

**ECOLOGY AND PHYSIOLOGY OF CALANOID COPEPODS
IN RELATION TO THE OXYGEN MINIMUM ZONE
IN THE EASTERN TROPICAL ATLANTIC**



LENA TEUBER

Dissertation zur Erlangung des akademischen Grades eines Doktors der
Naturwissenschaften (Dr. rer. nat.)

Vorgelegt an der Universität Bremen
Marine Zoologie
Fachbereich 2 Biologie/Chemie

1. Gutachter: PD Dr. Holger Auel
2. Gutachter: Prof. Dr. Claudio Richter

Kolloquium: 21. Mai 2014

CONTENTS

ABBREVIATIONS	I
SUMMARY	II
ZUSAMMENFASSUNG	IV
OUTLINE OF PUBLICATIONS	VI
1. SCIENTIFIC BACKGROUND AND OBJECTIVES	1
1.1 The oxygen minimum zone in the tropical Atlantic Ocean	1
1.2 Copepods of the eastern tropical Atlantic	5
1.3 Effects of hypoxia on marine organisms	8
1.4 Objectives	11
References	14
2. PUBLICATIONS	
Publication I	21
Teuber L, Kiko R, Séguin F, Auel H (2013): Respiration rates of tropical Atlantic copepods in relation to the oxygen minimum zone. <i>Journal of Experimental Marine Biology and Ecology</i> 448, 28-36.	
Publication II	31
Teuber L, Schukat A, Hagen W, Auel H (2013): Distribution and ecophysiology of calanoid copepods in relation to the oxygen minimum zone in the eastern tropical Atlantic. <i>PLoS ONE</i> 8 (11), e77590. doi: 10.1371/journal.pone.0077590.	
Publication III	45
Teuber L, Schukat A, Hagen W, Auel H (2014, accepted): Trophic interactions and life strategies of epi- to bathypelagic calanoid copepods in the tropical Atlantic. <i>Journal of Plankton Research</i> .	

3.	SYNOPTIC DISCUSSION	73
3.1	Distribution of calanoid copepods in relation to the oxygen minimum zone	73
3.2	Physiological adaptations to the oxygen minimum zone	78
3.3	Ecophysiology and life strategies of calanoid copepods	81
4.	PERSPECTIVES	89
	References	91
	DANKSAGUNG	99
	ERKLÄRUNG	101

ABBREVIATIONS

C	carbon
Chl <i>a</i>	chlorophyll <i>a</i>
CTD	conductivity-temperature-depth profiler
C5	copepodite stage 5
DM	dry mass
DO	dissolved oxygen
DVM	diurnal vertical migration
ETS	electron transport system
FA	fatty acid
FAlc	fatty alcohol
MOCNESS	multiple opening/closing net and environmental sensing system
n	number of individuals
N	nitrogen
OML	oxygen minimum layer
OMZ	oxygen minimum zone
OVM	ontogenetic vertical migration
O ₂	oxygen
<i>PO</i> ₂	oxygen partial pressure
SG	station group
SI	stable isotope
SST	sea surface temperature
TFA	total fatty acid
TFAlc	total fatty alcohol
TL	total lipid
WM	wet mass

SUMMARY

Calanoid copepods often contribute up to 95% of zooplankton communities and are key components in the energy transfer from primary producers to higher trophic level consumers such as fish, seabirds and whales. Through feeding, respiration and excretion, copepods considerably affect the cycling of organic and inorganic matter and thus essentially influence marine carbon fluxes. Increasing hypoxic conditions in tropical oceans influence the distribution, abundance and ecophysiology of pelagic organisms, but the effects of hypoxia on marine organisms are not yet fully understood and have become a field of increasing scientific interest. Since the oxygen minimum zone (OMZ) in the eastern tropical Atlantic is not yet as pronounced as in other tropical oceans, studies on zooplankton in relation to hypoxic conditions are still limited.

This study aims to analyse ecophysiological characteristics of calanoid copepods from the eastern tropical Atlantic to identify different life strategies. Special focus is given to the current impact of the OMZ on copepod distribution and metabolic processes as well as to possible ecophysiological adaptations to hypoxia. Different methodological approaches (assessment of abundance and vertical distribution by Multinet catches, measurement of respiration rates) and biochemical analyses (enzyme activities (ETS & LDH), total lipid, fatty acids and stable isotopes (^{13}C & ^{15}N)) were applied to assess specific ecophysiological characteristics.

Five different life strategies of abundant copepod species could be identified. The '*active converters*' are distributed in warm, well oxygenated epipelagic waters and are characterised by high activity levels and high metabolic rates as well as by low lipid content and low $\delta^{15}\text{N}$ ratios indicating continuous feeding and a herbivorous to omnivorous feeding behaviour. Mesopelagic copepods were classified as '*opportunistic predators*' with a predominantly carnivorous feeding mode indicated by high concentrations of the fatty acid 18:1(n-9), high $\delta^{15}\text{N}$ ratios and high carnivory ratio. Low metabolic rates but high lipid and wax ester contents represent valuable traits to cope with the food scarcity of mesopelagic habitats. Diurnal vertically migrating (DVM) copepods such as *Pleuromamma* spp. were categorised as '*adaptive migrants*'. This life strategy is primarily characterised by a bimodal distribution pattern, low Q_{10} values and variable metabolic rates. Trophic biomarkers point towards an omnivorous diet and increased LDH activity may support DVM and a temporal stay within the OMZ.

Ontogenetically vertically migrating (OVM) species (i.e. *Calanoides carinatus*) are described as 'dormant survivors' and have a bimodal distribution pattern. In *C. carinatus* this life strategy is characterised by diapausing copepodids C5 in meso- to bathypelagic depths that have low metabolic rates and high wax ester levels as lipid reserve. High concentrations of phytoplankton marker fatty acids, low $\delta^{15}\text{N}$ ratio and a low carnivory index point towards herbivorous feeding during active periods at the surface. Eucalanid copepods were identified as 'thrifty floaters', representing a distinctive life strategy among calanoid copepods. Their broad vertical distribution and high abundance especially within the OMZ as well as their low Q_{10} values point towards a high temperature and hypoxia tolerance. Sluggish movement, high lipid and wax ester contents as well as low metabolic rates may indicate dormancy, which possibly facilitates survival within OMZs. Lowered metabolic rates and potential dormancy in eucalanid copepods as well as DVM and increased LHD activities in *Pleuromamma* spp. were identified as possible ecophysiological and behavioural adaptations to hypoxic conditions.

In conclusion, this study identified five different life strategies of abundant calanoid copepods from epi- to bathypelagic depths in the eastern tropical Atlantic. It illuminates the current impact of hypoxic conditions and discusses specific ecophysiological and behavioural adaptations to the OMZ. The results imply that certain copepod groups can better cope with hypoxia than others and may thus be able to survive intensifying OMZs in the future.

ZUSAMMENFASSUNG

Calanoide Copepoden dominieren Zooplankton Gemeinschaften häufig mit bis zu 95% und stellen somit Schlüsselarten im Energietransfer zwischen Primärproduzenten und höheren trophischen Ebenen, wie z. B. Fischen, Meeresvögeln und Walen, dar. Durch biochemische Prozesse wie Nahrungsaufnahme, Atmung und Ausscheidung haben Copepoden daher einen wesentlich Einfluss auf den marinen Kohlenstoffzyklus. Kontinuierlich sinkende Sauerstoffkonzentrationen in tropischen Ozeanen beeinflussen die Verbreitung, Abundanz und Ökophysiologie pelagischer Organismen. Dennoch sind die Auswirkungen von Hypoxie auf marine Organismen noch nicht völlig verstanden, gelangen aber immer mehr in den Fokus der Wissenschaft. Da die Sauerstoffminimumzone im tropischen Ostatlantik bisher noch nicht so stark ausgeprägt ist wie in anderen tropischen Meeren, ist die Anzahl wissenschaftlicher Studien über atlantisches Zooplankton in Bezug auf Hypoxie noch sehr begrenzt.

Das Ziel dieser Studie ist die Analyse von ökophysiologischen Eigenschaften calanoider Copepoden aus dem tropischen Ostatlantik sowie die Identifizierung verschiedener Lebensstrategien. Der besondere Fokus dieser Arbeit liegt sowohl auf dem gegenwärtigen Einfluss der Sauerstoffminimumzone auf die Verteilung und metabolischen Prozesse der Copepoden als auch auf möglichen ökophysiologischen Anpassungen an geringe Sauerstoffkonzentrationen. Verschiedene Methoden (Bestimmung der Abundanz und Vertikalverteilung durch Multinetzfänge, Messung von Respirationsraten) und biochemische Analysen (Enzymaktivitäten (ETS & LDH), Gesamtlipid, Fettsäuren und Stabile ^{13}C und ^{15}N Isotope) wurden angewandt um spezifische ökophysiologische Merkmale und Eigenschaften zu charakterisieren.

Bei den untersuchten Copepoden-Arten konnten fünf verschiedene Lebensstrategien identifiziert werden. Die „*active converters*“ sind überwiegend im warmen, sauerstoffreichen Epipelagial verbreitet und zeichnen sich durch eine hohe Aktivität und hohe metabolische Raten sowie durch einen geringen Lipidgehalt und niedrige $\delta^{15}\text{N}$ Werte aus. Mesopelagische Copepoden wurden als „*opportunistic predators*“ klassifiziert, bei denen ein vorwiegend karnivores Fraßverhalten durch hohe Konzentrationen der Fettsäure 18:1(n-9), hohe $\delta^{15}\text{N}$ Werte und einen hohen Karnivorie-Index angezeigt wurde. Niedrige metabolische Raten aber hohe Lipid- und Waxestergehalte stellen physiologisch günstige Eigenschaften für ein geringes Nahrungsangebot im Mesopelagial dar. Vertikal wan-

dernde Copepoden, wie z. B. *Pleuromamma* spp., wurden als „*adaptive migrants*“ kategorisiert. Diese Lebensstrategie zeichnet sich hauptsächlich durch eine bimodale Vertikalverteilung, niedrige Q_{10} Werte und sehr variable metabolische Raten aus. Trophische Biomarker weisen auf eine omnivore Ernährungsweise hin und erhöhte LDH-Aktivitäten könnten sowohl die tägliche Vertikalwanderung als auch einen temporären Aufenthalt in der Sauerstoffminimumzone begünstigen. Ontogenetisch wandernde Arten, wie z. B. *Calanoides carinatus*, wurden als „*dormant survivors*“ beschrieben und haben ebenfalls eine bimodale Verbreitung. In *C. carinatus* äußert sich diese Lebensweise darin, dass sich Copepoditstadien C5 in größere Tiefen in Diapause begeben und einen reduzierten Stoffwechsel, aber sehr hohe Waxestergehalte als Lipidreserven aufweisen. Hohe Konzentrationen an Phytoplankton-Markerfettsäuren, niedrige $\delta^{15}\text{N}$ Werte und ein niedriger Karnivorie-Index deuten auf ein herbivores Fraßverhalten während aktiver Phasen an der Oberfläche hin. Eucalanide Copepoden wurden als „*thrifty floaters*“ bezeichnet und weisen eine besondere Lebensstrategie unter den calanoiden Copepoden auf. Ihre weiträumige vertikale Verbreitung und hohe Abundanz, besonders in der Sauerstoffminimumzone, sowie ihre niedrigen Q_{10} Werte weisen auf eine hohe Temperatur- und Hypoxietoleranz hin. Sowohl langsame Bewegungen, ein hoher Lipid- und Waxestergehalt als auch geringe metabolische Raten könnten auf eine dormante Phase hindeuten, die möglicherweise das Überleben in Sauerstoffminimumzonen begünstigt. Geringe Stoffwechselraten und eine potentielle Dormanz in eucalaniden Copepoden sowie tägliche Vertikalwanderungen und erhöhte LDH-Aktivitäten in *Pleuromamma* spp. können als mögliche ökophysiologische und verhaltensbedingte Anpassungen an Hypoxie gedeutet werden.

Zusammengefasst konnten in dieser Studie fünf verschiedene Lebensstrategien calanoider Copepoden aus epi- bis bathypelagischen Tiefen des tropischen Ostatlantiks identifiziert werden. Die vorliegende Arbeit zeigt den gegenwärtigen Einfluss hypoxischer Zonen auf die Copepoden-Gemeinschaft auf und diskutiert mögliche ökophysiologische und verhaltensbedingte Anpassungen an die Sauerstoffminimumzone im tropischen Atlantik. Die Ergebnisse deuten darauf hin, dass einige Copepoden-Gruppen besser mit Hypoxie zurechtkommen als andere und dadurch zukünftig in der Lage sein könnten, innerhalb von sich ausbreitenden Sauerstoffminimumzonen zu überleben.

OUTLINE OF PUBLICATIONS

The following list of publications gives an overview of the three first author publications written during my PhD thesis and states my contribution to each manuscript. The general idea for this PhD project was developed by PD Dr. H. Auel. The design of the studies on the Cape Verde Islands was realised in cooperation with Dr. R. Kiko from the GEOMAR Helmholtz Centre for Ocean Research, Kiel. I developed the concepts for the three manuscripts with scientific advice and assistance by Dr. H. Auel, Prof. Dr. W. Hagen and Dr. R. Kiko. Sampling and field experiments were conducted during two research trips to the Cape Verde Island São Vicente (2010), supported by GEOMAR and the University of Bremen, as well as during three research expeditions with *RRS Discovery* (2010) and *RV Maria S. Merian* (twice in 2011) within the framework of the GENUS project (Geochemistry and Ecology of the Namibian Upwelling System). All laboratory analyses were performed in the Marine Zoology Department at the BreMarE Institute (Bremen Marine Ecology, Centre for Research & Education), University of Bremen.

PUBLICATION I

Teuber L, Kiko R, Séguin F, Auel H

Respiration Rates of Tropical Atlantic Copepods in Relation to the Oxygen Minimum Zone

The idea of this study and the experimental design were developed by myself and H. Auel in close cooperation with R. Kiko. The sampling and experimental work was performed during two research trips to São Vicente, Cape Verde Islands, by myself and all co-authors. Zooplankton samples and data were analysed by myself with contributions from F. Séguin. I wrote the manuscript with scientific advice by H. Auel and R. Kiko. The article is published in *Journal of Experimental Marine Biology and Ecology* (2013).

Journal of Experimental Marine Biology and Ecology 448: 28-36, doi:10.1016/j.jembe.2013.06.012

PUBLICATION II

Teuber L, Schukat A, Hagen W, Auel H

Distribution and Ecophysiology of Calanoid Copepods in Relation to the Oxygen Minimum Zone in the Eastern Tropical Atlantic

The concept of this manuscript was developed by myself with assistance of H. Auel. The experimental setup was realised together with A. Schukat and H. Auel. Sampling and respiration experiments were performed on board with A. Schukat and H. Auel, while all laboratory analyses and data analyses were performed by myself. I wrote the manuscript with scientific support of all co-authors. The article is published in *PLoS ONE* (2013).

PLoS ONE 8(11): e77590. doi:10.1371/journal.pone.0077590

PUBLICATION III

Teuber L, Schukat A, Hagen W, Auel H

Trophic Interactions and Life Strategies of Epi- to Bathypelagic Calanoid Copepods in the Tropical Atlantic Ocean

I developed the concept for this study and performed the sampling together with A. Schukat and H. Auel. All laboratory analyses were performed by myself with assistance of A. Schukat. The data analysis was accomplished with support of A. Schukat. I wrote the manuscript with scientific contributions from all co-authors. The manuscript is accepted for publication in *Journal of Plankton Research*.

1. SCIENTIFIC BACKGROUND AND OBJECTIVES

1.1. The oxygen minimum zone in the tropical Atlantic Ocean

Dissolved oxygen (DO) in the ocean is a key factor determining the distribution of marine organisms throughout the oceans. In some areas of the world oceans, DO levels in intermediate water layers are exceptionally low and extensive hypoxic regions have developed (Fig. 1). These areas of permanently reduced DO concentrations are termed oxygen minimum zones (OMZ) or oxygen minimum layers (OML), which have become a field of scientific focus within the last few decades. Different thresholds defining the intensity of OMZs, have been described; slight hypoxia ($<90 \mu\text{mol O}_2 \text{ kg}^{-1}$), hypoxia ($<45 \mu\text{mol O}_2 \text{ kg}^{-1}$), suboxia ($<4.5 \mu\text{mol O}_2 \text{ kg}^{-1}$) and anoxia (absence of O_2) (Karstensen et al. 2008).

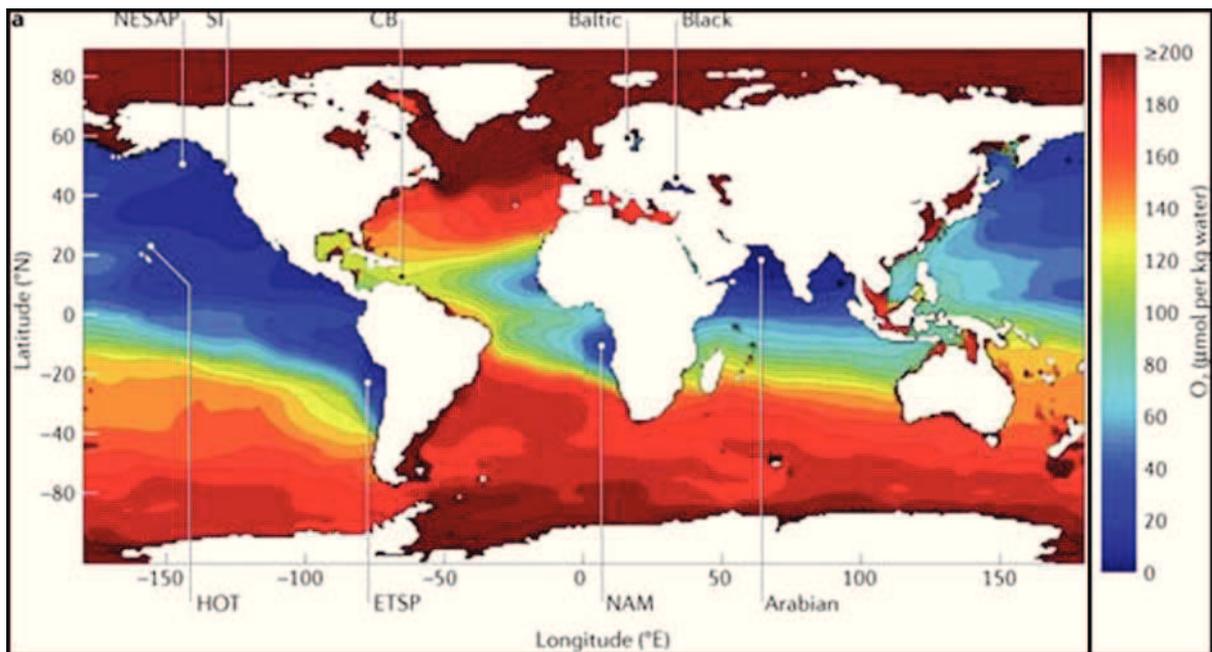


Figure 1. Global distribution of minimum dissolved oxygen concentrations (modified after Wright et al. (2012)). For a description of abbreviated regions see Wright et al. (2012).

The most pronounced OMZs are located in the eastern tropical North and South Pacific as well as in the Arabian Sea and Bay of Bengal (Fig. 1, Helly and Levin 2004, Stramma et al. 2008a, 2010). While the OMZs in the equatorial Pacific and northern Indian Ocean reach suboxic conditions and comprise large areas, the OMZ in the eastern tropical Atlantic is still more oxygenated and DO concentrations of $<45 \mu\text{mol O}_2 \text{ kg}^{-1}$ compose only $\leq 1\%$ of its

volume (Karstensen et al. 2008). Nevertheless, DO concentrations have most severely declined in the tropical Atlantic since the 1960s (Stramma et al. 2008a, 2009). Intermediate water layers with $<90 \mu\text{mol O}_2 \text{ kg}^{-1}$ DO have spread vertically by several hundred metres from 1960 to 2006 (Stramma et al. 2008a, b). In future years, the decline in DO and the spatial expansion of the OMZ has been predicted to be greatest in the Atlantic Ocean (Stramma et al. 2008a, 2009).

In the tropical Atlantic, areas affected by OMZs are located off the northern African coast, in the Angola Gyre and in the Benguela upwelling system (Fig. 2). Minimum O_2 concentrations of around $40 \mu\text{mol O}_2 \text{ kg}^{-1}$ were measured east of the Cape Verde Archipelago in the North Atlantic, whereas in the South Atlantic the O_2 minimum reaches $17 \mu\text{mol O}_2 \text{ kg}^{-1}$ (Karstensen et al. 2008, Stramma et al. 2008b).

The transport of water masses, which is associated with the formation of hypoxic zones in the eastern tropical Atlantic, is regulated by a complex current system (Fig. 2). The North and South Equatorial Under-Currents as well as the North and South Equatorial Counter-Currents transport relatively oxygen-rich water masses towards the African continent (Stramma and Schott 1999, Stramma et al. 2005). These eastward-flowing equatorial currents are advected polewards along the African coasts and turn into cyclonic circulation features in the northern and southern tropical basins (Stramma and Schott 1999). Within these cyclonic current systems, thermal domes are created by Coriolis forces and related to an uplift of isotherms (Mazeika 1967, Stramma and Schott 1999, Karstensen et al. 2008). In the northern hemisphere, the Guinea Dome is located at around 9°N , 25°W in boreal summer and at 10.5°N , 22°W in boreal winter, while the Angola Dome in the southern hemisphere is centred at 10°S , 8°E (Fig. 2, Mazeika 1967, Siedler et al. 1992, Karstensen et al. 2008).

Through stagnation of water transport and long residence times of water masses within the cyclonic gyres, the replenishment with oxygenated water is low, while oxygen consumption is continuous (Stramma et al. 2008b). These factors lead to oxygen-depleted waters within the domes (Voituriez and Herbland 1982). The Guinea and Angola Dome are thus associated with the centres of the OMZ in the tropical Atlantic containing minimum DO concentrations (Siedler et al. 1992, Mohrholz et al. 2001, Karstensen et al. 2008). Moreover, the Angola Gyre is supposed to be the 'source region of oxygen-depleted central water' for the surrounding areas (Mohrholz et al. 2008).

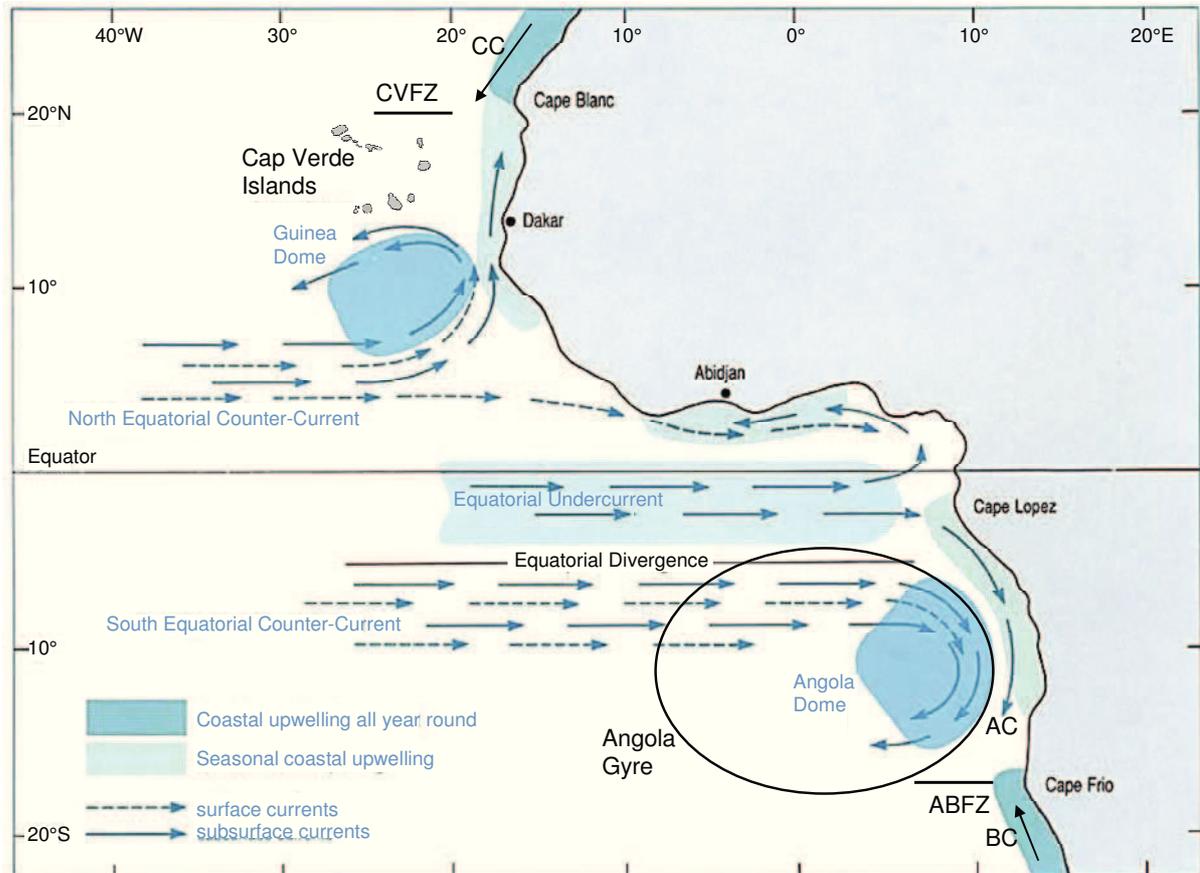


Figure 2. Hydrography and circulation patterns in the eastern tropical Atlantic Ocean (modified after Brown et al. (2001)). CVFZ Cape Verde Frontal Zone, ABFZ Angola-Benguela Frontal Zone, CC Canary Current, AC Angola Current, BC Benguela Current.

In eastern boundary upwelling systems, hypoxic conditions on the shelf are additionally caused by high primary production in surface layers and the subsequent remineralisation of sinking organic matter by bacteria, which consume large amounts of DO (Lass and Mohrholz 2008, Stramma et al. 2010). In the Benguela upwelling system off Namibia, these processes lead to an O_2 deficit in intermediate waters (60-500 m) on the shelf with DO concentration of $<60 \mu\text{mol } O_2 \text{ kg}^{-1}$ (Auel and Verheye 2007, Ekau et al. 2010). Moreover, oxygen-depleted water masses from the Angola Dome are transported southward by the poleward-flowing undercurrent, which sustains hypoxic conditions in the Benguela region (Mohrholz et al. 2008). The Angola-Benguela Frontal Zone (ABFZ, at around 17°S , Fig. 2) separates warm water masses of the Angola Current from the cool Benguela Current (Shannon et al. 1987).

The OMZ in the tropical North Atlantic is most prominent in the Canary Current upwelling region but has another centre east of the Cape Verde Islands (Stramma et al. 2008b). The islands are situated within a productive region characterised by mixing of different water masses and seasonal input of Saharan dust, which fuels primary production by introducing terrestrial iron to the marine environment (Lázaro et al. 2005, Medina et al. 2007). Two opposing current systems, the Canary Current and the Equatorial Counter-Current, separate North Atlantic Central Water from South Atlantic Central Water and generate a transition zone around the islands. This frontal system, the Cape Verde Frontal Zone (CVFZ), is a characteristic oceanographic feature in this area located at around 20°N, north of the Cape Verde Islands (Fig. 2, Stramma and Schott 1999).

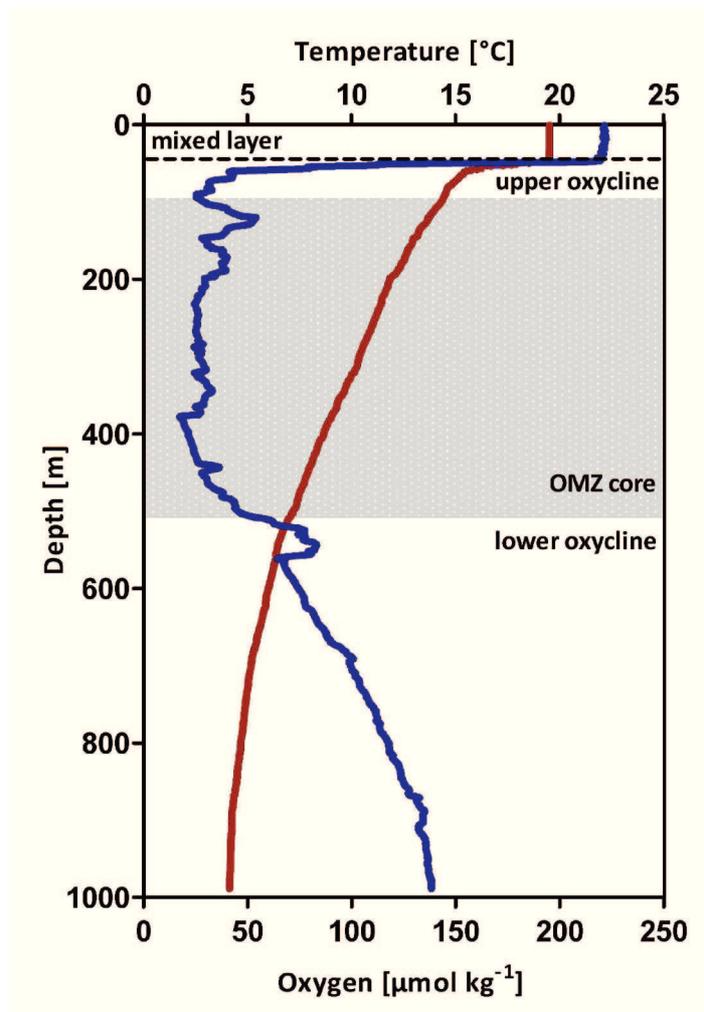


Figure 3. Typical vertical profiles of oxygen ($\mu\text{mol O}_2 \text{kg}^{-1}$, blue curve) and temperature ($^{\circ}\text{C}$, red curve) at a sampling station (St. 791) located at 12°S, 8°E in the Angola Gyre in the eastern tropical South Atlantic. Included is the vertical zonation suggested by Wishner et al. (2008) and Paulmier and Ruiz-Pino (2009).

OMZs are usually subdivided into three vertical layers (Fig. 3, Wishner et al. 2008, Paulmier and Ruiz-Pino 2009). The upper oxycline forms the lower boundary of the mixed, oxygen-saturated surface layer and has the strongest gradient in DO concentration. The core of the OMZ contains the lowest DO concentration, including the O₂ minimum, and may extend over several hundred metres. At the lower oxycline, DO concentrations begin to increase again above hypoxic levels (Wishner et al. 2008, Paulmier and Ruiz-Pino 2009).

In the future, tropical OMZs are predicted to intensify and expand in vertical and horizontal extent related to global warming and enhanced eutrophication (Luyten et al. 1983, Levin 2002, Stramma et al. 2008a, 2010, Keeling et al. 2010). Decreasing DO concentrations may act as barriers restricting the distribution and abundance of pelagic organisms such as zooplankton and fish (Ekau et al. 2010, Stramma et al. 2010). This will change the structure of pelagic communities, which in turn will have strong effects on global fisheries (Levin 2002, Stramma et al. 2012). In order to recognise changes at biological levels, it is fundamental to monitor influences of hypoxic conditions on pelagic key organisms such as copepods and to improve oceanographic models.

1.2 Copepods of the eastern tropical Atlantic

The physical oceanography and the abiotic environment of the tropical North and South Atlantic have extensively been studied within the last few decades. However, the pelagic zooplankton community is poorly known in these tropical offshore regions, since major biological research has so far concentrated on zooplankton of the coastal upwelling zones off northern Africa (Vives 1974, 1982, Binet 1978, 1983, Somoue et al. 2005) and off Namibia (Verheye et al. 1992, Auel et al. 2005, Loick et al. 2005, Schukat et al. 2013). The majority of studies on zooplankton from the central tropical Atlantic focused on distribution and taxonomic composition (Paiva 1963, 1970, Chahsavari-Archard and Razouls 1982, Woodd-Walker 2001, Woodd-Walker et al. 2002, Champalbert et al. 2005, Schnack-Schiel et al. 2010).

As major components of the zooplankton community, copepods are key organisms in marine pelagic environments and often contribute around 55 to 95% of all plankton to the mesozooplankton community worldwide (Longhurst 1985). Copepods are principal mediators in pelagic food webs, as they transfer energy from primary production to

higher trophic levels such as fish, seabirds and whales. Through feeding, respiration and excretion of fecal pellets, copepods considerably contribute to biogeochemical cycles in the open ocean (Longhurst 1991, del Giorgio and Duarte 2002, Wilson and Steinberg 2010). Especially diurnal vertically migrating (DVM) species enhance the vertical carbon flux, as they feed in surface waters at night and migrate to deeper layers for the day (Steinberg et al. 2000).

Tropical oceans are characterised by a pronounced vertical stratification, where a strong thermocline and a strong oxycline separate well-mixed, oxygenated surface water from cooler and less oxygenated waters below. Zooplankton communities are thus structured according to species-specific tolerance levels and may be limited in their vertical distribution by physical parameters and individual adaptations. In this context, OMZs present a challenging environment, potentially restricting the vertical distribution and influencing the ecophysiology of marine organisms (Parker-Stetter and Horne 2009, Ekau et al. 2010, Stramma et al. 2010, 2012, Wishner et al. 2013).

Among the copepod community, species of the order Calanoida comprise the major proportion of biomass (Raymont 1963). Calanoid copepods show a variety of life-cycle strategies, characterised by differences in vertical distribution and physiological as well as behavioural aspects in relation to their habitat.

The epipelagic copepod fauna in the tropical Atlantic is highly diverse and consists of small-sized species, often with a worldwide distribution in tropical and subtropical oceans (Chahsavari-Archard and Razouls 1982, Woodd-Walker 2001, Champalbert et al. 2005, Schnack-Schiel et al. 2010). Typical epipelagic species in the tropical Atlantic Ocean are *Undinula vulgaris*, *Temora stylifera*, *Neocalanus* spp., *Euchaeta marina*, *Candacia* spp. and *Scolecithrix danae* (Fig. 4). These species benefit from the continuous although scarce, food supply, have short life cycles and find adequate conditions for reproduction and growth year round (Mullin and Brooks 1970, Kattner and Hagen 2009).

Abundant members of the copepod community in tropical and subtropical oceans are specimens of the family Eucalanidae (Lang 1965, Saltzman and Wishner 1997a, Smith et al. 1998, Schnack-Schiel et al. 2008, Escribano et al. 2009). These copepods have a wide horizontal as well as vertical distribution from epi- to mesopelagic waters (Bradford-Grieve et al. 1999) and they occur frequently within OMZs in the tropical Pacific, Atlantic

and Arabian Sea (Flint et al. 1991, Smith et al. 1998, Madhupratap et al. 2001, Hidalgo et al. 2005, Auel and Verheye 2007, Schnack-Schiel et al. 2008, Escribano et al. 2009). Common species in the tropical and subtropical Atlantic are *Eucalanus hyalinus*, *Pareucalanus* spp., *Subeucalanus* spp., *Rhincalanus nasutus* and *R. cornutus* (Fig. 4), the latter being exclusively distributed in the Atlantic Ocean (Bradford-Grieve et al. 1999, Cass et al. 2011).

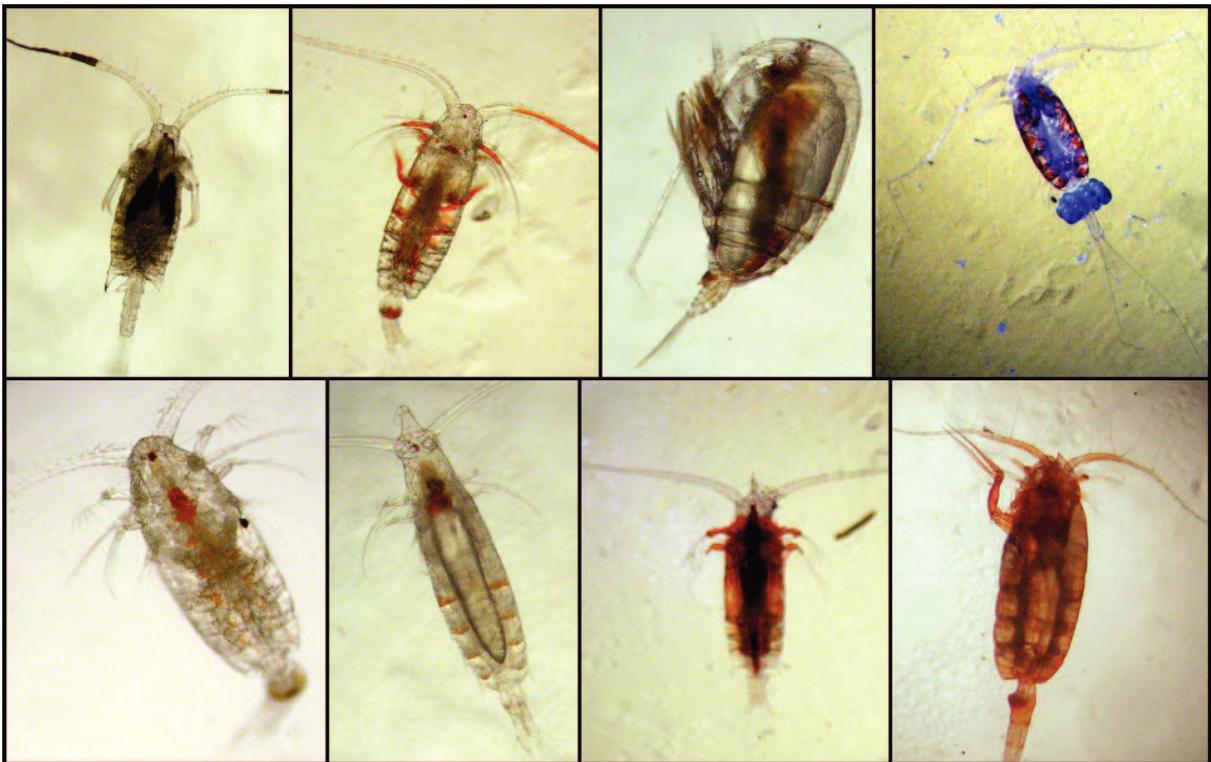


Figure 4. Common calanoid copepod species of the tropical Atlantic. From top left to bottom right: *Candacia pachydactyla* (male), females of *Neocalanus robustior*, *Scolecithrix danae*, *Euchaeta marina* (with egg sacs), *Pleuromamma robusta*, *Rhincalanus cornutus*, *Gaetanus pileatus*, *Paraeuchaeta hansenii*.

Many copepods that are distributed in the mesopelagic zone of the tropical Atlantic belong to the families Euchaetidae (e.g. *Paraeuchaeta* spp.) and Aetideidae (e.g. *Gaetanus* spp.), but also copepods such as *Megacalanus princeps* and *Gaussia princeps* are typical species of the mesopelagic copepod community (Fig. 4, Bradford-Grieve et al. 1999). These large-sized copepods often have a robust body structure; they experience colder temperatures and food paucity in the mesopelagic environment, associated with

longer generation times and longer life spans (Koppelman and Frost 2008, Kattner and Hagen 2009).

Copepods of the genus *Pleuromamma* are prominent species of the zooplankton community conducting intense diel vertical migrations (DVM) (Longhurst 1967, Champalbert and Gaudy 1972, Pavlova 1994, Loick et al. 2005). *Pleuromamma* spp. are distributed worldwide and some of the most abundant species in the tropical Atlantic are *P. abdominalis*, *P. robusta*, and *P. xiphias* (Fig. 4, Bradford-Grieve et al. 1999). As a result of their DVM, *Pleuromamma* spp. have a bimodal distribution pattern depending on time of day; they spend the night feeding in productive surface waters and migrate into deeper and cooler water layers for the day (Champalbert and Gaudy 1972, Mauchline 1998). In tropical Oceans, *Pleuromamma* often migrates into or through the OMZ and is apparently able to cope with very low DO concentrations (Vinogradov and Voronina 1962, Weikert 1982, Saltzman and Wishner 1997a, Smith et al. 1998, Auel and Verheye 2007, Escribano et al. 2009).

Among the copepods conducting ontogenetic vertical migrations (OVM), *Calanoides carinatus* is a key species in the upwelling regions off the west African Coast but also abundant in the tropical Atlantic and Arabian Sea (Verheye et al. 1991, 2005, Smith et al. 1998, Bradford-Grieve et al. 1999, Koppelman and Weikert 2005). Its life-cycle is characterised by a bimodal distribution of the population, including a resting state (diapause) of copepodids C5 in deeper water layers (Verheye et al. 1991, 2005, Auel et al. 2005, Schukat et al. 2013).

In order to characterise different life strategies of calanoid copepods, their vertical distribution, migration behaviour, dietary composition as well as their energy demand are important factors to consider. Species- or taxa-specific life strategies determine the particular role of different copepods within the zooplankton community and allow an estimation of their contribution to the pelagic carbon cycle.

1.3 Effects of hypoxia on marine organisms

Oxygen depletion is one of the most critical factors determining the distribution, behaviour and ecophysiology of marine organisms (Bograd et al. 2008, Ekau et al. 2010, Stramma et al. 2010). Marine animals rely on DO as terminal electron acceptor for the

oxidation of organic matter and extract DO from the surrounding water. The severe depletion or nearly absence of DO within OMZs may therefore represent a critical condition for survival of marine organisms. As a consequence, O₂ depletion may limit the vertical distribution and migration of many pelagic organisms and structures pelagic communities according to individual hypoxia tolerance levels (Saltzman and Wishner 1997a, Wishner et al. 2008).

The tolerance towards hypoxia varies considerably among different zooplankton and nekton species (Miller et al. 2002) and depends to a great extent on individual O₂ requirements and mobility levels (Vaquer-Sunyer and Duarte 2008, Seibel 2011). As defined in chapter 1.1, there are different O₂ thresholds describing the level of O₂ saturation in seawater, but physiological tolerance thresholds are species-specific (Miller et al. 2002, Seibel 2011).

Oxygen depletion is especially critical for zooplankton without the ability of independent horizontal movement to escape hypoxic environments. Since zooplankton organisms may only avoid oxygen-depleted water layers by vertical movement, zooplankton often exhibits a bimodal distribution pattern in OMZ regions (Saltzman and Wishner 1997a, Wishner et al. 1998, 2000, 2008, Escribano 2006). While zooplankton abundance is usually severely reduced within the core of the OMZ (Longhurst 1967, Wishner et al. 1998, 2008, Koppelman and Weikert 2005), areas of the upper and lower oxycline are often characterised by high zooplankton abundance and biomass (Wishner et al. 1990, Levin et al. 1991). Consequently, the OMZ may represent a barrier to the vertical distribution of less tolerant species and water layers above and below the OMZ are colonised by less hypoxia-tolerant organisms.

Crustaceans are usually quite tolerant to hypoxia and some euphausiid or copepod species may even survive DO concentrations of as low as 5-9 µmol O₂ kg⁻¹ (e.g. Sameoto et al. 1987, Flint et al. 1991). In contrast, large pelagic fish such as tuna, marlins and sailfish are generally more sensitive to O₂ depletion and do not tolerate hypoxic conditions of <60-120 µmol O₂ kg⁻¹ (Gray et al. 2002, Ekau and Verheye 2005, Vaquer-Sunyer and Duarte 2008). Fish and other nekton are able to horizontally escape hypoxic conditions, but may be restricted to the shallow, well oxygenated surface layer. Even though food availability for pelagic fish is enhanced in surface layers above the OMZ, the compression of their habitat by vertical extension of OMZs makes them more exposed to

fisheries (Prince and Goodyear 2006, Vaquer-Sunyer and Duarte 2008, Stramma et al. 2010, 2012, Wishner et al. 2013). Planktonic larvae of fish or crustaceans are usually even less hypoxia-tolerant and as a result, spreading OMZs may represent distributional boundaries especially to organisms with planktonic life stages (Ekau et al. 2010, Stramma et al. 2010).

In contrast, zooplankton and nekton organisms that temporarily tolerate hypoxic conditions and conduct DVM or OVM through or into the OMZ (Saltzman and Wishner 1997a, b, Prince and Goodyear 2006, Escribano et al. 2009, Maas et al. 2012) may find refuge from predation by less tolerant predators (Parker-Stetter and Horne 2009). Recently, increasing occurrences of gelatinous plankton in OMZ regions have been reported (Purcell et al. 2001, 2007, Lynam et al. 2006, Attrill et al. 2007). The higher tolerance levels of jellyfish and salps towards hypoxic conditions enables them to colonise areas with less than $60 \mu\text{mol O}_2 \text{ kg}^{-1}$ (Purcell et al. 2001, 2007, Thuesen et al. 2005, Wishner et al. 2008).

Different tolerance levels towards low DO may thus lead to a dominance of only a few hypoxia-tolerant species, which in turn reduces overall biodiversity in OMZ regions (Childress and Seibel 1998, Wishner et al. 2008, Levin et al. 2009, Stramma et al. 2010).

The effects of low DO concentrations on the metabolism of marine organisms are more difficult to predict. Hypoxic conditions could either lead to a reduction of metabolic rates in order to ensure survival within OMZs or to enhanced activity and increased metabolism caused by an escape response to leave areas with unfavourable environmental conditions (Pörtner and Grieshaber 1993).

In case of metabolic suppression as a response to low DO, two metabolic types can be distinguished. Some organisms reduce their metabolic rates under declining DO (metabolic conformers/oxyconformers), whereas others effectively maintain their metabolic activity until a species-specific threshold in DO concentration is reached (metabolic regulators/oxyregulators) (Pörtner and Grieshaber 1993, Childress 1995, Childress and Seibel 1998). Several jellyfish species have been categorised as metabolic conformers and show reduced metabolic rates within hypoxic zones (Rutherford and Thuesen 2005). In contrast, some midwater fishes, cephalopods and crustaceans naturally exposed to hypoxia, regulate their metabolism until the O_2 partial pressure of the water (PO_2) decreases below a critical threshold value of around 5 kPa ($\sim 60 \mu\text{mol O}_2 \text{ kg}^{-1}$)

(Childress 1995, Childress and Seibel 1998, Levin 2003, Seibel 2011). In this context, the PO_2 instead of the O_2 concentration of the water should be considered, since the PO_2 regulates the delivery of O_2 to organic tissues and thus determines the influence of low DO concentrations on the physiology of marine organisms (Seibel 2011).

At hypoxic conditions, the gradient is reduced that controls the diffusion of O_2 from the water to the mitochondria via respiratory epithelia (Pörtner et al. 2005, Seibel 2011). If the PO_2 drops below a critical threshold where routine metabolism cannot be supported by aerobic respiration alone, alternative energy pathways such as anaerobic metabolism may compensate for these energy losses (Pörtner and Grieshaber 1993, Childress and Seibel 1998, Seibel 2011). An appropriate indicator for anaerobic metabolism is an increased activity of the enzyme lactate dehydrogenase (LDH) or the accumulation of lactate, although this metabolite also accumulates during enhanced muscular activity (Childress and Somero 1979, Hochachka and Somero 2002). Nevertheless, the yield of ATP in anaerobic glycolysis is always considerably lower compared to aerobic metabolism (Hochachka and Somero 2002).

As outlined above, hypoxia affects the vertical and horizontal distribution, abundance and biomass as well as species composition and physiological processes of marine organisms (Childress and Seibel 1998, Levin 2003, Stramma et al. 2010). Changes in pelagic communities as well as increased mortalities of less hypoxia-tolerant taxa will lead to a loss of diversity in OMZ regions (Gilly et al. 2006, Stramma et al. 2010). In the long run, these changes may alter the structure of existing food webs as well as biogeochemical fluxes in pelagic environments.

1.4 Objectives

This study represents a first comprehensive assessment of the complex ecological and physiological characteristics of calanoid copepods from oceanic regions of the eastern tropical Atlantic. While distribution, physiology and some individual life cycles of a few tropical and subtropical copepod species have been well studied, ecophysiological data on the majority of copepods from the tropical Atlantic are still scarce. The overall aim of this study was therefore to characterise different life strategies of abundant calanoid copepod species with special focus on the current impact of the oxygen minimum zone (OMZ) on copepod distribution and ecophysiology.

The following main objectives will be addressed:

Impact of the oxygen minimum zone

Oxygen depletion in midwater layers of tropical oceans has been identified as one of the major challenges to marine life in the present time and near future. The concentration of dissolved oxygen (DO) in seawater determines horizontal and vertical distribution ranges as well as metabolic activity of marine organisms. Since OMZs in tropical oceans will expand in vertical and horizontal extent, it is essential to understand the effects of hypoxic conditions on zooplankton distribution patterns and physiological processes. In order to assess the impact of current low DO levels on the abundance, community structure and physiology of calanoid copepods, vertical distribution patterns were analysed and aerobic as well as anaerobic metabolic activities were experimentally determined (Publications I-II). The investigation of potential physiological adaptations to hypoxic conditions coupled with species-specific vertical distribution patterns and/or vertical migration behaviour should reveal potential tolerances of calanoid copepods towards low DO to survive within the OMZ.

Trophic interactions

Feeding preferences and dietary spectra of calanoid copepods from tropical open-ocean ecosystems are largely determined by depth of occurrence, food availability, foraging behaviour and individual metabolic strategy. Since phytoplankton production in epipelagic layers of tropical environments is limited, microzooplankton as well as picoplankton may serve as suitable alternative food sources and omnivory is often the prevailing feeding mode. Analyses of total lipid content, fatty acid and fatty alcohol compositions as well as stable ^{13}C and ^{15}N isotopic ratios (Publication III) help elucidate the feeding preferences and trophic interactions of epi- to bathypelagic calanoid copepods within the food web of the tropical Atlantic.

Life strategies

Tropical oceans are characterised by a strong vertical stratification with a pronounced thermocline separating the warm and well-mixed surface layer from the cool and more stable deeper water layers. These hydrographic features affect the DO concentration in the water as well as the food availability and thus influence the behaviour and physiology

of zooplankton organisms depending on their preferred depth of occurrence. To characterise different life strategies of tropical calanoid copepods and to determine typical behavioural as well as ecophysiological features, their distribution patterns and metabolic activity (respiration and enzyme activity measurements, Q_{10} values) as well as feeding mode and lipid storage strategies (total lipid content) were investigated (Publications I-III). Based on these analyses, various life-strategy types of calanoid copepods from the tropical Atlantic were identified and characterised.

This thesis consists of a local study on the copepod community of the Cape Verde Archipelago (Publication I) as well as two large-scale studies on calanoid copepods from three research expeditions to the eastern tropical Atlantic (Publications II and III). A variety of different methodological approaches, such as the assessment of horizontal and vertical distribution and abundance (Publication I-II), the measurement of respiration rates and enzyme activities (Publication I-II) as well as the analysis of lipids, fatty acids and stable isotopes (Publication III), led to a detailed investigation of different life strategies and ecophysiological adaptations to different physical environments in the eastern tropical Atlantic.

The results of this study in the tropical Atlantic will serve as a baseline assessment for evaluating the impact of hypoxic conditions on calanoid copepods as key components of the mesozooplankton community. Furthermore, the identification of different life strategies and possible metabolic or behavioural adaptations towards hypoxia will help to understand future changes in community structure and physiological responses of copepods caused by expanding and intensifying OMZs in tropical oceans.

REFERENCES

- Attrill MJ, Wright J, Edwards M (2007) Climate-related increases in jellyfish frequency suggest a more gelatinous future for the North Sea. *Limnol Oceanogr* 52: 480-485
- Auel H, Hagen W, Ekau W, Verheye HM (2005) Metabolic adaptations and reduced respiration of the copepod *Calanoides carinatus* during diapause at depth in the Angola-Benguela Front and northern Benguela upwelling regions. *Afr J Mar Sci* 27: 653-657
- Auel H, Verheye HM (2007) Hypoxia tolerance in the copepod *Calanoides carinatus* and the effect of an intermediate oxygen minimum layer on copepod vertical distribution in the northern Benguela Current upwelling system and the Angola-Benguela Front. *J Exp Mar Biol Ecol* 352: 234-243
- Binet D (1978) Analyse globale des populations de copépodes pélagiques du plateau continental ivoirien. *Cahiers ORSTOM Série Océanographie* 16: 19-61
- Binet D (1983) Zooplancton des régions côtières à upwellings saisonniers du Golfe de Guinée. *Océanographie Tropicale* 18: 357-380
- Bograd SJ, Castro CG, Di Lorenzo E, Palacios DM, Bailey H, Gilly W, Chavez FP (2008) Oxygen declines and the shoaling of the hypoxic boundary in the California Current. *Geophys Res Lett* 35: L12607, doi:10.1029/2008GL034185
- Bradford-Grieve JM, Markhaseva EL, Rocha CEF, Abiahy B (1999) Copepoda. In: Boltovskoy D (ed) *South Atlantic Zooplankton*. Backhuys Publishers, Leiden, pp 869-1098
- Brown E, Colling A, Park D, Phillips J, Rothery D, Wright J (2001) Chapter 5 - Other major current systems. *Ocean Circulation*. Butterworth-Heinemann, pp 143-189
- Cass CJ, Wakeham SG, Daly KL (2011) Lipid composition of tropical and subtropical copepod species of the genus *Rhincalanus* (Copepoda: Eucalanidae): a novel fatty acid and alcohol signature. *Mar Ecol Prog Ser* 439: 127-138
- Chahsavari-Archard V, Razouls C (1982) Les copépodes pélagiques au sud-est des îles du Cap Vert, I. Aspects qualitatifs (Mission Guidôme du N O «J Charcot» septembre-octobre 1976). *Vie Milieu* 32: 25-45
- Champalbert G, Gaudy R (1972) Etude de la respiration chez des copépodes de niveaux bathymétriques variés dans la région sud marocaine et canarienne. *Mar Biol* 12: 159-169
- Champalbert G, Pagano M, Kouame B, Riandey V (2005) Zooplankton spatial and temporal distribution in a tropical oceanic area off West Africa. *Hydrobiologia* 548: 251-265
- Childress JJ (1995) Are there physiological and biochemical adaptations of metabolism in deep-sea animals? *Trends Ecol Evol* 10: 30-36
- Childress JJ, Seibel BA (1998) Life at stable low oxygen levels: adaptations of animals to oceanic oxygen minimum layers. *J Exp Biol* 201: 1223-1232
- Childress JJ, Somero GN (1979) Depth-related enzymic activities in muscle, brain and heart of deep-living pelagic marine teleosts. *Mar Biol* 52: 273-283
- del Giorgio PA, Duarte CM (2002) Respiration in the open ocean. *Nature* 420: 379-384

- Ekau W, Auel H, Pörtner HO, Gilbert D (2010) Impacts of hypoxia on the structure and processes in pelagic communities (zooplankton, macro-invertebrates and fish). *Biogeosciences* 7: 1669-1699
- Ekau W, Verheye HM (2005) Influence of oceanographic fronts and low oxygen on the distribution of ichthyoplankton in the Benguela and southern Angola currents. *Afr J Mar Sci*: 629-639
- Escribano R (2006) Zooplankton interactions with the oxygen minimum zone in the eastern South Pacific. *Suplemento Gayana* 70: 19-21
- Escribano R, Hidalgo P, Krautz C (2009) Zooplankton associated with the oxygen minimum zone system in the northern upwelling region of Chile during March 2000. *Deep-Sea Res Part II* 56: 1049-1060
- Flint MV, Drits AV, Pasternak AF (1991) Characteristic features of body composition and metabolism in some interzonal copepods. *Mar Biol* 111: 199-205
- Gilly WF, Markaida U, Baxter CH, Block BA, Boustany A, Zeidberg L, Reisenbichler K, Robison B, Bazzino G, Salinas C (2006) Vertical and horizontal migrations by the jumbo squid *Dosidicus gigas* revealed by electronic tagging. *Mar Ecol Prog Ser* 324: 1-17
- Gray JS, Wu RSS, Or YY (2002) Effects of hypoxia and organic enrichment on the coastal marine environment. *Mar Ecol Prog Ser* 238: 249-279
- Helly JJ, Levin LA (2004) Global distribution of naturally occurring marine hypoxia on continental margins. *Deep-Sea Res Part I* 51: 1159-1168
- Hidalgo P, Escribano R, Morales CE (2005) Ontogenetic vertical distribution and diel migration of the copepod *Eucalanus inermis* in the oxygen minimum zone off northern Chile (20-21 degrees S). *J Plankton Res* 27: 519-529
- Hochachka PW, Somero GN (2002) Biochemical Adaptation: Mechanisms and Process in Physiological Evolution. Oxford University Press, Oxford
- Karstensen J, Stramma L, Visbeck M (2008) Oxygen minimum zones in the eastern tropical Atlantic and Pacific Oceans. *Prog Oceanogr* 77: 331-350
- Kattner G, Hagen W (2009) Lipids in marine copepods: latitudinal characteristics and perspectives to global warming. In: Arts MT, Brett M, Kainz M (eds) *Lipids in Aquatic Ecosystems*. Springer, Berlin, 377 pp
- Keeling RF, Kortzinger A, Gruber N (2010) Ocean deoxygenation in a warming world. *Annu Rev Mar Sci* 2: 199-229
- Koppelman R, Frost J (2008) The ecological role of zooplankton in the twilight and dark zones of the ocean. In: Mertens LP (ed) *Biological Oceanography Research Trends*. Nova Science Publishers, Inc., New York, pp 282
- Koppelman R, Weikert H (2005) Temporal and vertical distribution of two ecologically different calanoid copepods (*Calanoides carinatus* Krøyer 1849 and *Lucicutia grandis* Giesbrecht 1895) in the deep waters of the central Arabian Sea. *Mar Biol* 147: 1173-1178
- Lang BT (1965) Taxonomic review and geographical survey of the copepod genera *Eucalanus* and *Rhincalanus* in the Pacific Ocean. PhD Thesis, University of California, San Diego, 284 pp

- Lass HU, Mohrholz V (2008) On the interaction between the subtropical gyre and the Subtropical Cell on the shelf of the SE Atlantic. *J Mar Syst* 74: 1-43
- Lázaro C, Fernandes MJ, Santos AMP, Oliveira P (2005) Seasonal and interannual variability of surface circulation in the Cape Verde region from 8 years of merged T/P and ERS-2 altimeter data. *Remote Sens Environ* 98: 45-62
- Levin LA (2002) Deep-ocean life where oxygen is scarce. *Am Sci* 90: 436-444
- Levin LA (2003) Oxygen minimum zone benthos: adaptation and community response to hypoxia. *Oceanogr Mar Biol* 41: 1-45
- Levin LA, Ekau W, Gooday AJ, Jorissen F, Middelburg JJ, Naqvi SWA, Neira C, Rabalais NN, Zhang J (2009) Effects of natural and human-induced hypoxia on coastal benthos. *Biogeosciences* 6: 2063–2098
- Levin LA, Huggett CL, Wishner KF (1991) Control of deep-sea benthic community structure by oxygen and organic-matter gradients in the eastern Pacific Ocean. *J Mar Res* 49: 763-800
- Loick N, Ekau W, Verheye HM (2005) Water body preferences of dominant calanoid copepod species in the Angola-Benguela frontal zone. *Afr J Mar Sci* 27: 597-608
- Longhurst AR (1967) Vertical distribution of zooplankton in relation to the eastern Pacific oxygen minimum zone. *Deep-Sea Res* 14: 51-63
- Longhurst AR (1985) The structure and evolution of plankton communities. *Prog Oceanogr* 15: 1-35
- Longhurst AR (1991) Role of the marine biosphere in the global carbon cycle. *Limnol Oceanogr* 36: 1507-1526
- Luyten JR, Pedlosky J, Stommel H (1983) The ventilated thermocline. *J Phys Oceanogr* 13: 292-309
- Lynam CP, Gibbons MJ, Axelsen BE, Sparks CAJ, Coetzee J, Heywood BG, Brierley AS (2006) Jellyfish overtake fish in a heavily fished ecosystem. *Curr Biol* 16: R492-R493
- Maas AE, Wishner KF, Seibel BA (2012) Metabolic suppression in thecosomatous pteropods as an effect of low temperature and hypoxia in the eastern tropical North Pacific. *Mar Biol* 159: 1955-1967
- Madhupratap M, Gopalakrishnan TC, Haridas P, Nair KKC (2001) Mesozooplankton biomass, composition and distribution in the Arabian Sea during the fall intermonsoon: implications of oxygen gradients. *Deep-Sea Res Part II* 48: 1345-1368
- Mauchline J (1998) The biology of calanoid copepods. *Advances in Marine Biology*, Vol. 33. Academic Press, London, 710 pp
- Mazeika PA (1967) Thermal domes in the eastern tropical Atlantic Ocean. *Limnol Oceanogr* 12: 537-539
- Medina A, Brêthes JC, Sévigny JM, Zakardjian B (2007) How geographic distance and depth drive ecological variability and isolation of demersal fish communities in an archipelago system (Cape Verde, Eastern Atlantic Ocean). *Mar Ecol* 28: 404-417

- Miller DC, Poucher SL, Coiro L (2002) Determination of lethal dissolved oxygen levels for selected marine and estuarine fishes, crustaceans, and a bivalve. *Mar Biol* 140: 287-296
- Mohrholz V, Bartholomae CH, van der Plas AK, Lass HU (2008) The seasonal variability of the northern Benguela undercurrent and its relation to the oxygen budget on the shelf. *Cont Shelf Res* 28: 424-441
- Mohrholz V, Schmidt M, Lutjeharms JRE (2001) The hydrography and dynamics of the Angola-Benguela Frontal Zone and environment in April 1999. *S Afr J Sci* 97: 199-208
- Mullin MM, Brooks ER (1970) Growth and metabolism of two planktonic marine copepods as influenced by temperature and type of food. In: Steele JH (ed) *Marine food chains*. Oliver & Boyd, pp 74-95
- Paiva I (1963) Contribuição para o estudo dos copépodes Calanóides do arquipélago de Cabo Verde. *Estud Biol Mar* 42: 1-82
- Paiva I (1970) Nouvelle contribution à l'étude des Copépodes de l'Archipel du Cap Vert. *Rapports et Procès-verbaux des Réunion. Conseil International pour l'Exploration de la Mer*. 159: 116-117
- Parker-Stetter SL, Horne JK (2009) Nekton distribution and midwater hypoxia: a seasonal, diel prey refuge? *Est Coast Shelf Sci* 81: 13-18
- Paulmier A, Ruiz-Pino D (2009) Oxygen minimum zones (OMZs) in the modern ocean. *Prog Oceanogr* 80: 113-128
- Pavlova EV (1994) Diel changes in copepod respiration rates. *Hydrobiologia* 293: 333-339
- Pörtner HO, Grieshaber MK (1993) Critical PO_2 (s) in oxyconforming and oxyregulating animals: gas exchange, metabolic rate and the mode of energy production. In: Bicudo JEPW (ed) *Vertebrate gas transport cascade: adaptations to environment and mode of life*. CRC Press, Boca Raton, FL, pp 330-357
- Pörtner HO, Langenbuch M, Michaelidis B (2005) Synergistic effects of temperature extremes, hypoxia, and increases in CO_2 on marine animals: from Earth history to global change. *J Geophys Res C* 110
- Prince ED, Goodyear CP (2006) Hypoxia-based habitat compression of tropical pelagic fishes. *Fish Oceanogr* 15: 451-464
- Purcell JE, Breitburg DL, Decker MB, Graham WM, Youngbluth MJ, Raskoff KA (2001) Pelagic cnidarians and ctenophores in low dissolved oxygen environments: a review. *Coast Est Sci* 58: 77-100
- Purcell JE, Uye S, Lo WT (2007) Anthropogenic causes of jellyfish blooms and their direct consequences for humans: a review. *Mar Ecol Prog Ser* 350: 153-174
- Raymont JEG (1963) *Plankton and productivity in the oceans*. Pergamon Press, Oxford/New York, 660 pp
- Rutherford LD, Thuesen EV (2005) Metabolic performance and survival of medusae in estuarine hypoxia. *Mar Ecol Prog Ser* 294: 189-200
- Saltzman J, Wishner KF (1997a) Zooplankton ecology in the eastern tropical Pacific oxygen minimum zone above a seamount: 2. Vertical distribution of copepods. *Deep-Sea Res Part I* 44: 931-954

- Saltzman J, Wishner KF (1997b) Zooplankton ecology in the eastern tropical Pacific oxygen minimum zone above a seamount: 1. General trends. *Deep-Sea Res Part I* 44: 907-930
- Sameoto D, Guglielmo L, Lewis MK (1987) Day/night vertical distribution of euphausiids in the eastern tropical Pacific. *Mar Biol* 96: 235-245
- Schnack-Schiel SB, Mizdalski E, Cornils A (2010) Copepod abundance and species composition in the Eastern subtropical/tropical Atlantic. *Deep-Sea Res Part II* 57: 2064-2075
- Schnack-Schiel SB, Niehoff B, Hagen W, Böttger-Schnack R, Cornils A, Dowidar MM, Pasternak A, Stambler N, Stübing D, Richter C (2008) Population dynamics and life strategies of *Rhincalanus nasutus* (Copepoda) at the onset of the spring bloom in the Gulf of Aqaba (Red Sea). *J Plankton Res* 30: 655-672
- Schukat A, Teuber L, Hagen W, Wasmund N, Auel H (2013) Energetics and carbon budgets of dominant calanoid copepods in the northern Benguela upwelling system. *J Exp Mar Biol Ecol* 442: 1-9
- Seibel BA (2011) Critical oxygen levels and metabolic suppression in oceanic oxygen minimum zones. *J Exp Biol* 214: 326-336
- Shannon LV, Agenbag JJ, Buys MEL (1987) Large-scale and mesoscale features of the Angola-Benguela front. *S Afr J Mar Sci* 5: 11-34
- Siedler G, Zangenberg N, Onken R (1992) Seasonal-changes in the tropical Atlantic circulation - observation and simulation of the Guinea dome. *J Geophys Res C* 97: 703-715
- Smith S, Roman M, Prusova I, Wishner K, Gowing M, Codispoti LA, Barber R, Marra J, Flagg C (1998) Seasonal response of zooplankton to monsoonal reversals in the Arabian Sea. *Deep-Sea Res Part II* 45: 2369-2403
- Somoue L, Elkhiaiti N, Ramdani M, Lam Hoai T, Ettahiri O, Berraho A, Do Chi T (2005) Abundance and structure of copepod communities along the Atlantic coast of southern Morocco. *Acta Adriatica* 46: 63-76
- Steinberg DK, Carlson CA, Bates NR, Goldthwait SA, Madin LP, Michaels AF (2000) Zooplankton vertical migration and the active transport of dissolved organic and inorganic carbon in the Sargasso Sea. *Deep-Sea Res Part I* 47: 137-158
- Stramma L, Brandt P, Schafstall J, Schott F, Fischer J, Kortzinger A (2008b) Oxygen minimum zone in the North Atlantic south and east of the Cape Verde Islands. *J Geophys Res C* 113: C04014, doi:10.1029/2007JC004369
- Stramma L, Huttel S, Schafstall J (2005) Water masses and currents in the upper tropical northeast Atlantic off northwest Africa. *J Geophys Res C* 110: C12006, doi:10.1029/2005JC002939
- Stramma L, Johnson GC, Sprintall J, Mohrholz V (2008a) Expanding oxygen minimum zones in the tropical oceans. *Science* 320: 655-658
- Stramma L, Prince ED, Schmidtko S, Luo JG, Hoolihan JP, Visbeck M, Wallace DWR, Brandt P, Kortzinger A (2012) Expansion of oxygen minimum zones may reduce available habitat for tropical pelagic fishes. *Nature Climate Change* 2: 33-37

- Stramma L, Schmidtko S, Levin LA, Johnson GC (2010) Ocean oxygen minima expansions and their biological impacts. *Deep-Sea Res Part I* 57: 587-595
- Stramma L, Schott F (1999) The mean flow field of the tropical Atlantic Ocean. *Deep-Sea Res Part II* 46: 279-303
- Stramma L, Visbeck M, Brandt P, Tanhua T, Wallace D (2009) Deoxygenation in the oxygen minimum zone of the eastern tropical North Atlantic. *Geophys Res Lett* 36: L20607, doi:10.1029/2009GL039593
- Thuesen EV, Rutherford LD, Brommer PL, Garrison K, Gutowska MA, Towanda T (2005) Intragel oxygen promotes hypoxia tolerance of scyphomedusae. *J Exp Biol* 208: 2475-2482
- Vaquier-Sunyer R, Duarte CM (2008) Thresholds of hypoxia for marine biodiversity. *Proc Natl Acad Sci USA* 105: 15452-15457
- Verheye HM, Hagen W, Auel H, Ekau W, Loick N, Rheenen I, Wencke P, Jones S (2005) Life strategies, energetics and growth characteristics of *Calanoides carinatus* (Copepoda) in the Angola-Benguela frontal region. *Afr J Mar Sci* 27: 641-651
- Verheye HM, Hutchings L, Huggett JA, Painting SJ (1992) Mesozooplankton dynamics in the Benguela ecosystem, with emphasis on the herbivorous copepods. *S Afr J Mar Sci* 12: 561-584
- Verheye HM, Hutchings L, Peterson WT (1991) Life-history and population maintenance strategies of *Calanoides carinatus* (Copepoda, Calanoida) in the southern Benguela ecosystem. *S Afr J Mar Sci* 11: 179-191
- Vinogradov ME, Voronina NM (1962) Influence of the oxygen deficit on the distribution of plankton in the Arabian Sea. *Deep-Sea Res* 9: 523-530
- Vives F (1974) Le zooplancton et les masses d'eau des environs du Cap Blanc. *Téthys* 6: 313-318
- Vives F (1982) Sur les copépodes de la région CINECA (Parties nord et centrale). In: Hempel G (ed) *The Canary Current: studies of an upwelling system*. Rapp P-v Réunion, pp 289-296
- Voituriez B, Herbland A (1982) A comparative study of the productive systems of the tropical East Atlantic: thermal domes, coastal upwelling and equatorial upwelling. Rapp P-v Réunion. Conseil Permanent International pour l'Exploration de la Mer, pp 114-130
- Weikert H (1982) The vertical distribution of zooplankton in relation to habitat zones in the area of the Atlantis II Deep, central Red Sea. *Mar Ecol Prog Ser* 8: 129-143
- Wilson SE, Steinberg DK (2010) Autotrophic picoplankton in mesozooplankton guts: evidence of aggregate feeding in the mesopelagic zone and export of small phytoplankton. *Mar Ecol Prog Ser* 412: 11-27
- Wishner K, Levin L, Gowing M, Mullineaux L (1990) Involvement of the oxygen minimum in benthic zonation on a deep seamount. *Nature* 346: 57-59
- Wishner K, Outram DM, Seibel BA, Daly KL, Williams RL (2013) Zooplankton in the eastern tropical north Pacific: boundary effects of oxygen minimum zone expansion. *Deep-Sea Res Part I* 79: 122-140

- Wishner KF, Gelfman C, Gowing MM, Outram DM, Rapien M, Williams RL (2008) Vertical zonation and distributions of calanoid copepods through the lower oxycline of the Arabian Sea oxygen minimum zone. *Prog Oceanogr* 78: 163-191
- Wishner KF, Gowing MM, Gelfman C (1998) Mesozooplankton biomass in the upper 1000 m in the Arabian Sea: overall seasonal and geographic patterns, and relationship to oxygen gradients. *Deep-Sea Res Part II* 45: 2405-2432
- Wishner KF, Gowing MM, Gelfman C (2000) Living in suboxia: ecology of an Arabian Sea oxygen minimum zone copepod. *Limnol Oceanogr* 45: 1576-1593
- Woodd-Walker RS (2001) Spatial distributions of copepod genera along the Atlantic Meridional Transect. *Hydrobiologia* 453: 161-170
- Woodd-Walker RS, Ward P, Clarke A (2002) Large-scale patterns in diversity and community structure of surface water copepods from the Atlantic Ocean. *Mar Ecol Prog Ser* 236: 189-203
- Wright JJ, Konwar KM, Hallam SJ (2012) Microbial ecology of expanding oxygen minimum zones. *Nat Rev Micro* 10: 381-394

PUBLICATION I

RESPIRATION RATES OF TROPICAL ATLANTIC COPEPODS IN RELATION TO THE OXYGEN MINIMUM ZONE

TEUBER L, KIKO R, SEGUIN F, AUDEL H

Published in *Journal of Experimental Marine Biology and Ecology* (2013)

Dataset published in *PANGAEA* database doi:10.1594/PANGAEA.816158



Respiration rates of tropical Atlantic copepods in relation to the oxygen minimum zone



Lena Teuber^{a,*}, Rainer Kiko^b, François Séguin^a, Holger Auel^a

^a BreMarE Bremen Marine Ecology, University of Bremen, P.O. Box 330 440, 28334 Bremen, Germany

^b GEOMAR Helmholtz Centre for Ocean Research, Hohenbergstr. 2, 24105 Kiel, Germany

ARTICLE INFO

Article history:

Received 16 October 2012

Received in revised form 26 April 2013

Accepted 12 June 2013

Available online xxxx

Keywords:

Cape Verde

Hypoxia

Oxygen consumption

Respirometry

Zooplankton

ABSTRACT

Zooplankton respiration plays an important role in the carbon cycling of pelagic ecosystems. The rate of oxygen consumption in zooplankton is affected by the physical environment, vertical distribution range and species-specific behavior. Especially in tropical oceans, oxygen minimum zones (OMZs) may influence zooplankton metabolic processes and vertical distribution and thus structure zooplankton communities. Here we present respiration rates of tropical Atlantic copepods in relation to environmental factors, especially O_2 concentration, and species-specific characteristics. Copepods were sampled during two research stays on the Cape Verde Island São Vicente in March/April and May/June 2010. Minimum O_2 concentrations of $51 \mu\text{mol kg}^{-1}$ (pO_2 of 4.25 kPa) at 400 m depth were recorded within the OMZ. Respiration rates of epipelagic and mesopelagic calanoid copepods were measured by optode respirometry at three different ambient temperatures (13, 18, and 23 °C) to establish the effect of temperature on metabolic rates. Mass-specific oxygen consumption ranged from $27 \mu\text{mol } O_2 \text{ g}_{\text{DM}}^{-1} \text{ h}^{-1}$ in copepodids C5 of *Lophothrix* sp. at 13 °C to $774 \mu\text{mol } O_2 \text{ g}_{\text{DM}}^{-1} \text{ h}^{-1}$ in *Pleuromamma xiphias* copepodids C5 at 18 °C and was mainly controlled by body mass and temperature. Mass-specific respiration rates were highest in surface-dwelling organisms and decreased with increasing depth. To allow for a comparison of shallow and deep-living copepods, respiration rates were standardized to a common temperature of 18 °C and a mean body dry mass of 0.5 mg, applying a Q_{10} of 2.0 and a body mass exponent of -0.56 . Temperature- and body mass-corrected respiration rates did not decrease with increasing depth indicating that neither depth of occurrence, nor current hypoxic conditions within the OMZ had a fundamental, persistent effect on zooplankton respiration.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Respiration rates serve as a direct indicator of metabolic activity in marine organisms and help tracing energy pathways and carbon fluxes (Hernández-León and Ikeda, 2005; Ikeda, 1985). Zooplankton respiration plays an important role in the cycling of organic matter in marine ecosystems. In particular, vertically migrating species contribute to the vertical carbon flux and enhance the biological carbon pump (Longhurst, 1991). Respiration rates are often used to describe zooplankton activity in biogeochemical models (Pahlow et al., 2008). Zooplankton respiration is affected by the vertical distribution range of organisms and species-specific behavior, besides the two main factors, temperature and body mass (Hirche, 1987; Ikeda, 1985; Ikeda et al., 2001). About 95% of variation in copepod and zooplankton respiration can be attributed to these two factors (Bode et al., 2013; Ikeda, 1985; Ikeda et al., 2001). Other aspects affecting zooplankton respiration are activity level, depth of occurrence and the ambient

O_2 partial pressure (pO_2). In tropical oceans, temperature and oxygen concentration decrease abruptly below the mixed surface layer, whereas body mass of organisms generally increases towards deeper waters. These vertical changes in temperature and body mass, both, lead to a decrease in mass-specific respiration of zooplankton with increasing depth. In addition, metabolic rates of some visually-oriented midwater fishes, cephalopods and crustaceans seem to decline with increasing depth even independent of temperature and body mass trends (Childress, 1995; Childress and Seibel, 1998; Ikeda et al., 2006; Seibel, 2011). The effects of low O_2 concentrations on zooplankton metabolism, however, are more difficult to predict. Hypoxic conditions could either lead to reduced metabolic rates in order to ensure survival or to enhanced activity and increased metabolism triggered by an escape response to leave areas with unfavorable environmental conditions.

The most prominent OMZs are located in the eastern tropical Pacific, Arabian Sea and Indian Ocean (Karstensen et al., 2008; Stramma et al., 2008a). In these regions, oxygen concentrations frequently fall below $4.5 \mu\text{mol kg}^{-1}$ (0.1 ml l^{-1}) (Karstensen et al., 2008; Paulmier and Ruiz-Pino, 2009; Stramma et al., 2008a, 2008b), while the OMZ in the eastern tropical North Atlantic is not as pronounced yet. East of the Cape Verde Archipelago the O_2 minimum

* Corresponding author. Tel.: +49 421 21863037; fax: +49 421 21863055.
E-mail address: teuber@uni-bremen.de (L. Teuber).

of $\sim 50 \mu\text{mol kg}^{-1}$ is found at 400 to 500 m depth (Stramma et al., 2008b). Nevertheless, the Atlantic has been suffering from the most severe decline in oxygen concentration within the last 50 years ($\sim 0.5 \mu\text{mol kg}^{-1} \text{a}^{-1}$, Stramma et al., 2008a, 2009). OMZs are expected to expand vertically and horizontally (Stramma et al., 2008a, 2010), but the impacts of hypoxic conditions on marine organisms are not yet fully understood.

Only a few studies exist on the physiology of tropical Atlantic copepods and even less is known about consequences of low oxygen concentrations on their physiology and community structure. Metabolic activities were measured in bulk zooplankton samples off the Canary Islands (Aristegui and Montero, 2005; Hernández-León et al., 1998, 2004), whereas species-specific respiration rates are available for copepods in the Benguela upwelling system (Bode et al., 2013; Schukat et al., 2013). Pavlova (1994) linked metabolic rates of tropical copepods from the Indian Ocean to their general activity levels.

The effects of hypoxia on zooplankton have been studied in the eastern tropical Pacific, Arabian Sea and Indian Ocean (Auel and Verheye, 2007; Escribano, 2006; Saltzman and Wishner, 1997; Wishner et al., 2008). Results show that low $p\text{O}_2$ can have severe consequences on species distribution, physiology and metabolic processes and may alter migration behavior, community structure and trophic pathways (Bograd et al., 2008; Chan et al., 2008; Childress and Seibel, 1998; Ekau et al., 2010; Stramma et al., 2010, 2012). Changes in zooplankton biomass, species composition and metabolic activity may in turn have major impacts on ocean biogeochemistry, as mesopelagic zooplankton strongly contributes to biogeochemical cycles via feeding, fecal pellet production and respiration processes (del Giorgio and Duarte, 2002; Longhurst, 1991; Longhurst and Harrison, 1989; Wilson and Steinberg, 2010).

The Cape Verde Archipelago, located in the tropical northeast Atlantic Ocean, is known for its high biodiversity of zooplankton (Chahsavari-Archard and Razouls, 1982). Here we present the first physiological dataset on respiration rates and metabolic activities of dominant copepod species from this tropical region. The following research questions are addressed: (i) How are different copepod species physiologically and behaviorally adapted to their specific depth of occurrence? and (ii) How does the physical environment around the Cape Verde Archipelago influence zooplankton communities with special focus on low oxygen conditions within the OMZ?

2. Material and methods

2.1. Zooplankton sampling

Copepods were sampled at two sampling sites off the island of São Vicente, Cape Verde Archipelago, in spring (March/April) and early summer (May/June) of 2010. The inshore sampling site was located in Mindelo Bay (16.9°N , 15.01°W ; bottom depth of 22 m), while the offshore station was located south of the island around 8 km off the town of São Pedro (16.77°N , 25.12°W ; bottom depth of 800 m) (Fig. 1). Samples were collected on board the local fishing vessel 'Sinagoga' using a WP-2 net (Hydrobios, 0.26 m^2 mouth opening, $200 \mu\text{m}$ mesh size). The net was equipped with a flow meter to calculate the total volume of water filtered and a Conductivity-Temperature-Depth (CTD) profiler for vertical profiles of temperature, salinity and oxygen. Oxygen partial pressures (kPa) were calculated from these values using the R-package AquaEnv (Hofmann et al., 2010). Depending on the bottom depth at the respective station, the net was either applied as a driftnet (inshore station), drifting for 10 min in 22 to 0 m depth below the surface, or towed vertically (offshore station) with a towing speed of 0.5 m s^{-1} . In March/April 2010 maximum sampling depth at the offshore station was 560 m, and the net was deployed for stratified sampling with repetitive hauls from 560 to 210 m, from 210 to 80 m, and from 80 to 0 m, respectively, using a net closing mechanism triggered by a releaser weight from the vessel. In May/June 2010 a similar sampling strategy was applied at the same offshore station, but with different depth intervals of 600 to 300 m, 300 to 100 m, and 100 to 0 m. In addition, depth-integrated hauls were conducted from 600 to 0 m or from 500 to 0 m during both field campaigns. On board zooplankton samples were immediately transferred to buckets filled with seawater at ambient temperatures until the return to the laboratory in Mindelo. Seawater was collected on site for later use in laboratory experiments.

2.2. Respiration measurements

Respiration rates of abundant copepod species were measured in the land-based laboratory at the Instituto Nacional de Desenvolvimento das Pescas (INDP) in Mindelo. Oxygen consumption was measured

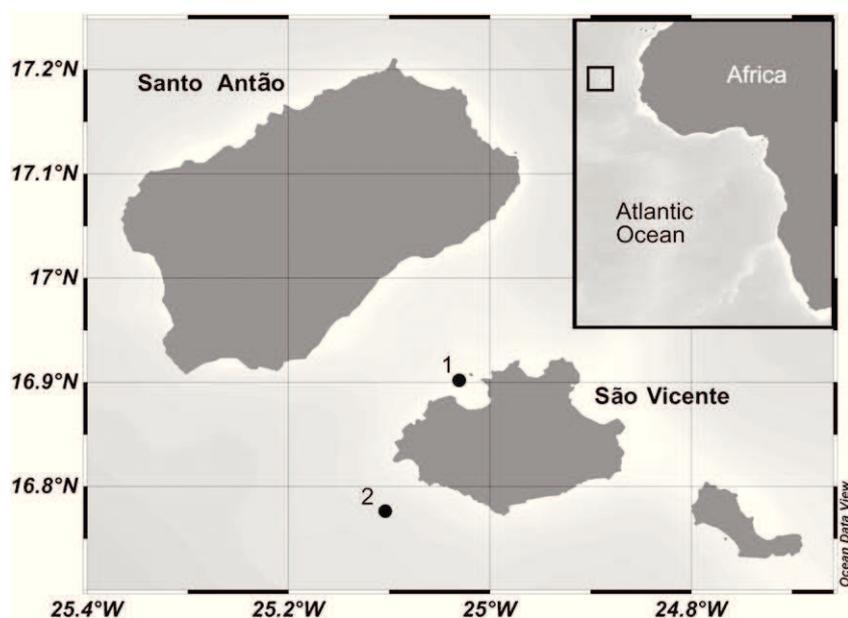


Fig. 1. Map of the northern islands of the Cape Verde Archipelago off the north-west African coast and location of the two sampling sites off São Vicente (1, inshore; 2, offshore).

non-invasively by optode respirometry with a 10-channel oxygen respirometer (Oxy-10 Mini, PreSens Precision Sensing GmbH, Regensburg, Germany). Oxygen-sensitive sensor spots were glued to the inner wall of ten gas-tight glass bottles (12–13 ml volume). Bottles were filled with oxygenated seawater, previously sterilized by UV-light (Aqua Cristal UV C 5 W, JBL) and filtered through a 0.2 μm Whatman GFF filter to reduce microbial respiration. Thus, the use of antibiotics could be avoided. Respiration measurements started within 5 to 70 h after sampling. Depending on the body size of each species, incubation bottles contained one to six individuals (0.08–0.5 ind. ml^{-1}). Only specimens in good condition were used for the experiments. All experiments were run in darkness in temperature-controlled incubators (LMS Cooled Incubator Series 1A, Model 280) equipped with water baths to ensure constant temperatures throughout the experiments. Incubators were set to three different temperatures (13, 18, and 23 $^{\circ}\text{C}$), tolerating a variation of ± 1 $^{\circ}\text{C}$. Experimental temperatures were chosen according to in situ conditions at the three sampling depths, i.e. surface layer, thermocline and deep water, respectively. For each experimental setup, two animal-free control bottles were run under the same conditions to compensate for microbial activity. Animals were not fed before or during the experiments.

Copepods were incubated at ambient temperatures to determine their oxygen consumption under natural conditions. Additional experiments were conducted at higher and/or lower temperatures (± 5 $^{\circ}\text{C}$) in order to observe the physiological response to changing temperatures and to calculate Q_{10} values. Specimens for temperature shift experiments were acclimated to the experimental temperatures. Experiments were terminated after 6 to 21 h. Individuals were separately preserved, either in 100% ethanol (samples March/April) or 4% buffered formaldehyde in seawater solution (samples May/June) for exact staging, species identification and dry mass determination in the home laboratory. Oxygen consumption rates were calculated by determining the slope of the decrease of oxygen concentration over time. The first 60 min of each experiment were discarded to exclude eventual temperature effects or handling stress.

2.3. Dry mass determination of preserved samples

In the home lab at the University of Bremen, preserved samples were dried in a heating cabinet at 60 $^{\circ}\text{C}$ for 48 h. Dry mass of each individual was determined on a SARTORIUS microbalance (MC21S). Samples preserved in ethanol and formaldehyde usually suffer a dissimilar weight loss of $\geq 25\%$ due to hyperosmotic pressure during preservation (Durbin and Durbin, 1978; Leuven et al., 1985; Omori, 1978). The losses in body mass by preservation in ethanol or formaldehyde, respectively, were quantified by collecting replicate samples of different copepod species from another trip to the Cape Verde Islands in 2011 and preserving them either in ethanol or in formaldehyde or immediately drying them. Dry mass (DM) values of the preserved samples were determined after a preservation period comparable to the original samples obtained in 2010. A linear regression analysis revealed that for ethanol-preserved samples $\text{DM}_{\text{ethanol}} = 0.6165 * \text{DM}_{\text{real}} + 0.0033$ ($R^2 = 0.898$, $n = 55$), whereas for formaldehyde-preserved samples $\text{DM}_{\text{formalin}} = 0.4194 * \text{DM}_{\text{real}} + 0.0431$ ($R^2 = 0.55$, $n = 56$). These regression equations were applied to calculate realistic and comparable dry mass values for ethanol and formaldehyde preserved samples.

2.4. Calculation of Q_{10} values

Mass-specific respiration rates of abundant copepod species measured at different temperatures were used to calculate species-specific Q_{10} values. Q_{10} values were derived from the regression of mass-specific respiration rate and experimental temperature and were only determined for species/stages with ≥ 4 replicates.

2.5. Temperature and body mass standardization

To compare respiration rates of copepod species from different bathymetric zones without the influence of ambient temperature and individual body mass differences, mass-specific respiration rates were standardized to a mean ambient temperature of 18 $^{\circ}\text{C}$ applying a general Q_{10} of 2 and the van't Hoff equation ($R_{18} = R_1 * Q_{10}^{(18-T_1)/10}$, where R_{18} is the respiration rate at 18 $^{\circ}\text{C}$, R_1 is the original respiration rate at the corresponding temperature T_1). The temperature of 18 $^{\circ}\text{C}$ was chosen as a mean habitat temperature between surface and deeper waters. The scaling coefficient b (-0.56), derived from the slope of the regression line (Fig. 2), was used to standardize respiration rates to a mean copepod dry mass of 0.5 mg, applying the allometric equation $R_{0.5; 18} = a_{18} * 0.5^b$, where $R_{0.5; 18}$ is the respiration rate at 18 $^{\circ}\text{C}$ and 0.5 mg body dry mass, a_{18} is the rate coefficient, 0.5 is the target dry mass and b is the derived exponential constant (-0.56) (compare Ikeda et al., 2006; Thuesen et al., 1998). The rate coefficient a_{18} was calculated for each respiration rate by the equation $a_{18} = R_{18}/(W^{-0.56})$.

3. Results

3.1. Hydrography

Environmental conditions at the offshore sampling site did not differ considerably between both sampling seasons (Fig. 3). The well-mixed surface layer extended down to about 80–100 m. The average sea surface temperature (SST) was around 25 $^{\circ}\text{C}$, while oxygen concentrations at the surface were on average between 204 and 208 $\mu\text{mol kg}^{-1}$ and sea surface salinity was between 35.9 and 36.8. Below the mixed surface layer, a strong thermocline extended to around 100 m, where gradients of all three parameters were strongest. Below 100 m depth, temperature decreased steadily from around 18 to 8.9 $^{\circ}\text{C}$ at 600 m depth, whereas salinity stabilized at about 35.2. The oxygen concentration decreased to a minimum of 51.4 $\mu\text{mol kg}^{-1}$ ($p\text{O}_2$ of 4.25 kPa) at 403 m in April and to 51.8 $\mu\text{mol kg}^{-1}$ ($p\text{O}_2$ of 4.29 kPa) at 355 m in May. Waters with O_2 concentrations below 70 $\mu\text{mol kg}^{-1}$ ($p\text{O}_2 < 6.0$ kPa) extended from 259 to 595 m in April and from 194 to 600 m in May. The 60 $\mu\text{mol kg}^{-1}$ isoline ($p\text{O}_2 < 5.0$ kPa) was located at 295 and 476 m depth in April and at 302 and 471 m in May.

3.2. Respiration rates

A total of 179 respiration measurements were conducted with 35 calanoid copepod species from the Cape Verde Archipelago. Table 1

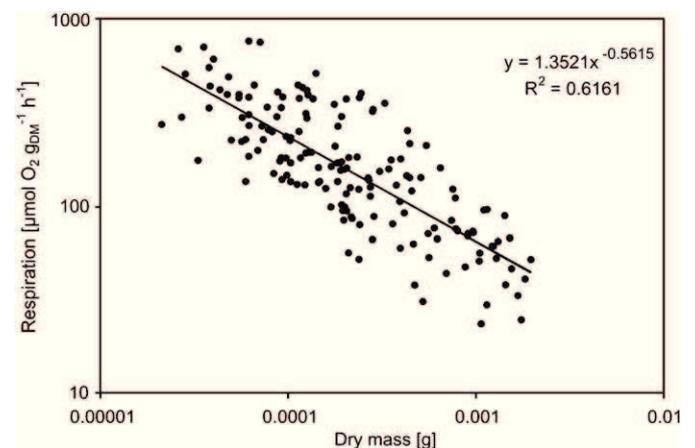


Fig. 2. Mass-specific respiration rates of copepods at a standardized temperature of 18 $^{\circ}\text{C}$ plotted against dry mass at logarithmic scale.

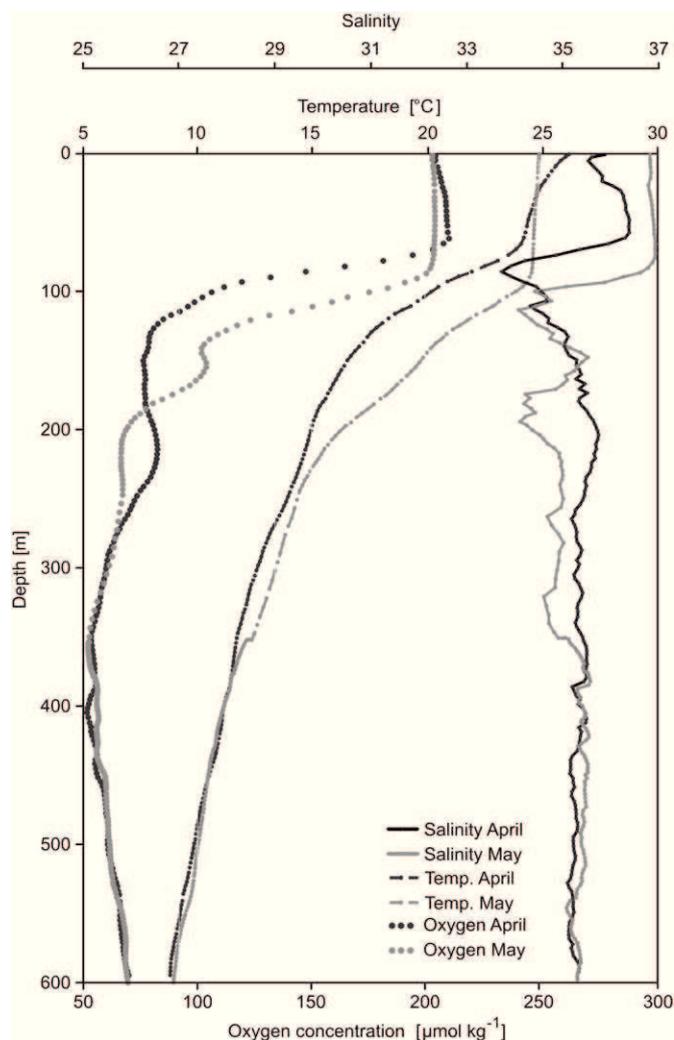


Fig. 3. Vertical CTD profiles of salinity, temperature and oxygen concentration at the off-shore sampling site south of São Vicente in April (black curves) and May (gray curves).

shows the results for all species with ≥ 2 replicates. Data from individual measurements are available online from the PANGAEA database (Teuber et al., in press). As expected, mass-specific respiration rates increased with decreasing body dry mass and increasing temperature. At 13 °C mass-specific respiration varied from $27 \mu\text{mol O}_2 \text{g}_{\text{DM}}^{-1} \text{h}^{-1}$ in copepodids C5 of *Lophothrix* sp. to $562 \mu\text{mol O}_2 \text{g}_{\text{DM}}^{-1} \text{h}^{-1}$ in females of *Nannocalanus minor*. At 18 °C mass-specific respiration ranged from $50 \pm 27 \mu\text{mol O}_2 \text{g}_{\text{DM}}^{-1} \text{h}^{-1}$ in *Pleuromamma xiphias* males to $774 \mu\text{mol O}_2 \text{g}_{\text{DM}}^{-1} \text{h}^{-1}$ in a copepodid C5 of the same species. At 23 °C *Scolecithrix danae* females displayed the lowest mass-specific respiration of $176 \mu\text{mol O}_2 \text{g}_{\text{DM}}^{-1} \text{h}^{-1}$, whereas males of *Subeucalanus subtenuis* showed the highest rate of $412 \pm 71 \mu\text{mol O}_2 \text{g}_{\text{DM}}^{-1} \text{h}^{-1}$.

3.3. Q_{10} values

Q_{10} values were calculated on the basis of mass-specific respiration rates derived from measurements at different temperatures (13, 18, and 23 °C). The Q_{10} values ranged from 1.0 in males of *P. xiphias* to 2.7 in *S. danae* females (Table 1). A median Q_{10} of 1.8 was determined for the whole set of 11 species.

3.4. Respiration rates of copepods vs. depth

Mass-specific respiration of calanoid copepods was highest at the surface and decreased with increasing depth (Fig. 4). Differences in

respiration rates between the surface layer (0–100 m) and deeper waters (100–500 m) were significant (Mann-Whitney U test, $p < 0.0001$), although respiration rates of the same species sampled in epi- and in mesopelagic waters were not significantly different from one another. In the epipelagic layer, *S. subtenuis* and egg-carrying females of *Euchaeta marina* showed high variability in respiration, as did females of *Candacia pachydactyla*, *Rhincalanus cornutus* and *Pleuromamma abdominalis* between 200 and 300 m depth. Below 300 m, females of *R. cornutus* and *Neocalanus robustior*, males of *P. xiphias*, and a C5 of *Neocalanus* sp. showed very low overall respiration rates and very low variability.

3.5. Respiration rates standardized for temperature and body mass

The scaling coefficient $b = -0.56$, derived from the regression equation of body mass versus respiration adjusted to 18 °C (Fig. 2), was used to standardize mass-specific respiration rates to a common mean body dry mass of 0.5 mg (Ikeda et al., 2006). After standardization for temperature and body-mass effects, no statistically significant depth-related differences in mass-specific respiration rate could be determined, indicating that neither depth of occurrence, nor lower oxygen concentration had an impact on copepod metabolism (Fig. 5A). For most copepod species, standardized mass-specific respiration rates did not differ significantly from the overall mean of $212 \pm 93 \mu\text{mol O}_2 \text{g}_{\text{DM}}^{-1} \text{h}^{-1}$ (Fig. 5B). However, females of *E. marina* with egg sacs had significantly higher respiration rates, whereas males of *S. danae* and *Subeucalanus crassus* showed significantly lower rates than the overall mean (Mann-Whitney U test, p -values < 0.05).

4. Discussion

This study presents a first assessment of respiration rates and metabolic demands of tropical calanoid copepods from the Cape Verde Archipelago in relation to habitat depth, hydrography and species-specific characteristics. Respiration rates were measured by optode respirometry, which allowed individual measurements of very small organisms like copepods with a body mass down to 25 μg . This method proved to be an adequate technique for simultaneous measurements of several replicates and is thus a successful and time-efficient method for field experiments. It has several advantages over conventional methods such as Winkler titration or Clark electrodes, e.g. continuous and non-invasive measurement, no consumption of oxygen by the optode itself and no use of chemicals (Klimant et al., 1995; Köster et al., 2008; Warkentin et al., 2007).

In all experiments the number of individuals per incubation bottle was chosen with respect to experimental temperature and body size and did not exceed 0.5 ind. ml^{-1} (range of 0.08 – 0.5 ind. ml^{-1} , median of $0.08 \text{ ind. ml}^{-1}$). At this density, a significant decrease in oxygen over time could be observed, while crowding effects were minimized. The effect of crowding may either increase or reduce zooplankton respiration (Razouls, 1972; Satomi and Pomeroy, 1965), while most studies report an increase in apparent respiration rate with increasing animal density (Satomi and Pomeroy, 1965; Zeiss, 1963). For *Calanus finmarchicus*, there was no crowding effect at densities of 0.1 to 0.4 ind. ml^{-1} (Zeiss, 1963). In the copepods *Temora stylifera* and *Centropages typicus* respiration rates only declined at densities $> 0.5 \text{ ind. ml}^{-1}$ (Razouls, 1972). Therefore, we are convinced that a maximum density of 0.5 ind. ml^{-1} in our experiments did not have significant negative effects on copepod metabolic rate.

Respiration rates from this study are comparable to published data from tropical and subtropical regions (Bode et al., 2013; Gaudy and Boucher, 1983; Ikeda, 1970; Ivleva, 1980; Rajagopal, 1962; Schukat et al., 2013) and on a global scale (Ikeda et al., 2007) when taking into account differences in temperature and body mass. Pavlova (1994; Table 2) measured higher mass-specific respiration rates in copepods from the Indian Ocean, which can be explained by the fact that body mass data were not corrected for bias by formaldehyde preservation.

Table 1
Individual and mass-specific respiration rates (R, mean \pm SD if $n \geq 3$, min and max values if $n = 2$), dry mass (DM) range and Q_{10} values of calanoid copepods. f: female, m: male, C5/C4: copepodite stages C5 and C4, and n: number of measurements.

Family Species	Stage	DM range [μg]	Sampling depth [m]	Exp. T. [$^{\circ}\text{C}$]	Individual R [$\text{nmol O}_2 \text{ ind.}^{-1} \text{ h}^{-1}$] \pm SD (n)	Mass-spec. R [$\mu\text{mol O}_2 \text{ g}_{\text{DM}}^{-1} \text{ h}^{-1}$] \pm SD (n)	Q_{10}
Calanidae							
<i>Nannocalanus minor</i>	f	48/71	500–0	13.5	18/40 (2)	367/562 (2)	
<i>Neocalanus gracilis</i>	f	353–444	500–0	13.6	47 \pm 1 (3)	116 \pm 13 (3)	
<i>Neocalanus robustior</i>	f	282–509	600–0	13.7	64 \pm 16 (5)	159 \pm 62 (5)	1.9
	f	393–741	600–0	18	48 \pm 11 (4)	84 \pm 17 (4)	
<i>Neocalanus</i> sp.	C5	116/184	5–0	23.3	30/71 (2)	262/394 (2)	
<i>Undinula vulgaris</i>	f	87–147	600–0	13.6	26 \pm 11 (4)	221 \pm 93 (4)	1.4
	f	178–210	5–0	23	51 \pm 5 (4)	270 \pm 31 (3)	
	C5		5–0	23	34/49 (2)		
Eucalanidae							
<i>Pareucalanus sewelli</i>	f	62–283	600–0	13.6	51 \pm 33 (3)	245 \pm 45 (3)	1.7
	f	112/200	500–0	17.9	15/32 (2)	131/158 (2)	
	m	33/62	500–0	17.9	11/6 (2)	184/168 (2)	
<i>Rhincalanus cornutus</i>	f	55–103	600–0	13.6	13 \pm 3 (4)	167 \pm 86 (4)	2.5
	f	28–133	500–0	17.7	23 \pm 10 (5)	406 \pm 211 (5)	
<i>Subeucalanus crassus</i>	m	38–93	300–0	17.9	13 \pm 4 (5)	229 \pm 94 (5)	
<i>Subeucalanus subtenuis</i>	f	113–128	500–0	13.8	35 \pm 5 (3)	286 \pm 54 (3)	1.1
	f	47–114	500–0	17.8	20 \pm 5 (7)	285 \pm 71 (7)	
	f	84–126	5–0	23.3	39 \pm 24 (8)	358 \pm 196 (6)	
	m	92/94	500–0	13.5	27/23 (2)	285/251 (2)	1.5
	m	73–82	5–0	23.1	32 \pm 6 (3)	412 \pm 71 (3)	
Euchaetidae							
<i>Euchaeta marina</i>	f	267–376	500–0	17.8	47 \pm 7 (4)	145 \pm 11 (4)	
	f + eggs	260–350	500–0	18	52 \pm 13 (5)	169 \pm 39 (5)	2.4
	f + eggs	230–257	5–0	23	90 \pm 31 (4)	373 \pm 129 (4)	
<i>Paraeuchaeta</i> sp.	C4	183/205	600–0	17.8	24/33 (2)	133/161 (2)	
	C5	545–1144	600–0	13.6	63 \pm 34 (3)	101 \pm 71 (3)	1.1
	C5	906–1674	600–0	17.7	62 \pm 7 (4)	58 \pm 18 (4)	
Scolecitrichidae							
<i>Lophothrix</i> sp.	C5	396/472	300–100	13	13/17 (2)	27/423 (2)	
<i>Scolecitrich danae</i>	f	200–286	210–0	13	16 \pm 3 (5)	73 \pm 14 (5)	2.7
	f	219–281	500–0	17.7	24 \pm 10 (3)	95 \pm 35 (3)	
	f	170/238	5–0	23	42/40 (2)	176/234 (2)	
	m	193/195	600–0	13	14/13 (2)	72/69 (2)	2.4
	m	169/210	500–0	17.7	12/17 (2)	55/99 (2)	
<i>Scottocalanus securifrons</i>	f	782–1440	600–0	17.8	68 \pm 11 (6)	63 \pm 14 (6)	
Metridinidae							
<i>Pleuromamma abdominalis</i>	f	233/244	600–0	13.6	32/74 (2)	136/303 (2)	
<i>Pleuromamma xiphias</i>	f	198–777	600–0	17.8	53 \pm 31 (4)	116 \pm 43 (4)	
	m	877–1285	600–0	13.1	55 \pm 24 (5)	50 \pm 19 (5)	1.0
	m	1423–1824	600–0	17.9	79 \pm 35 (4)	50 \pm 27 (4)	
	C5	62/61	500–0	17.9	48/24 (2)	774/386 (2)	
Temoridae							
<i>Temora stylifera</i>	f/m		500–0	13.5	23 \pm 25 (3)		
	f/m		5–0	23.3	46 \pm 22 (3)		
Candaciidae							
<i>Candacia pachydactyla</i>	f	38–274	500–0	17.9	29 \pm 7 (3)	265 \pm 250 (3)	
	m	90/217	500–0	17.9	16/19 (2)	175/88 (2)	

Copepod respiration rates were predominantly influenced by body mass and ambient temperature. These two factors have the greatest effect on metabolic rates in marine poikilotherms (Bode et al., 2013; Hirche, 1987; Ikeda, 1985; Ikeda et al., 2001; Ikeda et al., 2007). Oxygen consumption varied from 27 $\mu\text{mol O}_2 \text{ g}_{\text{DM}}^{-1} \text{ h}^{-1}$ in large C5 of *Lophothrix* sp. at 13 $^{\circ}\text{C}$ to 774 $\mu\text{mol O}_2 \text{ g}_{\text{DM}}^{-1} \text{ h}^{-1}$ in *P. xiphias* C5 at 18 $^{\circ}\text{C}$. As expected, mass-specific respiration rates generally increased with increasing temperature and decreasing body mass. High sea surface temperatures and the typically small body size of tropical epipelagic species result in higher mass-specific metabolic rates at the surface (Hernández-León and Ikeda, 2005) as opposed to lower mass-specific rates in deeper-living species with generally larger body size at lower temperature (Childress, 1975; Ikeda, 1970, 1985). The present dataset includes a wide range of copepod body masses covering about two orders of magnitude from small copepodids C5 of *R. cornutus* to large females of *Gaetanus pileatus*, and therefore represents a large variety of different metabolic rates.

Around 95% of variation in oxygen consumption of copepods and zooplankton in general can be explained by differences in body

mass and temperature (Ikeda, 1985; Ikeda et al., 2001). The scaling coefficient b , derived from the allometric equation linking mass-specific respiration rate to body dry mass, varies in the range of -0.1 to -0.4 in different organisms (Peters, 1983), but is usually close to -0.25 (Blueweiss et al., 1978; Fenchel, 1974; Moloney and Field, 1989). Our scaling coefficient of -0.56 , derived from the regression analysis of body mass and respiration rates standardized to 18 $^{\circ}\text{C}$, is lower than most published data (Table 2). Nevertheless, also other studies on tropical copepods measured at high temperatures delivered rather low values for b of -0.46 and -0.32 , respectively (Ikeda, 1970; Pavlova, 1994). In addition, we cannot completely rule out the possibility that the specific mix in this study of larger-sized copepods from greater depths and, hence, lower ambient temperature with smaller individuals from the surface and higher temperature may have lead to a comparatively low scaling coefficient b .

Besides the dominant effects of temperature and body mass on copepod respiration, differences in oxygen demand can also be related to other factors such as feeding mode, activity level, migration and developmental status (Bode et al., 2013; Hirche, 1987; Ikeda et al.,

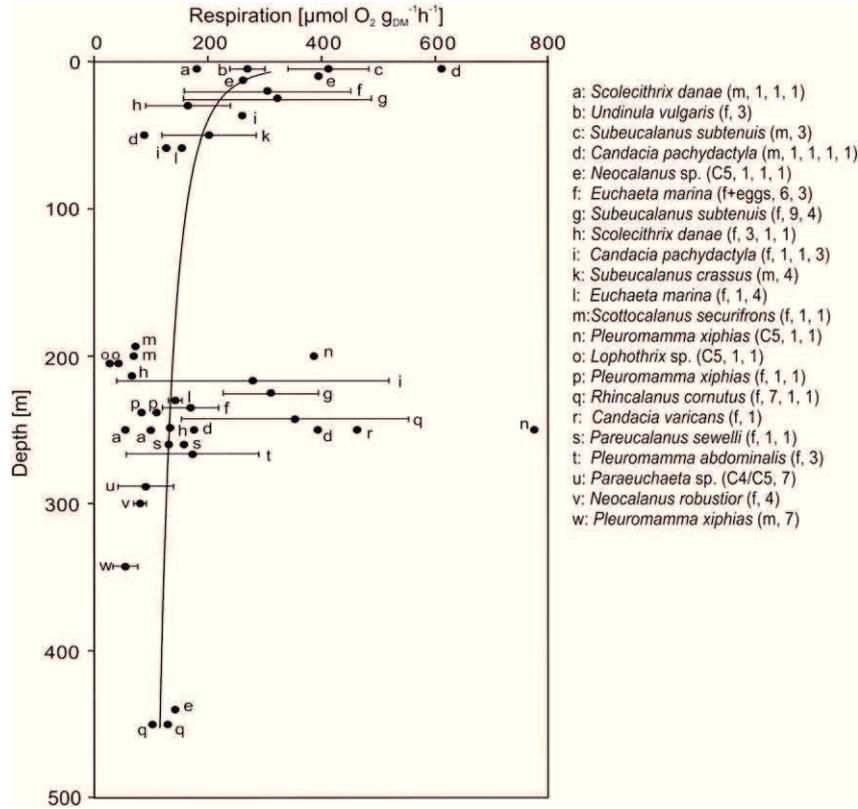


Fig. 4. Mass-specific respiration rates (mean ± SD if n ≥ 3) of 16 calanoid copepod species at ambient temperature plotted against mean mid-depth of the sampling interval. Individuals of the same species sampled at different depth layers are presented separately with their corresponding respiration rates. Number of individuals (n) is shown in parentheses, for species sampled in more than one depth layer n values are given separately for each depth layer. f: female, m: male, and C5: copepodite stage C5.

2006). Typical surface species of the Cape Verde region are *Undinula vulgaris*, *Neocalanus* sp., *T. stylifera*, *S. crassus*, *S. subtenuis*, *E. marina* and *C. pachyactyla*, which usually had the highest respiration rates in line with the high sea surface temperature. An exception was *S.*

danae with surprisingly low rates. The differences in respiration rate between egg-carrying and non-egg-carrying females of *E. marina* could be explained by enhanced energy requirements for locomotion in egg-carrying females or differences in reproductive status. Even

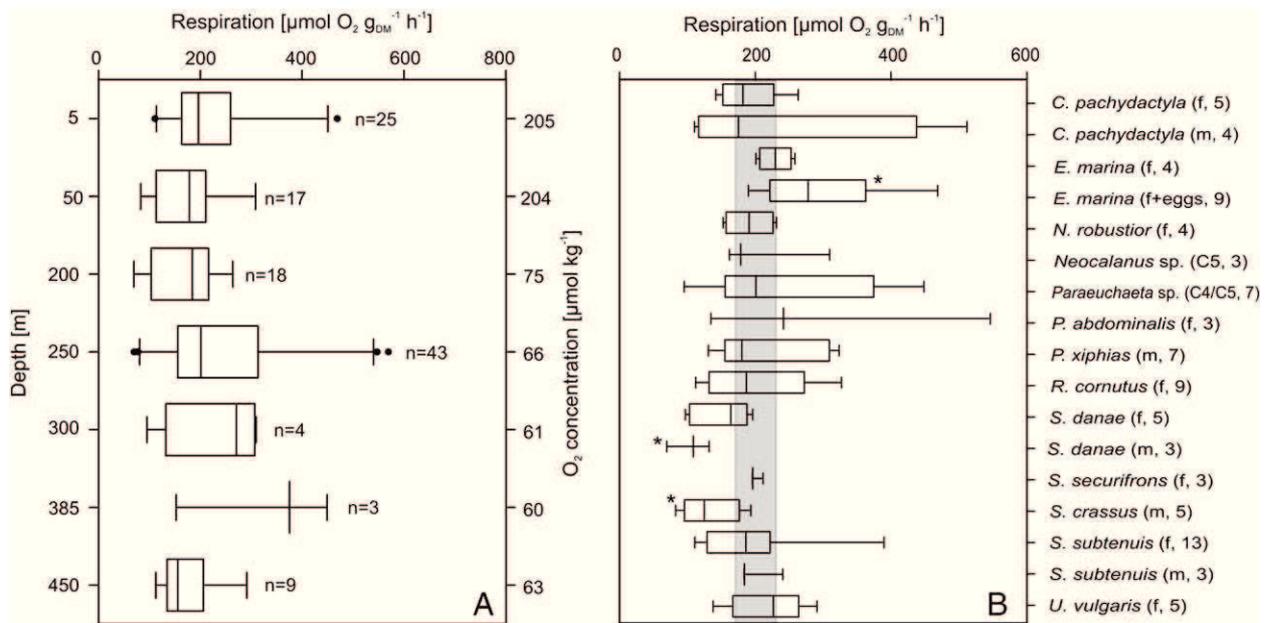


Fig. 5. (A) Standardized mass-specific respiration rates of copepods measured at simulated in situ temperatures, plotted against mid-sampling depth and corresponding ambient oxygen concentration (median, 5–95 percentile). Respiration rates have been standardized to a temperature of 18 °C (Q_{10} of 2) and a mean dry mass of 0.5 mg (b of -0.56). n: number of measurements. Depth-axis not drawn to scale. (B) Standardized mass-specific respiration rates of 13 copepod species (median, 5–95 percentile). Shaded area at 200 $\mu\text{mol O}_2 \text{gDM}^{-1} \text{h}^{-1}$ represents the overall mean respiration rate for those copepod species. Asterisks indicate significant differences from overall mean. f: female, m: male, C5: copepodite stage C5, and n is given in parentheses behind indication of stage.

Table 2

Compilation of the scaling coefficients a and b for the allometric function $R = aW^b$ linking mass-specific respiration rate R to body mass W . n : number of measurements and R^2 : correlation coefficient.

Taxon	Exp. T. [°C]	a	b	n	R^2	Authors
Study area						
Vertical range						
Copepods	13	0.695	−0.621	27	0.55	Present study
Tropical Atlantic	18	1.617	−0.525	62	0.68	
0–600 m	23	4.841	−0.458	21	0.24	
Zooplankton	5.1–12.2	1.054	−0.169	41	0.21	Ikeda (1970)
West Pacific	17–17.2	2.275	−0.309	14	0.66	
Epipelagic	27.9–30.3	7.482	−0.464	48	0.34	
Copepods	20	2.378	−0.099	16	0.07	Gaudy & Boucher (1983) ^a
Indian Ocean	28.5	3.164	−0.261	16	0.43	
0–400 m						
Copepods						Pavlova (1994) ^b
Indian Ocean	22	0.006	−0.321	52	0.10	
Epipelagic						
Copepods	11.3–14.8	1.19	−0.304	10	0.85	Ikeda et al. (2007) ^a
Global	15.1–20.2	2.319	−0.247	9	0.63	
Epi- to bathypelagic	22–26.4	4.502	−0.079	12	0.14	
Copepods						Thuesen et al. (1998) ^c
California	5	0.656	−0.214	226	0.11	
Deep sea						

^a Recalculation of available data from publication.

^b Dry mass of formalin-fixed samples without correction of dry mass.

^c Scaling coefficients for mass-specific respiration rates relative to wet mass.

after the correction for temperature and body-mass effects, respiration rates of egg-carrying females of *E. marina* were still significantly higher compared to other copepods, which may confirm that either ovogenesis increases metabolic demand (Arashkevich and Drits, 1997; Gaudy and Boucher, 1983) or the increased hydrodynamic drag of swimming with a large egg sac attached to the urosome indeed represents a substantial energetic burden. In contrast, males of *S. crassus* and *S. danae* showed significantly lower rates than other copepods after temperature and body-mass standardization. Similarly low metabolic rates in eucalanids have been documented for *Eucalanus inermis* and *Eucalanus elongatus* from the Humboldt and California Current upwelling systems (Flint et al., 1991). Oxygen uptake (measured as the activity of the electron transport system) of eucalanids was only 1/10 to 1/35 compared to other calanoid copepods. Flint et al. (1991) assign them to a physiologically distinctive group with a 'lethargic lifestyle', whose metabolic characteristics partly resemble those of jellyfish.

Mesopelagic organisms such as the copepods *Neocalanus gracilis*, *N. robustior*, *Candacia* spp., *Pleuromamma* spp., *Euchiarella* spp., and different eucalanid species are often characterized by highly variable respiration rates and comparatively low Q_{10} values. The variability in metabolic rates of these midwater species may be partly related to the wide depth intervals of the deeper net catches. The actual depth of occurrence of each individual could not be determined and the mean depth of the sampling interval was assumed as catching depth instead. Temperature drastically declines below the mixed surface layer, and lower temperatures at depth cause reduced metabolic activity in deeper-living species. Respiration rates and Q_{10} values of copepodids C4 and C5 of *Paraeuchaeta* spp. as well as males and females of *P. xiphias* are among the lowest values measured in this study. While *Paraeuchaeta* is a typical meso- and bathypelagic genus (Auel, 1999; Gaudy, 1975), *Pleuromamma* spp. are known to conduct extensive diel vertical migrations (Auel and Verheye, 2007; Champalbert and Gaudy, 1972; Loick et al., 2005; Pavlova, 1994). Since vertically migrating species encounter steep temperature gradients, they must be able to rapidly adapt their metabolic rates to changing conditions.

Copepods, which migrate to depths deeper than 200 m or permanently live at these depths, must temporarily or permanently cope

with low oxygen concentrations within the oxygen minimum zone (OMZ). Species of the families Eucalanidae and Metridinidae are frequent inhabitants of oxygen-poor waters in tropical oceans. *Rhincalanus nasutus*, *Eucalanus* spp. and *Pleuromamma* spp. reside in the OMZs off California and Peru, in the Arabian Sea and in the Benguela upwelling system and can tolerate fairly low oxygen concentrations of less than 1 ml l^{-1} (Auel and Verheye, 2007; Longhurst, 1967; Schnack-Schiel et al., 2008; Vinogradov and Voronina, 1962). Females of *R. cornutus* caught at 600 to 300 m showed drastically reduced respiration rates compared to individuals from shallower depths of 300 to 100 m. The deeper-living specimens were probably in a dormant state as observed for other *Rhincalanus* species (Schnack-Schiel et al., 2008; Schukat et al., 2013).

Copepod metabolic rates are closely related to the activity level (Bode et al., 2013; Ikeda, 1985; Paffenhöfer, 2006; Pavlova, 1994) and increase during feeding by up to 60% (Ikeda, 1977b; Kiørboe et al., 1985). Fast moving copepods had considerably higher respiration rates than slower ones (Paffenhöfer, 2006). Lower metabolic rates in deep-sea species are often caused by reduced locomotory abilities (Ikeda, 1988). Many epipelagic organisms such as *U. vulgaris* and *S. danae* are either herbivores or omnivores that constantly search for prey (Ikeda et al., 2006; Mullin and Brooks, 1970), while deep-sea species are often opportunistic predators (e.g. *Paraeuchaeta* sp.). There are also carnivorous species in tropical surface waters such as *E. marina*, since food supply for herbivores is limited in oligotrophic tropical oceans. In the present study, deeper-living carnivores such as *Paraeuchaeta* sp. had very low respiration rates, while surface-dwelling *E. marina* showed higher metabolic rates, most likely caused by higher temperature and smaller body mass.

In order to compare metabolic rates of surface-dwelling and deeper living organisms and to compensate for the dominating effects of temperature and body mass on copepod respiration, we standardized respiration rates to a common temperature of 18 °C and to a mean dry mass of 0.5 mg. This standardization removed all depth-related changes in respiration rate. This result is in line with the findings by Thuesen et al. (1998), but stands in contrast to some other studies that observed a significant decrease of metabolic rate in pelagic crustaceans and copepods (Childress, 1975; Ikeda et al., 2006, 2007). All authors had compensated respiration rates for temperature and body mass effects. A reason for these discrepancies may be that Ikeda et al. (2006, 2007) included copepods from bathypelagic depths to 3000 m, while our maximum sampling depth was only 600 m.

The ambient oxygen partial pressure (pO_2) is another potential factor affecting copepod respiration (Childress, 1977; Ikeda et al., 2007; Seibel, 2011). A reduction in metabolic rate within well pronounced OMZs can be an advantage to cope with hypoxic conditions (Childress and Seibel, 1998; Seibel, 2011), for instance in overwintering copepodids C5 of *Calanus pacificus* in the OMZ off California (Longhurst, 1967) or in vertically migrating *Calanus euxinus* in the Black Sea (Svetlichny et al., 2000). In oxygen-poor environments, many crustaceans maintain their aerobic respiration until a critical pO_2 is reached, below which respiration decreases ("metabolic regulators", Childress, 1975, 1995; Childress and Seibel, 1998). This threshold causing hypoxic stress and eventually mortality in marine organisms is species-specific (Childress and Seibel, 1998; Ekau et al., 2010; Ikeda, 1977a; Vaquer-Sunyer and Duarte, 2008), but often close to 5 kPa ($60 \mu\text{mol kg}^{-1} / 2 \text{ mg l}^{-1}$) (Gray et al., 2002; Seibel, 2011). The copepods *E. marina* and *U. vulgaris*, for instance, died at 15–30% oxygen saturation (2.5–5 kPa, Ikeda, 1977a).

In the present study, all experiments were conducted under normoxic conditions. Therefore, immediate or direct effects of hypoxia on copepod respiration could per se not be detected in our experimental setup. Nevertheless, respiration rates did not change abruptly, once oxygen concentration fell below a certain threshold during the closed-bottle incubations, and individuals sampled from above and within the OMZ did not differ in standardized respiration rate. Thus,

hypoxic conditions, currently observed in the OMZ of the eastern tropical North Atlantic, still do not seem to critically affect zooplankton physiology and community structure. It will be interesting to trace future changes, should the OMZ in the eastern tropical North Atlantic intensify and expand as predicted by oceanographic models.

5. Conclusions

Optode respirometry proved to be an effective method for respiration measurements of very small organisms like tropical copepods. Metabolic activity was mainly influenced by temperature, body mass and species-specific differences in behavior and habitat. After compensation for temperature and body-mass effects, depth of occurrence did not have a significant effect on respiration. Minimum oxygen concentrations of $51 \mu\text{mol kg}^{-1}$ within the core of the OMZ around the Cape Verde Archipelago did not yet have a fundamental impact on copepod ecophysiology, but may become more relevant under future scenarios of expanding and intensifying OMZ in tropical oceans. Establishing physiological thresholds of key species with regard to changing abiotic parameters, such as temperature and oxygen concentration, will be crucial to anticipate potential changes in pelagic biodiversity, productivity and ecosystem structure related to future scenarios of global change.

Acknowledgments

The authors would like to thank the director and staff of the Instituto Nacional de Desenvolvimento das Pescas (INDP) in Mindelo, São Vicente, Cape Verde for providing the laboratories and facilities to conduct our research, in particular our colleagues Óscar Melício, Carlos Santos, Anibal Medina, Oksana Tariche, Péricles Silva, Ivanice Monteiro, and Nuno Vieira. We are thankful to the crew of the fishing vessel 'Sinagoga' for their assistance during the sampling trips. Prof. Tsutomu Ikeda provided valuable advice with regard to the standardization method. This study was supported by the Deutsche Forschungsgemeinschaft DFG through the Cluster of Excellence "The Future Ocean" and through the Sonderforschungsbereich 754 "Climate-Biogeochemistry Interactions in the Tropical Ocean" at the University of Kiel. [ST]

References

- Arashkevich, E.G., Drits, A.V., 1997. Ecological-physiological indices of *Calanoides carinatus* (Krøyer) from the Benguela upwelling region: biochemical composition, respiration rate, and gonad development of various life stages. *Oceanology* 37, 513–519.
- Aristegui, J., Montero, M.F., 2005. Temporal and spatial changes in plankton respiration and biomass in the Canary Islands region: the effect of mesoscale variability. *J. Mar. Syst.* 54, 65–82.
- Auel, H., 1999. The ecology of Arctic deep-sea copepods (Euchaetidae and Aetideidae). Aspects of their distribution, trophodynamics and effect on the carbon flux. *Ber. Polarforsch.* 319, 1–97.
- Auel, H., Verheye, H.M., 2007. Hypoxia tolerance in the copepod *Calanoides carinatus* and the effect of an intermediate oxygen minimum layer on copepod vertical distribution in the northern Benguela Current upwelling system and the Angola-Benguela Front. *J. Exp. Mar. Biol. Ecol.* 352, 234–243.
- Blueweiss, L., Fox, H., Kudzma, V., Nakashima, D., Peters, R., Sams, S., 1978. Relationships between body size and some life history parameters. *Oecologia* 37, 257–272.
- Bode, M., Schukat, A., Hagen, W., Auel, H., 2013. Predicting metabolic rates of calanoid copepods. *J. Exp. Mar. Biol. Ecol.* 444, 1–7.
- Bograd, S.J., Castro, C.G., Di Lorenzo, E., Palacios, D.M., Bailey, H., Gilly, W., Chavez, F.P., 2008. Oxygen declines and the shoaling of the hypoxic boundary in the California Current. *Geophys. Res. Lett.* 35.
- Chahsavari-Archard, V., Razouls, C., 1982. Les copépodes pélagiques au sud-est des îles du Cap Vert, I. Aspects qualitatifs (Mission Guidôme du N.O. «J. Charcot», Septembre–Octobre 1976). *Vie Milieu* 32, 25–45.
- Champalbert, G., Gaudy, R., 1972. Etude de la respiration chez des copépodes de niveaux bathymétriques variés dans la région sud marocaine et canarienne. *Mar. Biol.* 12, 159–169.
- Chan, F., Barth, J.A., Lubchenco, J., Kirincich, A., Weeks, H., Peterson, W.T., Menge, B.A., 2008. Emergence of anoxia in the California Current large marine ecosystem. *Science* 319, 920.
- Childress, J.J., 1975. The respiratory rates of midwater crustaceans as a function of depth of occurrence and relation to the oxygen minimum layer off Southern California. *Comp. Biochem. Physiol.* 50, 787–799.
- Childress, J.J., 1977. Effects of pressure, temperature and oxygen on oxygen-consumption rate of midwater copepod *Gausssia princeps*. *Mar. Biol.* 39, 19–24.
- Childress, J.J., 1995. Are there physiological and biochemical adaptations of metabolism in deep-sea animals? *Trends Ecol. Evol.* 10, 30–36.
- Childress, J.J., Seibel, B.A., 1998. Life at stable low oxygen levels: adaptations of animals to oceanic oxygen minimum layers. *J. Exp. Biol.* 201, 1223–1232.
- del Giorgio, P.A., Duarte, C.M., 2002. Respiration in the open ocean. *Nature* 420, 379–384.
- Durbin, E.G., Durbin, A.G., 1978. Length and weight relationships of *Acartia clausi* from Narragansett Bay, R.I. *Limnol. Oceanogr.* 23, 958–969.
- Ekau, W., Auel, H., Pörtner, H.O., Gilbert, D., 2010. Impacts of hypoxia on the structure and processes in pelagic communities (zooplankton, macro-invertebrates and fish). *Biogeosciences* 7, 1669–1699.
- Escribano, R., 2006. Zooplankton interactions with the oxygen minimum zone in the eastern South Pacific. *Supl. Gayana* 70, 19–21.
- Fenchel, T., 1974. Intrinsic rate of natural increase: the relationship with body size. *Oecologia* 14, 317–326.
- Flint, M.V., Drits, A.V., Pasternak, A.F., 1991. Characteristic features of body composition and metabolism in some interzonal copepods. *Mar. Biol.* 111, 199–205.
- Gaudy, R., 1975. Etude de la respiration chez des copépodes pélagiques méditerranéens (bassin occidental et Mer Ionienne) et de ses variations en fonction de la bathymétrie des espèces et de leur origine géographique. *Mar. Biol.* 29, 109–118.
- Gaudy, R., Boucher, J., 1983. Relation between respiration, excretion (ammonia and inorganic phosphorus) and activity of amylase and trypsin in different species of pelagic copepods from an Indian Ocean equatorial area. *Mar. Biol.* 75, 37–45.
- Gray, J.S., Wu, R.S.S., Or, Y.Y., 2002. Effects of hypoxia and organic enrichment on the coastal marine environment. *Mar. Ecol. Prog. Ser.* 238, 249–279.
- Hernández-León, S., Ikeda, T., 2005. A global assessment of mesozooplankton respiration in the ocean. *J. Plankton Res.* 27, 153–158.
- Hernández-León, S., Aristegui, J., Gómez, M., Torres, S., Almeida, C., Montero, M.F., Ojeda, A., 1998. Mesozooplankton metabolism and its effect on chlorophyll and primary production in slope waters of the Canary Islands. *Ann. Inst. Oceanogr., Paris* 74 (2), 127–138.
- Hernández-León, S., Almeida, C., Becognee, P., Yebra, L., Aristegui, J., 2004. Zooplankton biomass and indices of grazing and metabolism during a late winter bloom in subtropical waters. *Mar. Biol.* 145, 1191–1200.
- Hirche, H.J., 1987. Temperature and plankton. 2. Effect on respiration and swimming activity in copepods from the Greenland Sea. *Mar. Biol.* 94, 347–356.
- Hofmann, A.F., Soetaert, K., Middelburg, J.J., Meysman, F.J.R., 2010. AquaEnv: an aquatic acid-base modelling environment in R. *Aquat. Geochem.* 16, 507–546. <http://dx.doi.org/10.1007/s10498-009-9084-1>.
- Ikeda, T., 1970. Relationship between respiration rate and body size in marine plankton animals as a function of the temperature of habitat. *Bull. Fac. Fish. Hokkaido Univ.* 21 (2), 91–112.
- Ikeda, T., 1977a. The effect of laboratory conditions on the extrapolation of experimental measurements to the ecology of marine zooplankton. II. Effect of oxygen saturation on the respiration rate. *Bull. Plankton Soc. Japan* 24, 19–28.
- Ikeda, T., 1977b. The effect of laboratory conditions on the extrapolation of experimental measurements to the ecology of marine zooplankton. IV. Changes in respiration and excretion rates of boreal zooplankton species maintained under fed and starved conditions. *Mar. Biol.* 41, 241–252.
- Ikeda, T., 1985. Metabolic rates of epipelagic marine zooplankton as a function of body-mass and temperature. *Mar. Biol.* 85, 1–11.
- Ikeda, T., 1988. Metabolism and chemical composition of crustaceans from the Antarctic mesopelagic zone. *Deep-Sea Res.* I 35, 1991–2002.
- Ikeda, T., Kanno, Y., Ozaki, K., Shinada, A., 2001. Metabolic rates of epipelagic marine copepods as a function of body mass and temperature. *Mar. Biol.* 139, 587–596.
- Ikeda, T., Sano, F., Yamaguchi, A., Matsuishi, T., 2006. Metabolism of mesopelagic and bathypelagic copepods in the western North Pacific Ocean. *Mar. Ecol. Prog. Ser.* 322, 199–211.
- Ikeda, T., Sano, F., Yamaguchi, A., 2007. Respiration in marine pelagic copepods: a global-bathymetric model. *Mar. Ecol. Prog. Ser.* 339, 215–219.
- Ivleva, I.V., 1980. The dependence of crustacean respiration rate on body mass and habitat temperature. *Int. Rev. Gesamten Hydrobiol.* 65, 1–47.
- Karstensen, J., Stramma, L., Visbeck, M., 2008. Oxygen minimum zones in the eastern tropical Atlantic and Pacific oceans. *Prog. Oceanogr.* 77, 331–350.
- Kjørboe, T., Mohlenberg, F., Hamburger, K., 1985. Bioenergetics of the planktonic copepod *Acartia tonsa*: relation between feeding, egg production and respiration, and composition of specific dynamic action. *Mar. Ecol. Prog. Ser.* 26, 85–97.
- Klimant, I., Meyer, V., Kuhl, M., 1995. Fiberoptic oxygen microsensors, a new tool in aquatic biology. *Limnol. Oceanogr.* 40, 1159–1165.
- Köster, M., Krause, C., Paffenhöfer, G.A., 2008. Time-series measurements of oxygen consumption of copepod nauplii. *Mar. Ecol. Prog. Ser.* 353, 157–164.
- Leuven, R.S.E.W., Brock, T.C.M., Vandruuten, H.A.M., 1985. Effects of preservation on dry- and ash-free dry weight biomass of some common aquatic macro-invertebrates. *Hydrobiologia* 127, 151–159.
- Loick, N., Ekau, W., Verheye, H.M., 2005. Water body preferences of dominant calanoid copepod species in the Angola-Benguela frontal zone. *Afr. J. Mar. Sci.* 27, 597–608.
- Longhurst, A.R., 1967. Vertical distribution of zooplankton in relation to the eastern Pacific oxygen minimum zone. *Deep-Sea Res.* 14, 51–63.
- Longhurst, A.R., 1991. Role of the marine biosphere in the global carbon cycle. *Limnol. Oceanogr.* 36, 1507–1526.
- Longhurst, A.R., Harrison, W.G., 1989. The biological pump – profiles of plankton production and consumption in the upper ocean. *Prog. Oceanogr.* 22, 47–123.
- Moloney, C.L., Field, J.G., 1989. General allometric equations for rates of nutrient uptake, ingestion, and respiration in plankton organisms. *Limnol. Oceanogr.* 34, 1290–1299.

- Mullin, M.M., Brooks, E.R., 1970. Growth and metabolism of two planktonic marine copepods as influenced by temperature and type of food. In: Steele, J.H. (Ed.), *Marine Food Chains*. Oliver & Boyd, pp. 74–95.
- Omori, M., 1978. Some factors affecting on dry weight, organic weight and concentrations of carbon and nitrogen in freshly prepared and in preserved zooplankton. *Int. Rev. Gesamten Hydrobiol.* 63, 261–269.
- Paffenhöfer, G.A., 2006. Oxygen consumption in relation to motion of marine planktonic copepods. *Mar. Ecol. Prog. Ser.* 317, 187–192.
- Pahlow, M., Vezina, A.F., Casault, B., Maass, H., Malloch, L., Wright, D.G., Lu, Y.Y., 2008. Adaptive model of plankton dynamics for the North Atlantic. *Prog. Oceanogr.* 76, 151–191.
- Paulmier, A., Ruiz-Pino, D., 2009. Oxygen minimum zones (OMZs) in the modern ocean. *Prog. Oceanogr.* 80, 113–128.
- Pavlova, E.V., 1994. Diel changes in copepod respiration rates. *Hydrobiologia* 293, 333–339.
- Peters, R.H., 1983. *The ecological implications of body size*. Cambridge University Press, Cambridge (329 pp.).
- Rajagopal, P.K., 1962. Respiration of some marine planktonic organisms. *Proc. Indian. Acad. Sci., B.* 55 (2), 76–81.
- Razouls, S., 1972. Influence des conditions expérimentales sur le taux respiratoire des copépodes planktoniques. *J. Exp. Mar. Biol. Ecol.* 9, 145–153.
- Saltzman, J., Wishner, K.F., 1997. Zooplankton ecology in the eastern tropical Pacific oxygen minimum zone above a seamount: 2. Vertical distribution of copepods. *Deep-Sea Res.* 1 44, 931–954.
- Satomi, M., Pomeroy, L.R., 1965. Respiration and phosphorus excretion in some marine populations. *Ecology* 46, 877–881.
- Schnack-Schiel, S.B., Niehoff, B., Hagen, W., Böttger-Schnack, R., Cornils, A., Dowidar, M.M., Pasternak, A., Stambler, N., Stübing, D., Richter, C., 2008. Population dynamics and life strategies of *Rhincalanus nasutus* (Copepoda) at the onset of the spring bloom in the Gulf of Aqaba (Red Sea). *J. Plankton Res.* 30, 655–672.
- Schukat, A., Teuber, L., Hagen, W., Wasmund, N., Auel, H., 2013. Energetics and carbon budgets of dominant calanoid copepods in the northern Benguela upwelling system. *J. Exp. Mar. Biol. Ecol.* 442, 1–9.
- Seibel, B.A., 2011. Critical oxygen levels and metabolic suppression in oceanic oxygen minimum zones. *J. Exp. Biol.* 214, 326–336.
- Stramma, L., Johnson, G.C., Sprintall, J., Mohrholz, V., 2008a. Expanding oxygen minimum zones in the tropical oceans. *Science* 320, 655–658.
- Stramma, L., Brandt, P., Schafstall, J., Schott, F., Fischer, J., Kortzinger, A., 2008b. Oxygen minimum zone in the North Atlantic south and east of the Cape Verde Islands. *J. Geophys. Res.* 113. <http://dx.doi.org/10.1029/2007JC004369> C04014.
- Stramma, L., Visbeck, M., Brandt, P., Tanhua, T., Wallace, D., 2009. Deoxygenation in the oxygen minimum zone of the eastern tropical North Atlantic. *Geophys. Res. Lett.* 36.
- Stramma, L., Schmidtke, S., Levin, L.A., Johnson, G.C., 2010. Ocean oxygen minima expansions and their biological impacts. *Deep-Sea Res.* 1 57, 587–595.
- Stramma, L., Prince, E.D., Schmidtke, S., Luo, J.G., Hoolihan, J.P., Visbeck, M., Wallace, D.W.R., Brandt, P., Kortzinger, A., 2012. Expansion of oxygen minimum zones may reduce available habitat for tropical pelagic fishes. *Nat. Clim. Chang.* 2, 33–37.
- Svetlichny, L.S., Hubareva, E.S., Erkan, F., Gucu, A.C., 2000. Physiological and behavioral aspects of *Calanus euxinus* females (Copepoda: Calanoida) during vertical migration across temperature and oxygen gradients. *Mar. Biol.* 137, 963–971.
- Teuber, L., Kiko, R., Séguin, F., Auel, H., 2013. Respiration rates of tropical Atlantic copepods from the Cape Verde Islands. DOI:10.1594/PANGAEA.816158, Supplement to: Teuber, L., Kiko, R., Séguin, F., Auel, H., 2013. Respiration rates of tropical Atlantic copepods in relation to the oxygen minimum zone. *J. Exp. Mar. Biol. Ecol.* <http://dx.doi.org/10.1016/j.jembe.2013.06.012> (in press).
- Thuesen, E.V., Miller, C.B., Childress, J.J., 1998. Ecophysiological interpretation of oxygen consumption rates and enzymatic activities of deep-sea copepods. *Mar. Ecol. Prog. Ser.* 168, 95–107.
- Vaquer-Sunyer, R., Duarte, C.M., 2008. Thresholds of hypoxia for marine biodiversity. *Proc. Natl. Acad. Sci. U. S. A.* 105, 15452–15457.
- Vinogradov, M.E., Voronina, N.M., 1962. Influence of the oxygen deficit on the distribution of plankton in the Arabian Sea. *Deep-Sea Res.* 9, 523–530.
- Warkentin, M., Freese, H.M., Karsten, U., Schumann, R., 2007. New and fast method to quantify respiration rates of bacterial and plankton communities in freshwater ecosystems by using optical oxygen sensor spots. *Appl. Environ. Microbiol.* 73, 6722–6729.
- Wilson, S.E., Steinberg, D.K., 2010. Autotrophic picoplankton in mesozooplankton guts: evidence of aggregate feeding in the mesopelagic zone and export of small phytoplankton. *Mar. Ecol. Prog. Ser.* 412, 11–27.
- Wishner, K.F., Gelfman, C., Gowing, M.M., Outram, D.M., Rapien, M., Williams, R.L., 2008. Vertical zonation and distributions of calanoid copepods through the lower oxycline of the Arabian Sea oxygen minimum zone. *Prog. Oceanogr.* 78, 163–191.
- Zeiss, F.R., 1963. Effects of population densities on zooplankton respiration rates. *Limnol. Oceanogr.* 8, 110–115.

PUBLICATION II

DISTRIBUTION AND ECOPHYSIOLOGY OF CALANOID COPEPODS IN RELATION TO THE OXYGEN MINIMUM ZONE IN THE EASTERN TROPICAL ATLANTIC

TEUBER L, SCHUKAT A, HAGEN W, AUER H

Published in *PLoS ONE* (2013)

Distribution and Ecophysiology of Calanoid Copepods in Relation to the Oxygen Minimum Zone in the Eastern Tropical Atlantic

Lena Teuber*, Anna Schukat, Wilhelm Hagen, Holger Auel

Bremen Marine Ecology, University of Bremen, Bremen, Germany

Abstract

Oxygen minimum zones (OMZs) affect distribution patterns, community structure and metabolic processes of marine organisms. Due to the prominent role of zooplankton, especially copepods, in the marine carbon cycle and the predicted intensification and expansion of OMZs, it is essential to understand the effects of hypoxia on zooplankton distribution and ecophysiology. For this study, calanoid copepods were sampled from different depths (0–1800 m) at eight stations in the eastern tropical Atlantic (3°47'N to 18°S) during three expeditions in 2010 and 2011. Their horizontal and vertical distribution was determined and related to the extent and intensity of the OMZ, which increased from north to south with minimum O₂ concentrations (12.7 μmol kg⁻¹) in the southern Angola Gyre. Calanoid copepod abundance was highest in the northeastern Angola Basin and decreased towards equatorial regions as well as with increasing depth. Maximum copepod biodiversity was observed in the deep waters of the central Angola Basin. Respiration rates and enzyme activities were measured to reveal species-specific physiological adaptations. Enzyme activities of the electron transport system (ETS) and lactate dehydrogenase (LDH) served as proxies for aerobic and anaerobic metabolic activity, respectively. Mass-specific respiration rates and ETS activities decreased with depth of occurrence, consistent with vertical changes in copepod body mass and ambient temperature. Copepods of the families Eucalanidae and Metridinidae dominated within the OMZ. Several of these species showed adaptive characteristics such as lower metabolic rates, additional anaerobic activity and diel vertical migration that enable them to successfully inhabit hypoxic zones.

Citation: Teuber L, Schukat A, Hagen W, Auel H (2013) Distribution and Ecophysiology of Calanoid Copepods in Relation to the Oxygen Minimum Zone in the Eastern Tropical Atlantic. PLoS ONE 8(11): e77590. doi:10.1371/journal.pone.0077590

Editor: Howard I. Browman, Institute of Marine Research, Norway

Received: July 9, 2013; **Accepted:** September 9, 2013; **Published:** November 5, 2013

Copyright: © 2013 Teuber et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This study was partly funded by the German Federal Ministry of Education and Research (<http://www.bmbf.de/en/index.php>) in the framework of the GENUS "Geochemistry and Ecology of the Namibian Upwelling System" project. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The additional part of the funding was provided by the University of Bremen.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: teuber@uni-bremen.de

Introduction

The vertical and horizontal expansion of oxygen minimum zones in tropical oceans is a crucial factor for marine organisms influencing their distribution ranges, behavioural patterns and metabolic rates [1–3]. Oxygen depletion has been identified as one of the major future hazards to marine ecosystems and global fisheries [2,3]. Since the 1960s, the tropical Atlantic has suffered from the most severe decline in oxygen concentration of ~0.5 μmol kg⁻¹ year⁻¹ and the area affected by oxygen minimum zones (OMZs) has expanded in spatial extent by 4.5 million km² [3–5]. Recently, minimum oxygen concentrations of 17 μmol kg⁻¹ have been measured at intermediate depths in the eastern tropical Atlantic [6,7]. Nevertheless, the OMZ in the Atlantic is not as pronounced as in the eastern tropical Pacific and Arabian Sea, where oxygen concentrations are often below 4.5 μmol kg⁻¹ [7,8].

Expanding OMZs are a severe problem restricting the horizontal and vertical distribution as well as migrations of marine organisms [9–12]. Zooplankton organisms are especially susceptible and sensitive to changing environmental factors [13] and zooplankton biomass is usually severely reduced within the centre of the OMZ [10]. In contrast, gradients at the upper and lower

margin of the OMZ are often hotspots of biological activity [9,10,14]. Interestingly, certain copepod and euphausiid species are able to live within or migrate through the OMZ [15–17] and the copepods *Calanoides carinatus*, *Rhincalanus nasutus* and *Pleuromamma robusta* frequently inhabit the OMZ in the Atlantic [11,18]. Vertical migration through the OMZ is apparently associated with a reduction of respiration rates at lower oxygen partial pressures [19] and a higher activity of the enzyme lactate dehydrogenase (LDH), which indicates anaerobic metabolism [20,21]. Zooplankton mainly survives aerobically within OMZs [22], but the anaerobic pathway may serve as an additional energy supply to support activity above routine metabolism [21,23].

The influence of low ambient oxygen concentrations on zooplankton metabolism has been investigated in the OMZs of the major eastern boundary upwelling systems off Peru, California and Namibia [11,23,24]. During hypoxia tolerance measurements, *C. carinatus*, the dominant copepod species in the Benguela upwelling system, survived surprisingly low oxygen concentrations of ≤1 ml l⁻¹, but could not tolerate hypoxic conditions below 0.8 ml l⁻¹ in the centre of the OMZ [11]. Apart from changes in body mass and temperature, the copepods *R. nasutus* and *Metridia lucens* from the OMZ off Namibia showed a reduction in respiration rate by 62% and 43%, respectively, as compared to

Table 1. Sampling data.

Cruise	Station	Date (D/M/Y)	Time gear at depth [UTC]	Position latitude	Position longitude	Sampling intervals [m]	O ₂ min [$\mu\text{mol O}_2 \text{ kg}^{-1}$]	O ₂ min depth [m]	SST [$^{\circ}\text{C}$]
D 355	1*	01.09.2010	15:43	7°15'S	0°45'W	800-500-200-100-50-0	n.d.	n.d.	n.d.
	2*	03.09.2010	14:30	12°42'S	4°26'E	800-500-220-120-80-0	n.d.	n.d.	n.d.
MSM 17/3	310	22.02.2011	17:07	17°15'S	10°30'E	1000- 500-200-100 -50-0	14.6	258	24.8
	316	24.02.2011	04:01	17°15'S	10°00'E	1000- 500-200-100 -50-0	15.1	363	24.4
	317*	25.02.2011	22:50	10°00'S	8°00'E	1000- 600-300 -100-50-0	17.6	427	28.9
	318*	28.02.2011	00:30	4°07'S	1°26'W	1000- 550-350 -100-50-0	42.6	417	28.4
	319*	04.03.2011	04:12	3°47'N	13°58'W	1000-400-200-100-50-0	56.1	283	29.6
MSM 18/4	782	25.07.2011	15:52	3°00'S	8°00'E	1800-1000-500-150-30-0	50.0	204	22.1
	784*	26.07.2011	06:43	5°00'S	8°00'E	1800-1000- 600-150 -30-0	39.8	248	24.1
	786	26.07.2011	21:54	7°00'S	8°00'E	1800-1000- 400-250 -30-0	21.5	336	23.1
	787*	27.07.2011	06:32	8°00'S	8°00'E	1800-1000- 450-200 -30-0	18.6	336	22.9
	789	27.07.2011	21:48	10°00'S	8°03'E	1800-1000- 600-250 -40-0	16.7	397	22.2
	791	28.07.2011	12:32	12°00'S	8°00'E	1800-1000- 600-250-50 -0	18.7	334	20.7
	835	05.08.2011	17:49	14°00'S	9°06'E	1800-1000- 450-200-50 -0	17.4	378	19.5
	840*	07.08.2011	06:08	18°00'S	8°00'E	1800-1000- 450-250-85 -0	16.4	324	17.9
	842	07.08.2011	16:42	17°00'S	8°34'E	1800-1000- 400-250-85 -0	22.6	331	17.8
	845	10.08.2011	23:08	13°00'S	9°05'E	1800-1000- 600-200 -30-0	12.7	410	20.5
847	12.08.2011	21:36	14°30'S	9°51'E	800- 500-300-150-50 -0	17.9	341	18.7	

Sampling intervals highlighted in bold numbers indicate the approximate vertical extent of the oxygen minimum zone ($\text{O}_2 \leq 45 \mu\text{mol kg}^{-1}$). D = *Discovery* cruise, MSM = *Maria S. Merian* cruises, UTC = universal time code, O₂ min = lowest oxygen concentration at the respective station, O₂ min depth = depth of the oxygen minimum at the respective station, SST = sea surface temperature, n.d. = no data, * = stations analysed for copepod abundance.

doi:10.1371/journal.pone.0077590.t001

specimens from the surface [25]. Besides metabolic suppression, an efficient removal of oxygen from the water or an additional energy supply via anaerobic metabolism allow pelagic crustaceans to survive in hypoxic regions [22]. Zooplankton organisms specifically adapted to hypoxic conditions may thus find refuge within the OMZ from predation and competition by less tolerant species [11,26,27].

Zooplankton studies in the tropical and subtropical Atlantic have often focused on the abundance and distribution of copepods [28,29]. There is an increasing number of studies on influences of hypoxia on marine species, but ecophysiological data of copepods from the tropical Atlantic, investigating the effects of OMZs on distribution patterns and metabolic rates, are still limited. It is essential to understand the physiological capacities of different copepod species to assess potential implications of expanding OMZs on zooplankton communities. The aim of this study is to analyse the impact of the OMZ in the eastern tropical Atlantic on the distribution and metabolic activity of common calanoid copepods. This paper fills a gap in the limited data set on the ecophysiology of tropical Atlantic copepods and contributes to a better understanding of the zooplankton community structure and adaptive processes in the light of predicted expanding OMZs in the future.

Materials and Methods

Ethics Statement

The present study on planktonic copepods does not include protected or endangered species. No specific permissions were required for sampling in the open tropical Atlantic Ocean, since sampled stations were positioned in international waters.

Sampling

Mesozooplankton was sampled in the eastern tropical Atlantic Ocean during three expeditions in September 2010 (RRS *Discovery*, D355), February/March 2011 (RV *Maria S. Merian*, MSM 17/3) and July/August 2011 (RV *Maria S. Merian*, MSM 18/4) (Table 1, Figure 1). Stratified vertical hauls were collected with a Multinet Midi equipped with two flow meters (HydroBios, Kiel, Germany; mouth opening: 0.25 m², mesh size: 300 μm). Some specimens were also collected from a double (18 nets) and single (9 nets) MOCNESS (Multiple opening/closing net and environmental sensing system, mouth opening: 1 m², mesh size: 333 μm , [30]). At each station, five discrete depth layers were sampled by Multinet selected according to the local hydrographical regime (temperature and oxygen) determined by CTD casts (Table 1). In particular, the vertical extent of the oxygen minimum zone (OMZ) was reflected in the depth intervals with discrete samples collected above, within and below the OMZ. The volume of water filtered by the Multinet was determined by calibrated flow meters and was usually considered 25 m³ per 100 m depth interval and, thus, ranged from 7.5 m³ for a 30 m depth interval at the surface to 200 m³ for the deepest depth interval from 1800 to 1000 m during expedition MSM 18/4 (Table 1). Vertical profiles of oxygen concentration, temperature and fluorescence were measured by corresponding CTD casts at each station, except for stations 1 and 2 in 2010. Water layers with $\leq 45 \mu\text{mol kg}^{-1}$ of dissolved oxygen were considered as OMZ according to [7].

Mesozooplankton samples were sorted under a dissecting microscope, and only calanoid copepods in apparently good condition were used for respiration experiments on board. Copepods were staged and identified according to [31]. Additional copepod specimens were deep-frozen at -80°C for later enzyme

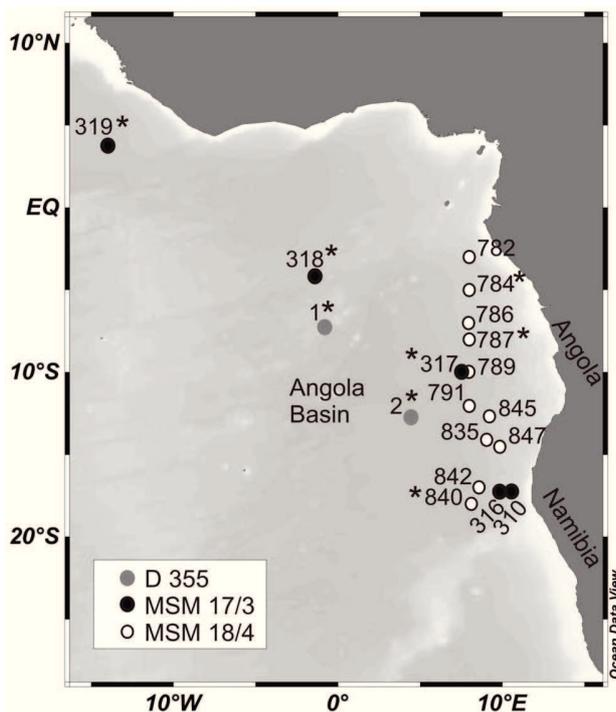


Figure 1. Sampling stations in the eastern tropical Atlantic Ocean. Copepods were sampled during three expeditions with RRS *Discovery* in 2010 (D355, gray circles) and with RV *Maria S. Merian* in 2011 (MSM 17/3, black circles and MSM 18/4, white circles). * = stations analysed for copepod abundance and species composition. doi:10.1371/journal.pone.0077590.g001

activity analyses of the electron transport system (ETS) and lactate dehydrogenase (LDH). The remains of the samples were preserved in a 4% buffered formaldehyde in seawater solution for the analysis of copepod abundance, vertical distribution and species composition.

Copepod Abundance and Data Analysis

Formaldehyde-fixed zooplankton samples of eight stations from three cruises (D 355: stns. 1 and 2 (day); MSM 17/3: stns. 317, 318, 319 (night); MSM 18/4: stns. 784, 787, 840 (dawn); Figure 1, Table 1) were chosen for the analysis of abundance and vertical distribution of calanoid copepods. The analysis focused on abundant and larger calanoid species that regularly occurred in the samples and were also used for respiration measurements and biochemical analyses. These copepods were counted from the entire sample and all specimens were staged and identified under a dissecting microscope to species or genus level according to [31]. The counted number of individuals per taxonomic category and sample ranged from a few individuals in rare deep-sea species to over 150 individuals per sample in abundant epipelagic taxa. For species that occurred only sporadically as single individuals in the samples, abundance values were not calculated. Instead, these species were listed as “single individuals” (s.i.). Specimens used for respiration measurements and/or biochemical analyses were added to the total number for the calculation of abundance.

To identify different biogeographical regions characterized by distinct copepod communities, a cluster analysis was performed with PRIMER v6 software [32] based on a species-station table compiling copepod abundance (no. of ind. $1000^{-1} m^{-2}$) in the upper 1000 m water column. Abundance data were fourth-root-transformed to minimize the dominance of highly abundant

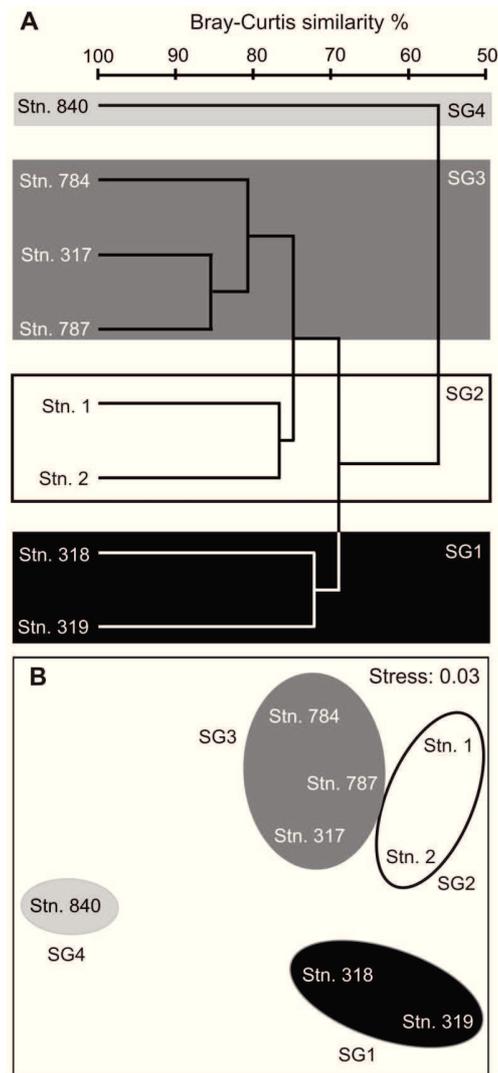


Figure 2. Cluster of stations with similar faunistic composition and derived station groups (SG1-4). A, dendrogram. B, multidimensional scaling (MDS) plot. doi:10.1371/journal.pone.0077590.g002

species and to increase the relevant impact of rare ones. Pair-wise similarity between each pair of stations was calculated by the Bray-Curtis similarity index based on the species-station table. For the cluster analysis a group average technique was applied. Results of the cluster analysis are presented in a dendrogram and a multidimensional scaling (MDS) plot (Figure 2). Based on the results of the cluster analysis, four station groups (SG1 to SG4) were derived and compared in terms of copepod abundance and distribution. Abundance data for each station will be made available via the PANGAEA database.

Respiration Measurements

Respiration measurements were performed on board of all three research vessels by optode respirometry with either three 1-channel or one 10-channel optode respirometer (Fibox 3 and Oxy-10 Mini, PreSens Precision Sensing GmbH, Regensburg, Germany). Oxygen concentration was measured via oxygen minisensors (ϕ 5 mm) attached to the inner wall of gas-tight

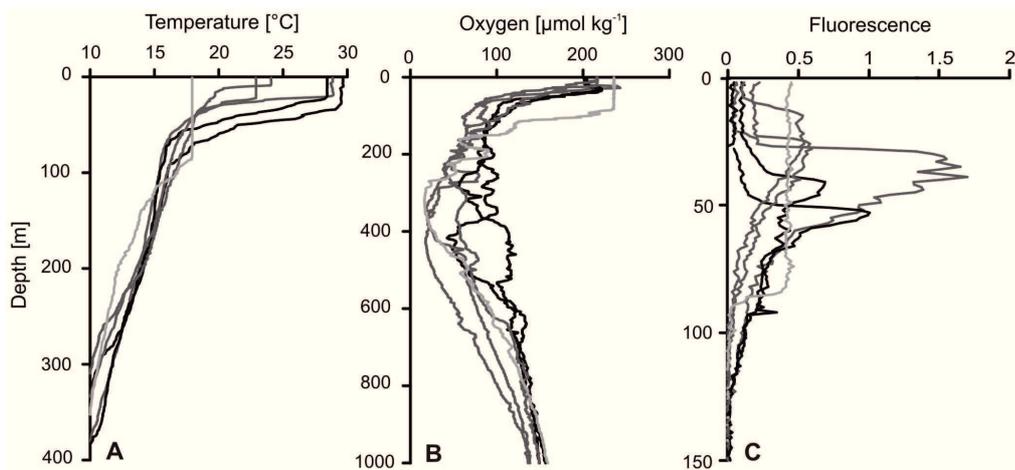


Figure 3. CTD profiles from stations analysed for copepod abundance. A, temperature. B, dissolved oxygen concentration. C, fluorescence (in relative units as proxy for chlorophyll *a* concentration). Shades of gray correspond to different station groups (SG1, 3, and 4) according to species composition derived from cluster analysis; SG1 = black, SG3 = dark gray, SG4 = light gray. CTD data for SG2 were not available. Note the different scaling of the y-axes.

doi:10.1371/journal.pone.0077590.g003

Winkler bottles (12–13 ml volume). Incubation bottles were filled with oxygenated seawater previously filtered (0.2 µm, Whatman GF/F filter) and UV-sterilised by slowly flowing through the container of an ultraviolet lamp (Aqua Cristal UV C5 W, JBL) to reduce microbial respiration. All specimens were acclimated at experimental temperature for several hours prior to the experiments and were not fed before and during experiments. Depending on the body size of each species, incubation bottles contained 1 to 10 individuals. Only specimens in apparently good condition were chosen for experiments. All experiments were run in darkness in water baths placed into temperature-controlled incubators to ensure constant temperatures throughout the experiments. Incubators were set to different target temperatures (5–20°C) according to *in situ* conditions at the corresponding sampling depths, tolerating a deviation of $\pm 1^\circ\text{C}$. Measurements lasted for at least 6 to 8 h. For each experimental setup, one to two animal-free controls were run under the same conditions to compensate for potential microbial respiration. After the experiments, all specimens were deep-frozen at -80°C for later dry-mass determination after lyophilisation for 48 h. Intra-specific differences in respiration between adults and copepodite stages as well as depth-related differences in respiration were statistically evaluated by Mann-Whitney U tests and Kruskal-Wallis tests, followed by a Dunn's multiple comparison test, respectively [33].

Enzyme Activities

Electron transport system (ETS) activity was measured according to standard methods [34,35] and optimized for copepod species [36]. The phosphate buffer (PHB, 0.1 M, pH 8.0) contained Triton X-100 (0.2% v/v), while the amount of polyvinylpyrrolidone (PVP) was reduced to 0.5 mg ml^{-1} in the homogenizing buffer (HOM, 75 µM). For the substrate solution (SUB), NADH (1.3 mM), NADPH (0.05 mM) and succinate (1 mM) were dissolved in PHB. The 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride (INT) solution (2.5 mM, pH 7.5) was dissolved in de-ionized water. Wet mass (WM) of frozen copepods was determined and specimens were immediately homogenized for 2 min in a reaction cup (2 ml, Eppendorf safe-lock tube) using a hand pistil. The homogenate contained 1 mg copepod per ml HOM. Homogenates were

centrifuged at 4700 g for 10 min at $0-4^\circ\text{C}$. All steps were conducted on ice. The final reaction mixture was reduced to 1 ml, containing 600 µl SUB, 200 µl INT and 200 µl supernatant. After a species-specific incubation time (10 to 60 min), absorbance was measured in 2 ml quartz cuvettes under non-limiting substrate conditions in a temperature-controlled photometer (490 nm wavelengths, Kontron Instruments, UVIKON 941) at *in situ* temperature and with distilled water as reference. No quench solution was used. For each sample, four replicates were measured as well as three sample blanks (600 µl PHB, 200 µl INT, 200 µl supernatant) and three substrate blanks (600 µl SUB, 200 µl INT, 200 µl HOM).

Lactate dehydrogenase (LDH) activity was measured according to standard methods [20,37] and optimized for copepod species. LDH assays were performed on a mixture of Tris/HCl buffer (80 mM, pH 7.2 at 20°C), pyruvate (2 mM), NADH (150 µM) and KCl (100 mM). The initial preparation of the homogenate was similar to the ETS activity assay, except that the homogenization dilution was usually 1 mg copepod per 24 µl homogenizing buffer (Tris HCl buffer, 0.01 M, pH 7.5 at 10°C). Homogenates were centrifuged at 6600 g for 10 min at $0-4^\circ\text{C}$. All steps were conducted on ice. The total volume of the assay medium in the cuvette was 1 ml. The reaction was started by adding 20 µl of the supernatant (final reaction volume 1.02 ml). All samples were analyzed in triplicate under substrate saturating conditions. The decrease in absorption was measured photometrically at 340 nm wavelength and 20°C every 18 seconds for a period of 3 minutes.

Ontogenetic differences in enzyme activity between adult and copepodite stages as well as depth-related differences in enzyme activity were statistically evaluated by Mann-Whitney U tests and Kruskal-Wallis tests, followed by a Dunn's multiple comparison test, respectively.

Results

Hydrographical Parameters

Sea surface temperature (SST) ranged from 17.8 to 29.6°C (Table 1, Figure 3A). SST was highest at stns. 318 and 319 near the equator (SG1, black curves) and at stn. 317 (SG3, dark gray curves), while the lowest SST was recorded at stn. 840 (SG4, light

Species abundance [Ind. 1000 ⁻¹ m ⁻³]	surface layer 0 m - ~100 m		above OMZ, epipelagic ~100 m - ~250 m		within OMZ, upper mesopelagic ~250 m - ~ 500 m		below OMZ, lower meso-/bathypelagic ~ 500 m - 1800 m	
<i>Scolecithrix danae</i> C5-C6	263-2000	1409-3000					SG1	SG2
		3000-4286					SG4	SG3
<i>Temora stylifera</i> C5-C6		0-91						
		5778-12846						
<i>Undinula vulgaris</i> C5-C6	7684-16692	0-1136	0-200					
		1231-6111						
Pontellidae C5-C6	1154-2211		s.i.		s.i.			
		0-1929						
<i>Candacia curta</i> C6f		45-174						
<i>Candacia pachydactyla</i> C5-C6	53-2231	130-364	s.i.					
		143-111		43-167				
<i>Candacia</i> spp. C5-C6	105-308	174-545	0-167					s.i.
		214-2111		21-250				
<i>Euchaeta marina</i> C3-C6	4789-24077	4957-5455	53-2083			0-83		
		5077-10111		0-1667		0-32		
<i>Euchaeta</i> sp. C4-C6f			0-286		0-63	0-42		
<i>Neocalanus</i> spp. C5-C6	77-947	2000-2545	0-250	0-250		0-125		14-28
		1077-3357	93	23-250	s.i.	0-111		0-14
<i>Arietellus aculeatus</i> C6				s.i.				
				0-333				
<i>Aetideus</i> spp. C5-C6f	231-632	0-136	100-667	417-1000		0-292		
		0-2714	s.i.	0-128	s.i.			
<i>Euchirella pulchra</i> C6f					0-95	s.i.	s.i.	15-28
				0-167	56	s.i.		
<i>Euchirella rostrata</i> C6f		45-174						
<i>Euchirella</i> sp. C4-C6f	s.i.	45-304	0-700	31-333			s.i.	s.i.
		0-444		0-49	74	0-48		
Eucalanidae ?C5	368-692	739-12045	48-417	31-750	0-167	42-240		84-231
		8000-14667		227-5488	574	618-1767		34-211
<i>Pareucalanus</i> spp. C4-C6	105-154	273-1174	18-100	83-100	47-83	31-42	27-210	27-112
		889-2000	93	43-146	537	0-16	24	11-189
<i>Rhincalanus cornutus</i> C5-C6	231-2789	826-2682	190-917	375-1333	354-930	250-1417	33-570	81-1400
		357-1615	s.i.	83-1585	296	127-221	34	7-136
<i>Rhincalanus nasutus</i> C5-C6f								
			47		722			
<i>Subeucalanus</i> spp. C5-C6	231-2421	652-17591	100-167	0-167	0-167		s.i.	
		5444-12286		68-2500	s.i.	0-238		0-63
<i>Eucalanus hyalinus</i> C5-C6								19-31
	s.i.		47	s.i.	4889	0-70	153	11-35
<i>Pleuromamma abdominalis</i> C5-C6*	316-923		330-2500	31-100	47-63	0-52		9-77
		0-357		0-1333	1296	29-571		0-14
<i>Pleuromamma quadrungulata</i> C6*			s.i.			s.i.		s.i.
					37			
<i>Pleuromamma robusta</i> C5-C6*	s.i.		167-190		s.i.		s.i.	0-31
				0-167	2759	0-79	16	0-21
<i>Pleuromamma xiphias</i> C5-C6*			s.i.			0-21	s.i.	0-62
				0-1000	s.i.	0-492		0-28
<i>Pleuromamma borealis/gracilis/piseki</i> C5-C6*	158-1000		123-5400	s.i.	70-146	52-83		0-46
		0-2286		49-917	1407	29-256	25	
<i>Scottocalanus securifrons</i> C5-C6	s.i.		35-571		s.i.			s.i.
	s.i.			s.i.		s.i.		
<i>Undeuchaeta major</i> C5-C6f			s.i.					0-18
<i>Gaetanus minor</i> C5-C6f	s.i.		83-600		0-42	0-21		9-31
				0-250		0-111		
<i>Gaetanus pileatus</i> C4-C6f					0-47		s.i.	s.i.
					s.i.	s.i.		s.i.
<i>Gaetanus tenuispinus</i> C5-C6f	s.i.		s.i.		0-42			s.i.
<i>Gaetanus kruppilii</i> C5-C6m							7-20	
<i>Gaetanus brevispinus</i> C5					56		7-10	
								s.i.
<i>Gaetanus</i> sp. C5						111		
<i>Aetideopsis carinata</i> C5-C6f						185		s.i.
<i>Chirundina streetsii</i> C5-C6f			s.i.			0-21		9-15
<i>Chiridius gracilis</i> C5-C6f			0-140		0-70			
<i>Calanoides carinatus</i> C5-C6				21-583	2222	0-235	672	0-27
							10-20	9-176
<i>Paraeuchaeta</i> spp. C4-C6			35-83		s.i.		16	19-54
		s.i.		s.i.	222	s.i.		s.i.
Augaptilidae C4-C6				0-1800		0-208		0-110
<i>Megacalanus princeps</i> C4-C6f								s.i.
							13	s.i.
<i>Lucicutia</i> sp. C5-C6m							s.i.	
<i>Valdiviella</i> sp. C5-C6f								0-11
								0-11
Total	91,878	68,895	3,521	1,625	2,490	3,933	977	1,304
	63	161,648	326	25,169	15,556	7,987	954	2,657

Figure 4. Abundance and vertical distribution of calanoid copepods in the eastern tropical Atlantic. Copepod abundance is presented for four different station groups (SG1–4) and for four different depth layers related to the vertical extent of the OMZ. Four cells per species and depth layer represent its abundance in each station group in clockwise order: top left = SG1 (black), top right = SG2 (white), bottom right = SG3 (dark gray), bottom left = SG4 (light gray), as depicted in the top right corner. * = species identified as diel vertical migrants. C3–C5 = copepodid 3–5, C6 = adult, f = female, m = male, s.i. = single individual, OMZ = oxygen minimum zone. Blank cells indicate absence. doi:10.1371/journal.pone.0077590.g004

gray curve) in the southern Angola Gyre. The depth of the mixed layer extended from 10 m at stn. 784 in the northeastern Angola Gyre to 86 m at stn. 840. Differences between temperature profiles were most pronounced within the upper few hundred meters, therefore, temperature profiles are only plotted down to 400 m depth.

The oxygen concentrations in surface waters exceeded $200 \mu\text{mol kg}^{-1}$ at all stations, while the depth of the upper oxycline differed greatly between stations (Figure 3B). The vertical extent and intensity of the oxygen minimum zone (OMZ, O_2 concentrations $\leq 45 \mu\text{mol kg}^{-1}$) was variable and increased from north to south (Table 1). At the equatorial stns. 318 and 319 (SG1, black curves) oxygen concentrations remained above $42 \mu\text{mol kg}^{-1}$, indicating an only weakly developed OMZ. At stns. 784, 787 and 317 in the northeastern Angola Gyre (SG3, dark gray curves), lower minimum O_2 values of 17.6 to $39.8 \mu\text{mol kg}^{-1}$ were determined between 240 and 430 m depth. In the southeastern Angola Gyre, the OMZ broadened and extended from about 50 to 400 m, with an overall O_2 minimum of $12.7 \mu\text{mol kg}^{-1}$ (Table 1). At stn. 840 (SG4, light gray curve), oxygen concentrations of $16.4 \mu\text{mol kg}^{-1}$ were measured at 324 m. Below 400 to 600 m, O_2 concentrations increased again above hypoxic levels.

Fluorescence, measured as proxy for chlorophyll *a* concentration, was highly variable at the different stations (Figure 3C). Lowest surface values (<0.2 in relative units) were detected at the northern tropical stns. 318 and 319 (SG1, black curves) and at the northeastern stns. 784, 787 and 317 (SG3, dark gray curves), while maximum values of >1.5 occurred in the subsurface layer (25 to 60 m) at stn. 317 (SG3). At the surface of stn. 840 (SG4, light gray curve), fluorescence was moderate (ca. 0.4 in relative units) and stayed constant down to 80 m.

Characteristics of Station Groups Revealed by Cluster Analysis

Cluster analysis grouped the eight stations according to their similarity in copepod abundance and species composition into four station groups (SG1 to SG4, Figure 2), which is also reflected by geographic location and oceanographic features (see also Figure 1, Table 1). SG1 included the two northern equatorial stations (stns. 318, 319) with highest SST ($>28^\circ\text{C}$) and only a weak OMZ ($\text{O}_2 \geq 42.6 \mu\text{mol kg}^{-1}$). SG2 comprised the two stations located in the central Angola Basin (stns. 1, 2). The tropical stations of SG1 and SG2 were similar in total copepod abundance, but biodiversity was higher in the deep layer of SG2 (Figure 4). Stations grouped into SG3 were all located closer to the coast in the northeastern Angola Gyre (stns. 784, 787, 317) and had intermediate SST ($23\text{--}29^\circ\text{C}$) and a moderate OMZ ($17.6\text{--}39.8 \mu\text{mol kg}^{-1}$). Total copepod abundance was highest in almost every depth layer of SG3 compared to the other station groups (Figure 4). SG4 consisted of only one station (stn. 840) located in the southern Angola Gyre near the Angola-Benguela front and had lowest SST (18°C) and lowest O_2 concentrations in the OMZ ($16.4 \mu\text{mol kg}^{-1}$). SG4 was different from all other stations due to the lowest copepod abundance in every depth layer except within the OMZ, where copepod abundance was highest of all stations (Figure 4).

Copepod Abundance and Distribution

In general, copepod abundance decreased with increasing depth. Abundances in the surface layer ranged from 69,000 in SG2 to $>160,000 \text{ ind. } 1000^{-1} \text{ m}^{-3}$ in SG3 and decreased to about 1,000 in SG1 to $2,700 \text{ ind. } 1000^{-1} \text{ m}^{-3}$ in SG3 below 500 m depth (Figure 4). In the central Angola Basin (SG2), the copepod abundance of $3,900 \text{ ind. } 1000^{-1} \text{ m}^{-3}$ in the upper mesopelagic zone (250 to 500 m depth) was two to threefold higher than those in the adjacent depth layers above and below. Stn. 840 (SG4) close to the Angola-Benguela front showed a completely different and very peculiar vertical distribution. In the upper 250 m, copepod abundance was extremely low with 63 to $326 \text{ ind. } 1000^{-1} \text{ m}^{-3}$, whereas maximum abundance occurred in the upper mesopelagic with $>15,500 \text{ ind. } 1000^{-1} \text{ m}^{-3}$. Regionally, copepod abundance was usually highest at stns. 784, 787 and 317 in the northeastern Angola Gyre (SG3). The highest biodiversity of copepods in the study area was recorded in the deepest layer of SG2 in the central Angola Basin.

Among the larger calanoid copepods, the most abundant species in the epipelagic was *Euchaeta marina* (up to $24,077 \text{ ind. } 1000^{-1} \text{ m}^{-3}$), followed by *Undinula vulgaris* and different eucalanid species (Figure 4). Eucalanidae were distributed throughout the water column and together with *Pleuromamma* spp. clearly dominated within the OMZ. Comparisons between day and night stations showed that *Pleuromamma* spp. conducted diel vertical migrations, with elevated abundance (up to $5,400 \text{ ind. } 1000^{-1} \text{ m}^{-3}$) in epipelagic waters at night (stns. 317 (SG3) and 318, 319 (SG1)) and higher abundance (up to $2,795 \text{ ind. } 1000^{-1} \text{ m}^{-3}$) within the centre of the OMZ during daytime (remaining stations). Species that exclusively occurred within the epipelagic layer were *Scolecithrix danae*, *Labidocera* spp., *Temora stylifera* and *U. vulgaris*. *Aetideus* spp., *Candacia* spp., *E. marina* and *Neocalanus* spp. were predominantly distributed at the surface, but also occurred in mesopelagic waters. *Euchirella* spp., *Gaetanus* spp., *Eucalanus hyalinus*, *Paraeuchaeta* spp. were principally distributed in the mesopelagial including the OMZ, while *Aetideopsis carinata*, *Lucicutia* sp., *Megacalanus princeps* and *Valdiviella* sp. mainly occurred at lower meso- to bathypelagic depths. *Calanoides carinatus* showed a bimodal distribution pattern at some stations (SG3); adults were found in epipelagic layers above the OMZ, while copepodids C5 exclusively dwelled below or within the OMZ.

The abundances of most of the typically epipelagic species were higher in the northeastern Angola Basin (SG3) compared to the central Angola Basin and equatorial area (SG1 and SG2). However, *U. vulgaris* and *E. marina* had their peak abundance at SG1 close to the equator with up to $16,692 \text{ ind. } 1000^{-1} \text{ m}^{-3}$ and $24,077 \text{ ind. } 1000^{-1} \text{ m}^{-3}$, respectively (Figure 4). Species of the families Eucalanidae (*Pareucalanus*, *Subeucalanus*, *Eucalanus* spp., *Rhincalanus cornutus*) and Metridiinae (*Pleuromamma* spp.) were distributed throughout the sampling area, except for *Rhincalanus nasutus*, which occurred only in the south (SG4). Highest abundance of eucalanids (up to $17,591 \text{ ind. } 1000^{-1} \text{ m}^{-3}$) was recorded in the epipelagic at SG2 and SG3, while metridiinae species were most abundant in the equatorial region at SG1 (up to $5,400 \text{ ind. } 1000^{-1} \text{ m}^{-3}$).

The following species only occurred as single individuals at some stations and do not appear in Figure 4: *Euchirella splendens*

Table 2. Individual and mass-specific respiration rates (mean \pm SD for $n \geq 3$ or range, if $n = 2$) of calanoid copepods from the tropical Atlantic.

Species	Stage	Sampling depth [m]	<i>in situ</i> T. [°C]	DM range [mg]	Ind. R [nmol h ⁻¹] mean \pm SD	DM-spec. R [μ mol g _{DM} ⁻¹ h ⁻¹] mean \pm SD	n
<i>Scolecithrix danae</i>	f	200-0	15–17.5	0.16–0.26	22.8 \pm 6.2	112.2 \pm 25.6	7
	f	50-0	18–20	0.16–0.22	32.9 \pm 10.7	169.5 \pm 49.2	6
	m	50-0	18–20	0.16/0.17	17.6/17.4	111.2/103.7	2
<i>Temora stylifera</i>	f	40-0	18–20	0.05–0.08	14.7 \pm 3.9	243.2 \pm 91.2	3
	m	40-20	18-20	0.03/0.06	8.8/3.1	273.9/51.3	2
<i>Labidocera acutifrons</i>	f	50-0	20	0.49–0.53	53.0 \pm 11.1	104.6 \pm 25.0	4
	m	50-0	20	0.48–0.54	57.0 \pm 8.6	111.4 \pm 12.3	4
<i>Undinula vulgaris</i>	f	50-0	15	0.17–0.20	31.5 \pm 8.5	174.9 \pm 59.9	4
	f	50-0	18.5–20	0.13–0.26	32.4 \pm 10.0	162.2 \pm 28.3	7
	m	50-0	15	0.18–0.20	27.9 \pm 7.7	144.0 \pm 34.7	4
	m	50-0	20	0.13/0.13	6.9/21.5	54.4/161.6	2
<i>Candacia bipinnata</i>	f	80-0	18–20	0.19–0.24	27.1 \pm 4.2	126.5 \pm 10.8	5
<i>Candacia curta</i>	f	50-0	20	0.16–0.20	15.4 \pm 4.7	83.6 \pm 21.9	3
<i>Candacia pachydactyla</i>	f	80-0	19–20	0.27/0.37	71.9/23.7	263.3/64.1	2
<i>Euchaeta marina</i>	f	220-0	13–16	0.14–0.32	18.3 \pm 10.6	86.9 \pm 64.9	6
	f	80-0	18–20	0.27–0.41	46.4 \pm 10.9	153.3 \pm 49.5	8
	f (ripe)	20-0	13	0.29–0.30	8.2 \pm 4.5	27.9 \pm 15.4	3
	f (ripe)	40-0	18–20	0.26–0.37	62.5 \pm 21.3	199.4 \pm 55.3	4
	f (eggs)	20-0	13	0.21/0.21	2.7/4.0	13.2/18.9	2
	f (eggs)	80-0	16–19	0.23–0.45	57.1 \pm 33.0	191.5 \pm 98.5	4
	m	80-0	14–16	0.23–0.25	21.7 \pm 6.7	89.7 \pm 25.3	3
	m	80-0	18–20	0.19–0.26	34.0 \pm 9.0	149.2 \pm 53.0	4
<i>Neocalanus gracilis</i>	f	800–500	7.5–8.2	0.27–0.44	19.4 \pm 6.3	50.7 \pm 11.6	10
	f	220-0	13.7–16	0.41–0.51	31.3 \pm 10.1	69.9 \pm 23.8	9
	f	80-0	18–19	0.32–0.43	61.5 \pm 30.1	161.3 \pm 86.0	5
<i>Neocalanus robustior</i>	C5	80-0	16	0.27/0.32	22.7/20.9	84.5/64.6	2
	f	220-0	14	0.51–0.63	32.6 \pm 5.7	59.3 \pm 4.5	3
	f	80-0	16–19	0.44–0.60	66.4 \pm 25.8	123.7 \pm 43.0	9
<i>Neocalanus</i> sp.	C5	100-0	14–16	0.23–0.37	15.9 \pm 4.3	58.1 \pm 9.4	3
<i>Arietellus aculeatus</i>	f	100-50	20	0.69/0.75	28.2/26.5	41.0/35.5	2
	m	100-50	20	0.69/0.79	16.4/25.0	23.6/31.6	2
<i>Aetideus</i> sp.	f	120-80	16	0.05/0.07	9.6/9.9	179.7/136.2	2
<i>Euchirella pulchra</i>	f	800-220	6.5–7.5	0.40–0.53	12.6 \pm 3.5	29.0 \pm 10.7	4
	f	500-30	10	0.40/0.52	13.6/16.0	34.0/30.8	2
<i>Euchirella rostrata</i>	f	600-50	17	0.38/0.47	45.6/49.9	119.9/105.7	2
	f	80-0	19–19.5	0.36/0.41	62.3/38.8	173.6/94.3	2
<i>Euchirella splendens</i>	f	800-40	5.5–8	0.77–1.27	27.8 \pm 7.2	28.2 \pm 7.1	5
<i>Euchirella</i> sp.	C4	80-0	16–18.5	0.20/0.24	10.6/37.3	52.4/152.9	2
	C5	800-30	7.5–9.6	0.21/0.79	5.6/21.9	26.1/27.9	2
	C5	50-100	14	0.66/0.71	57.2/51.9	86.3/73.7	2
	f	80-0	14–18.8	1.16/1.16	61.8/66.8	53.2/57.5	2
<i>Pareucalanus</i> spp.	C5	1800-1000	5–7.3	0.66–0.72	6.8 \pm 3.0	10.0 \pm 4.1	5
	C5	80-0	18.5	0.05/0.25	5.4/27.3	108.2/108.3	2
	f	220-0	13.7–16	0.17–0.41	10.3 \pm 6.7	40.0 \pm 31.8	4
	f	80-0	18–20	0.28–0.53	46.5 \pm 15.8	119.4 \pm 47.0	11
	m	50-0	18	0.65/1.08	46.6/66.8	72.2/61.9	2
<i>Rhincalanus cornutus</i>	C5	1000-200	6.5–7.5	0.09–0.20	2.8 \pm 1.1	22.9 \pm 11.5	4
	C5	220-0	14–16	0.04–0.22	10.0 \pm 3.9	83.0 \pm 36.0	8

Table 2. Cont.

Species	Stage	Sampling depth [m]	<i>in situ</i> T. [°C]	DM range [mg]	Ind. R [nmol h ⁻¹] mean ± SD	DM-spec. R [μmol g _{DM} ⁻¹ h ⁻¹] mean ± SD	n
	C5	50-0	20	0.11–0.17	17.6±4.0	129.6±30.4	5
	f	50-0	15	0.12–0.26	13.4±4.9	69.6±20.2	4
	f	50-0	18.5–20	0.15–0.26	18.5±9.8	95.6±70.8	7
<i>Pleuromamma abdominalis</i>	f	500-30	10	0.28–0.37	26.5±4.9	80.4±8.4	4
<i>Pleuromamma quadrangulata</i>	f	600-30	7.5–9.5	0.25–0.54	17.3±3.6	43.6±7.9	6
<i>Pleuromamma robusta</i>	f	100-50	15	0.25–0.34	33.3±5.4	107.3±11.1	7
<i>Pleuromamma xiphias</i>	f	1000-30	7.5–10	0.52–0.91	44.4±8.5	64.8±13.2	10
	f	200-100	14	0.35–0.61	26.2±19.9	53.2±34.9	4
	f	100-50	20	0.40–0.62	56.1±23.9	110.3±30.7	4
	m	1000-150	8–10	0.61/1.24	22.5/57.9	36.6/46.6	2
	m	100-50	20	0.46–1.10	83.7±19.5	137.2±55.2	3
<i>Gaussia princeps</i>	C5	1000-150	8	2.39–6.64	46.2±19.9	11.6±4.3	9
	f	800-200	8	8.54/9.54	103.6/101.2	12.1/10.6	2
	m	800-150	8	5.27–7.02	98.5±21.9	15.9±2.9	6
<i>Calanoides carinatus</i>	C5	1800-1000	4.7–6.2	0.11–0.14	2.1±0.9	17.0±8.7	6
	C5	400-300	10	0.10/0.11	6.6/7.4	64.4/64.9	2
<i>Chirundina streetsii</i>	f	800-400	6.5–8	1.38/1.46	36.6/26.7	26.6/18.3	2
<i>Gaetanus pileatus</i>	f	1000-250	8–10	1.02–1.72	19.7±2.7	15.0±4.3	3
<i>Paraeuchaeta gracilis</i>	f	600-400	8	1.41–2.16	41.5±29.7	23.9±18.0	4
<i>Paraeuchaeta hansenii</i>	f	800-400	8	5.89–5.98	71.8±25.0	12.0±4.1	4
<i>Paraeuchaeta</i> sp.	f	1800-500	5–7.5	1.25/1.42	19.5/18.7	15.6/13.2	2
<i>Megacalanus princeps</i>	C4/C5	1000-400	6.5–8	0.98–1.44	22.0±14.1	17.7±10.1	5
	f	1000-250	4.6	5.34/6.59	50.1/113.4	9.4/17.2	2
	f	1000-250	8	5.34–6.78	100.5±24.8	16.4±3.9	4
	m	600-400	8	3.81–4.56	49.0±11.6	11.5±1.9	3

f = female, m = male, C4/C5 = copepodid 4 and 5, T = temperature, DM = dry mass, Ind. R = individual respiration rate, DM-spec. R = mass-specific respiration rate, n = number of measurements.

doi:10.1371/journal.pone.0077590.t002

below the OMZ at SG2; *Arietellus plumifer* above and within the OMZ at SG1; *Nannocalanus minor* above the OMZ at SG1; *Scaphocalanus magnus* above the OMZ at SG3, within the OMZ at SG1 and below the OMZ at SG2; *Gaussia princeps* above the OMZ at SG3; *Gaetanus tenuispinus* within the OMZ at SG4; *Lophothrix* sp. below the OMZ at SG2 and *Pseudochirella* sp. above and within the OMZ at SG1.

Respiration Rates

A total of 342 individual respiration measurements were conducted with 40 calanoid copepod species. Respiration rates of copepods with ≥2 replicate measurements are shown in Table 2. Individual respiration rates varied between 2.1±0.9 μmol g_{DM}⁻¹ h⁻¹ in copepodids C5 of *C. carinatus* from below 1000 m and 206.9 μmol g_{DM}⁻¹ h⁻¹ in female *G. princeps* from 200–100 m depth. Mass-specific respiration ranged from 4 μmol g_{DM}⁻¹ h⁻¹ in female *Valdiviella oligarthra* from 1800–1000 m to 273.9 μmol g_{DM}⁻¹ h⁻¹ in male *T. styliifera* from the surface. Mass-specific respiration was significantly higher at the surface (Kruskal-Wallis test, p<0.0001) and decreased with increasing depth (Figure 5A, B). Minimum respiration rates were measured in copepods in deeper water layers below the OMZ. Figure 5B compares mass-specific respiration rates of nine copepod species from different depths above, within and below the OMZ. Mass-specific

respiration rates were considerably higher in species from the surface, while there were hardly any differences in respiration between the OMZ core and below the OMZ. *Pleuromamma xiphias* and *G. princeps* had slightly lower respiration rates within the OMZ than below, but differences were not significant. The eucalanid copepods *Pareucalanus* sp. and *R. cornutus* showed considerably lower respiration in deeper waters compared to the surface. For the comparison of respiration rates between species, measurements of all stages were grouped, since differences were not significant (Mann-Whitney U test, p<0.05).

Enzyme Activities

Enzyme activities of the electron transport system (ETS) and lactate dehydrogenase (LDH) were measured for 18 and 20 copepod species, respectively (Table 3). ETS activities varied from 3.9 μmol g_{WM}⁻¹ h⁻¹ in female *E. splendens* from 1800-1000 m depth to 170.0±22.8 μmol g_{WM}⁻¹ h⁻¹ in *S. danae* females from the surface. LDH activities ranged from 10.6 μmol g_{WM}⁻¹ h⁻¹ in female *Pareucalanus* sp. from 200-50 m to 375.8 μmol g_{WM}⁻¹ h⁻¹ in *P. xiphias* males from the OMZ in 500-150 m. ETS activity was significantly higher at the surface (Kruskal-Wallis test, p<0.0001) and declined with increasing depth (Figure 5C). Differences in mean LDH activity in relation to the OMZ were not significant, although maximum LDH activities were recorded within the core

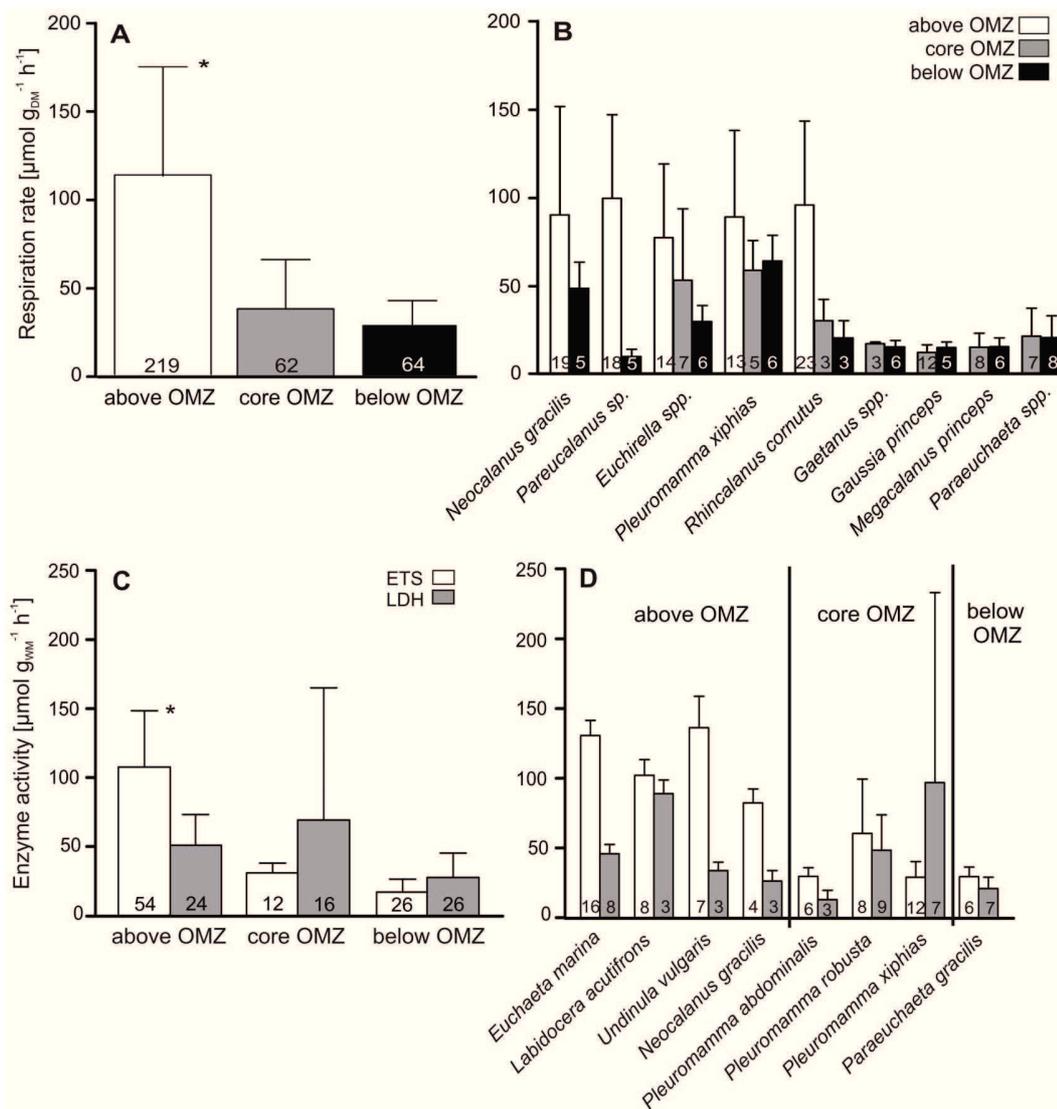


Figure 5. Metabolic rates of calanoid copepods in relation to the oxygen minimum zone (OMZ). A, mass-specific respiration rates. B, comparison of mass-specific respiration rates of nine copepod species (copepodids 5+adults) from different depths. C, enzyme activities of the electron transport system (ETS) and the lactate dehydrogenase (LDH). D, comparison of enzyme activities of eight copepod species (copepodids 5+adults) from different depths. Bars represent means with standard deviation; the number of measurements (n) is indicated in each bar. * = significantly higher respiration rate and ETS activity above the OMZ as compared to the core and below the OMZ. doi:10.1371/journal.pone.0077590.g005

of the OMZ (Figure 5C). *S. danae*, *E. marina*, *L. acutifrons* and *U. vulgaris* were among the epipelagic species with the highest ETS activities (Table 3, Figure 5D). *S. danae*, *L. acutifrons* and *E. marina* as well as *Candacia pachydactyla* and *R. cornutus* from above the OMZ had also high LDH activities (Table 3, Figure 5D). The overall maximum LDH activity ($375.8 \mu\text{mol g}_{\text{WM}}^{-1} \text{h}^{-1}$) was observed in *P. xiphias* within the OMZ. Moderate LDH activities were measured in *Pleuromamma robusta* and *Paraeuchaeta aequatorialis* from below the OMZ. For inter-specific comparison of ETS and LDH activities, measurements of all stages of one species were grouped, since differences were not significant (Mann-Whitney U test, $p < 0.05$).

Discussion

The most intense oxygen minimum zones (OMZs) are located in the eastern tropical Pacific as well as in the Arabian Sea, where hypoxic waters extend over depth ranges of several hundred metres and oxygen concentrations frequently drop below $4.5 \mu\text{mol kg}^{-1}$ [4,7,38]. Although the OMZ in the eastern tropical Atlantic is less pronounced than in other tropical regions, the Atlantic has a high potential to suffer from major losses in dissolved oxygen within the next decades [4,5]. The large-scale station grid sampled for the present study encompasses very diverse hydrographic regimes. The northernmost stations close to the equator were characterized by a weakly developed OMZ, whereas stations in the southern Angola Basin had a more

Table 3. Enzyme activities of the electron transport system (ETS) and lactate dehydrogenase (LDH) of calanoid copepods from the tropical Atlantic.

Species	Stage	ETS activities				LDH activities		
		Depth [m]	T. [°C]	WM range [mg]	ETS activity [$\mu\text{mol g}_{\text{WM}}^{-1} \text{h}^{-1}$] \pm SD (n)	Depth [m]	WM range [mg]	LDH activity [$\mu\text{mol g}_{\text{WM}}^{-1} \text{h}^{-1}$] \pm SD (n)
<i>Scolecithrix danae</i>	f	22-0	20	0.69–0.77	170.0 \pm 22.8 (3)	200-50	0.81	153.3 (1)
<i>Labidocera acutifrons</i>	f	50-0	20	2.20–2.49	100.0 \pm 8.3 (4)			
	m	50-0	20	1.98–2.14	104.4 \pm 14.7 (4)	50-0	1.97–2.27	89.1 \pm 9.7 (3)
<i>Undinula vulgaris</i>	f	50-0	20	0.77–0.88	150.2 \pm 5.0 (4)	50-0	0.80–0.84	33.9 \pm 6.0 (3)
	m	50-0	20	0.44–0.60	118.2 \pm 24.5 (3)			
<i>Candacia pachydactyla</i>	f	30-0	23	0.95–1.41	97.0 \pm 38.8 (4)	50-0	1.35/1.42	46.1/91.9 (2)
<i>Euchaeta marina</i>	C5	22-0	20	0.73–0.97	121.8 \pm 10.3 (3)			
	f	22-0	20	1.35–1.50	127.4 \pm 8.9 (4)	50-0	1.47/1.75	32.0/40.2 (2)
	f(ripe)	50-0	28	0.77–0.93	133.1 \pm 7.3 (5)	50-0	0.89–1.50	49.1 \pm 1.3 (3)
	f(eggs)	50-0	28	0.85–1.05	137.5 \pm 14.5 (4)	50-0	0.90–1.19	49.5 \pm 1.9 (3)
<i>Neocalanus gracilis</i>	f	300-100	15	1.80–2.06	82.4 \pm 10.0 (4)	300-100	1.95–2.10	26.4 \pm 7.5 (3)
<i>Rhincalanus cornutus</i>	f					250-50	0.76/1.02	20.1/49.8 (2)
<i>Pareucalanus</i> spp.	C5					1000-500	1.83–2.02	31.4 \pm 11.2 (3)
	f					1000-500	2.15–2.95	23.6 \pm 5.1 (3)
	f					200-50	2.46/3.42	16.5/10.6 (2)
<i>Pleuromamma abdominalis</i>	f	450-200	10	1.42–1.58	31.0 \pm 6.5 (4)	600-250	1.58–1.98	13.0 \pm 6.6 (3)
	m	450-200	10	1.00/2.10	22.9/31.8 (2)			
<i>Pleuromamma robusta</i>	f	100-50	15	1.40–1.50	96.7 \pm 7.3 (4)	100-50	1.38–1.39	66.4 \pm 4.6 (3)
	f	600-400	8	1.25–1.44	24.4 \pm 4.1 (4)	600-400	1.30–1.51	42.7 \pm 33.2 (4)
	f					450-200	1.62/1.68	22.3/43.7 (2)
<i>Pleuromamma xiphias</i>	f	100-50	20	2.64–3.47	28.0 \pm 15.3 (3)	100-50	3.72	39.1 (1)
	f	250-30	10	3.10–3.70	23.7 \pm 14.6 (3)	250-30	2.98	18.9 (1)
	f	500-150	10	2.60-3.40	35.5 \pm 8.0 (3)	500-150	3.25-3.6	21.2 \pm 7.3 (3)
	m	500-150	10	2.56-4.70	29.0 \pm 8.6 (3)	500-150	3.83/3.95	375.8/181.0 (2)
<i>Megacalanus princeps</i>	f	500-300	8	17.00/25.00	17.4/13.2 (2)	500-300	26.68/30.00	40.1/41.3 (2)
<i>Euchirella pulchra</i>	f	1000-450	5	2.41–2.64	10.3 \pm 3.8 (4)	450-200	2.46/2.67	48.4/80.2 (2)
<i>Euchirella rostrata</i>	f	200-30	14	1.88	100.7 (1)			
	f	1000-600	5	1.84	13.8 (1)			
<i>Euchirella splendens</i>	f	1800-1000	4	5.83/5.85	3.9/4.0 (2)	250-50	5.74	29.6 (1)
<i>Gaussia princeps</i>	f	200-100	14	12.40	32.6 (1)			
	f	1000-600	8	21.80	9.0 (1)	1000-600	35.00	16.5 (1)
	m	500-300	8	26.50	18.3 (1)	500-300	21.90	22.5 (1)
<i>Gaetanus brevicornis</i>	C5	500-300	8	2.81-3.20	11.1 \pm 1.3 (3)			
	f					500-300	5.67	15.3 (1)
<i>Gaetanus brevispinus</i>	C4/C5					1800-1000	0.91	17.8 (1)
	f	1800-1000	4	2.83	12.0 (1)			
<i>Paraeuchaeta aequatorialis</i>	f					1000-400	4.36	61.6 (1)
<i>Paraeuchaeta gracilis</i>	C5	600-400	8	3.86–4.17	26.4 \pm 8.2 (3)	600-400	3.50–3.84	20.1 \pm 13.3 (3)
	f	600-400	8	6.58–7.68	31.1 \pm 5.4 (3)	600-400	6.32–7.34	20.1 \pm 4 (4)
<i>Valdiviella</i> sp.	C3	1800-1000	4	4.90	6.0 (1)			

f = female, m = male, C3–C5 = copepodid 3–5, T = temperature, WM = wet mass, n = number of measurements.
doi:10.1371/journal.pone.0077590.t003

pronounced OMZ with a minimum oxygen concentration of 12.7 $\mu\text{mol kg}^{-1}$ at 410 m depth. The Angola Gyre is characterized by low oxygen concentrations in its centre and is suspected to be the source of hypoxic water for the southeastern Angola Basin

and Benguela region [39,40]. A shoaling of the OMZ in the southern Angola Basin may indicate the location of the Angola Dome (at about 10°S, 8°E), which is characterized by an uplift of

isotherms and low oxygen concentrations close to the surface [7,41].

The community analysis revealed four station groups (SG1 to SG4) that differed in copepod species composition and abundance. Abundance and distribution of calanoid copepods are comparable to other studies from the tropical Atlantic [28,29,42]. Calanoid copepod abundance was highest in the northeastern Angola Gyre (SG3) and decreased towards tropical waters at the equator and towards the central Angola Basin (SG1, 2). High zooplankton standing stocks in the Angola Gyre and off the coasts of Gabon and Congo have earlier been observed [43,44]. Maximum productivity occurred in August off Gabon [43], corresponding to the time of sampling at the northern stations of SG3. The uplift of the thermocline into the euphotic zone in the area of the Angola Dome increases nutrient supply and productivity in comparison to the surrounding oligotrophic tropical waters [41]. This is also evident in the shallower mixed layer depth and subsurface fluorescence maximum of SG3 compared to other stations (Figure 3). In addition, the discharge of the Congo River provides an extra supply of nutrients, which supports enhanced biological productivity in the northeastern part of the study area [41,45].

Copepod abundance was usually highest at the surface and continuously declined with increasing depth. This is in accordance with other findings from tropical oceans [42,46]. One station (stn. 840, SG4) deviated from all others and contained extremely few copepods in the surface layer, but high abundances within the OMZ. This station, located in the vicinity of the Angola-Benguela front, had a deeper mixed layer and low SST, which suggests an influence of colder waters from the Benguela Current [47]. In contrast to studies from the Benguela upwelling region, the eastern tropical Pacific and the Arabian Sea [9–11,26], we did not observe minimum copepod abundances within the OMZ, as compared to depth layers above and below. Biomass of pelagic organisms within OMZs does not seem to be affected by oxygen concentrations above $10 \mu\text{mol l}^{-1}$ [48], which suggests that current O_2 concentrations in the eastern tropical Atlantic do not yet influence copepod abundance and distribution.

The OMZ was frequently inhabited by various species of the two calanoid families Eucalanidae and Metridiidae that clearly dominated over others. These species are typical inhabitants of OMZs in the Peru and Benguela Current regions as well as in the Arabian Sea [11,17,49,50]. In the Arabian Sea, differences in hypoxia tolerance were observed among different eucalanid species, i.e. *Eucalanus attenuatus* and *Rhincalanus cornutus* were less common within the OMZ, while *Eucalanus elongatus* prevailed in hypoxic layers [10]. In the tropical North Pacific, *Eucalanus inermis* conducts extended ontogenetic vertical descents into the upper and lower boundary layers of the OMZ [26]. In the present study, *R. cornutus* was distributed throughout the water column, whereas *Pareucalanus* spp. and *Subeucalanus* spp. were less abundant within the OMZ but *Eucalanus hyalinus* more abundant. Several *Pleuromamma* species were identified as vertical migrants and regularly inhabited or temporally migrated into the OMZ. Since the OMZ did not seem to prevent vertical migrations of these species, they may find refuge within the OMZ from predation and competition by less tolerant species, such as fish [27].

Pelagic organisms that frequently inhabit OMZs cope with hypoxic conditions either via an efficient removal of oxygen from surrounding waters, via a reduction of metabolic rates or via additional energy from anaerobic metabolism [22]. In order to evaluate different metabolic strategies of calanoid copepods, we measured respiration rates as well as enzyme activities of the electron transport system (ETS) as a proxy for the potential aerobic rate [34,51], and lactate dehydrogenase (LDH) as an

indicator of anaerobic (glycolytic) metabolism [20]. Copepod aerobic respiration, in terms of actual respiration rate and ETS activity, was comparable to previous measurements of copepods from tropical and subtropical regions [25,36,52–54]. Mass-specific respiration rates and ETS activities were highest in copepods from surface waters and decreased with increasing depth, consistent with a decline in temperature and an increase in body mass [24,55,56]. While there was a rapid decline in aerobic activity below the oxygen-saturated epipelagic layer, the differences between the core of the OMZ and below were not as pronounced. Similar results have been reported from the eastern tropical Pacific [51] and Indo-Pacific [53].

A reduction of metabolic rates has been observed in organisms that inhabit OMZ regions and may be an advantage to survive within hypoxic zones [16,19,22,57,58]. Copepods of the family Eucalanidae seem to be particularly successful inhabitants of OMZs. They are often characterized by substantially reduced respiration rates and some species, such as *Rhincalanus nasutus* and *Eucalanus* spp., apparently enter a dormant state within or below the OMZ [25,52,54,59]. This could also be the case for *Rhincalanus cornutus* and *Pareucalanus* sp. investigated in this study, since specimens from deeper waters showed considerably reduced locomotory activity and metabolic rates. Certain physiological traits of the “lethargic lifestyle” of eucalanid copepods have been compared to the typical adaptive strategies of jellyfish [54], which allow eucalanid species to reduce their energy expenditure and permanently colonize waters of extreme hypoxia.

In contrast, the other successful group of OMZ colonizers includes the vertically migrating copepod *Pleuromamma xiphias*. This species migrates into the OMZ twice per day and is challenged by hypoxic conditions only for limited periods of time. Similarly, *Pleuromamma* spp. and the euphausiid *Euphausia mucronata* vertically migrate through or into the OMZ in the eastern Pacific [21,49,60]. In *E. mucronata*, this ability correlates with an increased activity of lactate dehydrogenase [21]. In the present study, *P. xiphias* showed the highest LDH activity of all copepods, which apparently is a successful strategy to migrate into hypoxic layers. Anaerobic metabolism may thus provide additional energy for a temporal stay in the OMZ [21–23], while the oxygen debt can be compensated during times spent in the oxygen-rich surface layer [15,22,26,61].

Increased LDH activity in surface species, e. g. *Scolecithrix danae*, *Euchaeta marina* and *Labidocera acutifrons* from this study, serves as an additional energy supply for enhanced locomotion and constant swimming [23]. Moreover, it may support the ‘sit-and-wait’ feeding strategy and burst swimming tactics typical of meso- to bathypelagic copepods such as *Paraeuchaeta aequatorialis* that also showed increased anaerobic activity [23,62]. In general, LDH activities of tropical Atlantic copepods from the present study are lower than those of copepods off California [23]. These authors measured maximum LDH activities of $70 \mu\text{mol g}_{\text{WM}}^{-1} \text{min}^{-1}$ ($4200 \mu\text{mol g}_{\text{WM}}^{-1} \text{h}^{-1}$), in contrast to our maximum value of $376 \mu\text{mol g}_{\text{WM}}^{-1} \text{h}^{-1}$. High LDH activities have also been recorded in bulk zooplankton of the pronounced OMZ in the Peru upwelling region [63]. These differences may indicate that animals from more intense OMZs, as in the subtropical Pacific, rely to a greater extent on an anaerobic energy supply [22].

In this study, we identified different copepod communities in the eastern tropical Atlantic Ocean in relation to hydrographic regimes and, in particular, the extent of the OMZ. While calanoid copepod abundance was highest in the northeastern Angola Gyre, maximum species diversity was found in the deep Angola Basin. Copepod abundance generally decreased with increasing depth, but was not drastically reduced within the OMZ. The OMZ harboured specifically adapted species including eucalanid cope-

pods that had a substantially reduced aerobic metabolism to permanently live within hypoxic zones, as well as diel vertical migrants such as *P. xiphias*, which showed elevated lactate dehydrogenase activity to temporarily cope with hypoxic conditions during migration through the OMZ. A detailed understanding of vertical distribution patterns and ecophysiological characteristics of tropical copepods is essential to identify possible changes in the zooplankton community structure caused by expanding OMZs in the eastern tropical Atlantic.

Acknowledgments

We thank Dr. Volker Mohrholz and Toralf Heene from the Leibniz Institute for Baltic Sea Research, Warnemünde, for providing the CTD

data from the RV *Maria S. Merian* cruises. We are grateful to Dr. Rolf Koppelman and Dr. Bettina Martin, who provided copepod samples from MOCNESS trawls. We also thank the captains and crew of the research vessels RRS *Discovery* and RV *Maria S. Merian* for their professional assistance during the cruises. Maya Bode kindly assisted with enzyme activity analyses.

Author Contributions

Conceived and designed the experiments: LT AS WH HA. Performed the experiments: LT AS HA. Analyzed the data: LT AS WH HA. Contributed reagents/materials/analysis tools: LT AS WH HA. Wrote the paper: LT AS WH HA. Took part in expeditions: LT AS WH HA.

References

- Bograd SJ, Castro CG, Di Lorenzo E, Palacios DM, Bailey H, et al. (2008) Oxygen declines and the shoaling of the hypoxic boundary in the California Current. *Geophysical Research Letters* 35: L12607, doi:10.1029/2008GL034185.
- Ekau W, Auel H, Pörtner HO, Gilbert D (2010) Impacts of hypoxia on the structure and processes in pelagic communities (zooplankton, macro-invertebrates and fish). *Biogeosciences* 7: 1669–1699.
- Stramma L, Schmidtko S, Levin LA, Johnson GC (2010) Ocean oxygen minima expansions and their biological impacts. *Deep-Sea Research Part I - Oceanographic Research Papers* 57: 587–595.
- Stramma L, Johnson GC, Sprintall J, Mohrholz V (2008a) Expanding oxygen minimum zones in the tropical oceans. *Science* 320: 655–658.
- Stramma L, Visbeck M, Brandt P, Tanhua T, Wallace D (2009) Deoxygenation in the oxygen minimum zone of the eastern tropical North Atlantic. *Geophysical Research Letters* 36: L20607, doi:10.1029/2009GL039593.
- Stramma L, Brandt P, Schafstall J, Schott F, Fischer J, et al. (2008b) Oxygen minimum zone in the North Atlantic south and east of the Cape Verde Islands. *Journal of Geophysical Research - Oceans* 113: C04014, doi:10.1029/2007JC004369.
- Karstensen J, Stramma L, Visbeck M (2008) Oxygen minimum zones in the eastern tropical Atlantic and Pacific oceans. *Progress in Oceanography* 77: 331–350.
- Morrison JM, Codispoti LA, Smith SL, Wishner K, Flagg C, et al. (1999) The oxygen minimum zone in the Arabian Sea during 1995. *Deep-Sea Research Part II - Topical Studies in Oceanography* 46: 1903–1931.
- Saltzman J, Wishner KF (1997) Zooplankton ecology in the eastern tropical Pacific oxygen minimum zone above a seamount: 2. Vertical distribution of copepods. *Deep-Sea Research Part I - Oceanographic Research Papers* 44: 931–954.
- Wishner KF, Gelfman C, Gowing MM, Outram DM, Rapien M, et al. (2008) Vertical zonation and distributions of calanoid copepods through the lower oxycline of the Arabian Sea oxygen minimum zone. *Progress in Oceanography* 78: 163–191.
- Auel H, Verheye HM (2007) Hypoxia tolerance in the copepod *Calanoides carinatus* and the effect of an intermediate oxygen minimum layer on copepod vertical distribution in the northern Benguela Current upwelling system and the Angola-Benguela Front. *Journal of Experimental Marine Biology and Ecology* 352: 234–243.
- Stramma L, Prince ED, Schmidtko S, Luo JG, Hoolihan JP, et al. (2012) Expansion of oxygen minimum zones may reduce available habitat for tropical pelagic fishes. *Nature Climate Change* 2: 33–37.
- Hays GC, Richardson AJ, Robinson C (2005) Climate change and marine plankton. *Trends in Ecology and Evolution* 20: 337–344.
- Bertrand A, Ballón M, Chaigneau A (2010) Acoustic observation of living organisms reveals the upper limit of the oxygen minimum zone. *PLoS ONE* 5 (4): e10330, doi:10.1371/journal.pone.0010330.
- Vinogradov ME, Voronina NM (1962) Influence of the oxygen deficit on the distribution of plankton in the Arabian Sea. *Deep-Sea Research* 9: 523–530.
- Longhurst AR (1967) Vertical distribution of zooplankton in relation to the eastern Pacific oxygen minimum zone. *Deep-Sea Research* 14: 51–63.
- Schnack-Schiel SB, Niehoff B, Hagen W, Böttger-Schnack R, Cornils A, et al. (2008) Population dynamics and life strategies of *Rhincalanus nasutus* (Copepoda) at the onset of the spring bloom in the Gulf of Aqaba (Red Sea). *Journal of Plankton Research* 30: 655–672.
- Loick N, Ekau W, Verheye HM (2005) Water body preferences of dominant calanoid copepod species in the Angola-Benguela frontal zone. *African Journal of Marine Science* 27: 597–608.
- Teal JM, Carey FG (1967) Respiration of a euphausiid from the oxygen minimum layer. *Limnology and Oceanography* 12: 548–550.
- Childress JJ, Somero GN (1979) Depth-related enzymic activities in muscle, brain and heart of deep-living pelagic marine teleosts. *Marine Biology* 52: 273–283.
- González RR, Quiñones RA (2002) Ldh activity in *Euphausia mucronata* and *Calanus chilensis*: implications for vertical migration behaviour. *Journal of Plankton Research* 24: 1349–1356.
- Childress JJ, Seibel BA (1998) Life at stable low oxygen levels: Adaptations of animals to oceanic oxygen minimum layers. *Journal of Experimental Biology* 201: 1223–1232.
- Thuesen EV, Miller CB, Childress JJ (1998) Ecophysiological interpretation of oxygen consumption rates and enzymatic activities of deep-sea copepods. *Marine Ecology Progress Series* 168: 95–107.
- Childress JJ (1975) The respiratory rates of midwater crustaceans as a function of depth of occurrence and relation to the oxygen minimum layer off southern California. *Comparative Biochemistry and Physiology* 50: 787–799.
- Schukat A, Teuber L, Hagen W, Wasmund N, Auel H (2013b) Energetics and carbon budgets of dominant calanoid copepods in the northern Benguela upwelling system. *Journal of Experimental Marine Biology and Ecology* 442: 1–9.
- Wishner K, Outram DM, Seibel BA, Daly KL, Williams RL (2013) Zooplankton in the eastern tropical north Pacific: Boundary effects of oxygen minimum zone expansion. *Deep-Sea Research Part I - Oceanographic Research Papers* 79: 122–140.
- Parker-Stetter SL, Horne JK (2009) Nekton distribution and midwater hypoxia: A seasonal, diel prey refuge? *Estuarine Coastal and Shelf Science* 81: 13–18.
- Wood-Walker RS (2001) Spatial distributions of copepod genera along the Atlantic Meridional Transect. *Hydrobiologia* 453: 161–170.
- Schnack-Schiel SB, Mizdalski E, Cornils A (2010) Copepod abundance and species composition in the Eastern subtropical/tropical Atlantic. *Deep-Sea Research Part II - Topical Studies in Oceanography* 57: 2064–2075.
- Wiebe PH, Morton AW, Bradley AM, Backus RH, Craddock JE, et al. (1985) New developments in the MOCNESS, an apparatus for sampling zooplankton and micronekton. *Marine Biology* 87: 313–323.
- Bradford-Grieve JM, Markhaseva EL, Rocha CEF, Abiahy B (1999) Copepoda. In: Boltovskoy D, editor. *South Atlantic Zooplankton*: Backhuys Publishers, Leiden. pp. 869–1098.
- Clarke KR, Warwick RM (1994) Changes in marine communities: an approach to statistical analysis and interpretation. Plymouth Marine Laboratory, NERC, Plymouth, UK.
- Mann H, Whitney D (1947) On a test of whether one of two random variables is stochastically larger than the other. *Annals of mathematical Statistics* 18: 50–60.
- Owens TG, King FD (1975) Measurement of respiratory electron-transport-system activity in marine zooplankton. *Marine Biology* 30: 27–36.
- Packard TT (1971) Measurement of respiratory electron-transport activity in marine phytoplankton. *Journal of Marine Research* 29: 235–244.
- Bode M, Schukat A, Hagen W, Auel H (2013) Predicting metabolic rates of calanoid copepods. *Journal of Experimental Marine Biology and Ecology* 444: 1–7.
- Somero GN, Childress JJ (1980) A violation of the metabolism-size scaling paradigm: activities of glycolytic enzymes in muscle increase in larger-size fish. *Physiological Zoology* 53: 322–337.
- Paulmier A, Ruiz-Pino D (2009) Oxygen minimum zones (OMZs) in the modern ocean. *Progress in Oceanography* 80: 113–128.
- Gordon AL, Bosley KT (1991) Cyclonic gyre in the tropical South Atlantic. *Deep-Sea Research Part I - Oceanographic Research Papers* 38: 323–343.
- Mohrholz V, Bartholomae CH, van der Plas AK, Lass HU (2008) The seasonal variability of the northern Benguela undercurrent and its relation to the oxygen budget on the shelf. *Continental Shelf Research* 28: 424–441.
- Lass HU, Mohrholz V (2008) On the interaction between the subtropical gyre and the Subtropical Cell on the shelf of the SE Atlantic. *Journal of Marine Systems* 74: 1–43.
- Champalbert G, Pagano M, Kouame B, Riandey V (2005) Zooplankton spatial and temporal distribution in a tropical oceanic area off West Africa. *Hydrobiologia* 548: 251–265.

43. Mahnken CVW (1969) Primary organic production and standing stock of zooplankton in tropical Atlantic Ocean - Equalant-I and Equalant-II. *Bulletin of Marine Science* 19: 550–567.
44. Piontkovski SA, Landry MR, Finenko ZZ, Kovalev AV, Williams R, et al. (2003) Plankton communities of the South Atlantic anticyclonic gyre. *Oceanologica Acta* 26: 255–268.
45. Mohrholz V, Schmidt M, Lutjeharms JRE (2001) The hydrography and dynamics of the Angola-Benguela Frontal Zone and environment in April 1999. *South African Journal of Science* 97: 199–208.
46. Le Borgne R, Champalbert G, Gaudy R (2003) Mesozooplankton biomass and composition in the equatorial Pacific along 180°. *Journal of Geophysical Research - Oceans* 108: C12, 8143, doi:10.1029/2000JC000745.
47. Shannon LV, Agenbag JJ, Buys MEL (1987) Large-scale and mesoscale features of the Angola-Benguela front. *South African Journal of Marine Science* 5: 11–34.
48. Seibel BA (2011) Critical oxygen levels and metabolic suppression in oceanic oxygen minimum zones. *Journal of Experimental Biology* 214: 326–336.
49. Escribano R, Hidalgo P, Krautz C (2009) Zooplankton associated with the oxygen minimum zone system in the northern upwelling region of Chile during March 2000. *Deep-Sea Research Part II - Topical Studies in Oceanography* 56: 1049–1060.
50. Smith S, Roman M, Prusova I, Wishner K, Gowing M, et al. (1998) Seasonal response of zooplankton to monsoonal reversals in the Arabian Sea. *Deep-Sea Research Part II - Topical Studies in Oceanography* 45: 2369–2403.
51. Packard TT, Healy ML, Richards FA (1971) Vertical distribution of activity of respiratory electron transport system in marine plankton. *Limnology and Oceanography* 16: 60–70.
52. Teuber L, Kiko R, Séguin F, Auel H (2013) Respiration rates of tropical Atlantic copepods in relation to the oxygen minimum zone. *Journal of Experimental Marine Biology and Ecology* 448: 28–36.
53. Schalk PH (1988) Respiratory electron transport system (ETS) activities in zooplankton and micronekton of the Indo-Pacific region. *Marine Ecology Progress Series* 44: 25–35.
54. Flint MV, Drits AV, Pasternak AF (1991) Characteristic features of body composition and metabolism in some interzonal copepods. *Marine Biology* 111: 199–205.
55. Childress JJ (1995) Are there physiological and biochemical adaptations of metabolism in deep-sea animals? *Trends in Ecology and Evolution* 10: 30–36.
56. Ikeda T, Kanno Y, Ozaki K, Shinada A (2001) Metabolic rates of epipelagic marine copepods as a function of body mass and temperature. *Marine Biology* 139: 587–596.
57. Maas AE, Wishner KF, Seibel BA (2012) Metabolic suppression in thecosomatous pteropods as an effect of low temperature and hypoxia in the eastern tropical North Pacific. *Marine Biology* 159: 1955–1967.
58. Svetlichny LS, Hubareva ES, Erkan F, Gucu AC (2000) Physiological and behavioral aspects of *Calanus euxinus* females (Copepoda: Calanoida) during vertical migration across temperature and oxygen gradients. *Marine Biology* 137: 963–971.
59. Ohman MD, Drits AV, Clarke ME, Plourde S (1998) Differential dormancy of co-occurring copepods. *Deep-Sea Research Part II - Topical Studies in Oceanography* 45: 1709–1740.
60. Saltzman J, Wishner KF (1997) Zooplankton ecology in the eastern tropical Pacific oxygen minimum zone above a seamount: 1. General trends. *Deep-Sea Research Part I - Oceanographic Research Papers* 44: 907–930.
61. Antezana T (2002) Adaptive behavior of *Euphausia mucronata* in relation to the oxygen minimum layer of the Humboldt Current. In: Färber-Lorda J, editor. *Oceanography of the Eastern Pacific II*. Mexico: CICESE, pp. 29–40.
62. Wong CK (1988) The swimming behavior of the copepod *Metridia pacifica*. *Journal of Plankton Research* 10: 1285–1290.
63. Escribano R (2006) Zooplankton interactions with the oxygen minimum zone in the eastern South Pacific. *Suplemento Gayana* 70: 19–21.

PUBLICATION III

TROPHIC INTERACTIONS AND LIFE STRATEGIES OF EPI- TO BATHYPELAGIC CALANOID COPEPODS IN THE TROPICAL ATLANTIC OCEAN

TEUBER L, SCHUKAT A, HAGEN W, AUJEL H

Accepted for publication in *Journal of Plankton Research*

TROPHIC INTERACTIONS AND LIFE STRATEGIES OF EPI- TO BATHYPELAGIC CALANOID COPEPODS IN THE TROPICAL ATLANTIC OCEAN

LENA TEUBER, ANNA SCHUKAT, WILHELM HAGEN, HOLGER AUDEL

ABSTRACT

Copepods play central roles in pelagic food webs linking primary production to higher trophic levels. Biomarkers (lipids, stable isotopes) provide modern approaches to study dietary preferences and trophic interactions. A cluster analysis based on the fatty acid and fatty alcohol compositions of 19 copepod species (copepodids C4/C5 and adult stages) from the southeastern tropical Atlantic identified five distinct groups according to lipid composition and storage strategy, coinciding with differences in vertical distribution from the surface to 1800 m depth. Most epipelagic species were characterised by low lipid levels (~10% of dry mass, DM), low quantities of wax esters (WE) and low $\delta^{15}\text{N}$ ratios indicating low trophic positions. In contrast, surface-dwelling *Rhincalanus cornutus* had higher lipid levels (>29% DM) and stored WE (>90% of total lipid), whereas vertically migrating *Pleuromamma* species did not store WE. Most mesopelagic copepods belonged to another cluster, defined by high lipid level (max. 47% DM), high amounts of the fatty acid 18:1(n-9) and high $\delta^{15}\text{N}$ ratios >9‰ indicating carnivorous feeding at a higher trophic level. Diapausing *Calanoides carinatus* (copepodids C5), collected at great depth, formed a separate cluster with low $\delta^{15}\text{N}$ ratios and high amounts of herbivory markers. The latter were apparently accumulated during active feeding on phytoplankton in surface waters and transferred to the deep sea during ontogenetic vertical migrations. In conclusion, these tropical calanoid copepod species from the surface to the deep sea have adopted diverse feeding strategies and occupy a wide range of ecological niches, affecting energy flux and carbon cycling in the tropical Atlantic.

KEYWORDS: plankton, lipids, fatty acids, stable isotopes, trophic markers, deep sea

INTRODUCTION

Copepods are central components of marine pelagic food webs as they mediate the energy flow from phytoplankton to higher trophic levels. Species that conduct diel vertical migration (DVM) enhance the vertical carbon flux by feeding at the surface at night and respiring and excreting in deeper layers during the day (Longhurst 1991). Food webs in tropical oceans are characterised by a high degree of omnivorous species and many opportunistic predators (Kleppel 1993, Calbet and Saiz 2005, Escribano and Pérez 2010), reflecting the scarcity of pelagic primary production in these oligotrophic waters. Important food sources for small epipelagic copepods are typically microzooplankton organisms such as ciliates and flagellates (Calbet and Landry 1999, Gaudy et al. 2003, Hernández-León et al. 2007).

In tropical surface layers, phytoplankton production is too limited to support the accumulation of large lipid reserves in epipelagic copepods. Since low primary production is present year-round, lipid storage is not as crucial as compared to congeners from highly seasonal environments, such as polar oceans, where primary production is only temporarily available. Tropical epipelagic copepods are rather characterised by permanent feeding, high metabolic rates as well as fast growth, intense reproduction and short life cycles (Kattner and Hagen 2009, Teuber et al. 2013a). This is in stark contrast to the life strategies of deep-sea species, usually opportunistic omnivores or predators with lower metabolic rates and longer life cycles (Kattner and Hagen 2009). Lipid deposition thus increases with increasing depth, as energy storage is a wide-spread strategy in deeper-living copepods worldwide that have to cope with a rather stochastic food supply (Lee et al. 2006). A third group of copepods, mainly prevailing in polar, boreal and upwelling regions, perform seasonal or ontogenetic vertical migrations and enter dormancy to overcome unfavourable conditions (Verheye et al. 2005, Shimode et al. 2012). The presence of wax esters (WE) often characterises these copepods, which developed adaptations to a temporarily fluctuating food supply such as energy storage and metabolic reduction (diapause) to endure long periods of starvation (Båmstedt et al. 1990, Hagen and Schnack-Schiel 1996). In the tropical Atlantic, this life strategy can be observed in *Calanoides carinatus*, a dominant copepod from the Benguela upwelling system, which also occurs in tropical regions.

In order to elucidate species-specific feeding behaviour and dietary preferences, the trophic biomarker approach has been applied to different zooplankton species (Auel et al. 2002, Dalsgaard et al. 2003, Schukat et al. 2014). Trophic marker fatty acids have commonly been used in marine food web studies to reveal major dietary components and thus indicate primarily herbivorous or carnivorous feeding (Dalsgaard et al. 2003). In addition, stable isotopic ratios of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) resolve the source of ingested organic carbon and the trophic position of a species in the food web, respectively (Minagawa and Wada 1984, Post 2002). Since the heavier ^{15}N isotope accumulates in the animal's body tissue, the isotopic composition of a consumer is enriched in ^{15}N by around 3.4‰ per trophic level (DeNiro and Epstein 1978, Hobson and Welch 1992, Bode and Alvarez-Ossorio 2004).

Published data on lipid content and compositions as well as trophic interactions of tropical Atlantic copepods are still very limited. The main objective of this study is therefore to elucidate dietary preferences and feeding strategies of various calanoid copepod species from the southeastern tropical Atlantic in relation to depth of occurrence and species-specific differences in life strategies. This study presents a comprehensive trophic data set of 32 copepod species (including copepodids C4/C5 and adult stages) from various stations throughout the southeastern tropical Atlantic, from the surface down to 1800 m.

MATERIAL AND METHODS

Zooplankton sampling

Zooplankton specimens were sampled during three expeditions to the southeastern tropical Atlantic in September 2010 (RRS *Discovery*, D355), February/March 2011 (RV *Maria S. Merian*, MSM 17/3) and July/August 2011 (RV *Maria S. Merian*, MSM 18/4). Samples were retrieved between 4°S and 20°S by stratified vertical hauls from 1800 to 0 m with a Multinet Midi (HydroBios, Kiel, Germany, mouth opening 0.25 m², mesh size 300 µm) (Table 1). Additional specimens were collected from a double (18 nets) and single (9 nets) MOCNESS (Multiple Opening/Closing Net and Environmental Sensing System, mouth opening 1 m², mesh size 333 µm, Wiebe et al. 1985). For a detailed description of the sampling method see Teuber et al. (2013b). Data on phytoplankton biomass (Table 1)

was provided by Wasmund et al. (pers. comm.) for the assessment of food availability for herbivorous copepods.

Table 1. Station data. D: *Discovery* cruise, MSM: *Maria S. Merian* cruises, D: day, M: month, Y: year, UTC: universal time code, SST: sea surface temperature, Phytopl. biomass: total phytoplankton biomass, n.d.: no data.

Cruise	Station	Date (D/M/Y)	Time gear at depth [UTC]	Position latitude	Position longitude	Maximum sampling depth [m]	SST [°C]	Phytopl. biomass [mg C m ⁻²]*
D 355	1	01.09.2010	15:43	7°15'S	0°45'W	800	n.d.	n.d.
	2	03.09.2010	14:30	12°42'S	4°26'E	800	n.d.	n.d.
MSM 17/3	275	13.02.2011	03:11	20°00'S	12°10'E	200	21.5	1020
	282	14.02.2011	05:29	19°00'S	12°15'E	100	19.7	360
	295	16.02.2011	15:11	19°05'S	11°01'E	1000	23.7	280
	298	17.02.2011	07:49	19°00'S	10°30'E	1000	23.8	n.d.
	306	19.02.2011	20:47	17°19'S	11°19'E	400	23.1	520
	308	20.02.2011	18:05	17°15'S	11°00'E	1000	23.9	1100
	309	21.02.2011	08:09	17°15'S	10°47'E	1000	23.0	180
	317	25.02.2011	22:50	10°00'S	8°00'E	1000	28.9	100
	318	28.02.2011	00:30	4°07'S	1°26'W	1000	28.4	50
	MSM 18/4	782	25.07.2011	15:52	3°00'S	8°00'E	1800	22.1
784		26.07.2011	06:43	5°00'S	8°00'E	1800	24.1	80
787		27.07.2011	06:32	8°00'S	8°00'E	1800	22.9	80
789		27.07.2011	21:48	10°00'S	8°03'E	1800	22.2	70
791		28.07.2011	12:32	12°00'S	8°00'E	1800	20.7	40
835		05.08.2011	17:49	14°00'S	9°06'E	1800	19.5	120
840		07.08.2011	06:08	18°00'S	8°00'E	1800	17.9	60
842		07.08.2011	16:42	17°00'S	8°34'E	1800	17.8	n.d.
845		10.08.2011	23:08	13°00'S	9°05'E	1800	20.5	140
847		12.08.2011	13:34	14°30'S	9°51'E	800	18.7	20

* Wasmund et al. (pers. comm.)

Calanoid copepods were sorted from zooplankton samples and identified according to Bradford-Grieve (1999). Live specimens of different species were deep-frozen at -80 °C for lipid, fatty acid and stable isotope analyses. In the home lab, frozen samples were lyophilised for 48 h and weighed for dry mass determination. Eventually, individuals of the same species and stage collected at the same station were pooled to have sufficient biomass for gravimetric total lipid measurements and stable isotope analyses.

Lipid analysis

Total lipid was essentially extracted after Folch et al. (1957) and modified by Hagen (2000) with dichloromethane:methanol (2:1 per vol.). A Potter homogenizer (Braun, Potter S) and

an ultrasonic cell disrupter (Bandelin electronic, UW 2070) were used to homogenize the samples. Lipid extracts were washed with aqueous KCl solution (0.88%) prior to centrifugation and phase separation.

After lipid extraction, subsamples were prepared for the gas-chromatographic determination of fatty acid (FA) and fatty alcohol (FAlc) compositions after Kattner and Fricke (1986). Subsamples of total FA were transesterified into methyl esters with hexane and methanol containing 3% concentrated sulphuric acid and heated at 80 °C for four hours. FA methyl esters were analysed with a gas chromatograph (Agilent Technologies 7890A), equipped with a DB-FFAP column (30 m length, 0.25 mm diameter) and helium as carrier gas. FAs and FAlcs were detected by flame ionisation and peaks were identified by comparing their retention times to those of a fish oil standard (Menhaden) and a natural copepod standard (*Calanus hyperboreus*) of known lipid composition. The wax ester (WE) percentage was estimated based on the FAlc content, assuming equal masses of the FA chain and the FAlc chain in the WE molecule (Kattner et al. 2003).

The composition of FAs was analysed applying the trophic marker approach of Dalsgaard et al. (2003). The FAs 16:1(n-7), 16:4(n-1) and 18:1(n-7) were considered diatom markers, whereas 18:4(n-3) is found in high amounts in dinoflagellates (Sargent et al. 1987, Graeve et al. 1994a, Dalsgaard et al. 2003) and thus served as an indicator of dinoflagellate feeding. The FAs 16:1(n-7) and 18:1(n-7) have also been suggested as bacterial markers (Cohen and Vonshak 1991, Dalsgaard et al. 2003, Brinis et al. 2004). A typical marker FA indicating carnivorous feeding is 18:1(n-9) (Falk-Petersen et al. 1990).

To evaluate the level of carnivory versus herbivory in copepods, two ratios were calculated; the traditional ratio 18:1(n-9)/18:1(n-7) (Auel et al. 2002, Dalsgaard et al. 2003) and the new and more specific ratio $18:1(n-9)/[16:1(n-7)+16:4(n-1)+18:1(n-7)+18:4(n-3)]$, in the following named 18:1(n-9)/ Σ herb. markers that includes additional diatom marker FAs as well as a dinoflagellate FA (Schukat et al. 2014). Specific FAs comprising less than 2% of TFA composition were not included in the analyses and tables.

Stable isotope analysis

Dried individuals of different copepod species were transferred to tin capsules and sent to TÜV Rheinland Agroisolab GmbH (Jülich, Germany) for stable isotope analysis. Stable

isotopic ratios of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) were determined by mass spectrometry (Carlo Erba Instruments, EA NA1500 Series 2) with the standards IAEA-VPDB (IAEA-C1, Vienna) and AIR (atmospheric air; IAEA-N1, Vienna) as reference, respectively, and helium as carrier gas. Isotopic ratios of ^{13}C and ^{15}N are expressed in ‰, calculated by the formula described in Hodum and Hobson (2000).

In this study, lipid levels varied greatly among tropical copepod species and according to Tieszen et al. (1983), differences in lipid content may affect the $\delta^{13}\text{C}$ ratio, since lipids are depleted in $\delta^{13}\text{C}$. To compensate for this variation and to obtain standardised lipid-corrected $\delta^{13}\text{C}$ values, a lipid-normalisation model was applied to $\delta^{13}\text{C}$ ratios based on molar C/N ratios (valid if $\text{C/N} \geq 4$), tested for several marine invertebrates (McConnaughey and McRoy 1979) and for zooplankton in particular (Smyntek et al. 2007). Lipid-corrected $\delta^{13}\text{C}$ ratios will be referred to as $\delta^{13}\text{C}'$ in the following.

Statistical analysis

Prior to statistical analyses, data were tested for normal distribution. Species- and stage-specific differences were tested using two-tailed Man-Whitney U tests as well as Kruskal-Wallis tests followed by Dunn's Multiple Comparison tests. All analyses were performed with GraphPad Prism 5.0 and considered significant if $p < 0.05$.

In order to identify similarities in FA and FAlc compositions between different copepod species, a cluster analysis based on a similarity matrix was performed applying the group average linkage technique (Primer v6 software, Clarke and Warwick 1994). Prior to the cluster analysis, FA and FAlc concentrations were arcsine-square-root-transformed in order to compensate for inhomogeneous variance of percentage data (Osborne 2002). Similarity in FA and FAlc compositions among species was calculated by the Bray-Curtis similarity index. The results of the cluster analysis are presented in a dendrogram.

RESULTS

Total lipid content

Total lipid contents (TL) of tropical Atlantic copepods ranged from 5.1% dry mass (DM) in *Temora stylifera* to 47% DM in *Paraeuchaeta hansenii* (Table 2, Fig. 1A). In general, TL

contents increased with increasing depth of occurrence. Typical epipelagic species such as *T. stylifera*, *Undinula vulgaris* and *Scolecithrix danae* had TL contents <10% DM, while species from mesopelagic depths showed increased lipid levels of usually >20% DM or even >30% DM, as in *Paraeuchaeta aequatorialis* and *P. hansenii* or copepodids C5 of *Calanoides carinatus*. Exceptionally high lipid contents of up to 43.5% DM were found in

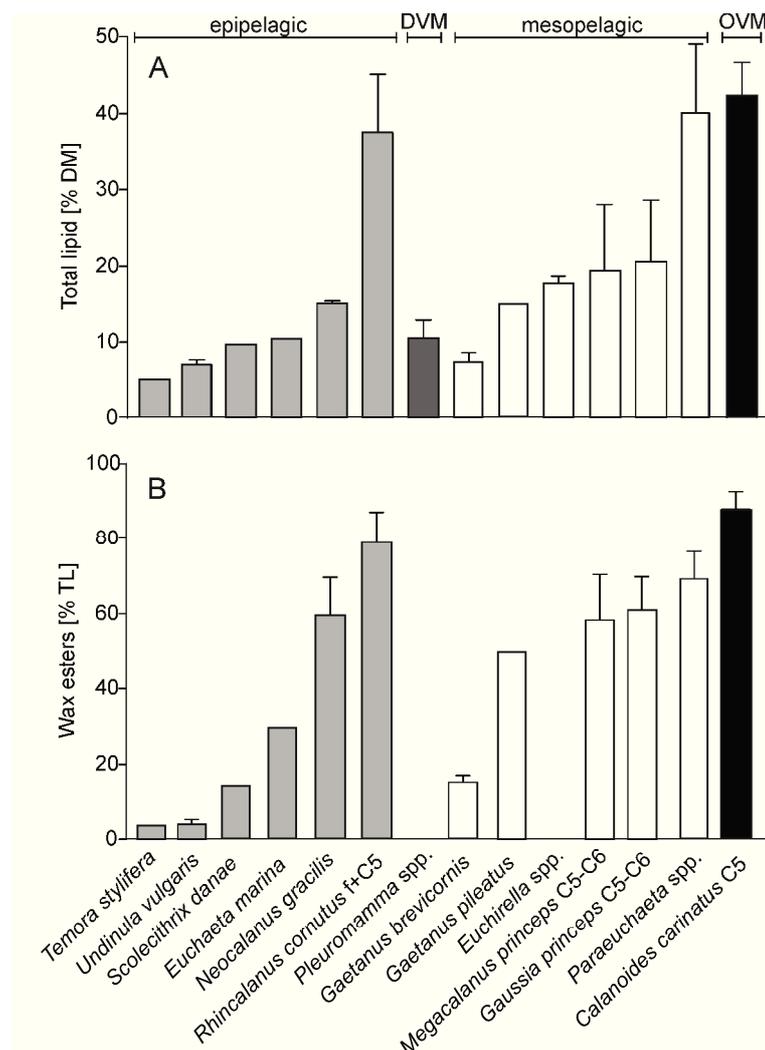


Figure 1. Total lipid (A, in % dry mass) and wax ester contents (B, in % total lipid, as calculated from fatty alcohols) of calanoid copepods from the tropical Atlantic. Bars are arranged according to increasing depth of occurrence from epi- to mesopelagic depths and increasing total lipid content within each group (light grey: epipelagic species, dark grey: diel vertical migrants, white: mesopelagic species, black: ontogenetic vertical migrants). The majority of copepods analysed were adult females, if not indicated otherwise. DM: dry mass, TL: total lipid, WE: wax ester, DVM: diel vertical migration, OVM: ontogenetic vertical migration, f: female, C5: copepodid 5, C6: adult stages (male and female). See Table 2 for the number of replicates.

females and copepodids C5 of *Rhincalanus cornutus* from the surface (Table 2, Fig. 1A). Diel vertical migrants such as *Pleuromamma* spp. showed TL contents of around 10% DM. *Megacalanus princeps* males displayed much higher TL contents ($27.7 \pm 3.9\%$ DM) as compared to females ($12.7 \pm 4.9\%$ DM) and copepodids C5 (13.9% DM) (Table 2). Males of *Gaussia princeps* had lower TL contents ($16.7 \pm 3\%$ DM) than females (21.3/23.4% DM), while TL in C5s was rather variable (12.5/36.3% DM). In contrast, *R. cornutus* did not display major differences in TL contents between adult females and copepodids C5 (29-44% DM).

Fatty acid and fatty alcohol compositions including wax ester content

The composition of fatty acids (FAs) and fatty alcohols (FALcs) in tropical calanoid copepods is summarized in Table 2 and was further analysed by cluster analysis applying the Bray-Curtis similarity index (Fig. 2). Only FAs and FALcs with maximum values of $\geq 2\%$ of total fatty acids (TFAs) and total fatty alcohols (TFALcs), respectively, were included in the analyses. Cluster analysis mainly differentiated between copepod genera and identified different subgroups of species that were distinct in their FA and FALc compositions. Wax ester (WE) contents, as calculated from fatty alcohols, are shown in Fig. 1B.

Females and copepodids C5 of *R. cornutus* from the surface differed from all other species and formed a separated cluster (Fig. 2). *R. cornutus* displayed the highest quantities of the short-chain saturated FAs 14:0 and 16:0 (Table 2) and contained extremely high amounts of WE (up to 96.8 % TL, Fig. 1B) that were dominated by the monounsaturated FALcs 16:1(n-7) and 18:1(n-9), together comprising ca. 90% of TFA (Table 2).

Several other epipelagic species (*Euchaeta marina*, *S. danae*, *T. stylifera* and *U. vulgaris*) formed a large cluster together with *Pleuromamma* spp. and *Euchirella* spp., which was characterised by little or no WE.

The epipelagic copepods formed a separate subgroup and contained high quantities of the FA 16:0, together with the long-chain polyunsaturated FAs 20:5(n-3) and 22:6(n-3) (Table 2). *E. marina* and *S. danae* were also high in 18:1(n-9) with 14-15% TFA. All these species contained lower amounts of WE (2.9-29.5% TL, Fig. 1B). While the shorter-chain saturated FALc 16:0 represented the major component of TFALc in *E. marina*, FALcs in the other three species mainly consisted of 14:0, 16:0 and 22:1(n-11) (Table 2).

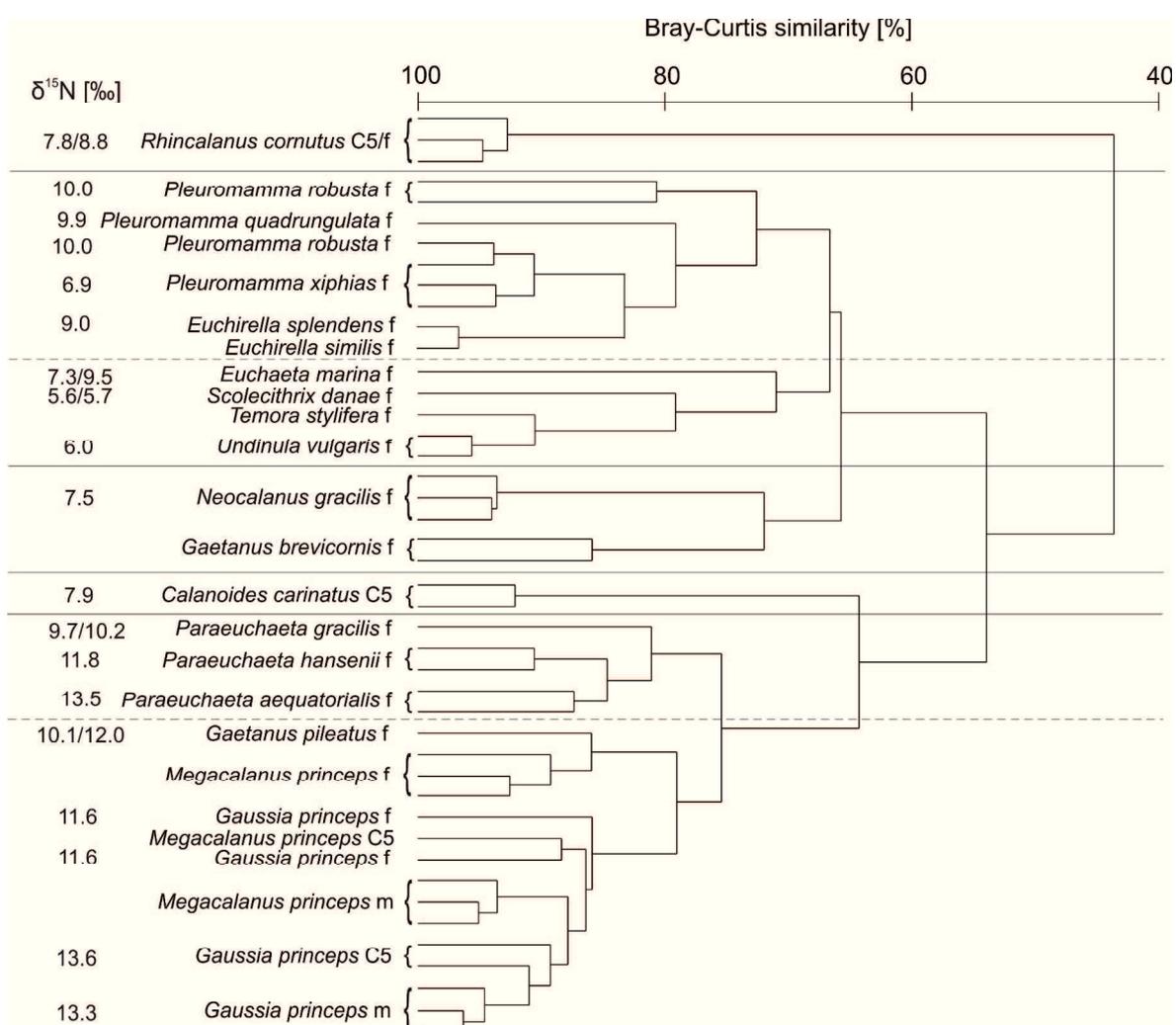


Figure 2. Cluster of calanoid copepods from the tropical southeastern Atlantic with similar patterns in fatty acid and fatty alcohol composition illustrated as a dendrogram. $\delta^{15}\text{N}$ ratios [‰] are indicated on the left as mean values. f: female, m: male, C5 copepodid 5.

Table 2. Total lipid and wax ester contents (calculated from fatty alcohols), fatty acid and fatty alcohol compositions (<2% not presented) as well as carnivory/herbivory ratios of tropical Atlantic calanoid copepods. Species are arranged according to increasing depth of occurrence. DM: dry mass, TL: total lipid, TFA: total fatty acids, TFAlc: total fatty alcohols, n (ind): number of samples (total number of individuals), f: female, m: male, C4/C5: copepodids 4 and 5. Σ herb. markers: sum of 16:1(n-7), 16:4(n-1), 18:1(n-7), 18:4(n-3). Values are given as mean \pm standard deviation for $n \geq 3$, if $n=2$; values are arranged according to the scheme: sample 1 data/sample 2 data.

Species	<i>Umbra</i> <i>magister</i>	<i>Tetraodon</i> <i>capitata</i>	<i>Stolechthrix</i> <i>daniconia</i>	<i>Eutrigla</i> <i>virgata</i>	<i>Nicotemna</i> <i>gracilis</i>	<i>Rhinichthys</i> <i>cornutus</i>	<i>Rhinichthys</i> <i>porphyreus</i>	<i>Pseudorasbora</i> <i>parva</i>	<i>Pseudorasbora</i> <i>parva</i>	<i>Pseudorasbora</i> <i>parva</i>	<i>Pseudorasbora</i> <i>parva</i>	<i>Eutrigla</i> <i>splendida</i>	<i>Eutrigla</i> <i>virgata</i>
	f	f	f	f (ind)	f	f	CP	f	f	f	f	f	f
Stage	50-0	50-0	25-0	40-0	50-0	50-0	50-0	100-0	100-0	100-0	100-0	250-0	400-0
Depth (m)	0.10/0.15	0.03	0.20	1.30	0.47/0.05	0.15/0.21	0.03	0.31/0.01	0.31/0.01	0.31/0.01	0.31/0.01	1.31	1.34
Dry mass (mg ind ⁻¹)	2 (90)	1 (56)	1 (15)	1 (9)	3 (17)	2 (2)	1 (19)	3 (2)	3 (2)	3 (2)	3 (2)	1 (2)	1 (2)
Total lipid (% DW)	4.5/7.4	5.1	9.6	10.3	14.9/10.4	28.5/10.0	43.5	5.3/6.6	5.3/6.6	5.3/6.6	15.4	18.3	17.0
Total fatty alcohol (% TC)	1.5/2.3	1.6	6.9	14.3	22.7/5.0	33.2/13.2	48.4	-	-	-	-	-	-
Water content (% TC)	3.9/6.6	3.1	13.7	19.5	58.4/33.9	79.9/36.4	96.8	-	-	-	-	-	-
Energy acids (% TE)	0.1/-	0.4	0.5	-	0.4/0.1	24.2/20.3	30.3	0.7/0.4	0.7/0.4	0.7/0.4	0.7/0.4	0.9	0.3
14-0	18.8/15.6	19.1	23.9	14.1	17.1/11.1	43.4/33.8	47.1	19.1/10.4	19.1/10.4	19.1/10.4	16.2	25.0	24.8
16-0	3.3/2.4	1.9	1.2	0.8	2.0/0.1	0.4/-	-	1.2/0.1	1.2/0.1	1.2/0.1	0.9	1.6	1.3
18-0	9.7/9.8	9.7	4.7	4.2	4.1/3.6	5.2/5.6	6.1	3.1/0.1	3.1/0.1	3.1/0.1	2.0	4.2	3.7
18:1(n-7)	4.7/0.5	0.4	4.7	1.2	1.1/0.3	1.7/0.5	2.4	2.1/0.2	2.1/0.2	2.1/0.2	3.1	3.3	2.8
18:2(n-7)	-	-	0.5	0.3	0.1/0.2	0.3/2.4	2.0	0.2/0.2	0.2/0.2	0.2/0.2	0.2	0.9	0.6
16:4(n-1)	-	-	-	-	-	0.8/0.7	-	-	-	-	-	-	-
18:1(n-7)	1.3/1.3	2.5	2.4	1.9	2.1/0.1	1.9/1.0	1.1	2.3/0.1	2.3/0.1	2.3/0.1	3.4	2.5	2.4
18:2(n-7)	3.0/4.4	2.0	14.3	14.8	8.8/6.6	5.8/7.4	9.1	4.2/0.8	4.2/0.8	4.2/0.8	20.8	13.7	15.0
18:2(n-6)	1.4/1.4	1.3	1.8	2.2	1.7/0.4	0.5/0.5	0.5	1.5/0.1	1.5/0.1	1.5/0.1	1.2	1.5	1.8
18:3(n-3)	1.8/1.8	0.3	0.5	3.1	1.1/0.1	0.4/-	-	1.2/0.2	1.2/0.2	1.2/0.2	0.2	1.4	2.1
20:1(n-7)	0.3/0.4	0.3	1.7	0.6	1.0/0.2	-	-	0.2/0.0	0.2/0.0	0.2/0.0	3.8	0.9	1.2
20:4(n-3)	0.9/0.9	1.5	0.4	0.6	1.9/0.5	-	-	0.9/0.1	0.9/0.1	0.9/0.1	0.4	0.5	0.4
20:5(n-3)	13.2/13.6	11.8	11.9	13.6	12.8/11.1	4.4/4.1	4.1	14.7/4.2	14.7/4.2	14.7/4.2	0.9	1.4	1.0
22:1(n-11)	-	0.2	0.2	0.3	1.0/0.2	-	-	0.2/0.2	0.2/0.2	0.2/0.2	2.8	0.2	0.2
22:2(n-11)	-	-	1.8	0.3	5.4/2.2	-	-	-	-	-	0.2	0.5	0.3
22:5(n-3)	3.5/0.8	1.4	0.2	0.6	1.0/0.2	-	-	0.8/0.0	0.8/0.0	0.8/0.0	0.7	1.0	0.7
22:6(n-3)	42.1/42.2	43.1	22.9	34.7	29.3/20.0	5.7/3.3	5.1	34.0/10.5	34.0/10.5	34.0/10.5	16.5	21.4	20.9
24:1(n-7)	3.9/5.0	2.7	2.7	3.1	3.1/0.3	0.7/0.7	0.8	5.2/0.2	5.2/0.2	5.2/0.2	10.9	2.2	2.2
18:1(n-7)/18:2(n-7)	3.0/3.5	0.6	5.9	7.8	4.1/0.3	5.7/7.2	4.7	2.3/0.1	2.3/0.1	2.3/0.1	6.1	5.4	6.2
18:1(n-7)/18:2(n-6)	1.0/2.5	0.6	1.9	2.4	2.0/0.2	1.8/1.4	1.4	0.8/0.0	0.8/0.0	0.8/0.0	3.1	1.9	2.1
Energy alcohol (% TE)	16.1	16.1	15.7	5.0	0.2/0.7	-	-	-	-	-	-	-	-
14-0	31.5/26.2	27.2	41.1	32.3	1.1/0.4	5.0/3.9	3.9	-	-	-	-	-	-
16-0	-	-	4.6	4.7	-	-	-	-	-	-	-	-	-
18-0	-	-	-	-	-	20.4/21.2	21.7	-	-	-	-	-	-
18:1(n-7)	-	-	-	-	-	1.5/2.4	3.1	-	-	-	-	-	-
18:2(n-7)	-	-	-	-	-	68.8/71.1	68.4	-	-	-	-	-	-
18:1(n-7)	-	-	-	-	0.7/0.6	-	-	-	-	-	-	-	-
20:1(n-7)	-	-	6.1	-	2.4/1.3	-	-	-	-	-	-	-	-
22:1(n-7)	-	-	-	-	4.8/0.2	-	-	-	-	-	-	-	-
22:1(n-6)	-	-	-	-	18.8/12.4	-	-	-	-	-	-	-	-
22:1(n-11)	39.5/25.2	56.7	32.6	-	71.5/33.3	3.4/1.9	2.9	-	-	-	-	-	-

Species	<i>Gryllus brevicornis</i>	<i>Stenobothrus pilicornis</i>	<i>Megaculicarpa princeps</i>	<i>Megaculicarpa princeps</i>	<i>Megaculicarpa princeps</i>	<i>Gryllus princeps</i>	<i>Gryllus princeps</i>	<i>Gryllus princeps</i>	<i>Paraculicarpa octonotata</i>	<i>Pteroculicarpa hirsutis</i>	<i>Paraculicarpa grisea</i>	<i>Catantops cornutus</i>
Stage	f	f	f	m	C4/C5	f	m	C5	f	f	f	C5
Depth [m]	609-400	1036-150	1000-350	600-400	600-400	600-200	800-200	600-200	1000-500	600-400	600-400	1800-1000
Dry mass [mg ind ⁻¹]	1.14/1.15	1.64	3.94/0.63	4.24/0.39	1.24	8.54/9.39	6.24/0.39	2.88/4.54	1.97/1.51	5.97/5.89	1.72	0.14/0.12
n (ind)	2 (4)	1 (3)	9 (3)	3 (3)	1 (3)	2 (2)	5 (3)	2 (3)	2 (3)	2 (2)	1 (2)	2 (54)
Total faty acids [% DW]	6.5/8.2	14.8	12.7/4.9	27.5/2.9	16.3	21.3/21.4	16.7/4.9	12.3/3.3	46.0/38.0	47.0/44.5	28.4	45.4/36.3
Total faty alcohol [% TI]	6.8/8.1	24.8	21.1/3.1	34.9/6.6	21.3	30.2/21.0	32.8/1.3	26.4/34.6	31.3/36.0	39.1/34.7	30.3	45.4/42.2
Wax ester [% TI]	13.8/16.2	49.9	32.3/6.1	63.9/1.2	54.7	60.4/45.2	65.7/5.6	52.2/69.3	62.6/73.2	78.2/69.4	65.6	90.9/84.4
Fatty acids [% TW]												
14:0	0.5/0.2	-	7.4/1.9	1.3/0.6	4.6	-	1.4/0.1	-	1.1/-	-/0.5	-	6.2/7.2
16:0	11.5/13.5	5.8	0.4/0.1	-	9.3	2.5/9.5	1.4/0.1	3.8/0.6	5.9/0.8	0.4/0.2	6.8	6.2/5.7
17:0	1.0/0.8	0.5	1.1/0.3	-	1.0	0.7/1.1	0.8/0.0	2.3/0.7	-	-	-	-
18:1(n-7)	3.8/2.7	1.2	3.1/0.8	5.0/0.4	9.0	4.9/5.8	6.5/1.8	4.5/5.8	22.5/34.7	19.3/16.8	12.8	15.6/14.6
16:2(n-4)	-/0.4	0.9	0.3/0.1	-	-	0.4/0.4	0.1/0.2	-/0.4	1.2/0.3	1.7/1.5	1.4	1.4/1.7
16:4(n-3)	-	0.5	0.4/0.1	0.9/0.0	0.3	1.3/0.3	1.3/0.2	1.3/1.5	-/0.5	0.7/0.5	1.0	1.8/2.7
18:1(n-7)	2.4/2.7	2.7	4.3/0.6	3.5/0.2	3.0	1.8/2.8	1.9/0.4	2.5/2.0	1.3/0.5	1.4/1.1	1.4	0.6/0.7
18:1(n-9)	9.6/20.4	30.9	39.4/8.5	45.8/1.5	46.7	51.3/45.5	55.0/1.2	36.5/66.5	38.3/53.6	51.0/46.3	35.8	4.4/4.9
18:2(n-6)	1.1/1.2	0.8	1.0/0.2	1.5/0.1	1.1	1.2/1.1	1.5/0.2	1.3/1.1	1.2/0.5	1.9/0.9	1.4	1.6/1.2
18:3(n-3)	-/0.3	0.8	0.4/0.1	0.9/0.1	-	0.5/0.3	0.4/0.2	-/0.7	-	1.3/0.3	1.5	1.9/1.7
20:1(n-9)	1.8/2.5	8.3	2.0/0.4	2.4/0.8	1.7	3.5/4.2	3.0/0.5	3.5/3.4	5.2/2.5	3.9/1.5	2.8	11.0/10.7
20:4(n-6)	-/0.3	0.7	0.3/0.1	0.3/0.1	-	0.8/0.6	0.7/0.2	-/0.7	-	-	0.5	0.8/0.4
20:4(n-3)	1.1/1.1	1.2	0.9/0.2	1.5/0.1	0.4	1.5/0.9	1.3/0.4	1.3/1.8	-	0.5/-	0.8	2.0/2.3
20:5(n-3)	9.2/7.4	2.4	6.8/2.0	9.2/6.3	9.3	8.2/7.3	8.1/1.1	8.8/11.0	2.3/1.8	4.3/2.0	9.7	12.8/16.6
22:1(n-9)	2.2/2.1	2.4	0.3/0.2	-	0.3	0.6/1.7	-	0.6/1.0	1.0/0.4	0.5/0.9	0.5	1.3/0.8
22:1(n-11)	1.3/1.7	1.1	3.7/1.2	3.1/0.5	3.0	1.8/2.8	1.5/0.1	1.1/0.8	9.8/5.4	4.8/2.2	9.7	17.9/11.7
22:5(n-3)	1.8/1.9	1.5	0.6/0.3	0.4/0.7	0.4	0.8/0.5	-	-/1.2	-	-	0.6	2.1/1.9
22:6(n-3)	40.6/27.8	27.5	30.4/4.4	20.2/1.3	16.2	12.7/10.1	11.9/4.0	32.2/17.4	4.8/4.8	2.2/2.2	12.4	6.0/8.1
28:1(n-9)	7.1/5.1	2.7	2.5/0.6	0.8/0.2	8.0	1.4/1.6	1.0/0.4	4.7/0.7	1.5/0.9	0.6/1.2	1.8	0.7/0.7
18:1(n-9)/22:1(n-7)	4.0/7.5	11.2	9.4/2.5	13.2/0.5	16.5	27.9/18.0	30.5/8.3	15.5/23.3	30.1/10.8	37.3/51.6	25.8	5.9/6.7
18:1(n-9)/2:hept. markers	2.2/2.9	4.0	4.8/0.6	4.4/0.1	7.8	6.0/5.4	5.8/1.2	4.5/4.7	1.6/2.1	2.3/3.0	2.2	0.2/0.2
Fatty alcohols [% TW/C]												
14:0	3.5/5.6	4.1	5.1/2.2	10.0/0.9	8.4	10.1/10.4	7.8/0.6	10.4/9.0	27.2/20.4	29.4/29.2	27.4	9.7/15.5
16:0	4.7/8.9	48.8	62.8/8.5	65.8/2.5	60.3	71.4/53.3	81.0/51.0	98.2/85.5	36.6/37.3	54.0/52.8	48.1	14.2/19.1
18:0	-	-	1.2/1.1	4.5/0.8	4.2	/2.7	2.7/0.2	3.4/3.6	-/1.0	1.3/1.1	2.4	1.2/1.8
16:1(n-7)	-	-	-	2.9/0.7	2.2	1.5/1.1	1.7/0.2	2.5/1.4	4.7/4.9	1.7/3.1	1.3	1.6/2.3
18:1(n-7)	-	-	-	4.0/0.3	4.6	-/4.5	5.5/0.6	6.4/5.1	4.3/4.0	3.0/3.0	-	-
18:1(n-9)	-	2.4	2.8/0.9	3.4/0.3	4.8	4.0/4.3	4.5/0.3	4.3/3.2	2.6/3.4	-/0.5	0.8	0.5/0.8
20:1(n-7)	-	1.2	3.0/1.6	0.9/0.5	1.8	6.9/1.6	0.7/0.6	-/0.9	-/0.5	-	0.6	-/0.8
20:1(n-9)	-/3.8	2.0	2.2/0.9	0.9/0.8	1.7	7.4/5.8	7.8/1.5	3.4/3.0	2.4/1.9	-	4.6	15.8/19.1
22:1(n-7)	14.2/9.8	3.8	2.4/0.7	-	7.9	2.4/5.3	2.5/1.2	3.0/2.1	6.6/5.7	3.1/4.8	3.8	1.8/2.8
22:1(n-9)	70.7/60.7	95.9	11.5/4.5	5.3/1.8	7.9	2.3/6.1	4.0/0.6	3.1/2.1	12.8/8.4	5.1/3.7	10.1	55.0/39.0
22:1(n-11)	-/10.6	1.0	6.9/2.1	3.2/0.8	6.3	2.3/6.1	4.0/0.6	3.1/2.1	12.8/8.4	5.1/3.7	10.1	55.0/39.0

The genera *Pleuromamma* and *Euchirella* formed the second subgroup within this cluster (Fig. 2), characterised by the absence of WE (Table 2, Fig. 1B). Their FA composition was dominated by higher levels of 16:0, 20:5(n-3) and 22:6(n-3). *Pleuromamma quadrangulata* and *Euchirella* spp. additionally contained higher amounts of 18:1(n-9) (Table 2).

Gaetanus brevicornis and *Neocalanus gracilis* formed a different cluster (Fig. 2). The FA and FALc compositions of *G. brevicornis* were similar to those of epipelagic species, with generally low amounts of WE (Table 2, Fig. 1B). Nevertheless, *G. brevicornis* was clearly enriched in the FA 18:1(n-9), while the long-chain monounsaturated FALcs 22:1(n-7) and 22:1(n-9) dominated its FALc composition (Table 2). *N. gracilis* differed from the other typical surface species due to its higher WE levels (~60% TL) (Table 2, Fig. 1B). The FA composition was similar to those of other epipelagic copepods, whereas the FALcs were dominated by the long-chain FALcs 22:1(n-11) and 22:1(n-9).

The largest cluster was formed by the deeper-living copepods, which could be further separated into three subgroups. Copepodids C5 of *C. carinatus* were distinct from all other mesopelagic copepods (Table 2, Fig. 2) due to their substantially higher WE levels (up to 90.9% TL) and different FA pattern (Table 2, Fig. 1B). Their FA composition was dominated by 16:1(n-7) as well as by the two long-chain monounsaturates 20:1(n-9) and 22:1(n-11) (Table 2). Likewise, the FALc composition mainly consisted of the long-chain monounsaturates 20:1(n-9) and 22:1(n-11).

P. aequatorialis and *P. hansenii* formed another subgroup within the mesopelagic species cluster, due to their high concentrations of the FA 16:1(n-7) (Table 2).

The large cluster of all other mesopelagic copepods contained species from different genera (Fig. 2) characterised by high amounts of WE (up to 78% TL) (Table 2, Fig. 1B). In contrast to all other species, the dominant FA in the mesopelagic *Gaetanus pileatus*, *M. princeps*, *G. princeps* and *Paraeuchaeta* spp. was the carnivory marker FA 18:1(n-9) with up to 56.9% of TFA. The FALc composition was characterised by highest concentrations of the short-chain moiety 16:0. The FALc 14:0 was also prominent in *M. princeps*, *G. princeps*, and *Paraeuchaeta* spp., while *G. pileatus* contained high portions of 22:1(n-9) (Table 2).

Trophic marker fatty acid ratios

The trophic marker FA ratios (carnivory/herbivory ratios), in particular 18:1(n-9)/ Σ herb. markers, serve as indices of the degree of carnivory in marine invertebrates (Table 2, Fig. 3). The copepods *T. stylifera*, *Pleuromamma robusta* and *C. carinatus* exhibited the lowest degree of carnivory with ratios <1 (Table 2, Fig. 3). While *T. stylifera* and *P. robusta* came from the epipelagic layer, copepodids C5 of *C. carinatus* were sampled between 1000 and 1800 m and showed the lowest ratio of all species investigated with 0.2. The majority of copepods distributed from the surface to mesopelagic waters displayed intermediate ratios between 1 and 3. A higher degree of carnivory (ratio >3) was determined in deeper-living species such as *M. princeps*, *G. princeps* and *G. pileatus*. *M. princeps* copepodids displayed the highest ratio of 7.8 (Table 2, Fig. 3).

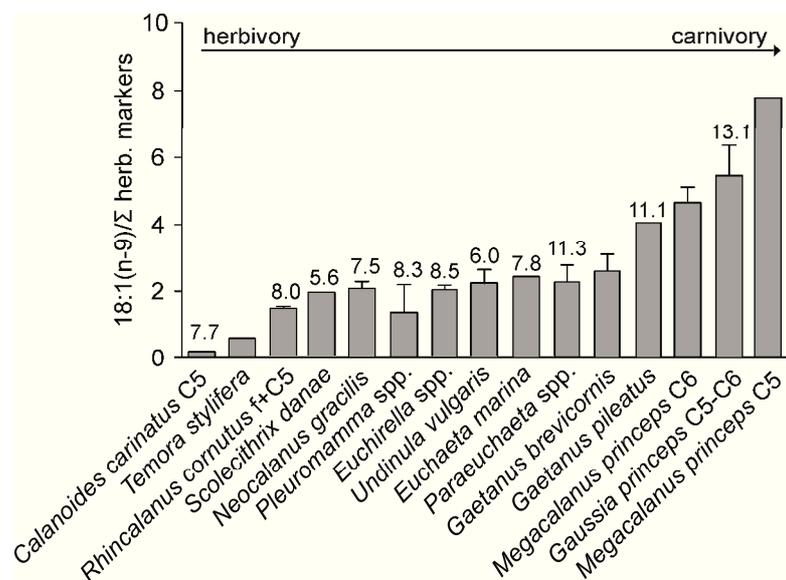


Figure 3. Carnivory/herbivory ratio 18:1(n-9)/ Σ herb. markers of tropical calanoid copepods in order of increasing degree of carnivory. $\delta^{15}\text{N}$ ratios [‰] are indicated above each bar as mean values. The majority of copepods analysed were adult females, if not indicated otherwise. f: female, C5 copepodid 5, C6: adult stages (male and female). See Table 2 and 3 for number of replicates.

The regression analysis showed (Fig. 4A) that the carnivory/herbivory ratio essentially increased with increasing depth of occurrence. Due to specialities in the FA compositions of *Paraeuchaeta* spp. (open circles) and due to the deviating life-cycle strategy of *C. carinatus* (open triangles) these data points were not included in the calculation of the regression coefficient. In *Paraeuchaeta* spp., high amounts of the FA 16:1(n-7) result in a

low carnivory/herbivory ratio, which does not reflect their actual carnivorous feeding behaviour. The FA 16:1(n-7) is most apparently retained in *Paraeuchaeta* species after feeding on herbivorous prey. In copepodids C5 of *C. carinatus*, it does not make sense to relate the low carnivory/herbivory ratio to its sampling depth, since high amounts of herbivory markers were incorporated by *C. carinatus* after feeding on phytoplankton in surface layers. At greater depth, *C. carinatus* rests in diapause and does not feed.

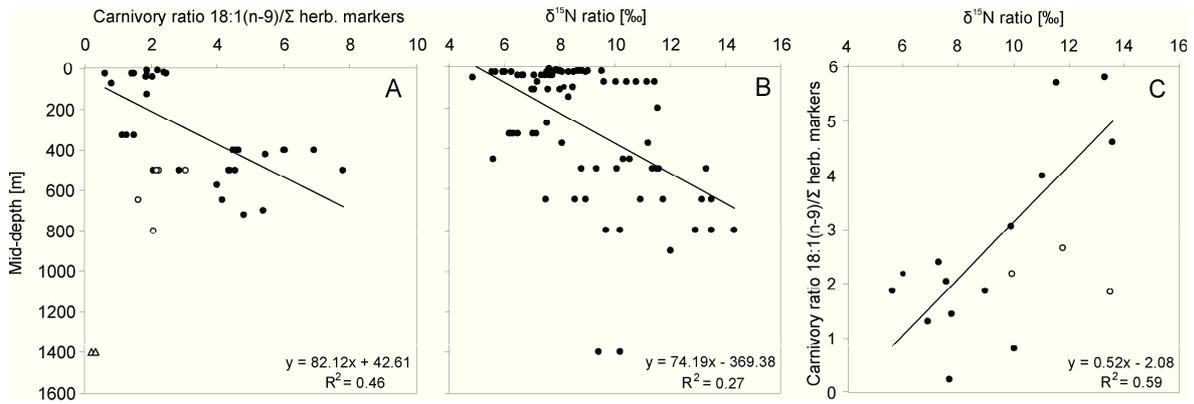


Figure 4. Regression analyses of (A) habitat depth (mid-depth of the sampling interval) and carnivory ratio, (B) habitat depth and $\delta^{15}\text{N}$ ratio and (C) carnivory ratio and $\delta^{15}\text{N}$ ratio of calanoid copepods from the tropical Atlantic. Black circles: majority of copepods used to calculate the regression equations, open circles: *Paraeuchaeta* spp., open triangles: copepodids C5 of *Calanoides carinatus*.

Stable isotope ratios

Stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopic signatures of different calanoid copepod species displayed a wide range of values (Table 3). Lipid-corrected $\delta^{13}\text{C}'$ ranged from -25.4‰ in females of *Pareucalanus sewelli* to -17.1‰ in females of *Candacia bipinnata*, although the majority of lipid-corrected $\delta^{13}\text{C}'$ were between -22‰ and -18‰. $\delta^{15}\text{N}$ varied from 5.6‰ in females of *S. danae* to 13.6 ± 0.7 ‰ in C5s of *G. princeps* (Table 3). Among species with low $\delta^{15}\text{N}$ ratios of around 6‰ were the epipelagic copepods *S. danae*, *U. vulgaris* and *Candacia* spp. as well as *Pleuromamma abdominalis* (Table 3). The other *Pleuromamma* species displayed $\delta^{15}\text{N}$ signatures between 6.9‰ and 11.1‰. The majority of mesopelagic copepods exhibited intermediate $\delta^{15}\text{N}$ ratios ranging from 7‰ to 10‰ (Table 3), while high $\delta^{15}\text{N}$ ratios above 10‰ were found in deeper-living species such as *Paraeuchaeta* spp., *G. pileatus* and *G. princeps* (Table 3, Fig. 3).

Table 3. Stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopic ratios as well as molar C/N ratios of calanoid copepods from the southeastern tropical Atlantic. $\delta^{13}\text{C}$: lipid-corrected $\delta^{13}\text{C}$ ratio, f: female, f (eggs): female carrying egg sac, m: male, C5: copepodid 5, n (ind): number of samples (total number of individuals). Values are given as mean \pm standard deviation for $n \geq 3$, if $n=2$; values are arranged according to the scheme: sample 1 data/sample 2 data.

Species	Stage	Depth [m]	$\delta^{13}\text{C}$ [‰]	$\delta^{13}\text{C}'$ [‰]	$\delta^{15}\text{N}$ [‰]	C/N	n (ind)
<i>Undinula vulgaris</i>	f	50-0	-20.0	-20.6	6.0 \pm 0.1	3.5	3 (16)
	m	50-0	-20.2	-20.8	6.3	3.5	1 (4)
<i>Scolecithrix danae</i>	f	50-0	-20.0/-19.7	-20.5/-20.1	5.7/5.6	3.6/3.6	2 (9)
<i>Candacia bipinnata</i>	f	50-0	-16.8/-16.8	-17.1/-17.2	8.9/8.8	3.7/3.7	2 (9)
<i>Candacia curta</i>	f	50-0	-18.3/-16.9	-18.6/-17.3	8.6/8.3	3.7/3.7	2 (8)
<i>Candacia pachydactyla</i>	m	30-0	-19.9	-20.4	7.9	3.6	1 (4)
<i>Calanoides carinatus</i>	f	40-0	-18.9 \pm 1.0	-17.9 \pm 0.5	7.9 \pm 0.1	5.2 \pm 0.7	3 (20)
	m	40-0	-20.8 \pm 1.2	-18.6 \pm 1.3	8.8 \pm 0.2	7.6 \pm 0.4	3 (21)
	C5	600-400	-20.0 \pm 0.3	-17.5 \pm 0.2	7.7 \pm 1.1	8.8 \pm 0.4	3 (44)
<i>Arietellus aculeatus</i>	f	120-80	-21.1/-20.7	-21.5/-21.2	8.2/8.5	3.7/3.6	2 (2)
<i>Euchaeta marina</i>	f	220-0	-21.3 \pm 0.3	-21.8 \pm 0.2	7.3 \pm 0.4	3.6 \pm 0.1	3 (8)
	f (eggs)	40-0	-18.9	-18.8	9.5	4.0	1 (3)
	C5	80-0	-21.8	-21.6	7.6	4.2	1 (4)
<i>Neocalanus gracilis</i>	f	220-0	-21.2 \pm 0.5	-21.6 \pm 0.3	7.5 \pm 1.0	3.7 \pm 0.2	3 (6)
<i>Neocalanus robustior</i>	f	220-0	-20.9 \pm 0.3	-21.3 \pm 0.3	7.6 \pm 0.5	3.7 \pm 0.4	3 (6)
<i>Pareucalanus sewelli</i>	f	40-0	-25.0/-18.4	-25.4/-18.2	7.6/7.6	3.7/4.2	2 (5)
<i>Pareucalanus langae</i>	f	220-0	-19.7 \pm 0.1	-20.2 \pm 0.1	7.2 \pm 0.7	3.7	4 (8)
<i>Rhincalanus cornutus</i>	f	150-0	-21.4 \pm 0.5	-19.2 \pm 0.5	7.8 \pm 0.7	7.7 \pm 0.1	3 (16)
	C5	800-200	-22.1	-19.7	8.8	8.5	1 (7)
<i>Pleuromamma abdominalis</i>	f	400-300	-20.1	-20.6	5.6	3.6	1 (3)
	m	500-150	-21.6	-22.2	6.2	3.5	1 (2)
<i>Pleuromamma quadrangulata</i>	f	500-150	-18.7 \pm 0.7	-18.9 \pm 0.7	9.9 \pm 1.6	3.9 \pm 0.1	4 (9)
<i>Pleuromamma robusta</i>	f	100-50	-19.6 \pm 0.6	-19.9 \pm 0.5	10.0 \pm 0.4	3.8 \pm 0.1	3 (10)
	m	100-50	-19.8 \pm 0.2	-19.7	11.1 \pm 0.3	4.2 \pm 0.2	3 (10)
<i>Pleuromamma xiphias</i>	f	600-150	-21.6 \pm 0.3	-22.1 \pm 0.2	6.9 \pm 1.1	3.6	3 (8)
	m	500-150	-21.8 \pm 0.5	-22.3 \pm 0.4	6.9 \pm 0.4	3.6 \pm 0.1	3 (6)
<i>Euchirella pulchra</i>	f	800-220	-20.3/-20.1	-21.1/-20.9	7.5/9.3	3.4/3.4	2 (4)
<i>Euchirella rostrata</i>	f	250-40	-22.4	-21.7	8.3	4.7	1 (2)
<i>Euchirella splendens</i>	f	800-500	-21.0	-21.7	9.0	3.4	1 (1)
<i>Gaetanus pileatus</i>	f	1000-400	-19.3/-21.9	-19.8/-20.3	10.1/12.0	3.6/6.1	2 (2)
<i>Gaussia princeps</i>	f	300-100	-21.4	-21.1	11.6	4.3	1 (1)
	m	600-400	-20.2	-19.6	13.3	4.7	1 (1)
	C5	1000-600	-21.2 \pm 1.1	-20.3 \pm 0.4	13.6 \pm 0.7	5.2 \pm 1.3	3 (3)
<i>Metridia princeps</i>	f	800-500	-20.7	-19.2	13.2	5.9	1 (2)
<i>Paraeuchaeta aequatorialis</i>	f	800-500	-21.9	-19.4	13.5	8.9	1 (1)
<i>Paraeuchaeta gracilis</i>	f	1000-600	-22.6/-22.5	-21.5/-21.2	10.2/9.7	5.3/5.6	2 (2)
	C5	600-400	-21.7 \pm 0.2	-19.2 \pm 0.2	11.5 \pm 0.1	9.1	3 (3)
<i>Paraeuchaeta hansenii</i>	f	800-500	-23.2	-20.9	11.8	8.4	1 (1)
<i>Chirundina streetsii</i>	f	800-500	-20.4	-20.7	10.9	3.8	1 (1)
<i>Lucicutia</i> sp.	f	1800-1000	-21.8	-21.0	9.4	4.8	1 (1)
	m	1800-1000	-22.3	-20.9	10.2	5.6	1 (1)

The $\delta^{15}\text{N}$ ratios of various species were related to their vertical distribution as well as to their degree of carnivory (Fig. 4B, C). The regression analyses showed that $\delta^{15}\text{N}$ ratios generally increased with increasing depth of occurrence (Fig. 4B) and that $\delta^{15}\text{N}$ ratios also correlate well with the degree of carnivory as quantified based on fatty acid patterns (Fig. 4C). Again, *Paraeuchaeta* spp. (open circles) was excluded from the calculation of the regression coefficient in Fig. 4C for reasons mentioned above.

DISCUSSION

This study presents a comprehensive set of lipid and stable isotope data reflecting dietary preferences as well as trophic interactions of tropical calanoid copepods from the southeastern Atlantic Ocean. Fatty acid (FA) and stable isotope biomarkers are extensively used for trophic studies in the world oceans and provide reliable estimates of dietary relationships and trophic levels (Graeve et al. 1994a, Auel et al. 2002, Dalsgaard et al. 2003, Schukat et al. 2014). Trophic biomarkers integrate dietary signals over longer time periods of days to several weeks depending on the species (Graeve et al. 1994a, Gentsch et al. 2009). Since lipid storage plays a rather minor role in tropical epipelagic zooplankton, certain concerns on the applicability of trophic biomarkers in tropical ecosystems have been raised. Lipid and FA compositions are highly dependent on the total lipid content of marine organisms and dietary signals are usually reflected best in depot (neutral) lipids (Hagen and Kattner 1998, Hagen et al. 2001, Lee et al. 2006). Tropical epipelagic copepods are characterised by constant feeding, rapid turnover and high metabolic rates (Kattner and Hagen 2009, Teuber et al. 2013a) and thus tend to contain rather low amounts of storage lipid, potentially limiting the validity of FA biomarkers.

Diatoms and dinoflagellates, which represent the typical primary producers in higher latitudes and eutrophic systems and for which characteristic marker FAs are available, are less abundant in oligotrophic tropical environments (Calbet and Landry 1999, Gaudy et al. 2003). In contrast, cyanobacteria, e.g. *Prochlorococcus* or *Trichodesmium*, may contribute up to 60% of total primary production in tropical offshore regions (Platt et al. 1983, Carpenter et al. 2004). *Trichodesmium* is the only cyanobacterium, of which a unique trophic marker is known, the FA 22:2(n-6) (Post et al. 2002). However, neither this FA nor other typical bacteria markers, e.g. FAs with an odd number of carbon atoms, were

detected in significant amounts in the tropical copepods. Therefore, we assume that 16:1(n-7) and 18:1(n-7), which are typical components of both, cyanobacteria and diatoms (Cohen and Vonshak 1991, Dalsgaard et al. 2003, Brinis et al. 2004), are largely of algal origin.

Although certain limitations exist in the use of the trophic biomarker approach for tropical copepods, the results of this study verify its applicability even in tropical species. In the present study, indices of carnivory derived from FA compositions (Table 2, Fig. 3.) agreed quite well with trophic positions determined by nitrogen stable isotope analysis, supporting the validity of the lipid biomarker concept (compare Fig. 4C). In addition, the long-chain monounsaturated FAs and FALcs 20:1 and 22:1, which are only synthesised by calanid copepods (see *C. carinatus* in Table 2), were also present in the carnivorous mesopelagic *Gaussia princeps* and *Paraeuchaeta* spp. This finding can be used as unambiguous evidence to reveal predator-prey relationships, indicating predation on *C. carinatus* by carnivorous copepods (Table 2) (Lee 1975, Kattner and Hagen 1995, Dalsgaard et al. 2003, Laakmann et al. 2009a; b).

Furthermore, cluster analysis grouped copepod species with similar FA and FALc compositions in broad clusters that were often characterised by similarities in vertical distribution and lipid storage, hence similar life strategies. Within each broader cluster, FA and FALc compositions largely reflected taxonomic relationships (Fig. 2), as clusters based on similarities in lipid composition often coincided with genera. This is obvious in the close clustering of the *Pleuromamma* species which rather reflects their taxonomic relationship, since these species conduct diel vertical migrations and cannot be related to a certain depth layer. In contrast, some other closely related species such as *Paraeuchaeta* spp. and *Euchaeta marina* or the two *Gaetanus* species were separated by cluster analysis and were predominantly grouped according to their habitat depth.

The parallel use of the two complementary and independent methods in the present study verifies that the fatty acid trophic marker concept is largely supported by similar results derived from stable nitrogen isotope analysis. Hence, this approach proved to be successful for tropical copepods.

Epipelagic copepods showed a primarily herbivorous to omnivorous feeding mode, which is in line with studies on other tropical epiplankton (Madhupratap and Haridas 1990, Kleppel et al. 1996, Escribano and Pérez 2010). Phytoplankton biomass (Table 1), an

indicator reflecting food availability for herbivorous copepods, was higher at the sampling sites in the eastern tropical Atlantic (this study) than previously measured in the central tropical Atlantic (Marañón et al. 2000). Huskin et al. (2001) calculated a copepod community ingestion rate of $49 \text{ mg C m}^{-2} \text{ d}^{-1}$ for the eastern tropical Atlantic, which suggests that existing phytoplankton biomass (compare Table 1) might be sufficient for herbivorous copepod consumption. Nevertheless, phytoplankton alone usually does not cover carbon demand in oligotrophic tropical regions (Zhang et al. 1995). The potentially limited availability of phytoplankton is therefore often compensated by feeding on microzooplankton (Kleppel 1993, Calbet and Landry 1999, Calbet and Saiz 2005), which results in a rather mixed diet and a more opportunistic and omnivorous feeding mode.

Most tropical copepods typically do not accumulate extensive lipid stores (e.g. Lee et al. 2006). They stay active throughout the year in this non-seasonal environment with a low but reliable food supply, which does not support but also does not require provisions for overwintering or resting phases (Lee and Hirota 1973). The absence or near absence of WE in most epipelagic species suggest the limited storage of triacylglycerols as short-term energy reserves (not WE, known as long-term energy depots), corresponding to faster turnover rates and shorter generation times (Lee and Hirota 1973, Hagen et al. 1993, Kattner and Hagen 2009). Dominating FAs in many epipelagic species were 16:0, 20:5(n-3) and 22:6(n-3), which are principal components of biomembranes (Lee et al. 2006, Kattner and Hagen 2009). Rather low levels of diatom and dinoflagellate biomarker FAs reflect the scarcity and rapid catabolism of these primary producers in tropical waters. While *S. danae* and *U. vulgaris* had rather low $\delta^{15}\text{N}$ ratios indicating lower trophic positions, *E. marina* showed intermediate $\delta^{15}\text{N}$ values, reflecting its carnivorous feeding mode (Morris and Hopkins 1983).

The lipid composition of *Rhincalanus cornutus* from the surface was considerably different from those of all other copepods and strongly deviated from the general characteristics of tropical epipelagic copepods as described above. *R. cornutus* contained very high amounts of lipid with up to 43.5% DM in copepodids C5, as well as remarkably high WE levels (80-97% TL), indicating the massive deposition of WE as important long-term lipid reserves (Lee and Hirota 1973, Cass et al. 2011). Its FA composition was dominated by the shorter-chain, saturated FAs 14:0 and 16:0, while the monounsaturated FALcs 16:1(n-7) and 18:1(n-9) represented the major WE moieties. *R. cornutus* is able to synthesize these FALcs via reduction from the corresponding FAs (Cass et al. 2011). The FA

and FAlc pattern of *R. cornutus* is similar to that of congeners from the Gulf of Mexico (Cass et al. 2011).

According to its $\delta^{15}\text{N}$ ratios (females 7.7‰, C5 8.5‰), *R. cornutus* occupies an intermediate trophic position in our study area, characterised by herbivorous to omnivorous feeding, which is also supported by its low carnivory/herbivory ratio. The storage of large amounts of lipid, especially as WE, is evident by its prominent oil sac, comprising most of its body volume. Females of other *Rhincalanus* species (*R. gigas*, *R. nasutus*) are assumed to conduct ontogenetic vertical migrations and enter a dormant stage in deeper waters (Schnack-Schiel et al. 2008, Shimode et al. 2012, Schukat et al. 2014), where they do not feed and rely on their extensive energy reserves accumulated at the surface. Dormancy has never been demonstrated for *R. cornutus*, but possibly females of *R. cornutus* (this study) were about to enter their resting phase or had just terminated it. Along with the observed sluggish movement, high lipid and WE levels may also help to contribute to neutral buoyancy (Jónasdóttir 1999) and save extra energy otherwise lost in constant locomotion to avoid sinking. These characteristics are also reflected in reduced metabolic rates in *R. cornutus* and other eucalanid species (Flint et al. 1991, Teuber et al. 2013a, b). Hence, Flint et al. (1991) have classified the special life-style of *Eucalanus* spp. as 'lethargic'.

Pleuromamma and *Euchirella* formed a separate subcluster, characterised by the absence of WE. *Pleuromamma* spp. are active diel vertical migrants (DVM) (Morris and Hopkins 1983, Madhupratap and Haridas 1990, Auel and Verheye 2007) and follow a different life-strategy in terms of energy metabolism (Teuber et al. 2013b). *Pleuromamma* spp. migrate to the surface at night to benefit from a richer food supply in epipelagic layers. The low C/N ratio implies a high protein content, which is necessary to support a strong musculature in DVM species (Morris and Hopkins 1983). These species occupy various trophic positions (Morris and Hopkins 1983, Longhurst 1985, Morales et al. 1993), as verified by their wide range of $\delta^{15}\text{N}$ ratios (6-11‰). While the FA compositions of *Pleuromamma xiphias* and *P. robusta* point towards herbivorous feeding, *P. quadrangulata* was characterised by a more carnivorous diet also reflected in its higher carnivory/herbivory ratio.

Mesopelagic copepods showed distinct lipid compositions. Both total lipid and WE levels increased with increasing depth of occurrence (present study; Lee and Hirota 1973,

Kattner and Hagen 2009). This coincides with a higher degree of carnivory in deep-sea species, as indicated by a high carnivory/herbivory ratio (3-8) and high amounts (>30% TFA) of the carnivory biomarker FA 18:1(n-9) (Falk-Petersen et al. 1990, Hagen et al. 1995). The FA 18:1(n-9) may also be synthesised *de novo* via desaturation of 18:0 (Kattner and Hagen 1995), but it seems rather unlikely as long as enough dietary resources of this FA are available (Dalsgaard et al. 2003). Supporting this view, higher $\delta^{15}\text{N}$ ratios of above 10‰ (maximum of 13.6‰) also underline carnivory as the dominant feeding type in *Paraeuchaeta* spp., *Megacalanus princeps* and *Gaussia princeps* from lower mesopelagic depths (Laakmann et al. 2009a, b). Predatory feeding in *Paraeuchaeta* spp. was also confirmed from feeding experiments, stomach content and from mouthparts morphology (Yen 1991, Øresland and Ward 1993, Olsen et al. 2000, Michels and Schnack-Schiel 2005).

Most deep-sea copepods contain large amounts of lipids, especially FALCs (as WE moieties, usually >20% TL) (Lee and Hirota 1973, Hagen et al. 1995, Lee et al. 2006). They are enriched in the short-chain FALCs 14:0 and 16:0, which typically point to a more opportunistic feeding mode (Graeve et al. 1994b, Kattner and Hagen 2009). In addition, large lipid stores serve as buoyancy aids (Sargent and Henderson 1986) to support the 'float and wait' feeding strategy of large-size deep-sea copepods (Auel and Hagen 2005, Laakmann et al. 2009a). Comparatively high amounts of the FA 16:1(n-7) were found in *Paraeuchaeta* spp., which has also been recorded in earlier studies (Hagen et al. 1995, Laakmann et al. 2009a). *Paraeuchaeta* probably accumulates this FA when feeding on herbivores with a high concentration of 16:1(n-7) as in *Calanoides carinatus*. Therefore *de novo* synthesis is not very likely (Hagen et al. 1995, Laakmann et al. 2009a, b). Furthermore, higher quantities of the calanid marker FAs 20:1 and 22:1 (Hopkins et al. 1993) were detected in some carnivorous deep-sea species (e.g. *Paraeuchaeta aequatorialis*, *Paraeuchaeta hansenii*), indicating predation on diapausing copepodids of *C. carinatus*, the only species synthesising these long-chain monounsaturated FAs in the region (present study, Schukat et al. 2014).

In the present study, *C. carinatus* occurred in large numbers as copepodids C5 at great depths between 1800 and 1000 m. This finding agrees well with the known ontogenetic vertical migration of the species, where non-feeding copepodids C5 descend to the deep sea and survive in diapause with severely reduced locomotion and metabolic suppression by 82% (Auel et al. 2005, Schukat et al. 2014). The high WE levels in C5s are advantageous for survival during periods of food shortage (Lee et al. 2006). Nevertheless, in this

extreme resting stage, *C. carinatus* probably utilises very little of its enormous lipid reserves previously accumulated during feeding in surface waters (Verheye et al. 2005). It is suggested that instead of fuelling maintenance, these energy depots are primarily invested in gonad maturation and reproductive processes towards termination of diapause and re-ascent to the surface. Freshly moulted females will be able to quickly produce new off-spring, which can make full use of the next phytoplankton bloom (Verheye et al. 1991).

Herbivorous feeding in *C. carinatus* is supported by high amounts of diatom biomarker FAs, especially 16:1(n-7) (present study, Verheye et al. 2005, Schukat et al. 2014). Although *de novo* synthesis of the FA 16:1(n-7) is possible in many marine animals, it has been confirmed that 16:1(n-7) in herbivorous calanoid copepods predominantly derives from dietary sources, i.e. diatoms (Graeve et al. 1994a, Dalsgaard et al. 2003). A herbivorous diet in *C. carinatus* is also supported by its rather low $\delta^{15}\text{N}$ ratio (7.7‰) and lowest FA carnivory/herbivory ratio of all copepods (0.2).

Besides large quantities of diatom biomarkers, the composition of its total lipid is characterised by the long-chain monounsaturated FAs 20:1(n-9) and 22:1(n-11), which are typical of calanid copepods, as they may synthesise these FAs *de novo* (Hopkins et al. 1993, Kattner and Hagen 1995). Since these FAs were also quite often found in considerable quantities in deep-sea species such as *Paraeuchaeta* spp. and *G. princeps*, this suggests *C. carinatus* C5s as possible prey items at depth. Applying an enrichment factor of around 3.4‰ per trophic level (Peterson and Fry 1987, Hobson and Welch 1992), deep-sea copepods with $\delta^{15}\text{N}$ ratios of around 11‰ may represent potential predators on *C. carinatus* copepodids. Similar interactions have been reported between *Paraeuchaeta* and *Calanus* species in other regions (Øresland and Ward 1993, Fleddum et al. 2001, Laakmann et al. 2009a).

In conclusion, the present study revealed a high diversity of species-specific life strategies with regard to feeding preferences and lipid storage mechanisms in tropical copepods from epipelagic to bathypelagic depths. Feeding modes and dietary preferences depend to a great extent on depth of occurrence, prey availability as well as individual metabolic strategy. Nevertheless, omnivory was the prevailing feeding mode, demonstrating a high degree of opportunistic feeding in tropical copepods. The two complementary trophic biomarker approaches, i.e. based on fatty acids and stable isotopes, lead to similar results and emphasise the applicability of lipid trophic biomarkers even in tropical regions, albeit

with certain limitations. Studies on zooplankton feeding strategies and dietary preferences are crucial to trace energy pathways in marine food webs and to identify trophic relationships. These data will improve our understanding of energy fluxes from the surface to the deep sea also in tropical pelagic food webs, which are still poorly investigated.

ACKNOWLEDGEMENTS

We would like to thank Anja Hansen and Dr. Norbert Wasmund from the Leibniz Institute for Baltic Sea Research, Rostock-Warnemünde, who provided data on phytoplankton biomass. We thank the captains and crews of RRS *Discovery* and RV *Maria S. Merian* for their skilful support at sea. We are thankful for the assistance of Petra Wencke during lipid and fatty acid analyses.

FUNDING

This work was supported in part by the German Federal Ministry of Education and Research (BMBF) in the framework of the GENUS “Geochemistry and Ecology of the Namibian Upwelling System” programme [03F0497E], and in part by the University of Bremen.

REFERENCES

- Auel H, Hagen W (2005) Body mass and lipid dynamics of Arctic and Antarctic deep-sea copepods (*Calanoida*, *Paraeuchaeta*): ontogenetic and seasonal trends. *Deep-Sea Res Part I* 52: 1272-1283
- Auel H, Hagen W, Ekau W, Verheye HM (2005) Metabolic adaptations and reduced respiration of the copepod *Calanoides carinatus* during diapause at depth in the Angola-Benguela Front and northern Benguela upwelling regions. *Afr J Mar Sci* 27: 653-657
- Auel H, Harjes M, da Rocha R, Stübing D, Hagen W (2002) Lipid biomarkers indicate different ecological niches and trophic relationships of the Arctic hyperiid amphipods *Themisto abyssorum* and *T. libellula*. *Polar Biol* 25: 374-383
- Auel H, Verheye HM (2007) Hypoxia tolerance in the copepod *Calanoides carinatus* and the effect of an intermediate oxygen minimum layer on copepod vertical

- distribution in the northern Benguela Current upwelling system and the Angola-Benguela Front. *J Exp Mar Biol Ecol* 352: 234-243
- Båmstedt U, Håkanson JL, Brenner-Larsen J, Bjørnsen PK, Geertz-Hansen O, Tiselius P (1990) Copepod nutritional condition and pelagic production during autumn in Kosterfjorden, western Sweden. *Mar Biol* 104: 197-208
- Bode A, Alvarez-Ossorio MT (2004) Taxonomic versus trophic structure of mesozooplankton: a seasonal study of species succession and stable carbon and nitrogen isotopes in a coastal upwelling ecosystem. *ICES J Mar Sci* 61: 563-571
- Bradford-Grieve JM, Markhaseva EL, Rocha CEF, Abiahy B (1999) Copepoda. In: Boltovskoy D (ed) *South Atlantic Zooplankton*. Backhuys Publishers, Leiden, pp 869-1098
- Brinis A, Mejanelle L, Momzikoff A, Gondry G, Fillaux J, Point V, Saliot A (2004) Phospholipid ester-linked fatty acids composition of size-fractionated particles at the top ocean surface. *Org Geochem* 35: 1275-1287
- Calbet A, Landry MR (1999) Mesozooplankton influences on the microbial food web: direct and indirect trophic interactions in the oligotrophic open ocean. *Limnol Oceanogr* 44: 1370-1380
- Calbet A, Saiz E (2005) The ciliate-copepod link in marine ecosystems. *Aquat Microb Ecol* 38: 157-167
- Carpenter EJ, Subramaniam A, Capone DG (2004) Biomass and primary productivity of the cyanobacterium *Trichodesmium* spp. in the tropical N Atlantic ocean. *Deep-Sea Res Part I* 51: 173-203
- Cass CJ, Wakeham SG, Daly KL (2011) Lipid composition of tropical and subtropical copepod species of the genus *Rhincalanus* (Copepoda: Eucalanidae): a novel fatty acid and alcohol signature. *Mar Ecol Prog Ser* 439: 127-138
- Clarke KR, Warwick RM (1994) *Changes in marine communities: an approach to statistical analysis and interpretation*. Plymouth Marine Laboratory, NERC, Plymouth, UK, 144 pp
- Cohen Z, Vonshak A (1991) Fatty acid composition of *Spirulina* and *Spirulina*-like cyanobacteria in relation to their chemotaxonomy. *Phytochemistry* 30: 205-206
- Dalsgaard J, St. John M, Kattner G, Muller-Navarra D, Hagen W (2003) Fatty acid trophic markers in the pelagic marine environment. *Adv Mar Biol* 46: 225-340
- DeNiro MJ, Epstein S (1978) Influence of diet on distribution of carbon isotopes in animals. *Geochim Cosmochim Acta* 42: 495-506
- Escribano R, Pérez CS (2010) Variability in fatty acids of two marine copepods upon changing food supply in the coastal upwelling zone off Chile: importance of the picoplankton and nanoplankton fractions. *J Mar Biol Ass U K* 90: 301-313
- Falk-Petersen S, Hopkins CCE, Sargent JR (1990) Trophic relationships in the pelagic, Arctic food web. In: M. B, N. GR (eds) *Trophic relationships in the marine environment*. Aberdeen University Press, Aberdeen, pp 315-333
- Fleddum A, Kaartvedt S, Ellertsen B (2001) Distribution and feeding of the carnivorous copepod *Paraeuchaeta norvegica* in habitats of shallow prey assemblages and midnight sun. *Mar Biol* 139: 719-726

- Flint MV, Drits AV, Pasternak AF (1991) Characteristic features of body composition and metabolism in some interzonal copepods. *Mar Biol* 111: 199-205
- Folch J, Lees M, Stanley GHS (1957) A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 226: 497-509
- Gaudy R, Champalbert G, Le Borgne R (2003) Feeding and metabolism of mesozooplankton in the equatorial Pacific high-nutrient, low-chlorophyll zone along 180°. *J Geophys Res C* 108: C12, 8144, doi:10.1029/2000JC0000743
- Gentsch E, Kreibich T, Hagen W, Niehoff B (2009) Dietary shifts in the copepod *Temora longicornis* during spring: evidence from stable isotope signatures, fatty acid biomarkers and feeding experiments. *J Plankton Res* 31: 45-60
- Graeve M, Hagen W, Kattner G (1994b) Herbivorous or omnivorous? On the significance of lipid compositions as trophic markers in Antarctic copepods. *Deep-Sea Res Part I* 41: 915-924
- Graeve M, Kattner G, Hagen W (1994a) Diet-induced changes in the fatty acid composition of Arctic herbivorous copepods: experimental evidence of trophic markers. *J Exp Mar Biol Ecol* 182: 97-110
- Hagen W (2000) Lipids. In: Harris R, Wiebe P, Lenz J, Skjoldal H, Huntley M (eds) *ICES Zooplankton Methodology Manual*. Academic Press, San Diego, pp 113-119
- Hagen W, Kattner G (1998) Lipid metabolism of the Antarctic euphausiid *Thysanoessa macrura* and its ecological implications. *Limnol Oceanogr* 43: 1894-1901
- Hagen W, Kattner G, Graeve M (1993) *Calanoides acutus* and *Calanus propinquus*, Antarctic copepods with different lipid storage modes via wax esters or triacylglycerols. *Mar Ecol Prog Ser* 97: 135-142
- Hagen W, Kattner G, Graeve M (1995) On the lipid biochemistry of polar copepods - compositional differences in the Antarctic calanoids *Euchaeta antarctica* and *Euchirella rostromagna*. *Mar Biol* 123: 451-457
- Hagen W, Kattner G, Terbrüggen A, Van Vleet ES (2001) Lipid metabolism of the Antarctic krill *Euphausia superba* and its ecological implications. *Mar Biol* 139: 95-104
- Hagen W, Schnack-Schiel SB (1996) Seasonal lipid dynamics in dominant Antarctic copepods: energy for overwintering or reproduction? *Deep-Sea Res Part I* 43: 139-158
- Hernández-León S, Gómez M, Arístegui J (2007) Mesozooplankton in the Canary Current System: the coastal-ocean transition zone. *Prog Oceanogr* 74: 397-421
- Hobson KA, Welch HE (1992) Determination of trophic relationships within a high Arctic marine food web using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis. *Mar Ecol Prog Ser* 84: 9-18
- Hodum PJ, Hobson KA (2000) Trophic relationships among Antarctic fulmarine petrels: insights into dietary overlap and chick provisioning strategies inferred from stable-isotope ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) analyses. *Mar Ecol Prog Ser* 198: 273-281
- Hopkins CCE, Sargent JR, Nilssen EM (1993) Total lipid content, and lipid and fatty acid composition of the deep-water prawn *Pandalus borealis* from Balsfjord, northern Norway: growth and feeding relationships. *Mar Ecol Prog Ser* 96: 217-228
- Huskin I, Anadón R, Woodd-Walker RS, Harris RP (2001) Basin-scale latitudinal patterns of copepod grazing in the Atlantic Ocean. *J Plankton Res* 23: 1361-1371

- Jónasdóttir SH (1999) Lipid content of *Calanus finmarchicus* during overwintering in the Faroe-Shetland Channel. *Fish Oceanogr* 8 (Suppl. 1): 61-72
- Kattner G, Albers C, Graeve M, Schnack-Schiel SB (2003) Fatty acid and alcohol composition of the small polar copepods, *Oithona* and *Oncaea*: indication on feeding modes. *Polar Biol* 26: 666-671
- Kattner G, Fricke HSG (1986) Simple gas-liquid chromatography method for the simultaneous determination of fatty acids and alcohols in wax esters of marine organisms. *J Chromatogr* 361: 263-268
- Kattner G, Hagen W (1995) Polar herbivorous copepods - different pathways in lipid biosynthesis. *ICES J Mar Sci* 52: 329-335
- Kattner G, Hagen W (2009) Lipids in marine copepods: latitudinal characteristics and perspectives to global warming. In: Arts MT, Brett M, Kainz M (eds) *Lipids in Aquatic Ecosystems*. Springer, Berlin, 377 pp
- Kleppel GS (1993) On the diets of calanoid copepods. *Mar Ecol Prog Ser* 99: 183-195
- Kleppel GS, Burkart CA, Carter K, Tomas C (1996) Diets of calanoid copepods on the West Florida continental shelf: relationships between food concentration, food composition and feeding activity. *Mar Biol* 127: 209-217
- Laakmann S, Kochzius M, Auel H (2009a) Ecological niches of Arctic deep-sea copepods: vertical partitioning, dietary preferences and different trophic levels minimize inter-specific competition. *Deep-Sea Res Part I* 56: 741-756
- Laakmann S, Stumpp M, Auel H (2009b) Vertical distribution and dietary preferences of deep-sea copepods (Euchaetidae and Aetideidae; Calanoida) in the vicinity of the Antarctic Polar Front. *Polar Biol* 32: 679-689
- Lee RF (1975) Lipids of Arctic Zooplankton. *Comp Biochem Physiol B* 51: 263-266
- Lee RF, Hagen W, Kattner G (2006) Lipid storage in marine zooplankton. *Mar Ecol Prog Ser* 307: 273-306
- Lee RF, Hirota J (1973) Wax esters in tropical zooplankton and nekton and geographical distribution of wax esters in marine copepods. *Limnol Oceanogr* 18: 227-239
- Longhurst AR (1985) The structure and evolution of plankton communities. *Prog Oceanogr* 15: 1-35
- Longhurst AR (1991) Role of the marine biosphere in the global carbon cycle. *Limnol Oceanogr* 36: 1507-1526
- Madhupratap M, Haridas P (1990) Zooplankton, especially calanoid copepods, in the upper 1000 m of the south-east Arabian Sea. *J Plankton Res* 12: 305-321
- Marañón E, Holligan PM, Varela M, Mouriño B, Bale AJ (2000) Basin-scale variability of phytoplankton biomass, production and growth in the Atlantic Ocean. *Deep-Sea Res Part I* 47: 825-857
- McConnaughey T, McRoy CP (1979) Food-web structure and the fractionation of carbon isotopes in the Bering Sea. *Mar Biol* 53: 257-262
- Michels J, Schnack-Schiel SB (2005) Feeding in dominant Antarctic copepods - does the morphology of the mandibular gnathobases relate to diet? *Mar Biol* 146: 483-495

- Minagawa M, Wada E (1984) Stepwise enrichment of ^{15}N along food chains - further evidence and the relation between $\delta^{15}\text{N}$ and animal age. *Geochim Cosmochim Acta* 48: 1135-1140
- Morales CE, Harris RP, Head RN, Tranter PRG (1993) Copepod grazing in the oceanic northeast Atlantic during a 6 week drifting station: the contribution of size classes and vertical migrants. *J Plankton Res* 15: 185-211
- Morris MJ, Hopkins TL (1983) Biochemical composition of crustacean zooplankton from the eastern Gulf of Mexico. *J Exp Mar Biol Ecol* 69: 1-19
- Olsen EM, Jørstad T, Kaartvedt S (2000) The feeding strategies of two large marine copepods. *J Plankton Res* 22: 1513-1528
- Øresland V, Ward P (1993) Summer and winter diet of four carnivorous copepod species around South Georgia. *Mar Ecol Prog Ser* 98: 73-78
- Osborne JW (2002) Notes on the use of data transformations. *Practical Assessment, Research & Evaluation* 8(6)
- Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. *Annu Rev Ecol Syst* 18: 293-320
- Platt T, Rao DVS, Irwin B (1983) Photosynthesis of picoplankton in the oligotrophic ocean. *Nature* 301: 702-704
- Post AF, Dedej Z, Gottlieb R, Li H, Thomas DN, El-Absawi M, El-Naggar A, El-Gharabawi M, Sommer U (2002) Spatial and temporal distribution of *Trichodesmium* spp. in the stratified Gulf of Aqaba, Red Sea. *Mar Ecol Prog Ser* 239: 241-250
- Post DM (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* 83: 703-718
- Sargent JR, Henderson RJ (1986) Lipids. In: Corner EDS, O'Hara SCM (eds) *The biological chemistry of marine copepods*. Clarendon Press, Oxford, pp 59-108
- Sargent JR, Parkes JR, Mueller-Harvey I, Henderson RJ (1987) Lipid biomarkers in marine ecology. In: Sleigh MA (ed) *Microbes in the Sea*. Ellis Horwood, Chichester, pp 119-138
- Schnack-Schiel SB, Niehoff B, Hagen W, Böttger-Schnack R, Cornils A, Dowidar MM, Pasternak A, Stambler N, Stübing D, Richter C (2008) Population dynamics and life strategies of *Rhincalanus nasutus* (Copepoda) at the onset of the spring bloom in the Gulf of Aqaba (Red Sea). *J Plankton Res* 30: 655-672
- Schukat A, Auel H, Teuber L, Lahajnar N, Hagen W (2014) Complex trophic interactions of calanoid copepods in the Benguela upwelling system. *J Sea Res* 85: 186-196
- Shimode S, Takahashi K, Shimizu Y, Nonomura T, Tsuda A (2012) Distribution and life history of two planktonic copepods, *Rhincalanus nasutus* and *Rhincalanus rostrifrons*, in the northwestern Pacific Ocean. *Deep-Sea Res Part I* 65: 133-145
- Smyntek PM, Teece MA, Schulz KL, Thackeray SJ (2007) A standard protocol for stable isotope analysis of zooplankton in aquatic food web research using mass balance correction models. *Limnol Oceanogr* 52: 2135-2146
- Teuber L, Kiko R, Séguin F, Auel H (2013a) Respiration rates of tropical Atlantic copepods in relation to the oxygen minimum zone. *J Exp Mar Biol Ecol* 448: 28-36

-
- Teuber L, Schukat A, Hagen W, Auel H (2013b) Distribution and ecophysiological characteristics of calanoid copepods in relation to the oxygen minimum zone in the eastern tropical Atlantic. PLoS ONE 8(11): e77590. doi:77510.71371/journal.pone.0077590
- Tieszen LL, Boutton TW, Tesdahl KG, Slade NA (1983) Fractionation and turnover of stable carbon isotopes in animal tissues - implications for $\delta^{13}\text{C}$ analysis of diet. Oecologia 57: 32-37
- Verheye HM, Hagen W, Auel H, Ekau W, Loick N, Rheenen I, Wencke P, Jones S (2005) Life strategies, energetics and growth characteristics of *Calanoides carinatus* (Copepoda) in the Angola-Benguela frontal region. Afr J Mar Sci 27: 641-651
- Verheye HM, Hutchings L, Peterson WT (1991) Life-history and population maintenance strategies of *Calanoides carinatus* (Copepoda, Calanoida) in the southern Benguela ecosystem. S Afr J Mar Sci 11: 179-191
- Wiebe PH, Morton AW, Bradley AM, Backus RH, Craddock JE, Barber V, Cowles TJ, Flierl GR (1985) New developments in the MOCNESS, an apparatus for sampling zooplankton and micronekton. Mar Biol 87: 313-323
- Yen J (1991) Predatory feeding behavior of an Antarctic marine copepod, *Euchaeta antarctica*. Polar Res 10: 433-442
- Zhang X, Dam HG, White JR, Roman MR (1995) Latitudinal variations in mesozooplankton grazing and metabolism in the central tropical Pacific during the U.S. JGOFS EqPac study. Deep-Sea Res Part II 42: 695-714

3. SYNOPTIC DISCUSSION

The main objectives of this thesis were to investigate ecophysiological characteristics of the copepod community in the eastern tropical Atlantic and to identify species- or taxa-specific life strategies in relation to vertical distribution and physical parameters. In particular, this study focused on the current impact of the oxygen minimum zone (OMZ) in the eastern tropical Atlantic on the distribution and ecophysiology of calanoid copepods. Special emphasis was laid on species-specific physiological adaptations to hypoxic conditions. The data set comprises distribution and abundance data as well as physiological measurements and biochemical analyses of 51 calanoid copepod species derived from two research trips to the Cape Verde Islands in the eastern tropical North Atlantic and from three research expeditions to the eastern tropical South Atlantic.

Chapter 3.1 examines the horizontal and vertical distribution and abundance of calanoid copepods, with special focus on distributional patterns in relation to the OMZ. Species-specific ecophysiological adaptations to hypoxic conditions are described and discussed in Chapter 3.2. Chapter 3.3 integrates all information on distribution patterns and feeding modes as well as ecological, physiological and behavioural aspects and adaptations and identifies different life strategies of tropical calanoid copepods.

3.1 Distribution of calanoid copepods in relation to the oxygen minimum zone

Intermediate OMZs structure pelagic communities in tropical oceans worldwide, as they influence horizontal and vertical distributions as well as behaviour and metabolic processes of marine organisms (Childress and Seibel 1998, Levin 2003, Ekau et al. 2010, Stramma et al. 2010). While larger nekton such as fish, marine mammals and cephalopods may escape from unfavorable environmental conditions (e.g. O₂ depletion), zooplankton, by definition, is not able of independent horizontal movement. Hypoxia may therefore present a hazardous experience to zooplankton organisms and they may only react by changes in their vertical distribution. In consequence, the zooplankton community should be vertically structured by species-specific hypoxia tolerance levels.

Although O₂ depletion in the tropical Atlantic is not as severe as in the Pacific and Arabian Sea, dissolved oxygen (DO) concentrations in the Atlantic have drastically declined within the last 50 years (Stramma et al. 2008a, 2009). The influence of hypoxic conditions on

zooplankton distribution has mainly been studied in the coastal Benguela upwelling system (Verheye et al. 2005, Auel and Verheye 2007, Schukat et al. 2013, Werner and Buchholz 2013), where the OMZ is more pronounced. Comparable studies from OMZ affected regions in the open tropical Atlantic are still limited.

Data on DO concentrations in the eastern tropical Atlantic derived from CTD casts from this study showed that the vertical extent and intensity of the OMZ was quite variable, but generally increased from areas north of the equator towards sampling stations in the southeastern Angola Basin. Minimum DO concentrations at the Cape Verde Archipelago were around $51 \mu\text{mol O}_2 \text{ kg}^{-1}$, while DO levels as low as $\sim 12 \mu\text{mol O}_2 \text{ kg}^{-1}$ were measured in the eastern Angola Gyre in the area of the Angola Dome (Publications I-II). In this area, the OMZ extended from around 50-400 m, while below 400 m, DO increased again above hypoxic levels. Although the OMZ in the tropical Atlantic is at present still more oxygenated compared to OMZs in the eastern Pacific and Arabian Sea ($\text{O}_2 \text{ min. } < 4.5 \mu\text{mol O}_2 \text{ kg}^{-1}$) (Karstensen et al. 2008, Stramma et al. 2008a, b, Paulmier and Ruiz-Pino 2009), this study of zooplankton distribution in relation to the current state of the OMZ represents a valuable baseline in view of the future scenario of increasing hypoxia in the Atlantic over the next decades (Stramma et al. 2008a).

In this study, maximum abundances of calanoid copepods ($> 160 \text{ ind. m}^{-3}$) were found in surface waters of the eastern Angola Gyre (Publication II). High zooplankton standing stocks in this area and off the coasts of Gabon and Congo have been observed earlier (Mahnken 1969, Piontkovski et al. 2003) and were related to an enhanced productivity of this region. At stations in the eastern Angola Gyre, the subsurface fluorescence maximum was very pronounced (CTD data from this study, Publication II), indicating increased phytoplankton productivity. An enhanced primary production is supported by additional input of nutrients from the Congo River as well as the uplift of the thermocline in the area of the Angola Dome, which provides an extra supply of nutrients (Mohrholz et al. 2001, Lass and Mohrholz 2008). Consequently, high copepod abundance can be sustained in this area.

Highest copepod abundance in the surface layer in the eastern Angola Gyre coincided with a very pronounced OMZ at these stations in the Angola Dome region (Publication II). Similarly, zooplankton biomass in the Costa Rica Dome in the eastern tropical Pacific was equally high or higher compared to the equatorial upwelling regions (Fernández-Álamo and Färber-Lorda 2006). Oxygen depletion in intermediate water layers at stations in the

Angola Dome might have compressed hypoxia-intolerant species into water layers above the OMZ and thus contributed to the enhanced copepod abundance in surface layers.

The vertical distribution of zooplankton abundance and biomass is strongly related to the physical environment (e.g. light and temperature) and food availability as well as species-specific tolerance levels (Fernández-Álamo and Färber-Lorda 2006). In particular, the concentration of DO within OMZ regions is a major factor influencing zooplankton vertical distribution patterns (Wishner et al. 2013). Although copepods are quite tolerant towards hypoxia as compared to other zooplankton (Childress and Seibel 1998, Wishner et al. 2008, Ekau et al. 2010), there seems to be a pronounced variability in hypoxia tolerance levels among calanoid copepods. The individual O₂ requirements of a species and its specific tolerance towards low DO thus determine its distributional range within OMZ regions (Publications I-II). The results of this study partly reflect patterns in copepod vertical distribution observed in relation to the OMZ in the tropical Pacific, which will be discussed in the following (Longhurst 1967, Saltzman and Wishner 1997a, Wishner et al. 2013).

The most common vertical distribution pattern of copepods in areas with extreme hypoxia is a bimodal distribution with maximum abundance in the surface layer and a secondary peak in abundance at the lower oxycline (Saltzman and Wishner 1997a, Fernández-Álamo and Färber-Lorda 2006, Wishner et al. 2013). In areas with pronounced intermediate OMZs as in the eastern tropical Pacific, water layers with severe hypoxia may extend vertically over several hundreds of meters. As a result, upper and lower OMZ boundaries are usually 'hotspots' of biological activity (Wishner et al. 1995, 2008, 2013), whereas zooplankton biomass is often severely reduced within the core of the OMZ (Wishner et al. 1995, 2008, 2013, Saltzman and Wishner 1997a, b, Bertrand et al. 2010). Minimum copepod abundances within the centers of pronounced OMZs have been observed in the Benguela upwelling region, the eastern tropical Pacific and the Arabian Sea (Vinogradov and Voronina 1962, Saltzman and Wishner 1997a, Koppelman et al. 2005, Auel and Verheye 2007, Wishner et al. 2008, 2013).

However, while maximum copepod abundance was usually observed in the surface layers above the OMZ in the present study, copepod abundance was not drastically reduced in the centre of the OMZ as compared to water layers above and below (Publication II). Copepod abundance rather continuously decreased with increasing

depth, which reflects the general decline of zooplankton biomass and abundance with depth and agrees with results of other studies from tropical oceans (Le Borgne et al. 2003, Champalbert et al. 2005).

A second peak in zooplankton biomass or abundance below the centre of the OMZ seems to be a characteristic of regions with very pronounced OMZs (Wishner et al. 2013), which is thus considerably different from zooplankton distribution in areas with a weaker OMZ as in the present study. According to Seibel (2011), biomass of pelagic organisms does not seem to be affected by DO concentrations $>10 \mu\text{mol O}_2 \text{ kg}^{-1}$.

Copepod genera that contributed to the abundance maximum at the lower oxycline in the tropical Pacific were *Clausocalanus*, *Oncaea*, *Oithona*, *Corycaeus* and *Euchaeta* (Saltzman and Wishner 1997a). These genera, with the exception of *Euchaeta*, were not analysed in this study, which might have contributed to deviating results. Consequently, it can be assumed that current DO concentrations in the eastern tropical Atlantic are not low enough to fundamentally reduce calanoid copepod abundance within the OMZ.

Another typical distribution pattern in OMZ regions is related to copepod species that conduct diurnal vertical migrations (DVM) in and out of the OMZ (Saltzman and Wishner 1997a). DVM species may temporarily pass through hypoxic water layers of the OMZ, among them often specimens of the genus *Pleuromamma* (Publication I-II, Vinogradov and Voronina 1962, Longhurst 1967, Saltzman and Wishner 1997a, Smith et al. 1998, Auel and Verheye 2007, Escribano et al. 2009). Differences in abundance of *Pleuromamma* spp. were evident between day and night samples (Publication II) and the daytime depth of these copepods often coincided with the core of the OMZ. Among the abundant species of this genus were the larger *P. xiphias*, *P. abdominalis* and *P. robusta* as well as small specimens of *P. gracilis*, *P. piseki* and *P. borealis*. An elevated abundance of *P. robusta* ($\sim 2.8 \text{ ind. m}^{-3}$) was observed within the OMZ in the southern Angola Gyre (Publication II).

Eucalanid copepods such as *Eucalanus hyalinus*, *Subeucalanus* spp., *Pareucalanus* spp. and *Rhincalanus cornutus* also showed a frequent occurrence within the OMZ of the tropical Atlantic (Publications I-II). Eucalanidae are distributed worldwide from coastal to oceanic regions and from surface to lower mesopelagic depths (Bradford-Grieve et al. 1999, Goetze 2003) and they are very successful inhabitants of hypoxic zones (Vinogradov and Voronina 1962, Flint et al. 1991, Auel and Verheye 2007, Wishner et al. 2008,

Escribano et al. 2009). While *Subeucalanus* spp., *R. cornutus* and copepodite stages of several eucalanids had their maximum abundance in the surface layer (up to 17.6 ind. m⁻³ for *Subeucalanus* spp.), *E. hyalinus* and *Rhincalanus nasutus* predominantly occurred within the OMZ layer (max. abundance of ~4.9 ind. m⁻³ for *E. hyalinus*, Publication II). Apparently, females of *R. nasutus* often rest within the OMZ in a dormant state characterised as quiescence (Schnack-Schiel et al. 2008, Schukat et al. 2013). In the tropical Pacific, *Eucalanus inermis* is a frequent inhabitant of the OMZ and migrates in and out of the OMZ (Saltzman and Wishner 1997a, Hidalgo et al. 2005, Wishner et al. 2013).

Calanoid copepods that were only occasionally found within the OMZ of the tropical Atlantic were representatives of the surface community such as *Euchaeta marina* and *Neocalanus* spp. (Publication I-II), with inconsistent DVM/OVM patterns (Pavlova 1994, Shimode et al. 2009). Typical mesopelagic species such as *Gaussia princeps*, *Euchirella* spp., *Gaetanus* spp. and *Paraeuchaeta* spp. also sporadically occurred within the OMZ (Publications I-II). *G. princeps* is known to tolerate quite low DO and conducts DVMs within the mesopelagic zone (Childress 1977).

Calanoides carinatus also occurred in higher abundance (2.2 ind. m⁻³) in the OMZ layer of the southern Angola Gyre, bordering the northern Benguela upwelling system (Publication II). This copepod is a key species of upwelling regions in the Benguela Current and Arabian Sea and its copepodite stages C5 diapause in deeper waters layers within or below the OMZ (Verheye et al. 1991, Koppelman and Weikert 2005, Auel and Verheye 2007, Wishner et al. 2008, Schukat et al. 2013). This finding suggests that hypoxic layers may even represent a potential refuge for diapausing *C. carinatus*, as they might reduce predation pressure.

Copepods that exclusively occurred within the mixed and well oxygenated surface layer above the OMZ were *Undinula vulgaris*, *Scolecithrix danae*, *Labidocera* spp., *Candacia* spp. and *Temora stylifera* (Publications I-II). The strong thermocline and drastically decreasing DO concentrations often present a barrier in vertical distribution of many epipelagic species (Longhurst 1967). Moreover, several deep-sea copepods (e.g. *Lucicutia* sp., *Valdiviella* sp. and *Megacalanus princeps*) were mainly distributed at lower meso- to bathypelagic depths below the OMZ (Publications I-II). The OMZ may thus represent a lower barrier for epipelagic copepods and an upper barrier for meso- to bathypelagic copepods (Wishner et al. 2008).

In conclusion, the results of this study demonstrate that the vertical distribution of calanoid copepods can be related to DO concentrations in the tropical Atlantic and apparently to taxon-specific differences in hypoxia tolerance levels. This resulted in the clear dominance of only a few copepod groups within the OMZ (i.e. eucalanid copepods and *Pleuromamma* spp.), whereas most species only occasionally occurred within the OMZ or seemed to avoid hypoxic water layers. While copepods that do not tolerate O₂ depletion may experience habitat compression under expanding hypoxia, eucalanids and *Pleuromamma* spp. may find protection from predators and experience reduced competition. Although the dominance of only a few species in the centre of the OMZ was obvious, the OMZ in the eastern tropical Atlantic did not yet seem to critically reduce overall abundances of calanoid copepods.

3.2 Physiological adaptations to the oxygen minimum zone

Oxygen depletion in intermediate water layers of OMZs may represent a critical environment for marine animals. The effects of hypoxia on marine organisms are not yet fully understood but this knowledge is of essential importance to predict possible changes to marine life under increasing hypoxic conditions. Although OMZs with severe O₂ depletion are uninhabitable for most organisms, several studies, including the present, have demonstrated that certain zooplankton groups can exist there.

Zooplankton and nekton that frequently or temporarily inhabit OMZ regions show different metabolic and behavioural responses to survive within O₂ depleted water. They deal with hypoxic conditions either by an efficient removal of O₂ from the water to assure O₂ delivery, by a reduction of metabolic rates to save energy or by additional energy from anaerobic metabolism at constant hypoxia (Childress and Seibel 1998, Wu 2002, Rosa and Seibel 2008, Seibel 2011, Maas et al. 2012, Wishner et al. 2013).

In fact, most pelagic organisms usually survive aerobically within OMZs until a species-specific critical oxygen partial pressure (PO_2) is reached (Childress and Seibel 1998). A physiological prerequisite that allows aerobic survival of marine organisms within OMZs is the ability to efficiently remove O₂ from the water (Childress and Seibel 1998). Due to the much higher energy yield of aerobic versus anaerobic metabolism, aerobic respiration should be maintained as long as possible (Childress and Seibel 1998). Critical PO_2 thresholds causing hypoxic stress are species-specific and often close to 60 $\mu\text{mol O}_2 \text{ kg}^{-1}$

for many larger fishes, but might also be as low as $\sim 6 \mu\text{mol O}_2 \text{ kg}^{-1}$ for some crustaceans, cephalopods and midwater fish (Childress and Seibel 1998, Gray et al. 2002, Seibel 2011). DO levels below these thresholds result in drastically decreasing aerobic respiration rates, the onset of anaerobic metabolism or even mortality (Childress and Seibel 1998, Seibel 2011).

In the euphausiid *Euphausia mucronata* the ability to temporarily survive within or migrate through OMZs is related to an increased activity of lactate dehydrogenase (LDH), a key enzyme of anaerobic energy metabolism (González and Quiñones 2002, Escribano et al. 2009). High LDH activities have also been measured in bulk zooplankton from the OMZ in the Peru upwelling region (Escribano 2006). Similarly, the large mesopelagic copepod *Gaussia princeps* covers up to 50% of its metabolic requirements at low DO anaerobically and also conducts DVM within the mesopelagic zone (Childress 1977). In the present study, *Pleuromamma xiphias* had the highest LDH activity of all copepods, corresponding to its DVM into the OMZ of the eastern tropical Atlantic (Publication II).

While enhanced metabolic costs during DVM may require additional energy from anaerobic metabolism (Thuesen et al. 1998, González and Quiñones 2002), the extra energy supply from glycolytic pathways may also enable organisms to temporarily survive in oxygen-depleted water layers (Childress and Seibel 1998, Thuesen et al. 1998, González and Quiñones 2002).

Metabolic suppression, as another strategy for survival at low PO_2 , has been reported in several pelagic organisms that inhabit hypoxic waters such as euphausiids and copepods as well as cephalopods and different pteropod species (Longhurst 1967, Teal and Carey 1967, Childress 1995, Svetlichny et al. 2000, Rosa and Seibel 2008, Maas et al. 2012). Euphausiids reduce their respiration rates when experimentally exposed to hypoxic conditions (Teal and Carey 1967) and some jellyfish survive DO levels below $60 \mu\text{mol kg}^{-1}$ by actively reducing their metabolic requirements and store and use intragel O_2 (Thuesen et al. 2005). The jumbo squid *Dosidicus gigas* shows a comparatively high tolerance towards hypoxia and frequently conducts DVM into the OMZ in the eastern tropical and subtropical Pacific (Rosa and Seibel 2008). *D. gigas* reduces its respiration by $\sim 80\%$, followed by an increase in anaerobic glycolysis, which considerably prolongs its stay within the OMZ supporting predator avoidance (Rosa and Seibel 2008).

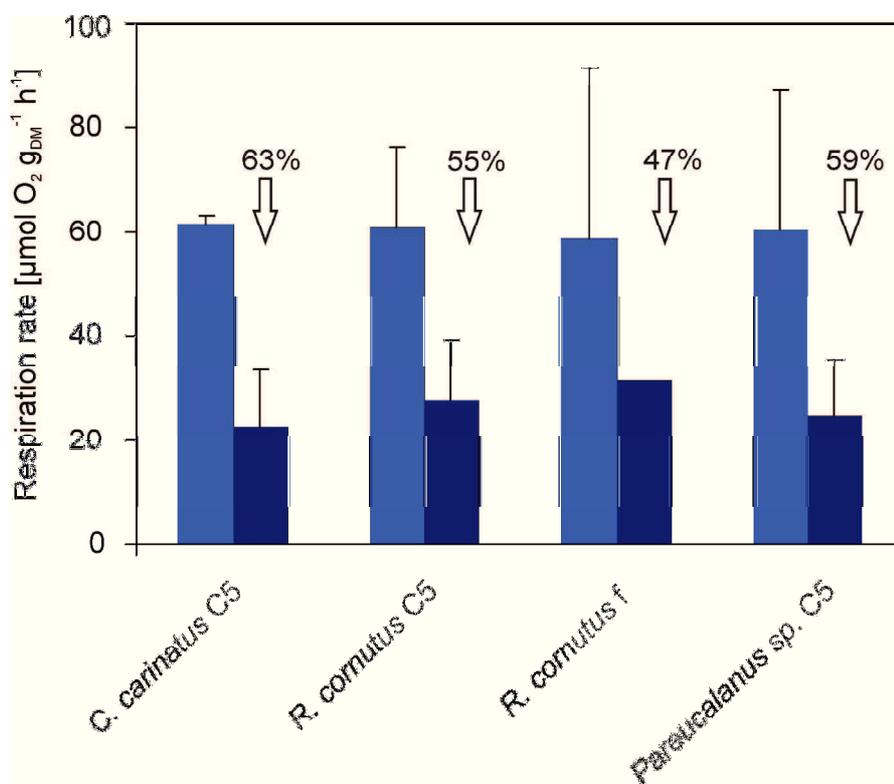


Figure 5. Reduction of respiration rates between surface (light blue bars) and deep water (dark blue bars) in three calanoid copepod species from the eastern tropical Atlantic. Respiration rates ($\mu\text{mol O}_2 \text{ g}_{\text{DM}}^{-1} \text{ h}^{-1}$) were previously standardised to a medium temperature of 18 °C and an average dry mass of 0.5 mg applying a Q_{10} of 2.0 and a scaling coefficient of -0.41. For methodological details on standardisation procedure compare Publication I. C5: copepodite stage C5, f: female.

The reduction of metabolic rates is especially pronounced in copepods that enter a dormant state such as quiescence or the even more extreme diapause to overcome unfavourable environmental conditions or better cope with hypoxic zones (Flint et al. 1991, Kleppel et al. 1996, Ohman et al. 1998, Auel et al. 2005, Schukat et al. 2013). *Calanoides carinatus* and *Rhincalanus nasutus* from the Benguela upwelling system reduced their metabolic rates during dormancy by up to 96% and 62%, respectively, as compared to active specimens from the surface (Auel et al. 2005, Schukat et al. 2013). Similarly, metabolic rates of the eucalanid copepods *Eucalanus inermis* and *E. elongatus* from the OMZ of the eastern tropical Pacific were only 1/10 to 1/35 compared to other copepods (Flint et al. 1991). In *Calanoides carinatus* from this study, a reduction in respiration rates between surface copepodids C5 and diapausing specimens of 63% was observed (Fig. 5). Similarly, the eucalanid species *Rhincalanus cornutus* and *Pareucalanus* sp. had considerably reduced respiration in deeper water layers by up to 59% (Fig. 5). All

rates were previously standardised to a common temperature and a mean copepod body mass, to exclude the prominent effects of temperature and body mass on respiration (Ikeda 1985, Ikeda et al. 2001, Bode et al. 2013).

Although lower O_2 consumption rates are advantageous for survival at low PO_2 , they are not an explicit adaptation to hypoxic conditions, since metabolic reduction with depth is not exclusively found in organisms from OMZ regions (Childress and Seibel 1998, Seibel 2011).

Since the decline in DO concentrations in the eastern tropical Atlantic has been very extreme and the OMZ covers a relatively small area as compared to other tropical oceans, the increase of hypoxic regions is expected to be greatest in the tropical Atlantic (Stramma et al. 2008a, Stramma et al. 2009). In this context, the influence of increasing hypoxia on tropical Atlantic zooplankton will presumably become more evident in future years. Zooplankton organisms that show certain physiological or behavioural strategies, which enable them to live within or migrate through OMZs, may therefore outcompete organisms that are less tolerant towards hypoxic conditions.

3.3 Ecophysiology and life strategies of calanoid copepods

Tropical environments are characterised by rather consistent conditions throughout the year and do not show extreme climatic or seasonal variations in productivity, as observed in polar or upwelling regions. As a consequence, studies on species-specific life strategies and ecophysiological characteristics of tropical copepods in relation to the physical environment are still scarce, while respective data on copepods from polar and temperate oceans as well as from upwelling regions are numerous (Verheye et al. 1992, Schnack-Schiel and Hagen 1994, Atkinson 1998, Schnack-Schiel 2001, Woodd-Walker et al. 2002). Nevertheless, tropical environments have gained increasing attention within the last decades, due to expanding and intensifying OMZs.

Zooplankton is very susceptible and sensitive to environmental conditions and especially copepods show species- or taxa-specific life strategies in relation to their habitat. These strategies are characterised by typical physiological and behavioural traits and can be related to ambient physical conditions. Among typical and abundant calanoid copepods of the tropical Atlantic, five different life-strategies types could be identified based on a

range of analyses of vertical distribution patterns, metabolic activities, and dietary preferences (Table 1).

'Active converters'

Oceanic surface layers are often distinguished into 'different epipelagic biogeographic provinces' (Fernández-Álamo and Färber-Lorda 2006, and references therein), hosting a characteristic composition of pelagic taxa influenced by current systems and water masses. Abundant epipelagic copepods of the tropical Atlantic are *Undinula vulgaris*, *Temora stylifera*, *Neocalanus* spp., *Euchaeta marina*, *Candacia* spp. and *Scolecithrix danae* (Publications I-II). Tropical epipelagic copepods usually have a small body size and are characterised by high metabolic rates (Publications I-II, Ikeda et al. 2001, Hernández-León and Ikeda 2005), which are associated with their short life cycles, continuous feeding and growth, and intense reproduction (Lee and Hirota 1973, Mauchline 1998, Kattner and Hagen 2009). Increased metabolic rates in surface species are predominantly attributed to their smaller body masses as well as to higher water temperatures and higher activity levels (Ikeda 1985, Hirche 1987, Ikeda et al. 2001, Paffenhöfer 2006, Bode et al. 2013).

Revealed by their elevated Q_{10} values of up to 2.7 (Publication I), epipelagic copepods are often weak metabolic regulators (Mullin and Brooks 1970) and thus have a low tolerance to changing thermal conditions. Due to a strong stratification of the water column in tropical oceans with a pronounced thermocline, these species are often restricted to the warm surface layers (Longhurst 1967, Lee et al. 1971) and only some conduct vertical migrations (e.g. *E. marina*, *Neocalanus* spp.; Pavlova 1994, Shimode et al. 2009). Some species such as *Labidocera acutifrons* and *Scolecithrix danae* showed increased activities of the enzyme lactate dehydrogenase (LDH) (Publication II), which seems to provide additional anaerobic energy to support enhanced locomotion and feeding in these active copepods (Thuesen et al. 1998).

Epipelagic species are often herbivorous and/or omnivorous suspension feeders, which occupy rather low trophic positions (Publication III, Madhupratap and Haridas 1990, Kleppel et al. 1996, Escribano and Pérez 2010). This is reflected in their low $\delta^{15}\text{N}$ values in the range of 5.6-8‰ (Publication III). Since carbon demands may not be covered alone by the usually low year-round phytoplankton production in tropical oligotrophic surface waters (Zhang et al. 1995, Gaudy et al. 2004), microzooplankton and picoplankton may

play an important role in the food spectrum of epipelagic copepods (Kleppel 1993, Calbet and Landry 1999, Calbet and Saiz 2005, Hernández-León et al. 2007). This represents an important link between classical food chains and the microbial loop (Landry 2002, Calbet and Saiz 2005). Omnivorous feeding is thus presumably the wide-spread feeding mode among tropical epipelagic species. Nevertheless, the proportion of carnivorous zooplankton is higher in tropical oceans as compared to higher latitudes, represented by typical carnivorous copepods such as *E. marina* and *Candacia* spp. (Publication III, Gaudy and Boucher 1983, Woodd-Walker et al. 2002, Gaudy et al. 2004). The low, but continuous food supply does neither require nor support large lipid reserves in tropical epipelagic species. Therefore, since food supply is limited but consistent, lipid storage does not play a significant role and lipid content and, in particular, wax ester content is correspondingly low (Publication III, Lee et al. 1971, Lee et al. 2006, Kattner and Hagen 2009). Due to the characteristics described above, epipelagic copepods are best described by the life strategy of ‘active converters’.

‘Opportunistic predators’

The mesopelagic zone below the thermocline layer is characterised by rather stable physical conditions, low temperature, lower DO as well as scarce food supply (Koppelman and Frost 2008, Robinson et al. 2010). This is reflected in the life cycles of mesopelagic organisms, which is in stark contrast to those of epipelagic species and characterised by long generation times, slow growth and occasional feeding (Mauchline 1998, Koppelman and Frost 2008, Kattner and Hagen 2009). Common copepods of the mesopelagic community in the eastern tropical Atlantic were *Paraeuchaeta* spp., *Megacalanus princeps*, *Gaussia princeps*, *Euchirella* spp., and *Gaetanus* spp. (Publication I-II).

Lower temperatures at depth cause reduced activity levels in mesopelagic copepods and metabolic rates, in terms of O₂ consumption and activity of the electron transport system (ETS), were correspondingly low (Publication I-II, Ikeda 1970, Childress 1971, 1975, Ikeda 1985). Nevertheless, during active foraging, respiration rates may temporarily be increased in these predatory copepods (Raymont 1959, Mauchline 1998). Low aerobic respiration is sometimes compensated by high anaerobic metabolic rates, reflected in an increased activity of the enzyme lactate dehydrogenase (LDH) (e.g. in *Paraeuchaeta*

aequatorialis from this study, Publication II), presumably used for spontaneous energy boosts during burst swimming and foraging attacks (Wong 1988, Thuesen et al. 1998).

Most mesopelagic copepods are active predators, although some species are opportunistic omnivores (Lee et al. 1971, Øresland and Ward 1993, Olsen et al. 2000, Laakmann et al. 2009a, b). Carnivorous copepods occupy higher trophic levels, which is reflected in their high $\delta^{15}\text{N}$ values (>10‰) and high amounts of the carnivory marker fatty acid 18:1(n-9) (Publication III). Due to the constantly low food supply, many mesopelagic species store large amounts of lipid, mainly wax esters, as energy reserves (Lee et al. 1971, 2006, Lee and Hirota 1973, Hagen et al. 1995, Kattner and Hagen 2009). The storage of lipid as wax esters is energetically very efficient (Morris 1972) and wax ester levels of up to 78% of total lipid were measured in mesopelagic copepods from this study (Publication III). Low metabolic rates as well as pronounced energy storage in mesopelagic copepods from the tropical Atlantic represent a strategy to cope with the scarce food supply and extreme environment of the mesopelagic realm and these copepods are thus characterised by the life strategy of ‘opportunistic predators’.

Table 1. Summary of main ecophysiological characteristics of calanoid copepods from the tropical Atlantic Ocean sorted according to designated life-strategy type. ETS: electron transport system, LDH: lactate dehydrogenase, WE: wax ester, n.d.: no data. The life style-description of ‘dormant survivors’ only refers to diapausing copepodids C5 of *Calanoides carinatus*.

Life style	<i>active converters</i>	<i>opportunistic predators</i>	<i>adaptive migrants</i>	<i>dormant survivors</i>	<i>thrifty floaters</i>
Habitat	epipelagial	mesopelagial	bimodal, epi- & mesopelagial	bimodal, epi- & mesopelagial	epi- to mesopelagial
Respiration rate	high	low	variable	very low	low to medium
Enzyme activity (ETS)	high	low	variable	n.d.	n.d.
Enzyme activity (LDH)	medium to high	low and high	medium to high	n.d.	low
Lipid storage	very low lipid, less WE	high lipid, mainly WE	low lipid, no WE	high lipid, mainly WE	high lipid, mainly WE
Feeding mode	herbivorous/ omnivorous	omnivorous/ carnivorous	omnivorous	herbivorous	omnivorous

'Adaptive migrants'

Diurnal vertically migrating (DVM) species predominantly belonged to the genus *Pleuromamma* (Publication I-II), which spend the night in warm, food-rich surface layers and dwell in deeper and cooler water layers during the day. *Pleuromamma* spp. were characterised by highly variable metabolic rates (Publications I-II), which can be attributed to changing activity levels during migration (Conover 1960, Pavlova 1994). Copepods may encounter temperature changes of up to 15 °C during their DVM and a high temperature tolerance, revealed by low Q_{10} values (1.0 in *Pleuromamma xiphias*), is therefore a characteristic of these species (Publication I, Champalbert and Gaudy 1972).

Vertically migrating species may exploit a variety of phytoplankton as well as microzooplankton food during their night-time stay in surface waters, whereas they reduce their metabolic requirements, respire and defecate in cooler waters at depth during the day (Steinberg et al. 2000, Fernández-Álamo and Färber-Lorda 2006, Wishner et al. 2008, Robinson et al. 2010). The variety of possible food items as well as the specific fatty acid composition of *Pleuromamma* spp. point towards an omnivorous diet (Publication III), which has been reported earlier for this genus (Mullin 1966, Hopkins 1985). Their intermediate trophic position is also reflected in low to medium $\delta^{15}\text{N}$ ratios in the range of 5.6-11‰ (Publication III). *Pleuromamma* spp. did not accumulate large lipid reserves and did not store wax esters (Publication III), which has also been observed in other DVM species (Lee et al. 1971, Morris and Hopkins 1983). These aspects may indicate that vertical migrants encounter sufficient food during their diurnal migration, and together with a metabolic reduction at depth, DVM may represent one possible survival strategy for life in oligotrophic oceans.

In tropical regions, the day-time depth of *Pleuromamma* spp. often coincides with the centre of the OMZ (Vinogradov and Voronina 1962, Longhurst 1967, Saltzman and Wishner 1997b, Escribano et al. 2009). Vertical migration behaviour is often coupled with an increased tolerance towards hypoxia, which secures survival within OMZs (e.g. Wishner et al. 2013). In this study, *P. xiphias* had slightly lower respiration rates within the OMZ than above and below (Publications I-II). During their temporal stay in the OMZ a reduced O_2 demand may be beneficial, while O_2 debts and high energetic costs for migration may be compensated in oxygen-rich and productive surface waters at night (Vinogradov and Voronina 1962, Childress and Seibel 1998, Wishner et al. 2013). This is

also reflected in the fact that *P. xiphias* had the highest LDH activity of all copepods (Publication II). The migrating life style and the apparent high thermal as well as hypoxia tolerance of *Pleuromamma* spp. are thus valuable traits for life in OMZ regions of tropical oceans and assign them the life strategy of 'adaptive migrants'.

'Dormant survivors'

Ontogenetic vertical migrations (OVM) were observed in *Calanoides carinatus*, which shows a bimodal distribution pattern. While females and one part of the copepodid C5 population remain in surface waters, feed, grow and reproduce, the other copepodids C5 descent to deeper water layers and enter diapause (Arashkevich et al. 1996, Verheye et al. 2005, Schukat et al. 2013). In this extreme resting state, copepodids C5 do not feed and severely reduce their metabolic rates by up to 96% compared to surface dwelling active copepodids (Arashkevich et al. 1996, Auel et al. 2005, Schukat et al. 2013). In this study, temperature- and body mass-standardised O₂ consumption rates of *C. carinatus* were reduced by 63% in deeper-living copepodids C5 (Fig. 5). Metabolic reduction will also be a useful strategy, when the diapausing depth coincides with the core of the OMZ.

At the surface, *C. carinatus* predominantly feeds on phytoplankton and its herbivorous feeding behaviour is reflected in high concentrations of typical diatom marker fatty acids such as 16:1(n-7), a low $\delta^{15}\text{N}$ ratio as well as a low carnivory index (Publication III, Schukat et al. 2014). Deeper-living copepodids C5 were characterised by high lipid content (up to 45% of total lipid), predominantly composed of wax esters as the major energy reserve (Publication III). The storage of wax esters is typical of primarily herbivorous copepods to overcome long starvation periods during diapause (Båmstedt et al. 1990, Hagen and Schnack-Schiel 1996). It is suggested that copepodids C5 do not only cover their energy demand during dormancy by these lipid reserves, but use them to start reproducing soon after they ascend to the surface and moult into adults (Arashkevich et al. 1996). The lipid content in *C. carinatus* seems to be inversely correlated with its respiration rate; while diapausing copepodids C5 with large lipid reserves have the lowest respiration rate, O₂ consumption increases as oil sacs become smaller (Arashkevich et al. 1996). The OVM of *C. carinatus* coupled with large lipid reserves as well as low metabolic rates during diapause thus represents another successful strategy to overcome critical conditions such as food limitation or O₂ depletion. *C. carinatus* is therefore described as a 'dormant survivor'.

'Thrifty floaters'

Copepods of the calanoid family Eucalanidae showed a distinct and very specific life strategy. The generally low to medium Q_{10} values of eucalanids (1.1-2.5; Publication I) suggest a temperature-independent metabolism and thus these copepods can be regarded as eurythermal (Gaudy et al. 2000), which may explain their wide distribution ranges. A characteristic of this family is their elongated and transparent body. Their slow and sporadic movements and the fact that they easily float in the water column, is reflected in generally low metabolic rates of eucalanid copepods (Publications I-II, Flint et al. 1991, Schukat et al. 2013). After compensation for temperature and body mass effects, *Subeucalanus crassus* still showed significantly lower respiration rates as compared to other copepods (Publication I).

Females of *Rhincalanus nasutus* and *Rhincalanus gigas* seasonally migrate into deeper water layers and rest in a dormant state with considerably reduced metabolism (Ohman et al. 1998, Hagen 1999, Schnack-Schiel et al. 2008, Shimode et al. 2012, Schukat et al. 2013). For *R. nasutus* from the Benguela upwelling system it is assumed that females enter quiescence to overcome unfavourable conditions, as they reduce their respiration by around 60% at depth (Schukat et al. 2013). Similarly, *Eucalanus* spp. of the eastern tropical Pacific migrate into the OMZ, and reduced metabolic rates enable these copepods to successfully live in hypoxic zones and survive within OMZs (Flint et al. 1991, Cass 2011). In the Atlantic species *Rhincalanus cornutus*, dormancy has never been investigated, but it is assumed that *R. cornutus* may also rest in a dormant state. Females from deeper water layers had reduced respiration rates by 47% as compared to surface-living specimens. This may additionally enable *R. cornutus* to better cope with hypoxic conditions within the OMZ (Fig. 5).

Moreover, dormancy is often associated with increased levels of wax esters (Ohman et al. 1998, Schnack-Schiel et al. 2008). Eucalanids store large amounts of lipids, mainly as wax esters, and their large oil sacs often comprise a major part of their body volume (Publication III, Morris and Hopkins 1983, Cass et al. 2011, Schukat et al. 2014). *R. cornutus* females from this study contained wax esters of up to 85% of total lipids (Publication III), which may support the assumption of a dormant state in this species. The Pacific congener *Rhincalanus rostrifrons* enters dormancy in shallower water layers (200-500 m) as compared to *R. nasutus* (500-1000 m; Shimode et al. 2012). This shallower-water dormancy (quiescence) in female copepods might be a beneficial strategy in

tropical and subtropical regions as a response to temporally and spatially irregular phytoplankton production due to local events (Shimode et al. 2012). The authors also suggest that dormancy might be a common characteristic of *Rhincalanus* species that is also observed in tropical and subtropical open-ocean species.

The low carnivory ratio as well as $\delta^{15}\text{N}$ ratios of 7.2-8.8‰ in *R. cornutus* and *Pareucalanus* spp. (Publication III) underline the herbivorous to omnivorous feeding behaviour of these eucalanids (Cass 2011, Schukat et al. 2014). The high lipid content does not only provide an important energy reserve to overcome periods of starvation during dormancy but also serves as a buoyancy aid to support their floating life style (Morris 1972, Jónasdóttir 1999). Eucalanid copepods often inhabit hypoxic zones and may survive DO concentrations of as low as $5 \mu\text{mol O}_2 \text{ kg}^{-1}$ (Flint et al. 1991, Smith et al. 1998, Hidalgo et al. 2005, Schnack-Schiel et al. 2008). Their survival strategy may thus include a considerable reduction of their overall metabolic needs as well as seasonal migrations and quiescent periods to cope with O_2 depletion, characteristics that describe the life strategy of ‘thrifty floaters’.

In conclusion, the analysis of different ecological and physiological characteristics of abundant calanoid copepod species from the tropical Atlantic revealed that epi- to bathypelagic copepods could be classified by five distinct life-strategy types (Table 1). The results suggest that these life strategies are predominantly influenced by species-specific temperature preferences, feeding behaviour and individual metabolic requirements. However, ambient temperature and food availability seemed to have the greatest impact. The identification of these strategies will lead to a better understanding of the copepod community structure in the eastern tropical Atlantic, general energetic requirements and trophic interactions within the food web. This knowledge will support a more realistic assessment of the contribution of these tropical copepods to the pelagic carbon cycle in the eastern tropical Atlantic. Moreover, reduced metabolic rates and potential dormancy in eucalanid copepods as well as DVM behaviour and increased LHD activities in *Pleuromamma* spp. are suggested as advantageous ecophysiological and behavioural adaptations to life in hypoxic zones.

4. PERSPECTIVES

The classification of ecophysiological characteristics of key species is a central issue in understanding interactions within a zooplankton community and with the physical environment. This study demonstrated that life strategies and metabolic processes of tropical calanoid copepods are to a great extent influenced by ambient physical parameters. Especially dissolved oxygen (DO) plays a prominent role in structuring zooplankton communities depending on individual hypoxia tolerance levels.

The present study is one of the first assessments of current hypoxia impacting copepods in the eastern tropical Atlantic and therefore represents a baseline for further and more detailed studies on the effects of increasing hypoxia on tropical Atlantic species. In view of expanding hypoxia in future years, further research should focus on the determination of species-specific hypoxia thresholds. For this purpose the measurement of respiration rates at *in situ* hypoxic conditions is fundamental. Simulating ambient DO concentration in laboratory experiments and analyses will provide valuable details on individual hypoxia tolerance levels and physiological adaptations to hypoxia.

Early developmental stages of marine animals are often the most vulnerable to hypoxic conditions due to their increased oxygen requirements (Ekau et al. 2010 and references therein, Stramma et al. 2010). In this study, nauplii and copepodite stages younger than copepodite stage C4 were not investigated. Since the survival of nauplii and copepodids is essential for successful recruitment and population development, it is of great importance to include these early-life stages in further studies on the impact of hypoxia on copepods.

A more extensive sampling of the zooplankton community in deeper water layers below the OMZ could reveal, if zooplankton biomass and abundance increase again at the lower oxycline, as it was reported from other OMZ regions (e.g. Saltzman and Wishner 1997a, Wishner et al. 2013). This finding would confirm a possible avoidance of hypoxic layers by certain organisms. Understanding distribution patterns, ecophysiological characteristics and specific adaptations of key organisms, that are likely to survive within OMZs, will help to assess the impact of increasing hypoxia on the structure of zooplankton communities and whole ecosystems as well as on the respective fisheries in the long run.

The study of metabolic processes, dietary preferences and trophic interactions of zooplankton organisms provides important details to calculate ingestion rates and carbon requirements for the implementation into food-web models. In addition to the results achieved in this study, supplementary information of dietary composition and feeding preferences in tropical copepods could be gained through gut content analyses and feeding experiments. Measurements of copepod excretion rates (in particular nitrogen excretion) would provide further data on metabolic processes. This is especially important considering biochemical nitrogen cycles such as denitrification and anammox (anaerobic ammonium oxidation) within OMZs (Paulmier and Ruiz-Pino 2009, Kalvelage et al. 2013).

Metabolic processes in zooplankton such as feeding, respiration and excretion essentially contribute to the marine carbon cycle. This data is of fundamental importance for the establishment of realistic biogeochemical models on carbon fluxes in tropical marine ecosystems. In order to validate existing models, wide-ranging field data and more detailed biochemical analyses need to be provided.

The present study concentrated on larger calanoid copepod species that could be directly identified and subsequently used for onboard experiments. In consequence, the most abundant copepods in the tropical Atlantic, the small-sized calanoids (e.g. Clausocalanidae, Paracalanidae) and cyclopoids (e.g. *Oithona* spp., *Oncaea* spp.) (Champalbert et al. 2005, Schnack-Schiel et al. 2010), were not considered. Therefore, it is essential that future investigations include these abundant small-sized copepods in community analyses and measurements of metabolic processes to assess their contribution to total copepod energy consumption and carbon demand.

Since zooplankton is very susceptible to environmental changes, it can be regarded as a suitable indicator of global climatic changes in the ocean (Hays et al. 2005). Increasing CO₂ concentrations and correspondingly intensifying acidification of seawater have to be considered in more detail in combination with declining DO and increasing temperatures. Altogether, these factors will have a strong impact on zooplankton physiology and distribution. Changes in zooplankton biomass, species and size composition and metabolic activity may in turn have major effects on the entire food web, including pelagic-benthic coupling processes as well as biogeochemical cycles.

REFERENCES

- Arashkevich EG, Drits AV, Timonin AG (1996) Diapause in the life cycle of *Calanoides carinatus* (Krøyer), (Copepoda, Calanoida). *Hydrobiologia* 320: 197-208
- Atkinson A (1998) Life cycle strategies of epipelagic copepods in the Southern Ocean. *J Mar Syst* 15: 289-311
- Auel H, Hagen W, Ekau W, Verheye HM (2005) Metabolic adaptations and reduced respiration of the copepod *Calanoides carinatus* during diapause at depth in the Angola-Benguela Front and northern Benguela upwelling regions. *Afr J Mar Sci* 27: 653-657
- Auel H, Verheye HM (2007) Hypoxia tolerance in the copepod *Calanoides carinatus* and the effect of an intermediate oxygen minimum layer on copepod vertical distribution in the northern Benguela Current upwelling system and the Angola-Benguela Front. *J Exp Mar Biol Ecol* 352: 234-243
- Båmstedt U, Håkanson JL, Brenner-Larsen J, Bjørnsen PK, Geertz-Hansen O, Tiselius P (1990) Copepod nutritional condition and pelagic production during autumn in Kosterfjorden, western Sweden. *Mar Biol* 104: 197-208
- Bertrand A, Ballón M, Chaigneau A (2010) Acoustic observation of living organisms reveals the upper limit of the oxygen minimum zone. *PLoS ONE* 5 (4): e10330, doi:10.1371/journal.pone.0010330
- Bode M, Schukat A, Hagen W, Auel H (2013) Predicting metabolic rates of calanoid copepods. *J Exp Mar Biol Ecol* 444: 1-7
- Bradford-Grieve JM, Markhaseva EL, Rocha CEF, Abiahy B (1999) Copepoda. In: Boltovskoy D (ed) *South Atlantic Zooplankton*. Backhuys Publishers, Leiden, pp 869-1098
- Calbet A, Landry MR (1999) Mesozooplankton influences on the microbial food web: direct and indirect trophic interactions in the oligotrophic open ocean. *Limnol Oceanogr* 44: 1370-1380
- Calbet A, Saiz E (2005) The ciliate-copepod link in marine ecosystems. *Aquat Microb Ecol* 38: 157-167
- Cass CJ (2011) *A Comparative Study of Eucalanoid Copepods Residing in Different Oxygen Environments in the Eastern Tropical North Pacific: An Emphasis on Physiology and Biochemistry*. Ph.D. Thesis, University of South Florida, Tampa, 165 pp
- Cass CJ, Wakeham SG, Daly KL (2011) Lipid composition of tropical and subtropical copepod species of the genus *Rhincalanus* (Copepoda: Eucalanidae): a novel fatty acid and alcohol signature. *Mar Ecol Prog Ser* 439: 127-138
- Champalbert G, Gaudy R (1972) Etude de la respiration chez des copépodes de niveaux bathymétriques variés dans la région sud marocaine et canarienne. *Mar Biol* 12: 159-169
- Champalbert G, Pagano M, Kouame B, Riandey V (2005) Zooplankton spatial and temporal distribution in a tropical oceanic area off West Africa. *Hydrobiologia* 548: 251-265

- Childress JJ (1971) Respiratory rate and depth of occurrence of midwater animals. *Limnol Oceanogr* 16: 104-106
- Childress JJ (1975) The respiratory rates of midwater crustaceans as a function of depth of occurrence and relation to the oxygen minimum layer off southern California. *Comp Biochem Physiol* 50: 787-799
- Childress JJ (1977) Effects of pressure, temperature and oxygen on oxygen-consumption rate of midwater copepod *Gaussia princeps*. *Mar Biol* 39: 19-24
- Childress JJ (1995) Are there physiological and biochemical adaptations of metabolism in deep-sea animals? *Trends Ecol Evol* 10: 30-36
- Childress JJ, Seibel BA (1998) Life at stable low oxygen levels: adaptations of animals to oceanic oxygen minimum layers. *J Exp Biol* 201: 1223-1232
- Conover RJ (1960) The feeding behavior and respiration of some marine planktonic Crustacea. *Biol Bull* 119: 399-415
- Ekau W, Auel H, Pörtner HO, Gilbert D (2010) Impacts of hypoxia on the structure and processes in pelagic communities (zooplankton, macro-invertebrates and fish). *Biogeosciences* 7: 1669-1699
- Escribano R (2006) Zooplankton interactions with the oxygen minimum zone in the eastern South Pacific. *Suplemento Gayana* 70: 19-21
- Escribano R, Hidalgo P, Krautz C (2009) Zooplankton associated with the oxygen minimum zone system in the northern upwelling region of Chile during March 2000. *Deep-Sea Res Part II* 56: 1049-1060
- Escribano R, Pérez CS (2010) Variability in fatty acids of two marine copepods upon changing food supply in the coastal upwelling zone off Chile: importance of the picoplankton and nanoplankton fractions. *J Mar Biol Ass U K* 90: 301-313
- Fernández-Álamo MA, Färber-Lorda J (2006) Zooplankton and the oceanography of the eastern tropical Pacific: a review. *Prog Oceanogr* 69: 318-359
- Flint MV, Drits AV, Pasternak AF (1991) Characteristic features of body composition and metabolism in some interzonal copepods. *Mar Biol* 111: 199-205
- Gaudy R, Boucher J (1983) Relation between respiration, excretion (ammonia and inorganic phosphorus) and activity of amylase and trypsin in different species of pelagic copepods from an Indian Ocean equatorial area. *Mar Biol* 75: 37-45
- Gaudy R, Cervetto G, Pagano M (2000) Comparison of the metabolism of *Acartia clausi* and *A. tonsa*: influence of temperature and salinity. *J Exp Mar Biol Ecol* 247: 51-65
- Gaudy R, Le Borgne R, Landry MR, Champalbert G (2004) Biomass, feeding and metabolism of mesozooplankton in the equatorial Pacific along 180°. *Deep-Sea Res Part II* 51: 629-645
- Goetze E (2003) Cryptic speciation on the high seas; global phylogenetics of the copepod family Eucalanidae. *Proc R Soc B* 270: 2321-2331
- González RR, Quiñones RA (2002) Ldh activity in *Euphausia mucronata* and *Calanus chilensis*: implications for vertical migration behaviour. *J Plankton Res* 24: 1349-1356
- Gray JS, Wu RSS, Or YY (2002) Effects of hypoxia and organic enrichment on the coastal marine environment. *Mar Ecol Prog Ser* 238: 249-279

- Hagen W (1999) Reproductive strategies and energetic adaptations of polar zooplankton. *Invertebr Reprod Dev* 36: 25-34
- Hagen W, Kattner G, Graeve M (1995) On the lipid biochemistry of polar copepods - compositional differences in the Antarctic calanoids *Euchaeta antarctica* and *Euchirella rostromagna*. *Mar Biol* 123: 451-457
- Hagen W, Schnack-Schiel SB (1996) Seasonal lipid dynamics in dominant Antarctic copepods: energy for overwintering or reproduction? *Deep-Sea Res Part I* 43: 139-158
- Hays GC, Richardson AJ, Robinson C (2005) Climate change and marine plankton. *Trends Ecol Evol* 20: 337-344
- Hernández-León S, Gómez M, Arístegui J (2007) Mesozooplankton in the Canary Current System: the coastal-ocean transition zone. *Prog Oceanogr* 74: 397-421
- Hernández-León S, Ikeda T (2005) A global assessment of mesozooplankton respiration in the ocean. *J Plankton Res* 27: 153-158
- Hidalgo P, Escribano R, Morales CE (2005) Ontogenetic vertical distribution and diel migration of the copepod *Eucalanus inermis* in the oxygen minimum zone off northern Chile (20-21 degrees S). *J Plankton Res* 27: 519-529
- Hirche HJ (1987) Temperature and plankton. 2. Effect on respiration and swimming activity in copepods from the Greenland Sea. *Mar Biol* 94: 347-356
- Hopkins TL (1985) Food web of an antarctic midwater ecosystem. *Mar Biol* 89: 197-212
- Ikeda T (1970) Relationship between respiration rate and body size in marine plankton animals as a function of the temperature of habitat. *Bull Fac Fish Hokkaido Univ* 21(2): 91-112
- Ikeda T (1985) Metabolic rates of epipelagic marine zooplankton as a function of body-mass and temperature. *Mar Biol* 85: 1-11
- Ikeda T, Kanno Y, Ozaki K, Shinada A (2001) Metabolic rates of epipelagic marine copepods as a function of body mass and temperature. *Mar Biol* 139: 587-596
- Jónasdóttir SH (1999) Lipid content of *Calanus finmarchicus* during overwintering in the Faroe-Shetland Channel. *Fish Oceanogr* 8 (Suppl. 1): 61-72
- Kalvelage T, Lavik G, Lam P, Contreras S, Artega L, Löscher CR, Oschlies A, Paulmier A, Stramma L, Kuypers MMM (2013) Nitrogen cycling driven by organic matter export in the South Pacific oxygen minimum zone. *Nature Geoscience* doi:10.1038/NGEO1739
- Karstensen J, Stramma L, Visbeck M (2008) Oxygen minimum zones in the eastern tropical Atlantic and Pacific Oceans. *Prog Oceanogr* 77: 331-350
- Kattner G, Hagen W (2009) Lipids in marine copepods: latitudinal characteristics and perspectives to global warming. In: Arts MT, Brett M, Kainz M (eds) *Lipids in Aquatic Ecosystems*. Springer, Berlin, 377 pp
- Kleppel GS (1993) On the diets of calanoid copepods. *Mar Ecol Prog Ser* 99: 183-195
- Kleppel GS, Burkart CA, Carter K, Tomas C (1996) Diets of calanoid copepods on the West Florida continental shelf: relationships between food concentration, food composition and feeding activity. *Mar Biol* 127: 209-217

- Koppelman R, Frost J (2008) The ecological role of zooplankton in the twilight and dark zones of the ocean. In: Mertens LP (ed) *Biological Oceanography Research Trends*. Nova Science Publishers, Inc., New York, pp 282
- Koppelman R, Weikert H (2005) Temporal and vertical distribution of two ecologically different calanoid copepods (*Calanoides carinatus* Krøyer 1849 and *Lucicutia grandis* Giesbrecht 1895) in the deep waters of the central Arabian Sea. *Mar Biol* 147: 1173-1178
- Koppelman R, Zimmermann-Timm H, Weikert H (2005) Bacterial and zooplankton distribution in deep waters of the Arabian Sea. *Deep-Sea Res Part I* 52: 2184-2192
- Laakmann S, Kochzius M, Auel H (2009a) Ecological niches of Arctic deep-sea copepods: vertical partitioning, dietary preferences and different trophic levels minimize inter-specific competition. *Deep-Sea Res Part I* 56: 741-756
- Laakmann S, Stumpp M, Auel H (2009b) Vertical distribution and dietary preferences of deep-sea copepods (Euchaetidae and Aetideidae; Calanoida) in the vicinity of the Antarctic Polar Front. *Polar Biol* 32: 679-689
- Landry MR (2002) Integrating classical and microbial food web concepts: evolving views from the open-ocean tropical Pacific. *Hydrobiologia* 480: 29-39
- Lass HU, Mohrholz V (2008) On the interaction between the subtropical gyre and the Subtropical Cell on the shelf of the SE Atlantic. *J Mar Syst* 74: 1-43
- Le Borgne R, Champalbert G, Gaudy R (2003) Mesozooplankton biomass and composition in the equatorial Pacific along 180°. *J Geophys Res C* 108: C12, 8143, doi:10.1029/2000JC000745
- Lee RF, Barnett AM, Hirota J (1971) Distribution and importance of wax esters in marine copepods and other zooplankton. *Deep-Sea Res* 18: 1147-1165
- Lee RF, Hagen W, Kattner G (2006) Lipid storage in marine zooplankton. *Mar Ecol Prog Ser* 307: 273-306
- Lee RF, Hirota J (1973) Wax esters in tropical zooplankton and nekton and geographical distribution of wax esters in marine copepods. *Limnol Oceanogr* 18: 227-239
- Levin LA (2003) Oxygen minimum zone benthos: adaptation and community response to hypoxia. *Oceanogr Mar Biol* 41: 1-45
- Longhurst AR (1967) Vertical distribution of zooplankton in relation to the eastern Pacific oxygen minimum zone. *Deep-Sea Res* 14: 51-63
- Maas AE, Wishner KF, Seibel BA (2012) Metabolic suppression in thecosomatous pteropods as an effect of low temperature and hypoxia in the eastern tropical North Pacific. *Mar Biol* 159: 1955-1967
- Madhupratap M, Haridas P (1990) Zooplankton, especially calanoid copepods, in the upper 1000 m of the south-east Arabian Sea. *J Plankton Res* 12: 305-321
- Mahnken CVW (1969) Primary organic production and standing stock of zooplankton in tropical Atlantic Ocean - Equalant-I and Equalant-II. *Bull Mar Sci* 19: 550-567
- Mauchline J (1998) The biology of calanoid copepods. *Advances in Marine Biology*, Vol. 33. Academic Press, London, 710 pp

- Mohrholz V, Schmidt M, Lutjeharms JRE (2001) The hydrography and dynamics of the Angola-Benguela Frontal Zone and environment in April 1999. *S Afr J Sci* 97: 199-208
- Morris MJ, Hopkins TL (1983) Biochemical composition of crustacean zooplankton from the eastern Gulf of Mexico. *J Exp Mar Biol Ecol* 69: 1-19
- Morris RJ (1972) The occurrence of wax esters in crustaceans from the north-east Atlantic Ocean. *Mar Biol* 16: 102-107
- Mullin MM (1966) Selective feeding by calanoid copepods from the Indian Ocean. In: Barnes H (ed) *Some contemporary studies in marine science*. Allen and Unwin, London, pp 545-554
- Mullin MM, Brooks ER (1970) Growth and metabolism of two planktonic marine copepods as influenced by temperature and type of food. In: Steele JH (ed) *Marine food chains*. Oliver & Boyd, pp 74-95
- Ohman MD, Drits AV, Clarke ME, Plourde S (1998) Differential dormancy of co-occurring copepods. *Deep-Sea Res Part II* 45: 1709-1740
- Olsen EM, Jørstad T, Kaartvedt S (2000) The feeding strategies of two large marine copepods. *J Plankton Res* 22: 1513-1528
- Øresland V, Ward P (1993) Summer and winter diet of four carnivorous copepod species around South Georgia. *Mar Ecol Prog Ser* 98: 73-78
- Paffenhöfer GA (2006) Oxygen consumption in relation to motion of marine planktonic copepods. *Mar Ecol Prog Ser* 317: 187-192
- Paulmier A, Ruiz-Pino D (2009) Oxygen minimum zones (OMZs) in the modern ocean. *Prog Oceanogr* 80: 113-128
- Pavlova EV (1994) Diel changes in copepod respiration rates. *Hydrobiologia* 293: 333-339
- Piontkovski SA, Landry MR, Finenko ZZ, Kovalev AV, Williams R, Gallienne CP, Mishonov AV, Skryabin VA, Tokarev YN, Nikolsy VN (2003) Plankton communities of the South Atlantic anticyclonic gyre. *Oceanologica Acta* 26: 255-268
- Raymont JEG (1959) The respiration of some planktonic copepods. 3. The oxygen requirements of some american species. *Limnol Oceanogr* 4: 479-491
- Robinson C, Steinberg DK, Anderson TR, Arístegui J, Carlson CA, Frost JR, Ghiglione JF, Hernández-León S, Jackson GA, Koppelman R, Queguiner B, Ragueneau O, Rassoulzadegan F, Robison BH, Tamburini C, Tanaka T, Wishner KF, Zhang J (2010) Mesopelagic zone ecology and biogeochemistry - a synthesis. *Deep-Sea Res Part II* 57: 1504-1518
- Rosa R, Seibel BA (2008) Synergistic effects of climate-related variables suggest future physiological impairment in a top oceanic predator. *Proc Natl Acad Sci USA* 105: 20776-20780
- Saltzman J, Wishner KF (1997a) Zooplankton ecology in the eastern tropical Pacific oxygen minimum zone above a seamount: 2. Vertical distribution of copepods. *Deep-Sea Res Part I* 44: 931-954
- Saltzman J, Wishner KF (1997b) Zooplankton ecology in the eastern tropical Pacific oxygen minimum zone above a seamount: 1. General trends. *Deep-Sea Res Part I* 44: 907-930

- Schnack-Schiel SB (2001) Aspects of the study of the life cycles of Antarctic copepods. *Hydrobiologia* 453/454: 9-24
- Schnack-Schiel SB, Hagen W (1994) Life cycle strategies and seasonal variations in the distribution and population structure of four dominant calanoid copepod species in the easter Weddell Sea, Antarctica. *J Plankton Res* 16: 1543-1566
- Schnack-Schiel SB, Mizdalski E, Cornils A (2010) Copepod abundance and species composition in the Eastern subtropical/tropical Atlantic. *Deep-Sea Res Part II* 57: 2064-2075
- Schnack-Schiel SB, Niehoff B, Hagen W, Böttger-Schnack R, Cornils A, Dowidar MM, Pasternak A, Stambler N, Stübing D, Richter C (2008) Population dynamics and life strategies of *Rhincalanus nasutus* (Copepoda) at the onset of the spring bloom in the Gulf of Aqaba (Red Sea). *J Plankton Res* 30: 655-672
- Schukat A, Auel H, Teuber L, Lahajnar N, Hagen W (2014) Complex trophic interactions of calanoid copepods in the Benguela upwelling system. *J Sea Res* 85: 186-196
- Schukat A, Teuber L, Hagen W, Wasmund N, Auel H (2013) Energetics and carbon budgets of dominant calanoid copepods in the northern Benguela upwelling system. *J Exp Mar Biol Ecol* 442: 1-9
- Seibel BA (2011) Critical oxygen levels and metabolic suppression in oceanic oxygen minimum zones. *J Exp Biol* 214: 326-336
- Shimode S, Hiroe Y, Hidaka K, Takahashi K, Tsuda A (2009) Life history and ontogenetic vertical migration of *Neocalanus gracilis* in the western North Pacific Ocean. *Aquat Biol* 7: 295-306
- Shimode S, Takahashi K, Shimizu Y, Nonomura T, Tsuda A (2012) Distribution and life history of two planktonic copepods, *Rhincalanus nasutus* and *Rhincalanus rostrifrons*, in the northwestern Pacific Ocean. *Deep-Sea Res Part I* 65: 133-145
- Smith S, Roman M, Prusova I, Wishner K, Gowing M, Codispoti LA, Barber R, Marra J, Flagg C (1998) Seasonal response of zooplankton to monsoonal reversals in the Arabian Sea. *Deep-Sea Res Part II* 45: 2369-2403
- Steinberg DK, Carlson CA, Bates NR, Goldthwait SA, Madin LP, Michaels AF (2000) Zooplankton vertical migration and the active transport of dissolved organic and inorganic carbon in the Sargasso Sea. *Deep-Sea Res Part I* 47: 137-158
- Stramma L, Brandt P, Schafstall J, Schott F, Fischer J, Kortzinger A (2008b) Oxygen minimum zone in the North Atlantic south and east of the Cape Verde Islands. *J Geophys Res C* 113: C04014, doi:10.1029/2007JC004369
- Stramma L, Johnson GC, Sprintall J, Mohrholz V (2008a) Expanding oxygen minimum zones in the tropical oceans. *Science* 320: 655-658
- Stramma L, Schmidtko S, Levin LA, Johnson GC (2010) Ocean oxygen minima expansions and their biological impacts. *Deep-Sea Res Part I* 57: 587-595
- Stramma L, Visbeck M, Brandt P, Tanhua T, Wallace D (2009) Deoxygenation in the oxygen minimum zone of the eastern tropical North Atlantic. *Geophys Res Lett* 36: L20607, doi:10.1029/2009GL039593

- Svetlichny LS, Hubareva ES, Erkan F, Gucu AC (2000) Physiological and behavioral aspects of *Calanus euxinus* females (Copepoda: Calanoida) during vertical migration across temperature and oxygen gradients. *Mar Biol* 137: 963-971
- Teal JM, Carey FG (1967) Respiration of a euphausiid from the oxygen minimum layer. *Limnol Oceanogr* 12: 548-550
- Thuesen EV, Miller CB, Childress JJ (1998) Ecophysiological interpretation of oxygen consumption rates and enzymatic activities of deep-sea copepods. *Mar Ecol Prog Ser* 168: 95-107
- Thuesen EV, Rutherford LD, Brommer PL, Garrison K, Gutowska MA, Towanda T (2005) Intragel oxygen promotes hypoxia tolerance of scyphomedusae. *J Exp Biol* 208: 2475-2482
- Verheye HM, Hagen W, Auel H, Ekau W, Loick N, Rheenen I, Wencke P, Jones S (2005) Life strategies, energetics and growth characteristics of *Calanoides carinatus* (Copepoda) in the Angola-Benguela frontal region. *Afr J Mar Sci* 27: 641-651
- Verheye HM, Hutchings L, Huggett JA, Painting SJ (1992) Mesozooplankton dynamics in the Benguela ecosystem, with emphasis on the herbivorous copepods. *S Afr J Mar Sci* 12: 561-584
- Verheye HM, Hutchings L, Peterson WT (1991) Life-history and population maintenance strategies of *Calanoides carinatus* (Copepoda, Calanoida) in the southern Benguela ecosystem. *S Afr J Mar Sci* 11: 179-191
- Vinogradov ME, Voronina NM (1962) Influence of the oxygen deficit on the distribution of plankton in the Arabian Sea. *Deep-Sea Res* 9: 523-530
- Werner T, Buchholz F (2013) Diel vertical migration behaviour in Euphausiids of the Northern Benguela Current: seasonal adaptations to food availability and strong gradients of temperature and oxygen. *J Plankton Res* 35: 792-812
- Wishner K, Outram DM, Seibel BA, Daly KL, Williams RL (2013) Zooplankton in the eastern tropical north Pacific: boundary effects of oxygen minimum zone expansion. *Deep-Sea Res Part I* 79: 122-140
- Wishner KF, Ashjian CJ, Gelfman C, Gowing MM, Kann L, Levin LA, Mullineaux LS, Saltzman J (1995) Pelagic and benthic ecology of the lower interface of the eastern tropical Pacific oxygen minimum zone. *Deep-Sea Res Part I* 42: 93-115
- Wishner KF, Gelfman C, Gowing MM, Outram DM, Rapien M, Williams RL (2008) Vertical zonation and distributions of calanoid copepods through the lower oxycline of the Arabian Sea oxygen minimum zone. *Prog Oceanogr* 78: 163-191
- Wong CK (1988) The swimming behavior of the copepod *Metridia pacifica*. *J Plankton Res* 10: 1285-1290
- Woodd-Walker RS, Ward P, Clarke A (2002) Large-scale patterns in diversity and community structure of surface water copepods from the Atlantic Ocean. *Mar Ecol Prog Ser* 236: 189-203
- Wu RSS (2002) Hypoxia: from molecular responses to ecosystem responses. *Marine Pollution Bulletin* 45: 35-45

Zhang X, Dam HG, White JR, Roman MR (1995) Latitudinal variations in mesozooplankton grazing and metabolism in the central tropical Pacific during the U.S. JGOFS EqPac study. *Deep-Sea Res Part II* 42: 695-714

DANKSAGUNG

Mein großer Dank geht an PD Dr. Holger Auel und Prof. Dr. Wilhelm Hagen, da sie mir die Möglichkeit gegeben haben, in der Marinen Zoologie sowohl meine Diplomarbeit, als auch meine Promotion zu verwirklichen. Ich bedanke mich für die wissenschaftliche Unterstützung in dieser Zeit, für viele wertvolle Diskussionen und zielführende Gespräche. Außerdem bedanke ich mich für die finanzielle Unterstützung durch die Universität Bremen, die Arbeitsgruppe Marine Zoologie und das GENUS-Projekt um diese Studie durchführen zu können.

Ich danke PD Dr. Holger Auel und Prof. Dr. Claudio Richter für die Begutachtung meiner Dissertation.

Bei Holger möchte ich mich besonders für die erstklassige Ausbildung auf See und auf den Studenten-Exkursionen bedanken, wo ich viele praktische Fähigkeiten erlangt habe und wertvolle Erfahrungen sammeln durfte.

Ich möchte mich bei Dr. Rainer Kiko für die gute Zusammenarbeit als Kooperationspartner und Kollege im Kapverden-Projekt bedanken und dafür dass er mich vor Ort in Mindelo tatkräftig und professionell bei den Experimenten angeleitet und unterstützt hat. Außerdem vielen Dank an den Masterstudenten François Séguin für seine Mitarbeit in diesem Projekt und die detaillierte Probenbearbeitung.

Ich danke ebenfalls auch allen Mitarbeitern und Kollegen des INDP in Mindelo, São Vicente, dafür, dass sie uns während unseres Aufenthalts unterstützt haben und uns die Möglichkeit gaben vor Ort mit ihnen unsere Proben zu nehmen und im Labor unsere Experimente durchzuführen. Vielen Dank an die Crew der ‚Sinagoga‘ für ihre Hilfe bei der Probennahme und für erlebnisreiche Ausfahrten. All dies hat unseren Aufenthalt auf São Vicente sehr besonders gemacht.

Des Weiteren möchte ich mich sehr bei allen Kollegen des GENUS-Projekts bedanken, für ihre Hilfe und Unterstützung an Bord und vor Allem für die Möglichkeit auf einigen GENUS-Expeditionen auch im tropischen Atlantik meine Proben sammeln zu können. Die Africana, Discovery und Merian Expeditionen waren sehr tolle und erlebnisreiche Fahrten mit euch zusammen, vielen Dank für die gute Zusammenarbeit und die schöne Zeit!

Mein großer Dank geht an alle meine Kollegen und Kolleginnen, die während meiner Zeit in der Marinen Zoologie gearbeitet haben - Willy, Holger, Anna, Bernadette, Sabine, Petra, Maya, Britta, Silke, Marina und Joy - bei ihnen möchte ich mich für ein tolles Arbeitsklima, die wertvolle wissenschaftliche und persönliche Unterstützung und eine schöne gemeinsame Zeit bedanken. Mein besonderer Dank geht dabei an Anna, für ihre zuverlässige und unmittelbare Unterstützung bei jeglichen Fragen und Problemen und dafür dass wir zusammen immer eine Lösung gefunden haben.

Ich danke Bernadette, Anna und Sabine für das Korrekturlesen meiner Texte und für viele hilfreiche Kommentare und super Unterstützung besonders in den letzten Wochen der Fertigstellung dieser Arbeit.

Meiner Familie, meinen Freunden und vor Allem Thomas danke ich herzlich für alles was neben der Arbeit im Leben wichtig ist.

Zu guter Letzt danke ich den Copepoden des tropischen Atlantiks.

ERKLÄRUNG

Eidesstattliche Erklärung

(Gem. § 6(5) Nr. 1-3 PromO)

Hiermit versichere ich, dass ich die vorliegende Arbeit mit dem Titel „Ecology and Physiology of Calanoid Copepods in Relation to the Oxygen Minimum Zone in the Eastern Tropical Atlantic“:

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.

Des Weiteren erkläre ich hiermit, dass es sich bei den drei abgegebenen Exemplaren dieser Arbeit um 3 identische Ausführungen handelt.

Bremen, den 04.04 2014

Lena Teuber

LOVE & PEACE

„Die Natur kennt das große Geheimnis und lüchelt“

Victor Hugo