & Field 1992, Timonin 1997). Females of *C. carinatus* can immediately start reproduction at favourable food conditions in the upper water layer. Females in surface waters (50-0 m) near the coast showed lower and highly variable wax ester levels (12-42% TL) as compared to diapausing copepodids C5 below 400 m (Chapter I, Verheye et al. 2005). These low to moderate wax ester contents probably reflect different reproductive phases of females. Apparently, some females had already spent most of their internal reserves to fuel gonad maturation and egg production in the surface layer to start a new generation. The high metabolic rates of these females also support intense reproductive processes (Chapter II, Arashkevich & Drits 1997).

Besides this general life-cycle pattern of *C. carinatus*, a bimodal distribution of copepodite stage C5 was reported with individuals inhabiting at the same time surface and depth layers greater than 200 m (Kosobokova et al. 1988, Arashkevich & Drits 1997, Loick et al. 2005, Verheye et al. 2005). During the sampling period of this study near the coast, copepodids C5 were also found at the surface (50-0 m) together with adult females and males. Arashkevich & Drits (1997) identified two distinct forms of copepodids C5 occurring simultaneously in the epipelagic layer: one part of the population showed small oil sacs, low lipid contents and well developed gonads, whereas the other fraction had large oil sacs, high lipid contents and small immature gonads. Only the latter form is supposed to descend to grater depth to form the diapausing stock. However, the underlying mechanisms and triggers involved remain unclear (Peterson 1998) and detailed studies are needed to clarify whether copepodids C5 at different depth belong to the same population.

In contrast to the life cycle of *C. carinatus*, the eucalanid copepod *Rhincalanus nasutus* does not perform ontogenetic vertical migrations, but its life history also includes dormancy (Schnack-Schiel et al. 2008). The resting stage is entered as adult females or as both, adult females and copepodids C5 and the specimens are found in intermediate depth layers (Schnack-Schiel 2008, Ohman et al. 1998). Females of *R. nasutus* characterised by high wax ester levels >80%TL and strongly reduced metabolic activities inhabited the upper mesopelagic layer between 200 m and 70 m (Chapter I, Chapter II). This water layer had oxygen concentrations below 1.5 ml O_2 l^{-1} (CTD data), hence comprised an oxygen minimum zone (OMZ). The occurrence of *R. nasutus* in OMZs is well documented for the Arabian Sea and Red Sea (Vinogradov & Voronina 1961, Weikert 1980, 1982, Beckmann 1984). In the permanently stratified part of the Red Sea, *R. nasutus* is described as remaining in the OMZ for most of the year without any migratory behaviour or feeding (Weikert 1980, 1982, Beckmann 1984, Weikert & Koppelmann 1993). In contrast, in the present study, females of *R. nasutus* also occurred in the surface layer (30-0 m). These females were more active and showed a significantly higher metabolic rate of 162% compared to individuals in the OMZ (Chapter II). Comparisons of sea surface temperatures (CTD data) indicated that upwelling was more intense at stations, where *R. nasutus* occurred in the surface layer (13°C), as compared to stations, where they were found within the OMZ (16-18°C). Apparently, females of *R. nasutus* occupy surface waters during intense upwelling and use this productive period to ingest phytoplankton and accumulate large lipid reserves. Towards the end of an upwelling event they migrate into the OMZ and enter a dormant stage to endure the period of food paucity (Fig. 5). It still has to be clarified though, whether late copepodite stages of *R. nasutus* follow the same strategy. Lower metabolic rates were also found in copepodids C5 at shallow depths of 50-25 m (chapter III).

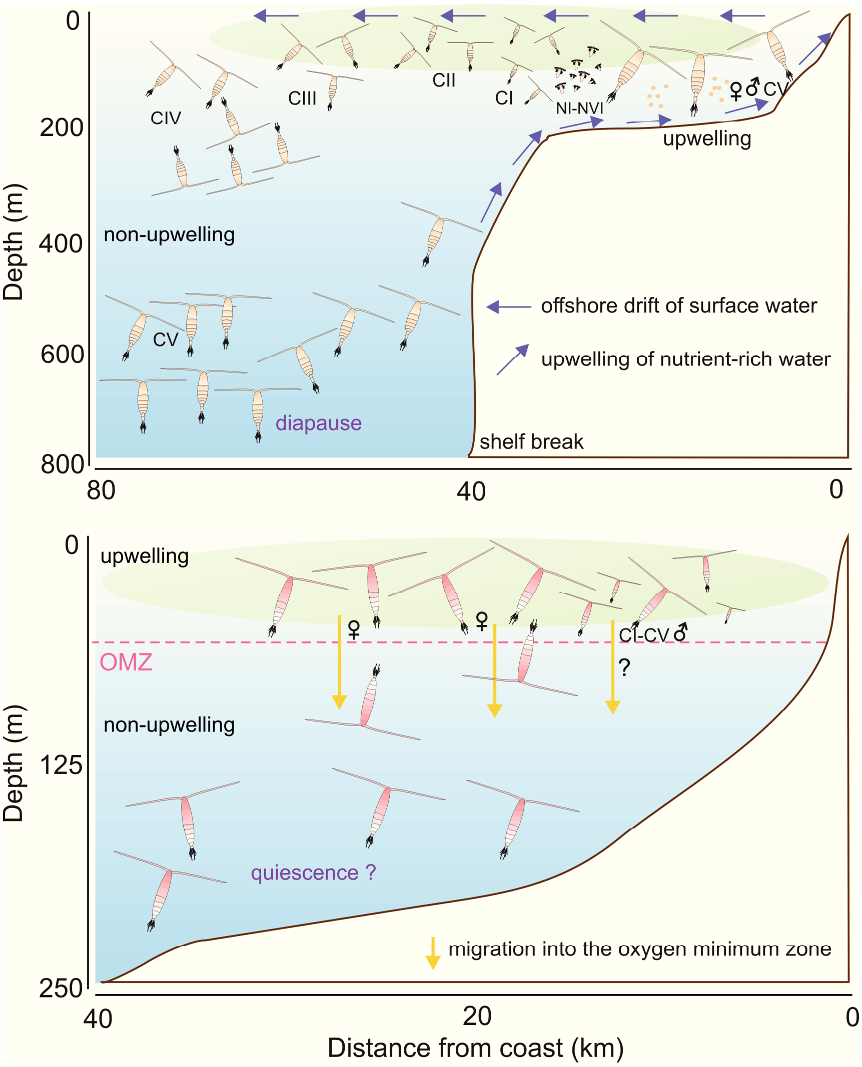

Figure 5: A) Life cycle of *Calanoides carinatus* with ontogenetic vertical migration and diapause in non-upwelling periods (modified after Auel & Hagen 2006). B) Vertical migration of *Rhincalanus nasutus* related to upwelling in the Benguela Current system. Note the different scales in A) and B). N = nauplii stages, C = copepodite stages, OMZ = oxygen minimum zone.

These results indicate that *C. carinatus* and *R. nasutus* follow different life strategies. *R. nasutus* does not seem to perform the extreme form of dormancy (diapause), but a rather moderate and more flexible dormancy, probably quiescence. Quiescence is defined as a spontaneous reaction of individuals in direct response to adverse conditions, resulting in arrested development, which is not fixed to a developmental stage and may occur repeatedly in the same individual. In contrast, diapause implies the suppression of development and growth under endocrine control at a predictable developmental stage. Diapause is induced by various environmental stimuli (Dahms 1995, Hirche 1996).

Despite these adaptive strategies (e.g. diel vertical migration, dormancy) to upwelling conditions, advective losses may be a major concern (Peterson et al. 1979). Escribano et al. (2001) calculated that 22% of the *Calanus chilensis* biomass may be lost to offshore waters during upwelling. Thus, it seems that only species with very high production rates may be able to compensate these advective losses, so that offshore transport does not have a major impact on their population.

In conclusion, the dominant species living in upwelling systems exhibit specialised strategies, which enable them to cope with the highly variable conditions of the ecosystem. However, some of the dominant copepod (e.g. *Eucalanus hyalinus*, *Aetideopsis carinata*, *Euchirella similis*, *Pleuromamma robusta*) and decapod (e.g. *Acanthephyra pelagica*, *Sergia robusta*) species are not only restricted to upwelling systems and reflect same life styles (e.g. diel vertical migration behaviour, lipid storage pattern) as they exhibit in polar, temperate or tropical regions (Lee & Hirota 1973, Kotani 2006). Moreover, species that are mainly associated with upwelling systems (e.g. *Calanoides carinatus*, *Rhincalanus nasutus*) also resemble characteristic life-history traits (e.g. wax ester storage, dormancy) as their Antarctic congeners (e.g., *Calanoides acutus* and *Rhincalanus gigas*). Thus copepods and decapods of the Benguela upwelling system seem to exhibit similar life-strategies as those species in other marine ecosystems.

3.2 Trophic interactions of copepods and decapods in the Benguela food web

The understanding of trophic interactions in ecosystems is a major concern to determine energy pathways and their efficiencies. The present study combined fatty acid trophic biomarkers and stable isotopes (C, N) as two complementary approaches to assess dietary preferences and trophic levels of different copepod and decapod species, as well as of other zooplankton and higher taxa of the food web in the northern Benguela upwelling region.

Trophic biomarkers integrate dietary signals over time spans of days to weeks (Graeve et al. 1994a, Gentsch et al. 2009) and supplement conventional gut-content analyses, which provide data for substantially shorter time scales ("snap-shots") and may be biased by cod-end feeding and regurgitation (Hyslop 1980).
This study focuses on the interpretation of marker fatty acids for specific taxa (e.g. diatoms, dinoflagellates, calanid copepods) and fatty acids ratios (herbivory vs. carnivory) as well as stable nitrogen isotope signatures (trophic levels). The combination of the two approaches produced suitable data; both methods resulted in rather similar trophic classifications of copepods and decapods. However, for some copepod (*Metridia lucens*, *Pleuromamma* sp., *Nannocalanus minor*) and decapod (*Acanthephyra pelagica*, *Oplophorus novazeelandiae*, *Plesionika carinata*) species, both approaches differed in their indication of the carnivory degree and trophic level (chapter I and chapter II). These differences may be a result of different sensitivities to changes in dietary composition, particularly with regard to component-specific turnover times and deviating integration of dietary signals by the different trophic biomarker approaches (Graeve et al. 1994a, Gentsch et al. 2009).

Trophic interactions in the Benguela food web have changed during the last decades from three principal dietary routs to a very complex food web (Fig. 2). Biomass of small pelagic fish (e.g., sardine, anchovy) decreased, whereas zooplankton biomass and diversity increased (Verheye et al. 1998). Copepods became a major component of the food web since the 1950s (Verheye & Richardson 1998). In contrast, pelagic decapods occur generally in smaller numbers in the Benguela Current (Kensley 2006). Copepods and decapod are expected to have different feeding behaviours. Copepods of coastal upwelling systems were traditionally considered as primarily herbivorous, acting as a key link between primary producers, pelagic fish and higher trophic levels (Lowndes 1935, Anraku & Omori 1963). In contrast, decapods are assumed to be predators focussing on zooplankton with a variety of prey items (including copepods), and thus occupy a higher trophic level than copepods (Heffernan & Hopkins 1981, Flock & Hopkins 1992). Based on the results of stable nitrogen isotopes and fatty acid biomarkers, a food web model of the northern Benguela system was developed (Fig. 5). Calanoid copepod species displayed a wide range of $\delta^{15}N$ values (4.8-12.8‰), whereas the range of nitrogen isotope ratios of pelagic decapod species was much narrower (6.5-8.8‰) (chapter I, chapter IV).

The classical scheme of copepods as key herbivores interlinking primary production with higher trophic levels fits only for two species: *Calanoides carinatus* and *Nannocalanus minor* both occupied the lowest trophic level (predominantly herbivorous) corresponding to high amounts of fatty acid markers for diatoms and dinoflagellates (chapter I). *C. carinatus* prevailed in nutrient-rich, recently upwelled water near the coast (chapter II) and accumulated high amounts of the diatom marker 16:1(n-7) (chapter I), emphasizing the dietary importance of diatoms for the nutrition of copepods within upwelling plumes (Smith 2001, Ceballos et al. 2006). *N. minor* occurred further offshore in generally warmer and nutrient-depleted waters (chapter II) and contained elevated levels of the dinoflagellate marker 18:4(n-3) (chapter I). These differences in the fatty acid patterns of both species agree with the general zonation pattern of phytoplankton across coastal upwelling systems. Diatoms generally dominate assemblages in nutrient-rich near-shore waters, whereas dinoflagellate communities prevail further offshore, where silicate has already been depleted (Gibbons & Hutchings 1996, Probyn et al. 2000).

C. carinatus and *N. minor* shared trophic positions with the salp *Salpa fusiformis* and the pteropod *Cymbulia* sp. (chapter I), two common herbivorous gelatinous zooplankter (Alldredge & Madin 1982). Due to very efficient budding (asexual reproduction), salp populations sometimes exponentially increase under advantageous conditions and dominate the plankton, leading to scarcity of other zooplankton taxa (Lee et al. 2010). This phenomenon was also observed in the Benguela region (pers.

obs.). At stations with high salp abundances, essentially no other zooplankton occurred. Salps are important primary consumers and thus are a direct competitor to herbivorous zooplankton (e.g. *C. carinatus* and *N. minor*). They are considered as trophic ‘cul-de-sac’ (dead end), since they are not known as common prey of fish or crustaceans at higher trophic levels.

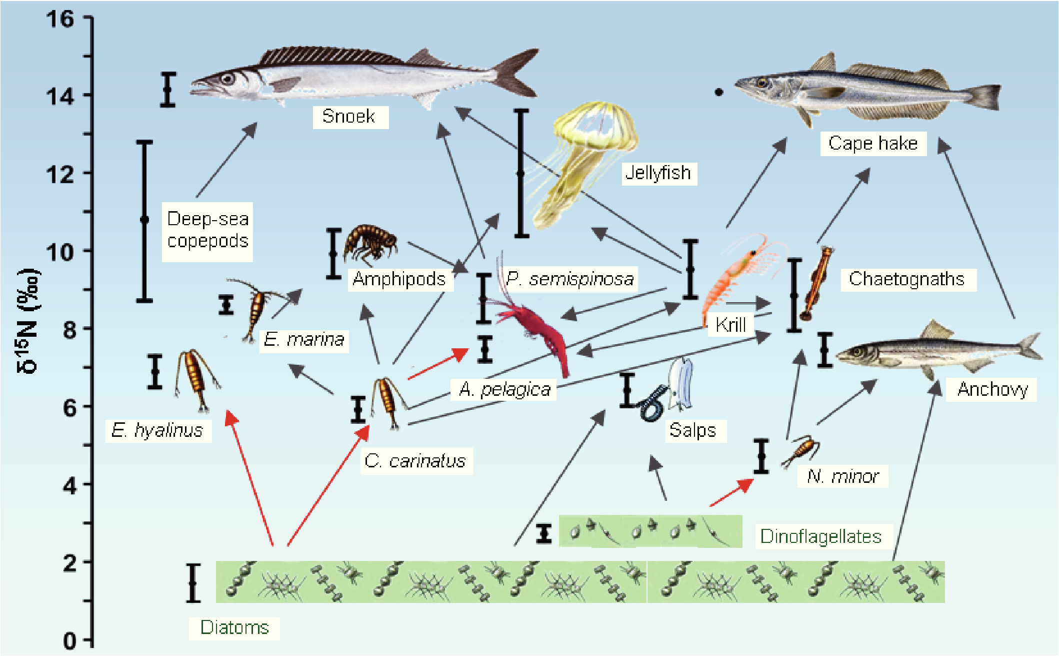

Figure 5: Model of the complex northern Benguela food web based on stable nitrogen isotopes and trophic marker fatty acids. Red arrows indicate dietary preferences revealed via fatty acids and gray arrows signify trophic interactions described in literature.

The majority of the copepods, as well as the decapods occupied trophic levels as secondary (trophic level >2.5) or even tertiary consumers (e.g. some deep-sea copepods), suggesting an omnivorous to carnivorous feeding behaviour. Several studies have shown that supposedly herbivorous zooplankton taxa rather have an omnivorous diet depending on local conditions (e.g. Gifford 1993, Vargas et al. 2006). Moreover, many copepod species are considered to be omnivorous with picoplankton, nanoplankton and microzooplankton as major components of their diet (e.g. Kleppel et al. 1996, Calbet et al. 2007, Escribano & Pérez 2010). These findings are supported by the fact that in general pure herbivory is rarely found in copepods and zooplankton of the northern Benguela upwelling system, whereas omnivory is very common (chapter I). High clearance rates or even a preference for microheterotrophs such as ciliates compared to phytoplankton were recorded for some copepods (Fessenden & Cowles 1994, Zeldis et al. 2002). Such omnivorous feeding behaviour by copepods may thus play an important role by establishing a link between the microbial loop and the 'classical food' chain (Calbet & Saiz 2005). On the other hand, some of the copepods (e.g. Calanidae) may contribute significantly to the nutrition of higher trophic level organisms such as decapods. The long-chain monounsaturated fatty acids and alcohols 20:1 and 22:1 are typical components of wax esters synthesised de novo by herbivorous calanid copepods (Hopkins et al. 1993 and references therein). Thus, high amounts of these components in carnivores imply feeding on calanid copepods. The wax esters of all caridean decapods were clearly dominated by the alcohols 20:1 and 22:1 (chapter IV), suggesting a consumption of calanid copepods such as *C. carinatus*. In addition, two decapod species, *Acanthephyra pelagica* and *Gennadas brevirostris* contained elevated amounts of the fatty acid 20:1(n-9) and occurred at same stations and depths as diapausing C5s of *C. carinatus* (chapter IV, chapter II). Accordingly, *C. carinatus* may represent an important, energy-rich food source for pelagic decapods in the Benguela Current.

Other dominant decapod species (e.g. *P. semispinosa* and *S. robusta*) as well as the predatory copepods *Euchaeta marina* and *Euchirella similis* occupied trophic levels of 3.1-3.2, corresponding to high fatty acid ratios of 18:1(n-9)/18:1(n-7) and Σ carnivory marker/Σ herbivory marker clearly indicating carnivorous feeding (chapter I, chapter IV). These species are known to feed on comparably large prey items such as other mesozooplankton organisms (Heffernan & Hopkins 1981, Yen 1988, Flock & Hopkins 1992) and they shared similar trophic positions with other predatory zooplankton (e.g. amphipods and chaetognaths).

The fourth trophic level (tertiary consumer) was occupied by large fish species such as 'Snoek' and cape hake. These species, as well as other hake species, horse mackerels, pilchards and pelagic gobies represent the dominant fishes in the northern Benguela region (Cury & Shannon 2004, van der Lingen et al. 2006, FAO annual report 2011). The high trophic level of fish indicates that, in comparison to the classical food web of upwelling systems, the steps from phytoplankton to fish have increased. The food web model of Ryther (1969) had on average 1.5 steps to pelagic fish. Moloney (1992) suggested at least 2 steps to fish, whereas the present study indicates that 3 steps to fish are very common in coastal upwelling systems. However, the short food chain towards fish is still present in the northern Benguela region. Adult anchovy showed a rather low trophic level (2.6). This small pelagic swarmfish is able to directly filter large chain-forming diatoms with its fine gill rakers (Bornbusch & Lee 1992) and most likely preyed on phytoplankton (chapter I). Fish feeding directly on phytoplankton but avoiding zooplankton yield a very efficient transfer of energy to higher trophic levels (Fenchel 1988, Cushing 1989). However, due to the lower abundance of small pelagic fish as compared to earlier times (e.g. 1960s), this pathway of energy seems to be less dominant nowadays.

In conclusion, most copepod species in the Benguela system occupy higher trophic positions than expected and cover nearly the whole range of δ^{15}N ratios, as it is found for all other higher taxa from salps to adult anchovy. This shows that the trophic role of calanoid copepods is far more complex than just interlinking primary producers with pelagic fish. In contrast, decapods represent a more homogeneous group regarding their carnivorous feeding habits. Furthermore, the study showed that the northern Benguela system comprise a very complex food web with different efficiencies of energy transfer.

3.3 Metabolic features and carbon budgets of copepods and deapods of the Benguela Current system

Copepods and decapods are major components of marine pelagic ecosystems and play an essential role in the cycling of organic matter in the ocean. Through their diel and ontogenetic vertical migrations they enhance the vertical flux of organic matter and dissolved inorganic carbon from the euphotic zone to deeper layers (Longhurst et al. 1990, Steinberg et al. 2008, Wilson & Steinberg 2010). Therefore, understanding the metabolism of these species is of vital importance to estimate their contribution to oceanic biogeochemical cycles.

Physiological processes such as ingestion rates are difficult to assess in zooplankton, because their natural food composition is still not well characterized and experimental approaches are very time-consuming. In contrast, direct measurements of oxygen consumption rates of zooplankton species provide a robust estimate of metabolic demands and energy expenditures (Ikeda 1985, Brown et al. 2004). *In vivo* respiration rates are physiologically closely coupled to the activity of the electron transport system (ETS). Hence, the ETS assay may be used as an alternative estimate of potential metabolic rates to approximate respiration (Packard et al. 1971, Owens & King 1975). ETS activities of the present study were significantly positively correlated with respiration rates of both, calanoid copepod and pelagic decapod species (chapter III+IV), confirming the findings of King & Packard (1975) and Packard & Williams (1981). Thus, especially for pelagic decapods, which are difficult to obtain in healthy condition from net hauls for experiments or to circumvent long incubation times, the ETS assay represents a practical alternative approach to the estimate respiration rates.

Metabolic rates are highly influenced by temperature and body mass. Up to 96% of the variability in individual respiration rates of zooplankton are in fact due to differences in these two parameters (Ikeda 1985, Ikeda et al. 2001). The contribution of temperature and body mass to the variance of individual respiration were lower in the present study with 69-72% (chapter II+III). Copepod respiration is dependent on intrinsic factors, e.g. general activity and maturity level, which may cause considerable inter- and intraspecific variations (Arashkevich & Drits 1997) as seen for *Calanoides carinatus* and *Rhincalanus nasutus* with their highly variable metabolic rates related to life cycle traits and upwelling conditions (see section 3.1). Locomotion as well as feeding behaviour have also been identified to influence metabolism (Conover 1960, Pavlova 1994, Paffenhöfer 2006). Thus, another life history trait, which can result in intraspecific variations, is the performance of diel vertical migrations (DVM). The most common form of DVM is the nocturnal migration; copepods feed in the upper surface layers at night and migrate into deeper layers, when light intensities increase, presumably to avoid predation (Mauchline 1998). Several copepod and decapod species of the Benguela system perform this kind of DVM (chapter IV, Timonin 1997). The more intense these migrations, the more pronounced the range of locomotion and thus, the more energy-consuming mode of swimming is required, leading to higher respiration rates at night as shown for *Pleuromamma xiphias* (Pavlova 1994).

Variations in abiotic factors such oxygen concentrations can also cause intraspecific differences in metabolic rates. Oxygen minimum zones (OMZs), occurring in depth layers of 60-500 m with oxygen concentrations of less than 1.4 ml O_2 l^{-1} are very pronounced in the Benguela upwelling system (Verheye & Ekau 2005). Some copepod (e.g. *Metridia lucens*, *Pleuromamma* spp.) as well as decapod species (e.g. *Acanthephyra pelagica*, *Oplophorus novazeelandiae*, *Gennadas brevirostris*, *Sergia robusta*) of the Benguela Current system have been reported to migrate through the OMZ and thus have to down-regulate their metabolic rates to exist at such low oxygen levels (Childress 1975, Weikert 1980, Seibel 2011). Females of *M. lucens* from intermediate depths with low oxygen concentrations (0.3-1.5 ml O_2 l^{-1}, CTD data) exhibited a 43% reduced respiration rate as compared to females at surfaces layers with higher oxygen concentrations (3.8-5.9 ml O_2 l^{-1}, CTD data) (chapter II).

Moreover, interspecific variations can be related to different feeding patterns. Carnivorous species are supposed to have higher oxygen requirements, owing to their more active life style (searching and catching prey) compared to herbivorous and omnivorous species (Conover 1960, Thuesen et al. 1998). However, this expectation could not be confirmed by the present data. Primarily herbivorous active species such as *C. carinatus* and *Nannocalanus minor* showed similar or even higher metabolic demands as compared to predominantly carnivorous taxa, such as *Euchirella* (chapter II).

Besides their application as proxy for metabolic activities, respiration rates also serve to trace energy pathways and carbon fluxes. Ingestion rates can be calculated based on respiration rates via an energy budget approach (chapter II, Pakhomov & Perissinotto 1996, Pakhomov et al. 1999, Auel & Werner 2003). Such ingestion rates agreed well with published data of copepod ingestion rates (via feeding experiments or gut evacuation) from the southern Benguela Current and the Humboldt Current (Dagg et al. 1980, Peterson 1989, Vargas & Gonzalez 2004) indicating the reliability of the energy budget approach.

Ingestion rates for predominantly herbivorous species (e.g. calanid copepods) were about 80 µg C mg_{DM} d^{-1}, whereas the majority of copepods exhibited lower ingestion rates (<50 µg C mg_{DM} d^{-1}). Ingestion by pelagic decapods was higher with 130-610 µg C mg_{DM} d^{-1} (chapter II, chapter IV), however, community consumption rates of decapods were generally lower (7-26 mg C m^{-2} d^{-1}) compared to consumption by copepods (neritic: 77 mg C m^{-2} d^{-1}, oceanic: 21 mg C m^{-2} d^{-1}). Total consumption rates of copepod communities in the western subarctic Pacific were higher with 214-375 mg C m^{-2} d^{-1} (Yamaguchi et al. 2002, Kobari et al. 2008) as well as for copepod communities of the Humboldt Current with ~450 mg C m^{-2} d^{-1} for neritic and ~60 mg C m^{-2} d^{-1} for oceanic regions (Grunewald et al. 2002). However, the grazing pressure of copepods upon phytoplankton biomass (e.g. <6%) and primary production (e.g. 4-17%) in the Humboldt Current was not higher as compared to the northern Benguela system. Daily consumption of phytoplankton biomass by primarily herbivorous copepods ranged between 0.1 to 30% in *C. carinatus* and from 0.1 to 16% in *N. minor* for different stations in the northern Benguela Current (Fig. 7, chapter II).

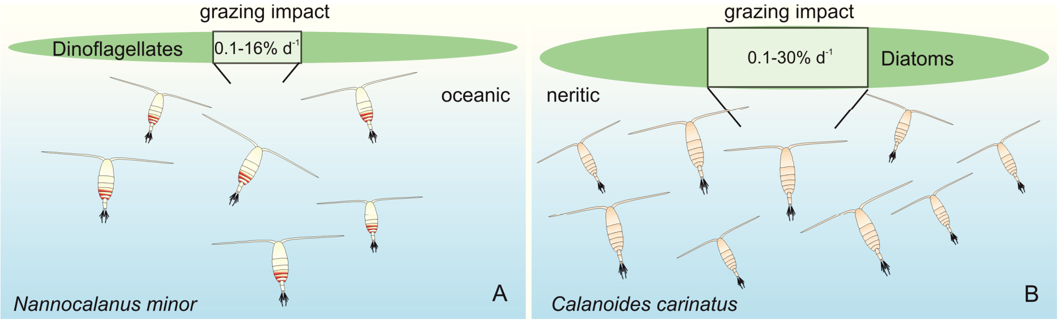

Figure 7: Grazing impact of primarily herbivorous copepods on phytoplankton in oceanic and neritic regions. A) *Nannocalanus minor* (oceanic phytoplankton dominated by dinoflagellates) and B) *Calanoides carinatus* (neritic phytoplankton dominated by diatoms).

Furthermore, very high abundances (100-170 x 10^3 ind. m^{-2}) of *C. carinatus* (Hansen et al. 2005, Verheye et al. 2005) indicate that up to 27% of daily primary production could be consumed by this species at maximum primary production levels (e.g. 6.7 g C m^{-2} d^{-1}) in the northern Benguela. The grazing impact of the copepod community on phytoplankton in the southern Benguela is usually less than 25% (Verheye et al. 1992). Generally, estimates of grazing pressure by zooplankton on primary production often vary over a wide range; zooplankton grazed between 5-40% of daily primary production off Peru, 6-58% off northern Chile and 16-44% off California (Dagg et al. 1980, Boyd & Smith 1983, Herman 1984, Landry et al. 1994, González et al. 2000). While data are rather similar for copepods, however, grazing impact by pelagic decapods is rarely estimated. This may be a result of their patchy distribution and the decapods´ ability to avoid nets*.* A potential consumption by pelagic decapods of 2 and 13% d^{-1} of the standing stock of *C. carinatus* was calculated for the 17°S and 19°S transect, respectively (chapter IV).

In conclusion, different metabolic demands related to species-specific life cycle characteristics (e.g. dormancy, diel vertical migration) could be revealed for copepods and decapods. Furthermore, the carbon budget data imply that *C. carinatus* may have an important grazing impact on phytoplankton biomass and primary production during upwelling plumes in neritic waters, whereas the other primarily herbivorous copepod, *N. minor*, exhibits a much lower grazing impact. Moreover, pelagic decapods apparently play a more prominent role in the energy transfer of the Benguela Current marine ecosystem than previously assumed, exerting a considerable predation impact on calanid copepods.

4 PERSPECTIVES

This study revealed several new aspects in life-cycle strategies, trophic interactions and energetics of dominant calanid copepods and pelagic decapods from the northern Benguela Current upwelling system. During the present study, some new question emerged, which could not be completely addressed within the framework of this PhD thesis but provide promising perspectives for future research. This section will shortly outline these research perspectives.

The knowledge of physiological aspects of pelagic decapods is generally limited. Reasons for this scarcity are their patchy distribution and ability to avoid nets. Another difficulty is that the condition of decapods after capture is often not good enough for subsequent experiments. Nevertheless, this study yielded new information on metabolic demands and trophic interactions of pelagic decapod species from the Benguela Current system. However, the analyses were focused on adult specimens. For the understanding of population dynamics and life cycles of pelagic decapods, studies on larvae and juvenile stages should be included. A suitable candidate for these investigations is *Pasiphaea semispinosa*. This species turned out to be the most important decapod in the Benguela Current, reaching similar biomasses as compared to euphausiids and mesozooplankton. Detailed studies of the distribution and abundance, as well as of physiological features (lipid storage, metabolic activity) of the younger stages will be helpful for the understanding of their life cycles and their importance in the Benguela food web.

Copepod species exhibited different life-cycle patterns in the northern Benguela region. *Calanoides carinatus* copepodids C5 enter the extreme form of dormancy, diapause, during non-upwelling periods. In contrast, for female *Rhincalanus nasutus* a more flexible dormancy (quiescence) in between upwelling events was hypothesised. Timing and stimuli for entering and emergence into dormancy are not clearly described. For the better understanding of the driving forces in population dynamics of species which have resting stages, studies address the physiological mechanisms that trigger the onset and even more important the termination of those resting phases. The assumption of quiescence in female *R. nasutus* has to be clarified. In addition, it has to be examined, whether copepodite stages of *R. nasutus* follow the same strategy by migrating into oxygen minimum zone and enter a resting stage. Changes in light, temperature, food availability, lipid levels and predation have been invoked as possible triggers for dormancy. Thus, *in situ* experiments combining different food levels with abiotic factors (e.g. light, temperature, oxygen concentration) of different developmental stages might be a promising approach to evaluate the mechanisms for entering and emerging of resting phases. This knowledge will be crucial to predict the ecological response on potentially changing climatic conditions.

Another topic, which could only be touched within this thesis, is the contribution of copepod and decapod species to the carbon flux in the Benguela Current ecosystem. Energy requirements and consumption rates were estimated and represent essential data for the modelling of carbon budgets of the Benguela Current. However, to assess the impact of zooplankton species on carbon fluxes, further information about vertical migrating (diel and ontogenetic) species are required. Migrating species enhance the downward carbon flux from the euphotic zone to deeper water layers, since they consume small particulate material in surface waters and respire and produce faecal pellets at depth or are eaten by deeper-living carnivores. The estimation of faecal pellet production and egestion rates in addition to oxygen consumption rates of abundant vertical migrating species, such as the copepod *Metridia lucens*, could improve the understanding of the role of specific zooplankton taxa in the carbon flux of the Benguela Current system.

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Eidesstattliche Erkärung

(Gem. § 6(5) Nr. 1-3 PromO)

Hiermit versichere ich, dass ich die vorliegende Arbeit mit dem Titel „Trophic interactions and energetics of key components in the Benguela Current Ecosystem: The role of calanoid copepods and pelagic decapods“:

1. ohne unerlaubte, fremde Hilfe angefertigt habe,
2. keine anderen, als die von mir im Text angegebenen Quellen und Hilfsmittel benutzt habe,
3. die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Ebenfalls erkläre ich hiermit, dass es sich bei den von mir abgegebenen Arbeiten um 3 identische Exemplare handelt.

Bremen, Oktober 2012

Anna Schukat