Master Thesis

Metabolism and response to short-term starvation in three Euphausiid species of the Northern Benguela Current

presented by

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Abstract

The first part of this study dealt with the comparison of specific physiological parameters of the three most abundant and therefore ecologically important Euphausiid species *Euphausia hanseni*, *Nematoscelis megalops* and *Nyctiphanes capensis*, which are typical of the Northern Benguela Current. For comparison the oxygen consumption rates, ammonium excretion rates, specific citrate synthase activity as well as proximate biochemical composition were analysed at four different experimental temperatures (5°C, 10°C, 15°C and 18°C/20°C). The species adjust their overall metabolic rates to a prevailing temperature gradient by approximately following the Q_{10}-rule. There was no significant difference among species. The same applied to specific enzyme activity and biochemical composition. Nevertheless, strong distinctions between and also within species could be found with respect to ammonium excretion. The results underlined the assumption that species were confronted to different trophic conditions concerning the region of catch and that *N. megalops* is depending on a predominant carnivorous feeding.

The second part of this thesis concerned the physiological reaction to short-term starvation in *E. hanseni*. Additionally, enzyme efficiency of citrate synthase as well as total lipid and protein content was analysed in order to determine the species' physiological performance to adapt to short-term food deprivation of up to seven days. Integrated to the starvation experiments, intermoult period and growth increment was measured. The experiments were conducted at three different temperatures (5°C, 10°C and 15°C). *E. hanseni* specimens were divided into two groups, which were defined by the region of catch (namely the "Benguela" and "Angola" regions) as it was assumed that the specimens were influenced by different water masses originating in these regions. The observations indicated a continuous reduction of overall metabolic rates with regard to the period of food deprivation. Respiration rates as well as ammonia excretion rates were reduced by about 50%. Decreasing trends were also suggested for the other measured parameters. It was concluded that all observations indicated an adaptation of *E. hanseni* specimens to short-term starvation in order to remain metabolically efficient. Furthermore, differences in the metabolic sensitivity with regard to starvation were found between groups. The differences in trophic condition may have contributed to this effect.

Moult parameters intermoult period and growth increment at moult appeared to be strongly correlated to temperature changes as the intermoult period decreased with increasing experimental temperature. The growth increment at moult showed high variances and ranged on average from shrinkage to 1.8 %.

1 Introduction

The Benguela Current ecosystem is located along the southwest Atlantic coast of Africa and belongs to one of the four major eastern boundary upwelling systems of the world oceans (Hutchings et al. 2009). It is bordered by the warm Angola Current in the north (at about 16°S) and by the warm Agulhas Current System (at about 34°S) in the south (Shannon and O'Toole M.J. 2003). Due to the strong perennial Lüderitz upwelling cell (27°S-28°S), the Benguela Current is clearly separated in a northern and a southern part (Boyer et al. 2000).

Coastal Upwelling regions are characterized by equatorward winds, cool nutrient-rich rising water masses and high plankton biomass. As a consequence, they are ecologically important fishing grounds.

In contrast to the Southern Benguela the Northern Benguela Current experienced some profound changes in the ecosystem structure during the last decades (Heymans et al. 2004; Hutchings et al. 2009). These were primarily a consequence of the dramatic decline of economically important fish stocks, which were initially accounted to the extreme exploitation of resources by the fishing industry between 1970 and 1990. However, later on, environmental changes in the ocean were identified (Boyer et al. 2000; Hutchings et al. 2009), that are now believed to have mainly contributed to ecosystem changes in the Benguela Current system and include the effects of the ongoing global climate change. For instance, the extension of oxygen depleted waters and the production of hydrogen sulfide due to high anoxic bacterial activity were detected (Ekau et al. 2009; Hutchings et al. 2009), as well as a decline in upwelling-generating winds in the Lüderitz-Orange River Cone (Hutchings et al. 2009).

The German Federal Ministry of Education and Research is funding a project aiming at investigating the complex Northern Benguela Current system and to detect a relation between climate change and ecosystem structure: the GENUS-Project (*G*eochemistry & *E*cology of the *N*amibian *U*pwelling *S*ystem; for further information see http://genus.zmaw.de/). In this project more than 10 international institutions are working together in studying the environmental context of the sensitive Northern Benguela Current off the coast of Namibia. Among others, one approach of this interdisciplinary project is to identify biological ecosystem components (key species) that are playing a role in the physically and biogeochemically changing Benguela Current system.

Among the zooplankton, the Benguela Current can be dominated by krill species. Krill are generally seen as key species in ocean’s food-webs and are distributed in high

abundances in all oceans. They occupy a central role as being consumers and furnish a major food source to fish, birds, seals and whales. A change in a krill population may thus have dramatic impacts on ecosystems. Nevertheless, there is little knowledge about the ecophysiology of krill. The state of science is still rather patchy and data are missing, which are fundamental for an understanding of the ecology and ecological changes in the oceans, the Benguela Current system in this case.

The whole life of krill is affected by performing diel vertical migration. Krill principally stay in the deep during day and migrate to the surface in order to feed during the night. This behavior is not only due the reaction to different light regimes and to the avoidance of predators, but also, especially in upwelling regions, believed to permit species to remain in favourable water masses and thus to decrease the effects of advection to food depleted offshore regions (Barange 1990). Diel vertical migration implies a physiological challenge, because species pass through a wide range of different abiotic environmental conditions, like strong changes in temperature, salinity and different oxygen concentrations. Krill therefore have to overcome dramatic variations and to adapt their overall metabolism to survive under sometimes extreme conditions.

In the Benguela system, common Euphausiid genera are *Stylocheiron*, *Thysanopoda*, *Nyctiphanes*, *Nematoscelis*, *Thysanoessa* as well as *Euphausia* (Gibbons 1997).

Among these, the species *Euphausia hanseni*, *Nematoscelis megalops* and *Nyctiphanes capensis* are dominating the Northern Benguela Current (Olivar and Barange 1990; Barange and Stuart 1991; Barange et al. 1991). Compared to krill species of other climate zones (like polar regions), the knowledge about Benguela krill is still scarce and there are no studies yet on species' metabolism or chemical composition although this is important in determining the role of krill in the energy flow of the ecosystem (Kim et al. 2010).

E. hanseni and *N. megalops* are the most abundant species at or close to the shelf break, partly sharing one habitat in the Northern Benguela Current (Barange et al. 1991). They differ in their lifestyle and migration behaviour, as *E. hanseni* is known as a filtering species above the thermocline and *N. megalops* as an actively hunting species below the thermocline (Barange et al. 1991). *N. capensis*, by contrast, lives in shallow coastal waters (< 200 m) and is a filter-feeder like *E. hanseni* (Barange and Stuart 1991).

The aim of this Master thesis is to contribute to the GENUS-project by collecting ecophysiological data of these three abundant and therefore ecologically important krill species of the Northern Benguela upwelling region.

All three Euphausiid species share one ecosystem and each of them developed a different lifestyle and habitat to avoid competition (Barange and Stuart 1991). Due to

these differences, questions arise about metabolic needs and possible specific adaptations, which are necessary to each species to persist in the challenging Northern Benguela Current system.

Following questions shall therefore be tackled:

1. How does each species metabolically react to temperature changes?
2. Is there a difference in metabolic adaptation with respect to a temperature gradient?
3. To what extend do the three Euphausiid species (*E. hanseni, N. megalops* and *N. capensis*) differ with respect to specific physiological parameters, like respiration and excretion rates, proximate biochemical composition and enzyme?

Furthermore, specific life strategy adaptations due to living in a nutritional polypulsed and stratified environment like the Northern Benguela Current system shall be investigated. The species rely on upwelling pulses that lead to rich plankton patches as a food source. The investigation time was in late austral summer, which is typically characterized by minimum upwelling events (Hagen et al. 2001), and therefore short-term starvation would not be untypical. Accordingly, on the example of *E. hanseni*, additional questions will be addressed:

1. How does *E. hanseni* metabolically react to a short-term starvation of seven days?
2. How far will metabolic parameters be regulated?
3. Are there differences in the reaction to starvation with respect to different temperature conditions?

For answering these questions, a range of metabolic parameters will be analysed. A major focus will be set on respiration measurements, as a significant expression of overall metabolism (Buchholz and Saborowski 2000), and determination of ammonium excretion, as a major end-product of protein catabolism (Jawed 1969). In addition, atomic ratios of oxygen and nitrogen as well as of atomic carbon and nitrogen will be measured as an indication of metabolic adaptability and in order to reflect changes and differences of lipid and protein metabolism (Ikeda 1974). Being a key enzyme in metabolism, the specific activity of citrate synthase will be measured, as it indicated physiological adjustments to different environmental conditions (Buchholz and Saborowski 2000). Furthermore, and related to the starvation experiments the enzyme kinetics of citrate synthase will be studied in *E. hanseni*, flanked by analysis of the proximate chemical composition, i.e. body protein and total body lipids, in order to assess the results to energy storage of the specimens aiming at gaining further information about its mode of life and environmental adaptation within the plankton community of the Benguela Current system.

2 Materials and Methods

The following chapter contains the detailed experimental procedures and the single analyses that were conducted on the respective krill species (*Euphausia hanseni, Nematoscelis megalops* and *Nyctiphanes capensis*). Methods from subchapter 2.5.6 on were only applied to *E. hanseni* that was used in the starvation experiments.

For detailed information of the used chemicals see list of chemicals in appendix (9.1).

2.1 Investigation area

All specimens were caught on board of the research vessel Maria S. Merian (MSM) during leg 17/3 in the coastal upwelling area off the coast of Namibia (Figure 1). The expedition was in late austral summer from January 30th to March 7th, 2011.

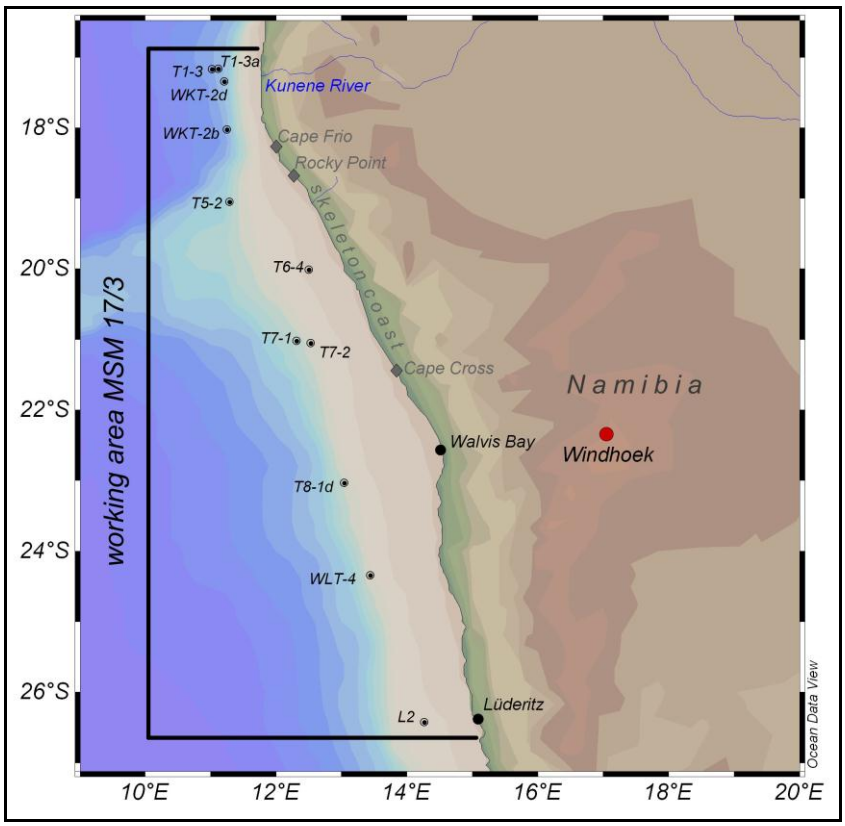

Figure 1: Working area of research cruise of MSM leg 17/3

The animals were collected on 11 stations between latitudes of 17° 15, 60’ S and 26° 41, 75’ S and accordingly between longitudes of 14° 26, 14’ E and 11° 00, 83’ E (Table 1).

At all stations, except station T6-4, a 1 m^2 MOCNESS was employed (Multiple Opening-Closing Net and Environmental Sampling System; Wiebe et al. 1976). It was equipped with eight nets with a mesh-size of 2000 µm with a soft cod-end net bucket. In order to facilitate a gentle catch to prevent damaging of the animals, the hauls were mostly done during night (when krill was closer to the surface) and were kept as short and shallow as possible. Furthermore, the net was operated at low speeds through the water (2 knots) and was veered and heaved at speeds around 0.3 m s^{-1}.

Station T6-4 was sampled by the GENUS-subproject "Ichthyoplankton" (Ph.D. student Simon Geist, ZMT Bremen) using a 1 m^2 Tucker Trawl with mesh sizes of 1000 µm and a 500 µm cod-end bucket. The haul was done within a depth of 20 m and the net was gently towed horizontally for 5 minutes with 2 knots through the water (personal communication Ph.D. student Simon Geist, ZMT Bremen).

Table 1: Sampled stations with informations on position, maximum depth, sampling depth and date of sampling (Animals from station T6-4 were taken out of the Tucker Trawl, which belonged to the GENUS-subproject "Ichthyoplankton")

Station name	Position		Maximum depth [m]	Sampling depth [m]	Date of sampling
L-2	26° 41,75' S	14° 26,14' E	326	100	February 2nd, 2011
WLT-4	24° 30,73' S	13° 42,34' E	356	60	February 4th, 2011
T8-1d	23° 00,12' S	13° 03,02' E	411	50	February 7th, 2011
T7-2	21° 04,94' S	12° 52,49' E	142	100	February 12th, 2011
T7-1	21° 00,85' S	12° 30,44' E	434	360	February 12th, 2011
T6-4	20° 01,67' S	12° 50,29' E	171	30	February 13th, 2011
T5-2	19° 03,89' S	11° 28,26' E	380	350	February 16th, 2011
WKT-2b	18° 01,60' S	11° 24,39' E	427	250	February 18th, 2011
WKT-2d	17° 33,57' S	11° 20,51' E	430	100	February 18th, 2011
T1-3a	17° 15,60' S	11° 11,12' E	932	150	February 20th, 2011
T1-3	17° 15,78' S	11° 00,83' E	3386	300	February 20th, 2011

2.2 Collection and maintenance of specimens

Immediately after each catch, one fraction of freshly caught animals (at least n=10) were directly put in single Eppendorf tubes (1.5 ml), frozen in liquid nitrogen and stored at -80°C for later analysis of body length and weight as well as physiological parameters after the cruise at Alfred Wegener Institute (AWI) Bremerhaven, Germany.

Another fraction of the freshly caught individuals were separated into species and transferred into 26 litre plastic aquaria (450x250x260 mm) that were filled with cooled filtered seawater (0.2 µm, CHROMAFIL® disposable filter, Germany). The aquaria were kept in special converted freezers for krill maintenance (Figure 2), at 8°C in the dark in order to allow the animals to acclimate for about 12 hours before being employed in experiments (see subchapters 2.3 and 2.4).

Figure 2: Modified freezer for krill maintenance equipped with temperature regulation device (arrow).

Aquaria containing *E. hanseni* or *N. capensis* were aerated by using natural air stones connected to an aquaria pump (Tetra*tec*® Aps300, Germany). Aquaria with *N. megalops* were not aerated according to previous observations, which showed a better condition of specimens without aeration (personal communication Thorsten Werner, Ph.D. student at AWI Bremerhaven).

2.3 Respiration measurements

In order to measure the oxygen (O_2) consumption of krill in resting state, four respiration chambers (special design for krill with a volume of 20 ml; Figure 3B), equipped with one oxygen microsensor each (PreSens GmbH, Germany), were used. The signals (value of oxygen concentration in mg L^{-1}; every 15 seconds) of the microsensors were transmitted to a computer by an Oxy-4micro transmitter (PreSens GmbH, Germany). The oxygen consumption was recorded using Oxy-4micro software (PreSens GmbH, Germany).

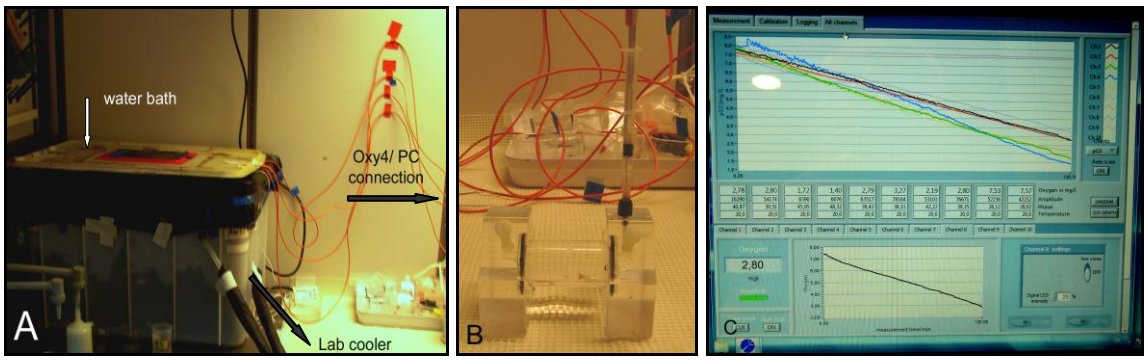

Figure 3: Experimental set-up. A) Plastic box serving as water bath and "dark room" for respiration experiments. B) Respiration chamber equipped with microsensor. C) Screenshot of OXY10-software recording oxygen consumption.

The single microsensors were calibrated in oxygen saturated air (for 100% of oxygen saturation, by measuring the oxygen conditions close to a moistened piece of cotton wool, which was put in a falcon tube) and in saturated sodium sulfite solution (for 0% of oxygen saturation).

Individual krill was employed after acclimation at a minimum of 12 hours (but not more than 48 hours) after catch. One hour before start of measurement specimens were already subjected to experimental temperature conditions in order to acclimate to these. Experiments on each krill species were conducted at four temperatures (5°C, 10°C, 15°C and 20°C). From these temperatures it was known, that the natural range is covered by taking the extent of vertical migration patterns to account (from about 500 m to 25 m) that was investigated in previous research cruise of MSM in 2008 (personal communication Prof. Dr. F. Buchholz and Ph.D. student Thorsten Werner, AWI Bremerhaven). The results of the hydrographic temperature distribution (demonstrated on the example of the Walvis Bay transect; Figure 4) confirm the selection of the defined experimental temperatures.

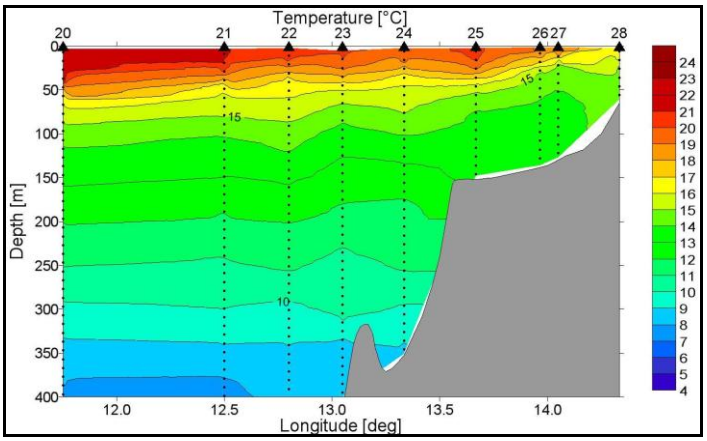

Figure 4: Depth profile of temperature at the Walvis Bay transect (06.-09.02.2011). Picture was taken from hydrographic cruise report of MSM 17/3 (Mohrholz et al. 2011). Image is based on CTD data and was created by GENUS-subproject "Physical Oceanography".

A 29 litre aquarium (450x250x260 mm) filled with filtered seawater (0.2 µm; AcroPak^{TM} 1000, Pall Filtersystems GmbH, Germany) of defined experimental temperature facilitated the fixing of the respiration chambers: the chambers were filled under water (avoiding air bubbles while filling) and the specimens were carefully transferred by using a small plastic beaker (also containing tempered seawater). Per run three chambers were equipped with one animal each and one chamber served as a control by only being filled with filtered seawater. During measurement the respiration chambers were stored in a poly-ethylene box (600x400x400 mm) that was filled with as much water as was needed to slightly cover the chambers. The box was covered with black plastic foil and closed by a lid to maintain dark experimental conditions (Figure 3A). Using a lab cooler (Julabo F25, Germany) the water inside the poly-ethylene box was permanently kept at defined experimental temperature.

The measurement was stopped as soon as the oxygen concentration inside the respective chamber reached 60% of the start concentration. After this, the specimen was put in an Eppendorf cup (1.5 ml), frozen in liquid nitrogen and stored at -80°C in order to be kept for later analysis of physiological parameters at AWI, Bremerhaven (Germany). In addition a sample of the experimental water (out of each chamber) was taken and stored in an Eppendorf cup (2 ml) at -80°C for further analysis of ammonia content (see subchapter 2.5.1) at AWI, Bremerhaven (Germany).

The respiration measurements were done in the air-conditioned salinometer room on board of the research vessel Maria S. Merian.

Some measurements were taken using the set-up of the GENUS-subproject "Marine Zooplankton" (Ph.D. students Anna Schukat and Lena Teuber, University of Bremen).

In contrast to our experimental set-up, the respiration chambers consisted of ten Winkler bottles (mean volume of 13.27 ± 0.40 ml; Figure 5) equipped with non-invasive oxygen sensors (PreSens GmbH, Germany) instead of oxygen microsensors. The Winkler bottles were also filled with filtered seawater (0.2 µm; AcroPak^{TM} 1000, Pall Filtersystems GmbH, Germany). The animals were carefully transferred into the bottles by using featherweight forceps. To maintain constant temperature the single Winkler bottles were immersed in a water bath. Compared to our experimental set-up the water bath was stored in a modified refrigerator (in the dark at controlled temperatures).

Up to 8 specimens were measured in single chambers in one run. At least two Winkler bottles served as control. The sensor signals (value of oxygen concentration in mg L^{-1}; every 15 seconds) were transmitted to a computer by an Oxy-10mini transmitter (PreSens GmbH, Germany) and the oxygen consumption was recorded using Oxy-10mini software (Figure 3C; PreSens GmbH, Germany).

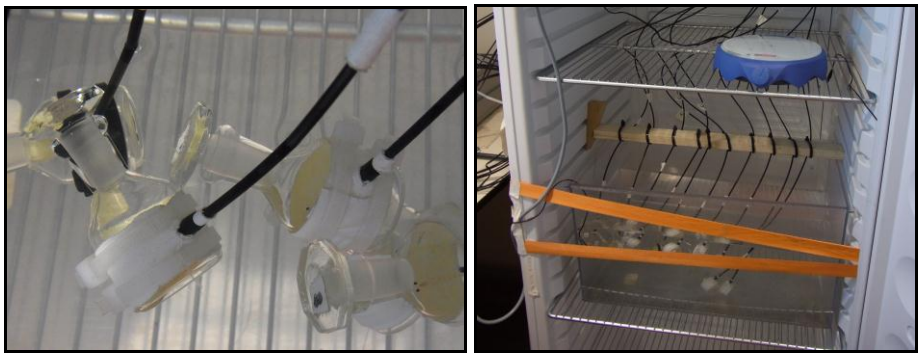

Figure 5: Experimental set-up of sub-project "marine zooplankton". Left: Winkler bottles connected to the non-invasive oxygen sensor (PreSens GmbH, Germany). Right: Arrangement of the Winkler bottles in the water bath. During measurement the door was closed.

Data handling after measurement

The data collected of oxygen consumption was transferred to Excel (Microsoft Office 2003) and plotted in x/y- Axis. The oxygen consumption rate [mg L^{-1} Ind^{-1} min^{-1}] in each chamber was determined by linear regression of the negative slope. The first 30 to 45 minutes were excluded from the evaluation as acclimation time of the animal to the experiment. The rates of the controls, as an indicator for microbial respiration, were subtracted afterwards.

For later comparison the results were then referred to hour (h), to the respective volume of the respiration chamber and to gram fresh weight (gFW) of the particular animal to standardized the units of respiration (O_2 [mg gFW^{-1} h^{-1}]), which could be additionally transformed to the molecular weight of oxygen (O_2 [µmol gFW^{-1} h^{-1}]).

Q_{10}-values of respiration rates were calculated by applying the equation according to van't Hoff (1884): $Q_{10}= (R_2/R_1)^{10/ (T2-T1)}$, in which R_1 and R_2 represent the respiration rates at the temperatures T_1 and T_2.

2.4 Starvation experiments

The detailed starvation experiments were only carried out on individuals of *E. hanseni*. Two main groups of animals were studied: animals that were caught south of 20°00,00' S, which were referred to as "Benguela"- Individuals as it was assumed that they had spent longer times in the Southern Benguela Current system (Figure 7A) and animals that were caught north of this latitude, which were referred to as "Angola"- Individuals, because it was assumed that they were more strongly influenced by the warm Angola water masses, coming from the north (Figure 6). Both starvation groups contained three sets (n=48 each) of single kept individuals that were maintained at different temperatures (5°C, 10°C and 15°C) over a period of 7 days without being fed.

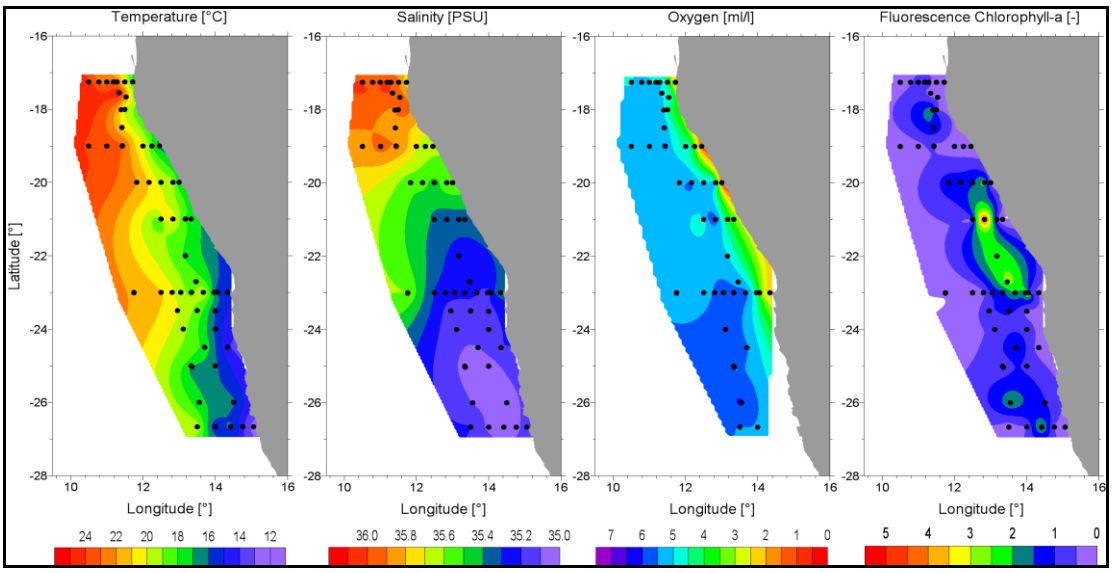

Figure 6: Horizontal distribution of temperature, salinity, oxygen and chlorophyll-a fluorescence off the coast of Namibia at 20 m depth. Picture was taken from hydrographic cruise report of MSM 17/3 (Mohrholz et al. 2011). Image is based on CTD data and was created by GENUS-subproject "Physical Oceanography".

The experiments were started after 12 hours of acclimation after catch. Only undamaged animals in an apparently good condition were chosen. The animals were placed individually in 48 wide neck plastic bottles (1 L; transparent, square, Kautex) and were then stored in two plastic boxes (600x400x200 mm) each containing 24 bottles. The bottles were filled with filtered seawater (0.2 µm; CHROMAFIL® disposable filter,

Germany) that was kept at the respective experimental temperature. The water level was at the upper rounding of the bottle so that every individual stayed in almost the same volume of water (about 900 ml).

The aeration of the bottles was ensured by using special aeration frames (Figure 7C), which were connected to an aquaria air pump (Tetra*tec*® Aps300, Germany). The frames were constructed in this way that each frame fitted on one plastic box having 24 exits equipped with pipette tips that exactly fitted in the Kautex bottles. The pipette tip did not enter the water but stopped close to the surface, whereby the water was slightly set into motion by an escape of air.

The experimental temperature was maintained by storing the boxes either in special temperature controlled rooms on board of the research vessel (cool room and salinometer room with temperature control accuracy of ± 0,5°C) or in the modified freezer with temperature control device (Figure 7B). The boxes were covered with dark lightproof plastic foil in order to ensure dark experimental conditions.

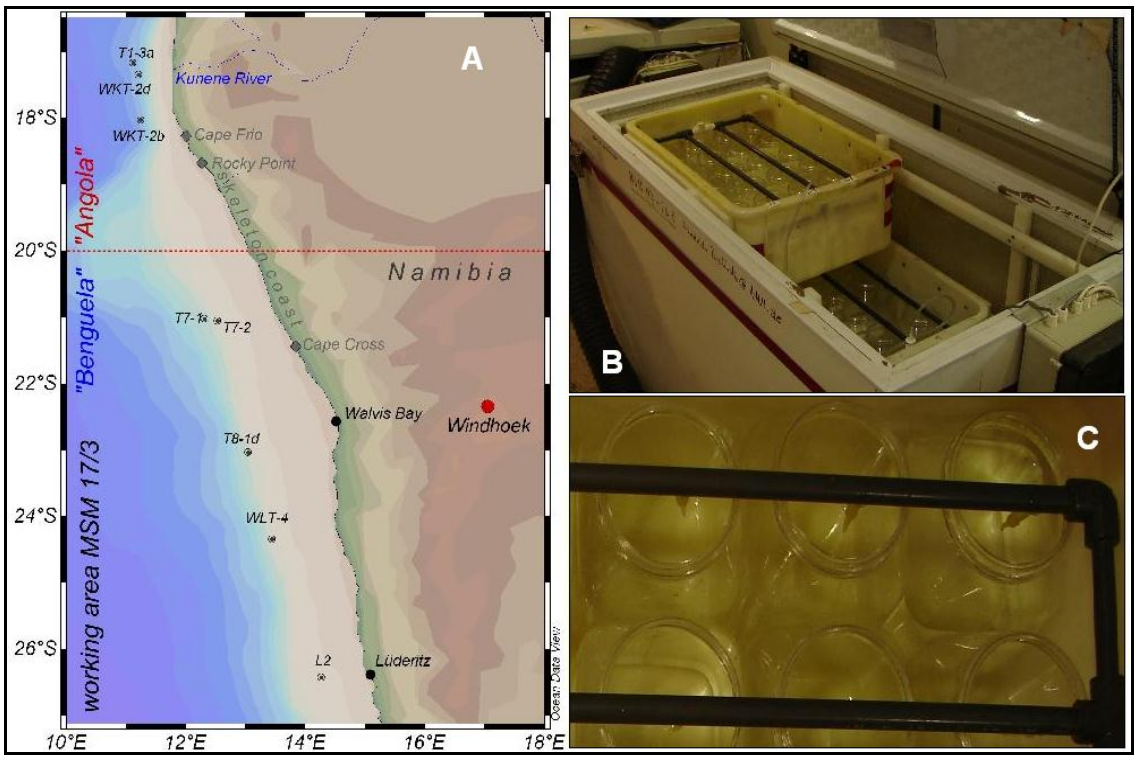

Figure 7: Starvation experiment. A) Map of working area and stations sampled separated in "Benguela" and "Angola". B) Example for maintenance of *E. hanseni* in modified freezer during experiment. C) Aeration of single bottles in detail.

For the first three days the specimens were checked twice a day for moults and mortality. The moults were immediately removed and individually put in an Eppendorf cup (1.5 ml)

then frozen and stored at -80°C for further analysis (see subchapter 2.5.8) at the home laboratory at AWI, Bremerhaven (Germany). After three days, subsamples were taken every day by conducting respiration measurements (see subchapter 2.3) on at least three animals out of the particular experiment. The animals were measured at defined experimental temperatures. After the experiment, the animals and the water were treated like described in subchapter 2.3.

In order to get information about the respiration of the first three days of each experiment an additional set of animals (n≈20) was kept next to the starvation experiment in 11 litre plastic aquariums (340x172x190 mm) containing filtered seawater (0.2 µm; CHROMAFIL® disposable filter, Germany). Respiration measurements (at least n=3) and further treatment of specimens were performed as mentioned before.

2.5 Analyses

All measurements were conducted in the laboratories of the Alfred-Wegener-Institute in Bremerhaven (Germany) from March 10th to June 20th, 2011.

The lipid analyses (see subchapter 2.5.7) were done in the laboratories of the "Marine Zoology"- Department of Prof. Dr. W. Hagen, University of Bremen.

2.5.1 Biometry

For correlation of fresh weight to length the body length of at least 20 animals per species and gender was determined by measuring the distance between the front of the eyes to the tip of the telson by using millimetre paper under a stereomicroscope. For this the respective animals had to be thawed. Hence only animals were taken, on which no further analysis were conducted (only animals that were frozen directly after catch). The weight was determined on a microbalance (Sartorius LA 230 S, Germany; d=0.1 mg). The relation between length and weight was later characterized by regression analysis.

In contrast to body length-analysis the animals had not to be thawed for weighing. Thus the fresh weight was determined on all animals. The specimens were carefully dipped in ice cold Milli-Q water. With tweezers the ice crust was gently removed having the animals in a Petri dish on a cooling device. Non-fuzzing paper was used to remove excessive water.

Additionally, the gender of each specimen was defined. If it was not possible to determine the sexual organs (thelycum or petasma) the animals were defined as juvenile.

2.5.2 Excretion

As an indication of excretion the ammonium (NH_4^+) content of the water from the respiration chambers was determined photometrically according to Solorzano (1969).

The standard was given by a defined ammonium working solution ($c_{(ammonium)}$=1g L^{-1}; ammonium chloride in Milli-Q). The total volume of sample always consisted of 500 µl. Also the dilution series was made in defined concentrations at 500 µl of sample (Table 2). Volumes of ammonium working solution were diluted with sodium chloride solution (c=36g L^{-1}, see appendix 9.2 for detailed dilution series). All samples were measured in triplicates.

For analysing, 500 µl of sample was mixed in Eppendorf cups (1.5 ml) with 750 µl Milli-Q water and 100 µl phenol solution (8 g Phenol + 30 ml Ethanol + 60 ml Milli-Q water) and incubated for 2 minutes at room temperature. Afterwards, 50 µl of citrate buffer (24 g tri-Sodium citrate dehydrate in 50 ml Milli-Q water) and 100 µl of DTT-reagent (25 mg Dichlorisocyanuric acid sodium salt dehydrate in 10 ml 0.5 M sodium hydroxide solution) were added. The samples were mixed and incubated for about one hour at 40°C and 400-500 rpm in a laboratory mixer (Thermomixer comfort, Eppendorf, Germany).

Table 2: Pipetting scheme for ammonium analysis according to Solorzano (1969)

	Standard series	Samples
Ammonium standard	0 – 500 µl	
Sample		500 µl
NaCl solution	ad. 500 µl	
Milli- Q	750 µl	750 µl
Phenol solution	100 µl	100 µl
Incubation	2 minutes at room temperature	
Citrate buffer	50 µl	50 µl
DTT-reagent	100 µl	100 µl
Incubation	1 hour, 40°C, 400-500 rpm in laboratory mixer	
Measurement	at wavelength of 630 nm	

After incubation the samples were analysed photometrically in a plastic cuvette (1 cm light path) at a wavelength of 630 nm (UV/VIS Spectrometer Lambda2, Perkin Elmer™ instruments, England).

The ammonium content of each sample was calculated by means of the slope of the straight calibration line, which was achieved from the measurement of the dilution series. For later comparison the results were referred to one hour (h), the respective volume of the respiration chamber and gram fresh weight (gFW) of the particular animal to standardize units of excretion (NH_4-N [mg gFW^{-1} h^{-1}]), which could be additionally transformed to molecular weight (NH_4-N [µmol gFW^{-1} h^{-1}]).

In order to calculate the ratio of atomic oxygen to atomic nitrogen (O:N-ratio) the obtained oxygen consumption (see subchapter 2.3) was divided by the excretion of each specimen.

2.5.3 Sample preparation

In further experiments (see subchapters 2.5.4, 2.5.5, 2.5.6 and 2.5.7) the associated set of test specimens was specifically prepared.

In the study of citrate synthase (CS; EC 2.3.3.1) the second abdominal segment was severed. This was done on a cooling device by using a scalpel and micro scissors. The chitinous exoskeleton was removed and the segment was placed in a preweighed ice-cool Eppendorf cup (1.5 ml) and weighed on a microbalance (Sartorius LA 230 S, Germany, d=0.1 mg). Until further treatment, the segment was stored at -80°C.

The rest of the respective animal, i.e. the cephalothorax with first abdominal segment, was also weighed and stored at -80°C until it was freeze-dried for 24 hours (ALPHA 1-4 LSC freeze-drier, Christ GmbH, Germany).

2.5.4 Elemental composition

The total carbon (C) and total nitrogen content (N) of the lyophilised cephalothorax and first segment was determined for each specimen.

The samples were pestled to powder by using a tapered glass pestle. From this homogenate aliquots of 1-2 mg of powder were weighed into tin weighing containers (5x9 mm; HEKAtech GmbH, Germany) by using an ultra microbalance (Mettler Toledo UMX2, Switzerland; d=0.1 µg).

The CN- content was determined by the use of an elemental analyser (Euro EA CHNS- O elemental analyzer, HEKAtech GmbH, Germany). Acetanilide (HEKAtech GmbH, Germany; C=71.09%, H=6.71%, N=10.36, O=11.84%) served as the standard for the measurement.

The values were calculated by the associated software (Callidus) and given in microgram per sample content that was weighed into the respective container. For comparison the atomic ratio of carbon to nitrogen was calculated.

2.5.5 Enzyme activity: Citrate synthase (CS)

The measurement of CS activity was conducted according to Stitt (1984) using the modifications mentioned in Meyer et al. (2010).

In order to measure the enzyme activity a homogenate of the second abdominal segment of defined weight was used. For this 500 µl ice-cold CS- buffer (0.05 M Tris + 0.1 M KCL + 1 mM EDTA; adjusted at room temperature with 0.1 M HCL-solution to pH 8) was added to each Eppendorf cup containing a segment. By the use of an Eppendorf pestle (tapered and made of stainless steel) the segment was carefully homogenized. The Eppendorf cups were permanently kept on crushed ice while doing this. Afterwards, the homogenates were additionally treated by ultrasound for efficient cell disruption. The Eppendorf cups were therefore sealed with Parafilm, so as to avoid spilling sample, and homogenized (3x5 seconds with 5 seconds break at 35% output) in an ice bath by using an ultrasound sonotrode (BRANSON SONIFIER® cell disruptor B15, Germany). Afterwards the samples were centrifuged for 10 minutes at 4°C and 10 000 g (Eppendorf centrifuge 5417R, Germany). The supernatant was taken and stored in a clean ice-cold Eppendorf cup to avoid resuspension of the pellet until measurement.

The measurement of CS was carried out photometrically (UV/VIS Spectrometer Lambda2 with Peltier Temperature Programmer PTP-6, Perkin Elmer™ instruments, England) at a wavelength of 412 nm. The CS activity at 25°C was established.

For analysis 30 µl of sample (supernatant), 30 µl of Acetyl-Coenzyme A (-CoA) solution (6 mM), 30 µl DTNB- solution (6 mM) and 780 µl of CS- buffer were combined in a glass cuvette (HELLMA® Type 104B-OS, Germany; light path of one cm) and incubated for 3 to 5 minutes at 25°C in the photometer. The reaction was initiated by adding (with stirring with a plastic stick) 30 µl of oxaloacetic acid solution (12 mM). At the same time the software for measuring the enzyme activity was started (UV KinLab, Perkin Elmer™ instruments, England). The measurement was set to monitor continuously for 3 minutes. All samples were analysed in duplicates.

The UV KinLab-software automatically calculated the change of absorption over time (dA_{412} min^{-1}). Then the volume activity (U ml^{-1}) using the Beer-Lambert Law (Figure 8) was calculated.

The specific enzyme activity (U gFW^{-1} or rather U $mgProtein^{-1}$) was obtained by referring the CS- volume activity to gram fresh weight (from the concentration of homogenate) or rather to milligram protein content of the respective segment by analysing the protein concentration of the homogenate according to Bradford (1976), as given below.

$$CS_{\text{volume activity}}\left(\frac{U}{ml}\right) = \frac{dA_{412}}{min} \cdot \frac{V_{cuvette}}{\varepsilon \cdot d \cdot V_{sample}}$$

Figure 8: Beer-Lambert Law

$CS_{volume\ activity}$ = CS activity in units (U) per ml
$dA_{412}\ min^{-1}$ = Change of absorption over time
$V_{cuvette}$ = Total volume in cuvette (900 µl)
ε = Extinction coefficient (13.61 L mol^{-1} cm^{-1})
d = Light path through cuvette (1 cm)
V_{sample} = Total volume of added sample (30 µl)

Protein analysis according to Bradford (1976)

For protein determination the homogenates of the segments were diluted 1:50 (v:v) with Milli-Q water. The analysis was carried out photometrically by using a microplate reader (Thermo Scientific, Multiscan FC, Finland) with associated software (SkanIt 2.5.1, Research Edition for Multiscan FC, Finland). The samples were analysed in triplicates. The measurement was conducted in a multiwell plate (96 well format).

Bovine Serum Albumin solution (BSA; c=0.1 mg ml^{-1}) was used as the starting solution for preparing the protein standard curve. Standards were measured in duplicates. Volumes of 0, 10, 20, 30, 40 and 50 µl BSA were used for calibration.

The reaction wells always contained 50 µl of standard/sample before 250 µl of diluted Protein Assay (1:5 with Milli-Q; Bio-Rad 500-0006) was added. 20 µl of sample was used for measurement, to which therefore 30 µl of Milli-Q was added in order to get a total volume of 50 µl (Table 3).

Table 3: Pipetting scheme for protein analysis according to Bradford (1976)

	Standard series	Samples
BSA standard	0 – 50 µl	
Sample		20 µl
Milli- Q	ad. 50 µl	30 µl
Protein Assay (1:5 diluted with Milli-Q)	250 µl	250 µl
Incubation	5- 15 minutes at room temperature	
Measurement	at wavelength of 600 nm and a temperature of 25°C	

Before measurement the plate was incubated at room temperature for 5-15 minutes and then measured at 25°C at a wavelength of 600 nm.

The software calculated the protein content of the reaction charge (in µg). The dilution had to be taken into account in order to obtain the correct protein content in the sample (of 20 µl). For comparison, the results were referred to milligram protein per millilitre sample homogenate.

2.5.6 Enzyme kinetics of CS

The Michaelis-Menten constants (K_m) for CS were measured of *E. hanseni*-specimens from the starvation experiments at 10°C.

The measurement was conducted for CS activity towards Acetyl-CoA concentration as described in Saborowski and Buchholz (2002) and Vetter (1995a, 1995b).

The measurements were conducted the same way as mentioned in subchapter 2.5.5 with the only difference that the Acetyl-CoA concentration in the reaction mixtures varied from 0 to 50 µmol L^{-1} (0, 5, 10, 20, 40 and 50 µmol L^{-1}).

The results were referred to Units per milligram fresh weight and transferred to GraphPad Prism5 (GraphPad Software, Inc., USA). The program plotted Michaelis-Menten curves and calculated the K_m values.

2.5.7 Proximate biochemical composition

The biochemical composition with respect to total lipid and total protein content was determined of *E. hanseni*-specimens from the starvation experiments at 10°C.

For observation of possible changes in lipid and protein content the cephalothoraxes (with first abdominal segment) of animals of the first day of starvation as well as animals of the seventh day of starvation were compared. The analysis of total lipid content was conducted gravimetrically according to Hagen (2000). Total protein content was analysed by applying the method according to Lowry et al. (1951). Respective methods will be described in the following paragraphs.

Determination of lipids according to Hagen (2000)

The lyophilized samples were weighed on a semi-microbalance (Sartorius R200D, Germany; d=0.01 mg) and pestled to powder by using a tissue grinder (glass, tapered style with glass pestle; 3 ml). At least 3mg of powder was taken for elemental analysis and protein analysis according to Lowry et al. (1951), weighed into an Eppendorf cup (1.5 ml) and stored for further measurements. The difference of the two weights (primary material less taken powder taken for other analyses) resulted in the amount of powder that remained in the tissue grinder and, accordingly, in that amount that was taken for lipid analysis.

For the experiment two sample vials (with Teflon faced cap, 8ml) and one centrifuge tube (culture tube with Teflon faced cap, 12 ml) were needed for each sample. Furthermore, three additional reference vials (A, B, C; same type as sample vials) were required.

Before start of procedure one sample vial per specimen was labelled and weighed after 30 minutes of evacuation in an exsiccator, in order to obtain the tare weight. These vials were later used for gravimetric determination of lipids and therefore named “lipid vials”. The reference vials were treated the same way like the lipid vials.

As first step, the tissue grinder, which contained the powder of the respective sample, was rinsed three times with 1ml dichloromethane : methanol-solution (2:1; v:v). The resulting mixture was collected in an 8 ml sample vial after every rinsing. Afterwards the vials were shaken for one minute and were then treated by ultrasound (Bandelin SONOPLUS ultrasonic homogenizer HD 2070, Germany) for efficient cell disruption (30 seconds, cycle 3, 30%). The solid parts were allowed to settle down (by waiting for 30 seconds) before the dichloromethane : methanol-mixture was transferred to a centrifuge tube by using a glass Pasteur pipette (115 mm). Then another 3 ml of dichloromethane : methanol-

solution was added to the respective sample vial and the vial was shaken again for 1minute and the procedure was repeated accordingly.

The centrifuge tube, now containing 6ml of dichloromethane : methanol-mixture, was treated further by adding 1.5 ml of potassium chloride solution (0.88%). After 30 seconds of shaking, the sample was centrifuged for 10 minutes at 2°C and 2500 rpm (Sigma 6K15 centrifuge, United Kingdom). Three phases developed during centrifugation: an upper clear, colourless aqueous-phase and a lower clear, orange lipid-phase that were separated by a thin layer of solid particles. The aqueous-phase was discarded by using a Pasteur pipette (115 mm). By using a 230 mm Pasteur pipette the lower lipid-phase was carefully picked up and transferred to a lipid vial.

For the last step the lipid vials were clamped to an analytical nitrogen evaporator (N-EVAP™ 112, USA) to let the lipid phase evaporate by a continuous nitrogen flow.

After evaporation, the lipid vials (as well as the reference vials) were again evacuated for 30 minutes in an exsiccator before they were weighed on the semi-microbalance.

The amount of lipid was determined by subtracting the initial tare weight of the respective vial from the weight gained of the last weighing step. The procedure of the reference vials served as a measurement for a possible drift of the balance. The variation, as an average loss or respectively average gain in weight, had to be added or subtracted accordingly.

For later comparison the analysed lipid content was referred to the percent to the weight of powder, which was applied to the measurement.

Determination of proteins according to Lowry (1951)

For this analysis, 1ml of sodium hydroxide solution (1 M NaOH in Milli-Q water) was added to an Eppendorf cup (1.5 ml) containing about 1 mg of krill powder (weighed on laboratory microbalance “Sartorius LA 230 S”, Germany; d=0.1 mg). Then the Eppendorf cups were sealed with Parafilm to avoid spilling sample, and homogenized (3x5 seconds with 5 seconds break at 35% output) in an ice bath by using an ultrasound sonotrode (BRANSON SONIFIER® cell disruptor B15, Germany). Afterwards the samples were first incubated for about 2 hours at 63°C and 300 rpm in a laboratory mixer (Thermomixer comfort, Eppendorf, Germany) and after that centrifuged for 10 minutes at room temperature at 10 000 g (Eppendorf centrifuge 5417R, Germany). The supernatant was taken and stored in a clean Eppendorf cup to avoid resuspension of the pellet until measurement.

The analysis was carried out photometrically (UV/VIS Spectrometer Lambda2, Perkin Elmer™ instruments, England). The samples were analysed in triplicates.

Bovine Serum Albumin solution (BSA; c=1mg ml^{-1}) was diluted 1:2 with sodium hydroxide solution (2 M NaOH in Milli-Q water) and then used as the standard solution for preparing the protein calibration curve. Standards were measured in duplicates. Volumes of 0, 12, 24, 36, 48 and 60 µl BSA were used for preparing the standard calibration series (always a certain amount of Milli-Q water was added to obtain the needed amount of 60 µl test volume).

The reaction tubes were prepared by mixing 60 µl of sample/standard, 60 µl of Reagent A (D_c Protein Assay; Bio-Rad, 500-0113) and 900 µl of Reagent B (D_c Protein Assay; Bio-Rad, 500-0114) in an Eppendorf cup (1.5 ml). Afterwards, the cups were incubated for 15 minutes at 25°C and 300 rpm in a laboratory mixer (Thermomixer comfort, Eppendorf, Germany). The absorbance was measured at a wavelength of 750 nm (Table 4).

The total protein content of each sample was calculated by means of the slope of the straight calibration line, which was achieved from the measurement of the standard calibration series.

Table 4: Pipetting scheme for protein analysis according to Lowry (1951)

	Standard series	Samples
BSA standard	0 – 60 µl	
Sample		60 µl
Milli- Q	ad. 60 µl	
Reagent A	60 µl	60 µl
Reagent B	900 µl	900 µl
Incubation	in laboratory mixer (15 minutes at 25°C and 300 rpm)	
Measurement	at wavelength of 750 nm	

For later comparison the analysed protein content was first referred to the initial volume of homogenate and then to the percentage of the defined weight of krill powder, which was applied to the measurement.

2.5.8 Intermoult period and growth increment

During each starvation experiment the first three days were particularly dedicated to collect parameters (as shortly mentioned in 2.4) on both the intermoult period (IMP) and the growth increment at moult (GI). The methodology was conducted according to Tarling et al. (2006).

For each of the experiments the IMP was calculated by following equation:

$$IMP = \frac{N \cdot d}{m}$$

IMP = Intermoult period in days
N = Specimens that survived this part of the experiment
d = Duration of experiment (3 days in this case)
m = Number of animals, which had moulted during the experiment

The GI was obtained by measuring the size of the right uropods of the moult and the associated animal. The measurement was done by using a dissecting microscope, which was connected to a computer by a camera (Leica DFC 295, Leica Microsystems; Germany). Pictures were taken and the uropod lengths were determined by use of a special photo software programme (Leica Application Suite, Germany; Figure 9).

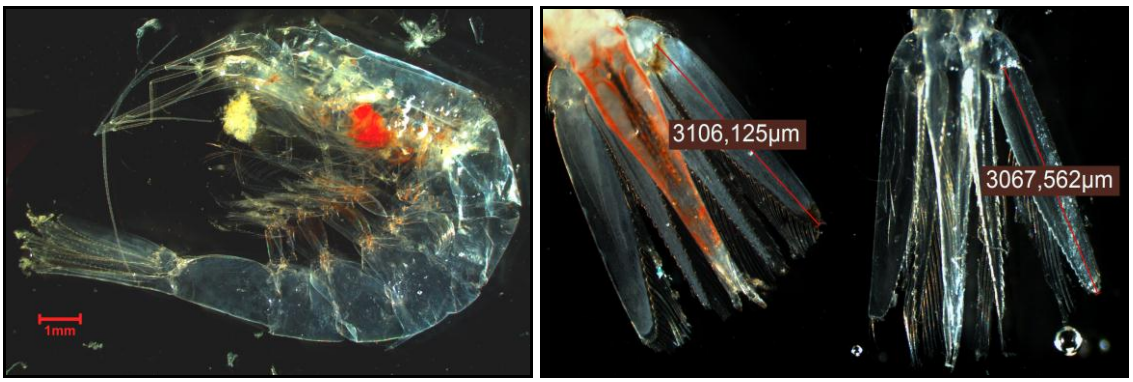

Figure 9: Analysing moults under a binocular. Left: Entire moult of an animal of about 20mm in size. Right: Example of measurement of uropod length of animal (left) and moult (right) by using the photo software.

By use of the following equation, the percentaged growth per moulted animal was described:

$$GI\ (\%) = \frac{U_a - U_m}{U_m} \cdot 100$$

GI (%) = Growth increment at moult described as percentaged growth
U_a = Uropod length of the animal
U_m = Uropod length of the associated moult

2.6 Data evaluation and statistical analysis

All collected data were primarily processed in Excel (Microsoft Office 2003). Further analysis and creation of graphs was conducted in GraphPad Prism5 (GraphPad Software, Inc., USA). All tables were created in Word (Microsoft Office 2003).

The maps were created by the use of Ocean Data View (ODV 4 software package, provided by Alfred Wegener Institute for Polar and Marine Research).

Mathematical equations were constructed by using Math Type 6 (Equation editor, Design Science, Inc. Company, USA).

For statistical analysis a t-test or a one-way analysis of variance (one-way ANOVA) with Tukey's multiple comparison post hoc test was performed in order to test for significant differences of the data collected. The significance level was generally set at $p < 0.05$.

3 Results

The following chapter contains the results of the analyses that were conducted on the three Euphausiid species. The chapter is divided in two parts. The first part deals with the results concerning the comparison of the species, the second part with the results that were received from the starvation experiments with *Euphausia hanseni.*

3.1 Inter-species comparison

Animals investigated were caught at different stations. *Nyctiphanes capensis* was only found at station T6-4. For technical reasons on board, *E. hanseni* from Benguela upwelling was only analysed at experimental temperatures of 5°C, 10°C and 15°C. *N. capensis* was analysed at 18°C instead of 20°C, because of the same reason.

Results of the metabolic parameters respiration rate, excretion rate, atomic O:N-ratio, CS-activity as well as atomic C:N-ratio are described and compared among specimens of *E. hanseni* (Benguela)*, E. hanseni* (Angola), *Nematoscelis megalops* and *N. capensis*.

3.1.1 Morphometric data

Table 5 gives an overview of the morphometrics situation of animals analysed in each Euphausiid species. A more detailed summary, in which specimens are separated into analysed temperatures, is given in appendix (9.3).

Among all species, *N. capensis* was the smallest and lightest having also the highest amount of juveniles of 86% among all tested animals. *E. hanseni* was the biggest and heaviest species. Specimens out of the Benguela upwelling were in average almost twice as big in length than *N. capensis* and more than 10 times heavier. There was a significant difference (T-Test $p < 0.0001$) among the animals of *E. hanseni* coming from Benguela and Angola with regard to mean length and mean fresh weight. *E. hanseni* Angola specimens were in average 4.9 mm smaller and 44.7 mg lighter than specimens of *E. hanseni* Benguela.

The percentage of female specimens was predominating for both *E. hanseni* and *N. megalops* specimens.

Table 5: Length, weight and sex distributions of the three species investigated with related stations. The mean values ± standard deviations are given. Sex is given in % of juvenile (j), male (m) and female (f) specimens. The lengths were calculated by use of the regression parameters of each species' length-weight relation given in appendix (9.4-9.7).

Species	Station	Length [mm]	Fresh weight [mg]	Sex [%] j/m/f	n
E. hanseni Benguela	T8-1d, T7-1, T7-2	25.3 ± 1.4	115.8 ± 20.8	0/33/67	18
E. hanseni Angola	WKT2b/d, T1-3a, T1-3	21.4 ± 2.2	71.1 ± 12.1	0/21/79	24
N. megalops	T1-3, T1-3a, T5-2	16.0 ± 1.7	28.3 ± 8.4	8/25/67	36
N. capensis	T6-4	12.5 ± 0.9	10.2 ± 1.9	86/14/0	7

3.1.2 Respiration rates

Among the species, *E. hanseni* Benguela had the highest respiration rates at all experimental temperatures (Figure 10). Apart for 5°C, *N. megalops* had the lowest respiration rates.

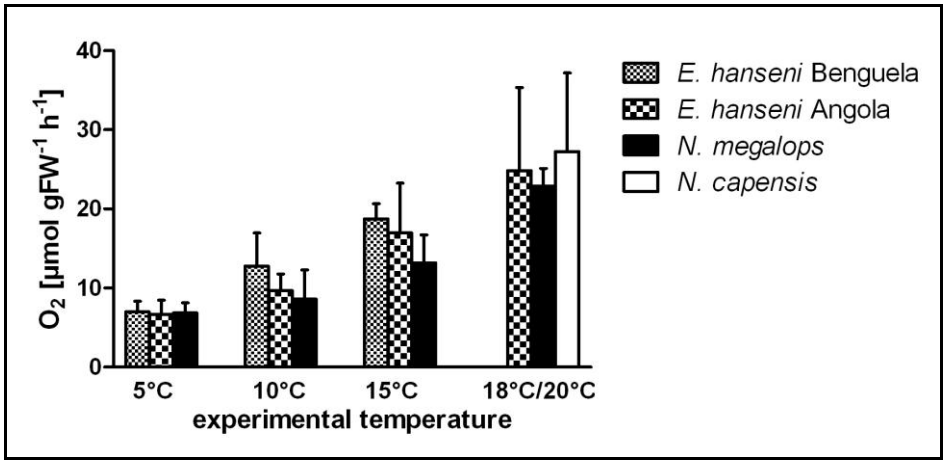

Figure 10: Respiration rates of each species at different experimental temperatures. The comparison of species in each temperature group is shown. Single groups were tested by one-way ANOVA. Values are given in means ± standard deviation. (n=3-11)

However, there were no significant differences between the species with respect to the different experimental temperatures.

Within the species the different experimental temperatures (respectively temperature increase) resulted in significant differences (Table 6). In all species, an increase of temperature by 10°C (from 5°C to 15°C) leaded to an increase of mean respiration rates by a factor of about 2. For E*. hanseni* this factor was even higher than 2 by comparing the mean respiration rates. For example the respiration rates of *E. hanseni* Angola were increasing from a mean value of 6.7 µmol O_2 gFW^{-1} h^{-1} to 17 µmol O_2 gFW^{-1} h^{-1}, which is equivalent to an increase by a factor of 2.54. The mean respiration rates of *N. megalops*, on the other hand, were increasing from 6.8 to 13.2 µmol O_2 gFW^{-1} h^{-1} by a smaller factor of 1.94.

Table 6: Average respiration rates ± standard deviation given in O_2 [µmol gFW^{-1} h^{-1}] for each species at different temperatures. In the outer right column the p-value for each row is given (tested by one-way ANOVA). Superscript letters indicate significant differences for each species' respiration rates with respect to changing temperature. (n=3-11)

		experimental temperature [°C]				
		5	**10**	**15**	**18/20**	**ANOVA**
species	***E. hanseni*** Benguela	7^{a} ± 1.3	$12.7^{a,b}$ ± 4.2	18.7^{b} ± 1.9		**0.0063**
	E. hanseni Angola	6.7^{a} ± 1.8	$9.6^{a,b}$ ± 2.1	$17^{a,b}$ ± 6.3	24.8^{b} ± 10.5	**0.0283**
	N. megalops	6.8^{a} ± 1.3	8.6^{a} ± 3.7	13.2^{b} ± 3.5	22.9^{c} ± 2.3	**<0.0001**
	N. capensis				27.2 ± 9.9	

The temperature effect on the respiration rates including standard deviations is plotted in Figure 11. The relationship between temperature increase and respiration rate of each species was best described by exponential regression. Analysis of covariance indicated no significant differences between the three regression lines (p=0.8944).

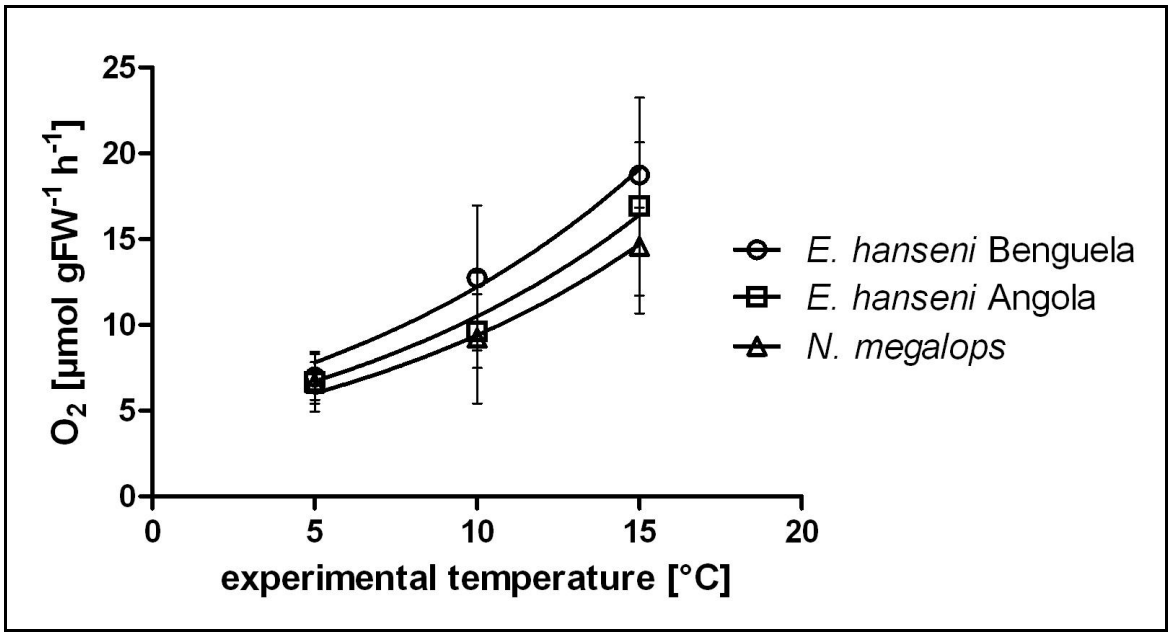

Figure 11: Graphical presentation of regression lines. Analysed were the respiration rates at three different experimental temperatures for *E. hanseni* Benguela, *E. hanseni* Angola and *N. megalops*. Given are the means ± standard deviations.

Parameters of each regression are listed in Table 7. Q_{10}-values ranged from 2.29 for *N. megalops* to 2.44 for *E. hanseni* Benguela and 2.70 for *E. hanseni* Angola.

Table 7: Regression parameters of exponential regressions given in Figure 11. Outer column gives the calculated Q_{10}-values for each species.

	exponential regression			
	Y_0	K	r^2	**Q_{10}**
***E. hanseni* Benguela**	5.00	0.09	0.66	**2.44**
***E. hanseni* Angola**	3.80	0.10	0.63	**2.70**
N. megalops	4.20	0.08	0.60	**2.29**

3.1.3 Excretion rates

In the comparison of species, *E. hanseni* Benguela had the highest mean ammonium excretion rate in all experimental temperature groups. There were significant differences compared to *E. hanseni* Angola and *N. megalops* (Figure 12). The mean rates were more than twice as high compared to *E. hanseni* Angola and up to seven times as high compared to N. megalops (at 5°C temperature group).

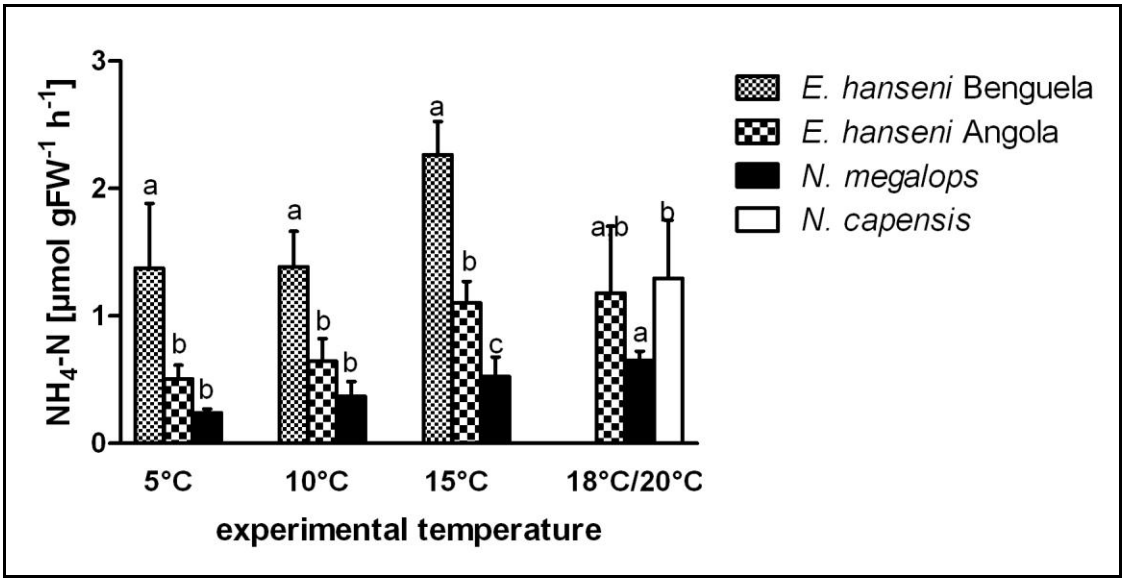

Figure 12: Excretion rates of each species at different experimental temperatures. The comparison of species in each temperature group is shown. Single groups were tested by one-way ANOVA. Letters indicate significant differences between species in each temperature group. Values are given in means ± standard deviation. (n=3-12)

The mean excretion rate of *E. hanseni* Angola was between the mean rates of *E. hanseni* Benguela and *N. megalops*, with significant differences compared to *N. megalops* at 15°C temperature group. At 18°C/20°C temperature group, no significant difference was indicated with respect to mean excretion rates of *E. hanseni* Angola and *N. capensis* (Figure 12), but in comparison to *N. megalops* the mean excretion rates of *N. capensis* differed significantly.

As given in Table 8, the increasing experimental temperature resulted in an increase of mean excretion rates in each species. Mean excretion rates almost doubled with an increase in temperature of 10°C in all specimens. There were significant differences among the temperature groups with respect to mean excretion rates for *E. hanseni* Benguela and *N. megalops*.

Table 8: Average excretion rates ± standard deviation given in NH_4-N [µmol gFW^{-1} h^{-1}] for each species at different temperatures. In the outer right column the p-value for each row is given (tested by one-way ANOVA). Superscript letters indicate significant differences for each species' excretion rates with respect to changing temperature. (n=3-12)

		experimental temperature [°C]				
		5	**10**	**15**	**18/20**	**ANOVA**
species	***E. hanseni*** Benguela	1.4^{a} ± 0.5	1.4^{a} ± 0.3	2.3^{b} ± 0.3		**0.0120**
	E. hanseni Angola	0.5 ± 0.1	0.6 ± 0.2	1.1 ± 0.2	1.2 ± 0.5	0.0740
	N. megalops	0.2^{a} ± 0.03	0.4^{a} ± 0.1	$0.5^{b,c}$ ± 0.2	0.6^{c} ± 0.1	**<0.0001**
	N. capensis				1.3 ± 0.5	

3.1.4 Atomic O:N-ratio

The atomic O:N-ratio of each species with respect to the different experimental temperatures is given in Figure 13.

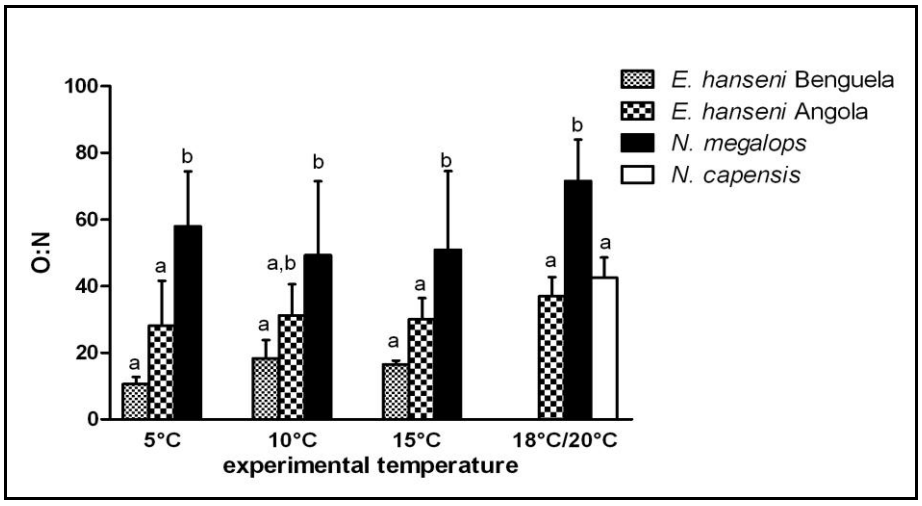

Figure 13: Atomic O:N-ratio of each species at different experimental temperatures. The comparison of species in each temperature group is shown. Single groups were tested by one-way ANOVA. Letters indicate significant differences between species in each temperature group. Values are given in means ± standard deviation. (n=3-13)

Among all species, *N. megalops* had the highest ratio with significant differences compared to the O:N-ratios of *E. hanseni* (exception *E. hanseni* Angola in 10°C temperature group) and of *N. capensis*. The mean values ranged from 49.3 to 71.5. On average, *E. hanseni* Benguela had the lowest O:N-ratio with mean values from 10.6 to 16.6. Compared to these, mean O:N-ratios of *E. hanseni* Angola specimens were higher with values from 28.1 to 37.

At different temperatures the atomic O:N-ratio for each species was relatively constant as seen in Table 9. There were no significant differences in the O:N-ratio of each species with respect to experimental temperature.

Table 9: Average atomic O:N-ratio ± standard deviation for each species at different temperatures. In the outer right column the p-value for each row is given (tested by one-way ANOVA). (n=3-13)

		experimental temperature [°C]				
		5	**10**	**15**	**18/20**	**ANOVA**
species	***E. hanseni*** Benguela	10.6 ± 2.1	18.4 ± 5.5	16.6 ± 1.1		0.0793
	E. hanseni Angola	28.1 ± 13.5	31.3 ± 9.3	30.1 ± 6.3	37 ± 5.8	0.6019
	N. megalops	57.9 ± 16.5	49.3 ± 22.2	50.9 ± 23.6	71.5 ± 12.4	0.1719
	N. capensis				42.6 ± 6.0	

3.1.5 CS activity

As shown in Figure 14, the comparison of species resulted in significant differences in the temperature groups of 5°C and 10°C. The CS activity of *N. megalops* was significantly lower than in *E. hanseni* specimens; the CS activity of *E. hanseni* Angola was significantly higher than in *E. hanseni* Benguela and in *N. megalops*.

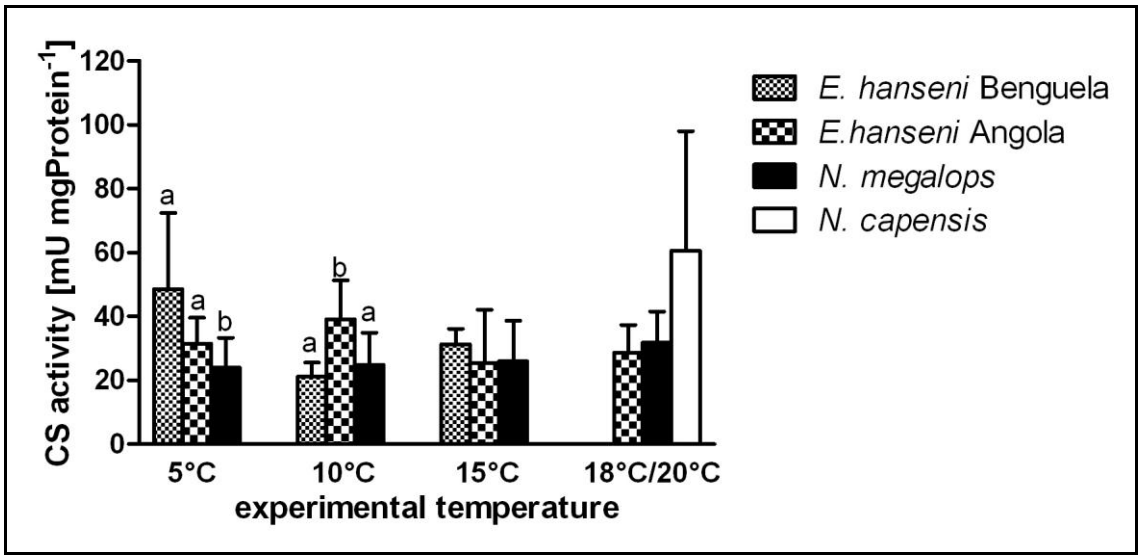

Figure 14: CS activity in second abdominal segment of each species at different experimental temperatures. The comparison of species in each temperature group is shown. Single groups were tested by one-way ANOVA. Letters indicate significant differences between species in each temperature group. Values are given in means ± standard deviation. (n=6)

Nevertheless, no patterns of differences among the species could be realized. Specific enzyme activities per gram fresh weight were also calculated for better comparison of past and future data (Table 10). Generally, in between species no significant differences of CS activity, measured at 25°C, were found, although *N. megalops* indicated a tendency of having higher activity per gram fresh weight.

Also within each species, the temperature effect had no significant influence on specific enzyme activity, measured at the standard temperature 25°C (Table 10). Values varied from 21.2 mU mgProtein^{-1} in *E. hanseni* Benguela to about 60 mU mgProtein^{-1} in *N. capensis* (with relatively high standard deviations).

Table 10: Mean specific enzyme activity ± standard deviation (n=6) given in $CS_{activity}$ [mU $mgProtein^{-1}$] for each species at different temperatures. In the outer right column the p-value for each row is given (tested by one-way ANOVA). Superscript letters indicate significant differences for each species' excretion rates with respect to temperature. Numbers in brackets give the enzyme activity in U gFW^{-1}.

		experimental temperature [°C]				
		5	**10**	**15**	**18/20**	**ANOVA**
species	***E. hanseni*** Benguela	48.5 ± 23.9 (3.8±1.3)	21.2 ± 4.3 (2.9±0.5)	31.3 ± 4.8 (4.1±1.4)		0.1541
	E. hanseni Angola	31.5 ± 8.2 (4.0±0.5)	39.2 ± 12.2 (3.2±0.7)	25.4 ± 16.7 (3.6±0.7)	28.7 ± 8.7 (3.4±0.8)	0.3699
	N. megalops	24.1 ± 9.3 (3.3±1.8)	24.8 ± 10.1 (5.5±2.0)	25.9 ± 12.7 (5.4±2.3)	31.9 ± 9.7 (5.1±0.6)	0.5712
	N. capensis				60.7 ± 37.4 (6.9±5.0)	

3.1.6 Elemental composition

The elemental composition, with respect to mean atomic C:N-ratio in each species, varied from lowest ratio of 4.1 in *E. hanseni* Angola to 4.4 in *N. capensis*. The values were grouped around 4.2 as shown in Figure 15. There was no significant difference of the mean C:N-ratio between *E. hanseni* and *N. megalops* in the first three temperature groups. In 18°C/20°C temperature group, *E. hanseni* differs significantly from *N. megalops* and *N. capensis*.

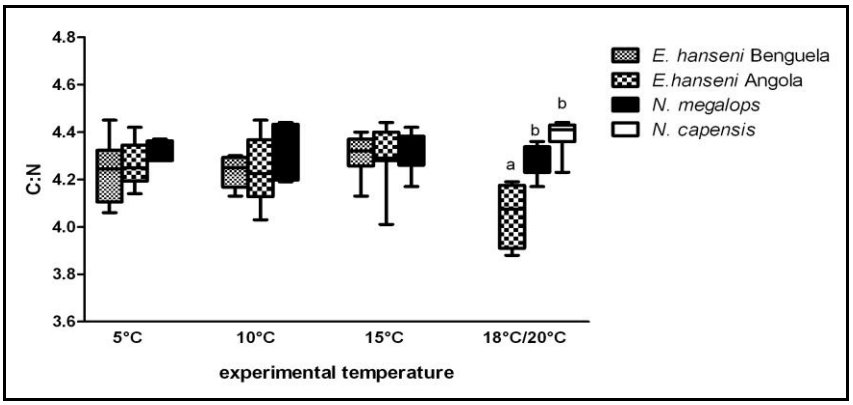

Figure 15: Atomic C:N-ratio of each species at different experimental temperatures. Presented is the comparison of species in each temperature group. Single groups were tested by one-way ANOVA. Letters indicate significant differences between species in each temperature group. Values are given in means ± standard deviation. (n=6-7)

The mean C:N-ratio also differed significantly within the experimental temperatures of the experiments that were conducted on *E. hanseni* Angola (Table 11), while generally no difference was found in the other specimens.

Table 11: Average atomic C:N-ratio ± standard deviation for each species at different temperatures. In the outer right column the p-value for each row is given (tested by one-way ANOVA). Superscript letters indicate significant differences for each species' atomic O:N-ratio with respect to changing temperature. (n=6-7)

		experimental temperature [°C]				
		5	**10**	**15**	**18/20**	**ANOVA**
species	***E. hanseni*** Benguela	4.2 ± 0.14	4.2 ± 0.07	4.3 ± 0.09		0.4042
	E. hanseni Angola	4.3^{a} ± 0.10	$4.2^{a,b}$ ± 0.15	4.3^{a} ± 0.14	4.1^{b} ± 0.13	**0.0157**
	N. megalops	4.3 ± 0.04	4.3 ± 0.11	4.3 ± 0.09	4.3 ± 0.07	0.8027
	N. capensis				4.4 ± 0.07	

3.2 Short-term starvation of *Euphausia hanseni*

Animals investigated were caught at different stations. For technically reasons on board, the starvation experiment on *E. hanseni* Angola at 5°C was only carried out until the end of the sixth day of starvation.

In this part of the results, the metabolic parameters respiration rate, excretion rate, atomic O:N-ratio, CS-activity and –kinetics as well as atomic C:N-ratio and proximate biochemical composition are described with regard to short-term starvation at different experimental temperatures and compared among specimens of *E. hanseni* Benguela and *E. hanseni* Angola. Additionally, results on intermoult period and growth increment are given for each group of animals maintained at different experimental temperatures.

3.2.1 Morphometric data

Table 12 provides an overview of the morphometric composition of each starvation experiment conducted on both *E. hanseni* Benguela and *E. hanseni* Angola.

Table 12: Length, weight and sex distributions of *E. hanseni* divided into region and single starvation experiments with related stations. The mean values ± standard deviations are given. Sex is given in % of juvenile (j), male (m) and female (f) specimens. The lengths were calculated by use of the regression parameters of each region's length-weight relation given in appendix (9.4 and 9.5).

Species	exp. T. [°C]	Station	Length [mm]	Fresh weight [mg]	Sex [%] j/m/f	n
E. hanseni Benguela	5	L2, WLT4,T8-1d	24.9 ± 2.2	113.4 ± 29.9	0/45/55	29
	10	T7-2	23.6 ± 1.4	93.3 ± 18.9	0/55/45	38
	15	T8-1d, T7-1	23.0 ± 1.3	87.0 ± 14.2	0/71/29	45
E. hanseni Angola	5	WKT2b/d	22.2 ± 2.0	78.8 ± 21.1	0/22/78	18
	10	WKT2b/d	21.9 ± 2.1	76.2 ± 21.3	0/25/75	24
	15	T1-3a	20.6 ± 1.8	65.0 ± 16.5	0/62/38	24

Among both regions, *E. hanseni* Benguela was on average greater in length and heavier in weight than *E. hanseni* Angola. When comparing the temperature experiments of each specimen group, the differences in both parameters were highly significant (T-Test p <0.0001). Also within the starvation experiments, which were conducted on *E. hanseni* Benguela, differences in size and fresh weight were found with regard to larger size and greater weight of specimen from the 5°C experiment compared to the 10°C and 15°C experiments (one-way ANOVA p <0.0001). *E. hanseni* Angola only showed differences in the length distribution between 5°C and 15°C of the starvation experiments. Here the specimen in 5°C were significantly greater in mean length (one-way ANOVA p=0.0183).
In experiments on *E. hanseni* Benguela, the proportion of female and male specimen was almost equal at the 5°C and 10°C maintenance temperature, but was dominated by 71% of male specimens in the 15°C experiment. For *E. hanseni* Angola, the starvation experiments of 5°C and 10°C were dominated by females and the 15°C starvation experiment by 62% males.

3.2.2 Respiration

On both specimens of *E. hanseni* Benguela and specimens of *E. hanseni* Angola the different maintenance temperatures resulted in different respiration rates with higher rates in 15°C starvation experiments at almost all days of starvation (Figure 16). Results were significant in both regions.

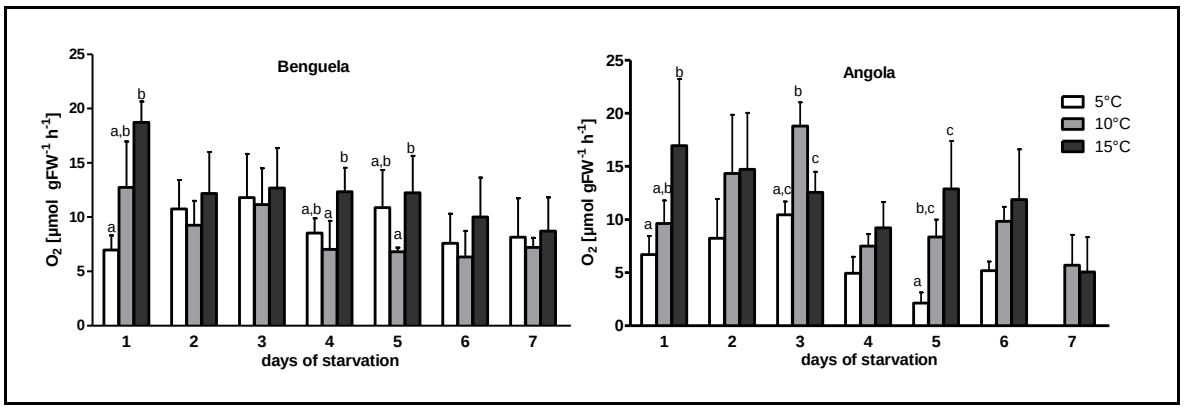

Figure 16: Respiration rates of specimens from each region sampled on different days of starvation. Given are the means ± standard deviations. Each graph gives the comparison of the different experimental temperatures grouped by days of starvation. Single groups were tested by one-way ANOVA. Letters indicate significant differences between species in each starvation group. (n=3-10)

Respiration rates measured on specimens of *E. hanseni* Benguela from 5°C and 10°C starvation experiment showed no significant differences, while the same experiments

conducted on *E. hanseni* Angola resulted in little lower oxygen consumption rates at 5°C compared to the 10°C experiment.

In Figure 16, all starvation experiments show a decreasing tendency of mean oxygen consumption rates with respect to starvation period. The only exceptions were specimens from 5°C Benguela experiment. In all other experiments, respiration rates were higher at the beginning of starvation and were reduced until the last starvation days. The starvation effect on mean respiration rate was significantly for E*. hanseni* Benguela from the 10°C and 15°C experiments and for E. hanseni Angola for the 5°C and 10°C experiments (Table 13).

Table 13: Mean respiration rates ± standard deviation for specimens of each region with respect to different experimental temperatures and starvation period. Values are given in O_2 [µmol gFW^{-1} h^{-1}]. Significant differences (in bold) with regard to days of starvation are given in column next to each temperature (tested by one-way ANOVA). Superscript letters indicate significant differences within the period of starvation. Rows below each temperature group present statistical comparison of regions (T-Test). (n=3-16)

		days of starvation							
		1	**2**	**3**	**4**	**5**	**6**	**7**	**p**
5°C	*E. hanseni* **Benguela**	7.0 ± 1.3	10.7 ± 2.7	11.8 ± 4.0	8.5 ± 1.4	10.9 ± 3.5	7.6 ± 2.7	8.2 ± 3.6	0.2837
	E. hanseni **Angola**	$6.7^{a,b,c}$ ± 1.8	$8.2^{a,b}$ ± 3.7	10.4^{a} ± 1.3	$4.9^{b,c}$ ± 1.6	2.1^{c} ± 1.0	$5.2^{a,b,c}$ ± 0.9		**0.0036**
	T-Test	n.s.	n.s.	n.s.	**0.0394**	**0.0043**	n.s.		
10°C	*E. hanseni* **Benguela**	12.7^{a} ± 4.2	$9.3^{a,b}$ ± 2.3	$11.2^{a,b}$ ± 3.3	$7.0^{a,b}$ ± 2.6	6.8^{b} ± 0.4	6.3^{b} ± 2.4	7.2^{b} ± 0.9	**0.0017**
	E. hanseni **Angola**	$9.6^{a.c.d}$ ± 2.1	$14.3^{a,b,c}$ ± 5.5	18.8^{b} ± 2.3	$7.5^{c,d}$ ± 1.1	$8.4^{c,d}$ ± 1.6	$9.8^{c,d}$ ± 1.4	5.7^{d} ± 2.8	**0.0003**
	T-Test	n.s.	n.s.	**0.0098**	n.s.	n.s.	n.s.	n.s.	
15°C	*E. hanseni* **Benguela**	18.7^{a} ± 1.9	$12.2^{a,b}$ ± 3.8	$12.7^{a,b}$ ± 3.7	$12.3^{a,b}$ ± 2.2	$12.2^{a,b}$ ± 3.4	10.0^{b} ± 3.7	8.7^{b} ± 3.1	**0.0026**
	E. hanseni **Angola**	17.0 ± 6.3	14.7 ± 5.3	12.6 ± 1.9	9.2 ± 2.5	12.9 ± 4.5	11.9 ± 4.7	5.1 ± 3.3	0.0790
	T-Test	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	

In the comparison of regions there mostly was no significant difference among specimens of *E. hanseni* with regard to days of starvation within all experimental temperatures.

Figure 17 illustrates the effect of starvation in each starvation experiment separated in *E. hanseni* Benguela and *E. hanseni* Angola. As mentioned before there was no evident difference within both regions. Analysis of regression showed negative slopes in all starvation experiments (Table 14). An effect of starvation was not indicated in the 5°C starvation experiment of *E. hanseni* Benguela, as the slope was not significantly different from zero.

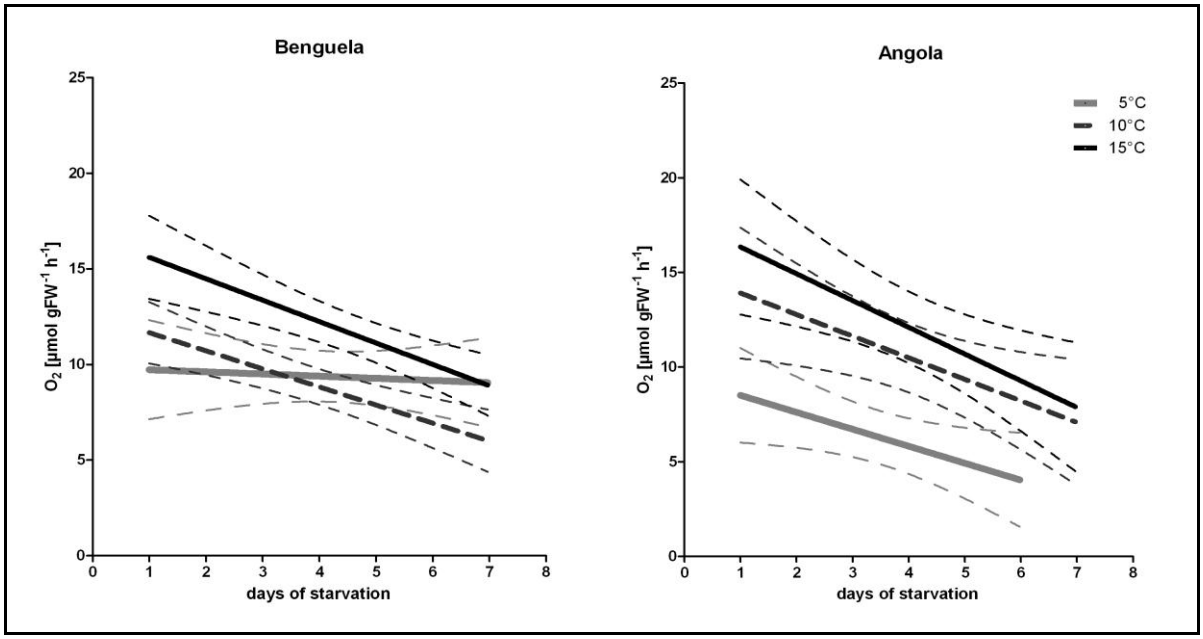

Figure 17: Linear regressions with confidence intervals. Given are the trends of the respiration rates with respect to starvation at different temperatures. Graphs show trends for both *E. hanseni* Benguela (left) and *E. hanseni* Angola (right).

From the regression parameters given in Table 14, the percentage of decrease in mean oxygen consumption was calculated of all experiments that indicated an effect of starvation.

Table 14: Regression parameters of linear regressions given in Figure 17. Parameters are presented for all temperature experiments that were conducted on specimens from respective regions. The outer column gives the percentaged decrease of the respiration rate from first to last day of starvation (calculated from regression parameters).

species	Exp. T. [°C]	regression parameter a	b	r^2	p	ΔO_2 [%]
E. hanseni	**5**	9.842	-0.1124	0.0048	0.7413	
Benguela	**10**	12.61	-0.9446	0.3505	**0.0001**	**49**
	15	16.72	-1,119	0.3111	**0.0001**	**43**
E. hanseni	**5**	9.405	-0.8953	0.2490	**0.0350**	**63**
Angola	**10**	15.05	-1.139	0.2289	**0.0209**	**49**
	15	17.75	-1.413	0.3001	**0.0068**	**52**

Accordingly, specimens of *E. hanseni* Angola reacted more strongly to starvation (decrease in oxygen consumption from 49% to 63% during short-term starvation) than specimens of *E. hanseni* Benguela (43% to 49% lower respiration rate with respect to first and last day of starvation).

3.2.3 Excretion

In the comparison of regions, the excretion rate of specimens was highest in the 15°C starvation experiment during most of the starvation period. In both *E. hanseni* Benguela and *E. hanseni* Angola this effect was mostly significant (Figure 18). The lowest excretion rates were primary analysed on animals out of the 5°C starvation experiments.

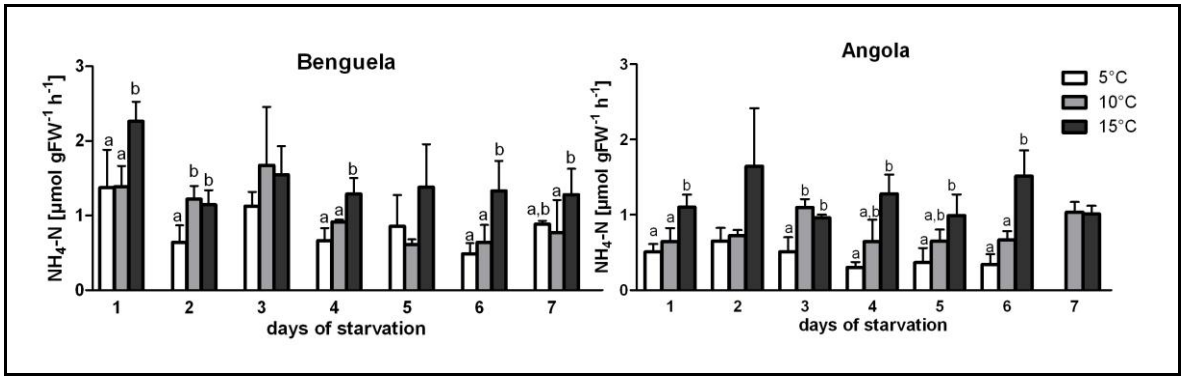

Figure 18: Excretion rates of specimens from each region sampled on different days of starvation. Given are the means ± standard deviations. Each graph gives the comparison of the different experimental temperatures grouped by days of starvation. Single groups were tested by one-way ANOVA. Letters indicate significant differences between species in each starvation group. (n=3-12)

Obviously the effect of starvation resulted in a decrease of excretion rate over time in *E. hanseni* Benguela at all experimental temperatures when comparing the first and the last days of starvation. There were significant differences among excretion rates of specimens (Table 15). In contrast, *E. hanseni* Angola showed no change in excretion rates over the period of starvation. Although there was a significant difference among means of excretion rates in the 10°C starvation experiment, the rates did not show a clear trend from the first to the last day of starvation. Comparing single starvation experiments with regard to experimental temperature and regions, specimens of *E. hanseni* Angola had a lower excretion compared to *E. hanseni* Benguela with significant values in all temperature groups (Table 15).

Table 15: Mean excretion rates ± standard deviation for specimens of each region with respect to experimental temperatures and starvation periods. Values are given in NH_4-N [µmol gFW^{-1} h^{-1}]. Significant differences (in bold) with regard to days of starvation are given in column next to each temperature (tested by one-way ANOVA). Superscript letters indicate significant differences within the period of starvation. Rows below each temperature group present statistical comparison of regions (T-Test). (n=3-12)

		days of starvation							
		1	**2**	**3**	**4**	**5**	**6**	**7**	**p**
5°C	*E. hanseni* **Benguela**	1.38^{a} ± 0.51	$0.6^{a,b}$ ± 2.7	$1.1^{a,b}$ ± 4.0	$0.7^{a,b}$ ± 1.4	$0.9^{a,b}$ ± 0.4	0.5^{b} ± 0.1	$0.9^{a,b}$ ± 0.05	**0.0252**
	E. hanseni **Angola**	0.5 ± 0.1	0.7 ± 0.2	0.5 ± 0.2	0.3 ± 0.1	0.4 ± 0.2	0.3 ± 0.1		0.1109
	T-Test	**0.0449**	n.s.	**0.0170**	**0.0247**	n.s.	n.s.		
10°C	*E. hanseni* **Benguela**	$1.4^{a,c}$ ± 0.3	$1.2^{a,b,c}$ ± 0.2	1.7^{a} ± 0.8	$0.9^{a,b,c}$ ± 0.03	0.6^{c} ± 0.1	0.6^{b} ± 0.2	0.8^{c} ± 0.4	**0.0005**
	E. hanseni **Angola**	$0.6^{a,c}$ ± 0.2	$0.7^{a,b,c}$ ± 0.1	1.1^{b} ± 0.1	0.6^{c} ± 0.3	0.7^{c} ± 0.1	$0.7^{a,b,c}$ ± 0.1	$1.0^{a,b,c}$ ± 0.1	**0.0064**
	T-Test	**0.0043**	**0.0023**	n.s.	n.s.	n.s.	n.s.	n.s.	
15°C	*E. hanseni* **Benguela**	2.3^{a} ± 0.3	1.2^{b} ± 0.2	$1.6^{a,b}$ ± 0.4	1.3^{b} ± 0.2	1.4^{b} ± 0.4	1.3^{b} ± 0.4	1.3^{b} ± 0.3	**0.0055**
	E. hanseni **Angola**	1.1 ± 0.2	1.6 ± 0.8	1.0 ± 0.04	1.3 ± 0.3	1.0 ± 0.3	1.5 ± 0.3	1.0 ± 0.1	0.1455
	T-Test	**0.0030**	n.s.	**0.0409**	n.s.	n.s.	n.s.	n.s.	

Figure 19 illustrates the effect of starvation in each starvation experiment in *E. hanseni* Benguela and *E. hanseni* Angola. As mentioned before there was an evident difference in both regions. Excretion rates of *E. hanseni* Angola were lower and the effect of starvation was stronger on *E. hanseni* Benguela as negative slopes of regression lines were higher than in *E. hanseni* Angola experiments (Table 16).

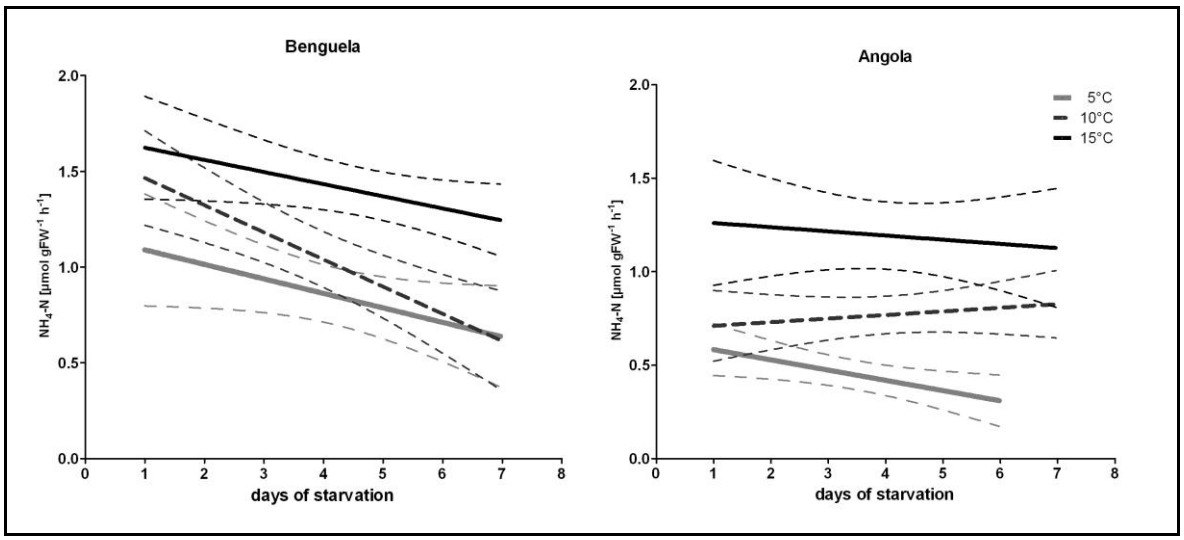

Figure 19: Linear regressions with confidence intervals. Given are the trends of the excretion rates with respect to starvation at different temperatures. Graphs show trends for both *E. hanseni* Benguela (left) and *E. hanseni* Angola (right).

In regression analysis the effect of starvation was only significant in the 10°C experiment of *E. hanseni* Benguela and the 5°C experiment of *E. hanseni* Angola. The calculated percentage change of ammonium excretion from the first to the last days of starvation resulted in a decrease of 58% and 56% in the two experiments (Table 16).

Table 16: Regression parameters of linear regressions given in Figure 19. Parameters are presented for all temperature experiments that were conducted on specimens from respective regions. The outer column gives the percentaged decrease of the excretion rate from first to last day of starvation (calculated from regression parameters).

species	Exp. T. [°C]	regression parameter a	b	r^2	p	ΔNH_4-N [%]
E. hanseni	**5**	1.165	-0.07564	0.1471	0.0584	
Benguela	**10**	1.607	-0.1418	0.3463	**0.0002**	**58**
	15	1.686	-0.06334	0.08838	0.0500	
E. hanseni	**5**	0.6378	-0.05476	0.2870	**0.0219**	**56**
Angola	**10**	0.6910	0.01934	0.02781	0.4469	
	15	1.283	-0.02229	0.01288	0.6150	

3.2.4 Atomic O:N-ratio

In both regions, at all experimental temperatures there was no difference in atomic O:N-ratio at defined days of starvation, with the only exception of *E. hanseni* Benguela. At the second and sixth day of the 5°C starvation experiment, specimens showed a significantly lower ratio than those of the 10°C and 15°C experiment (Figure 20).

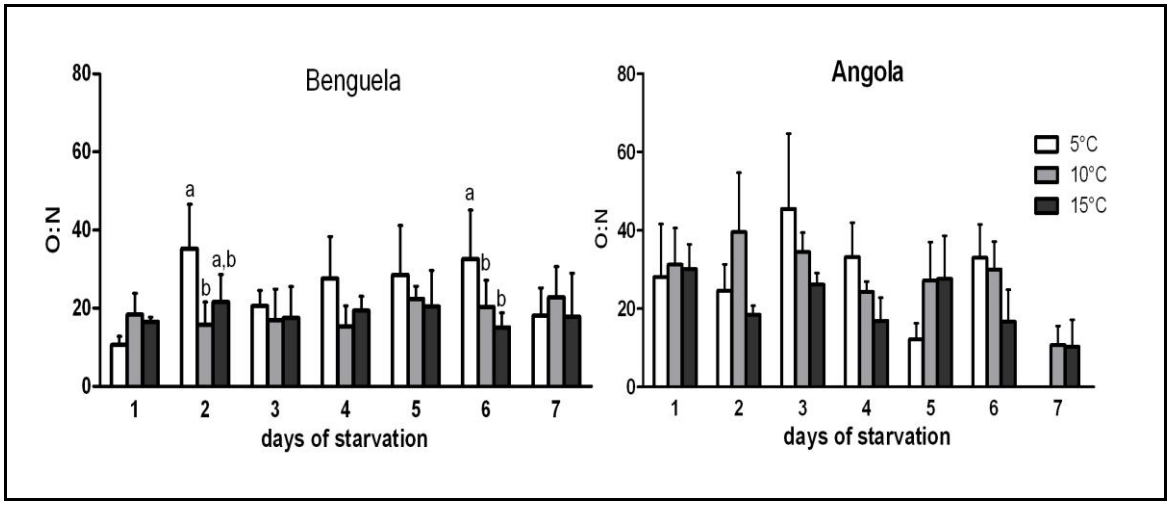

Figure 20: Atomic O:N-ratio of specimens from each region sampled on different days of starvation. Given are the means ± standard deviations. Each graph gives the comparison of the different experimental temperatures grouped by days of starvation. Single groups were tested by one-way ANOVA. Letters indicate significant differences between species in each starvation group. (n=3-12)

For *E. hanseni* Benguela, the atomic O:N-ratios were very variable and the effect of starvation showed no significant influence on the specimens. The atomic O:N-ratios were more or less equal. Compared to these, the effect of starvation showed a significant difference of O:N-ratios in *E. hanseni* Angola at experimental temperatures of 10°C and 15°C (Table 17). During starvation the atomic O:N-ratio decreased in both experiments.

Among the regions it is remarkable that at all experimental temperatures *E. hanseni* Angola showed significantly higher O:N-ratios in the first day of starvation compared to *E. hanseni* Benguela. The significant difference remained in the 10°C starvation experiment until the fourth day of starvation (Table 17).

Table 17: Mean atomic O:N-ratio ± standard deviation for specimens of each region with respect to different experimental temperatures and starvation periods. Significant differences (in bold) with regard to days of starvation are given in column next to each temperature (tested by one-way ANOVA). Superscript letters indicate significant differences within the period of starvation. Rows below each temperature group present statistical comparison of regions (T-Test). (n=3-12)

		days of starvation							
		1	**2**	**3**	**4**	**5**	**6**	**7**	**p**
5°C	*E. hanseni* **Benguela**	10.6 ± 2.1	35.3 ± 11.41	20.5 ± 4.0	27.5 ± 10.8	28.6 ± 12.6	32.6 ± 12.5	18.1 ± 7.0	0.0781
	E. hanseni **Angola**	28.1 ± 13.5	24.6 ± 6.7	45.5 ± 19.2	33.2 ± 8.7	12.1 ± 4.2	33.0 ± 8.5		0.0609
	T-Test	**0.0482**	n.s.	n.s.	n.s.	n.s.	n.s.		
10°C	*E. hanseni* **Benguela**	18.4 ± 5.5	15.8 ± 5.8	17.0 ± 8.0	15.3 ± 5.3	22.4 ± 3.3	20.3 ± 6.9	22.8 ± 7.8	0.4175
	E. hanseni **Angola**	$31.3^{a,b}$ ± 9.3	39.6^{a} ± 15.1	$34.4^{a,b}$ ± 5.0	$24.3^{a,b}$ ± 2.6	$27.2^{a,b}$ ± 9.9	$30.0^{a,b}$ ± 7.1	10.7^{b} ± 4.9	**0.0272**
	T-Test	**0.0315**	**0.0092**	**0.0150**	n.s.	n.s.	n.s.	n.s.	
15°C	*E. hanseni* **Benguela**	16.6 ± 1.1	21.5 ± 7.1	17.5 ± 8.1	19.4 ± 3.7	20.4 ± 9.3	15.1 3.7	17.8 ± 11.2	0.8330
	E. hanseni **Angola**	30.1 ± 6.3	18.4 ± 2.3	26.2 ± 2.9	16.9 ± 5.8	27.6 ± 11.1	16.7 ± 8.2	10.3 ± 6.9	**0.0451**
	T-Test	**0.0219**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	

3.2.5 Enzyme activity and enzyme kinetics: CS

Figure 21 gives an overview of specific citrate synthase activity with respect to different experimental temperature and starvation period. For *E. hanseni* Benguela means of CS activity from specimens from the different temperature experiments were significantly different within each starvation group (tested by one-way ANOVA). CS activity out of the 5°C starvation experiment was significantly higher than those of specimens maintained at 10°C and 15°C (p=0.0153). Tukey's multiple comparison test indicated no significant differences between temperatures tested on the last days of starvation, but means showed a significant difference (p=0.0476; see asterisk in Figure 21). Among experiments conducted on *E. hanseni* Angola significant differences between maintenance temperatures were found only in the last days of starvation. Specimens that were kept at 15°C showed significant lower CS activity compared to those kept at 5°C and 10°C.

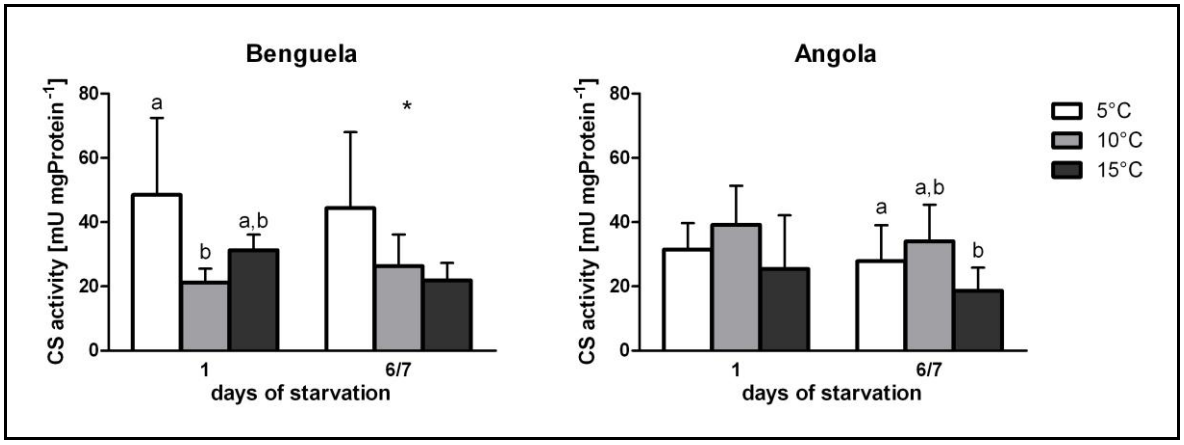

Figure 21: Specific enzyme activity (CS) of specimens from each region sampled on first and last days of starvation. Given are the means ± standard deviations. Each graph gives the comparison of the different experimental temperatures grouped by days of starvation. Single groups were tested by one-way ANOVA. Letters indicate significant differences between species in each starvation group. (n=6)

In all experiments, but the 10°C starvation experiment of *E. hanseni* Benguela, a decreasing trend in mean CS activity in relation to the period of starvation could be seen (Figure 21). For both *E. hanseni* Benguela and *E. hanseni* Angola, average CS activity was decreasing from the first to the last days of starvation with a statistically significant effect on animals of *E. hanseni* Benguela at the 15°C of starvation temperature (Table 18).

Table 18: Mean specific enzyme activity ± standard deviation given in $CS_{activity}$ [mU $mgProtein^{-1}$] for specimens of each region with respect to different experimental temperatures and starvation period. Numbers in brackets give the enzyme activity in U gFW^{-1}. Significant differences with regard to days of starvation are given in column next to each temperature (tested by T-Test). Bottom row presents statistical comparison of regions (T-Test). Significance is highlighted in bold. (n=6)

	days of starvation								
	5°C			10°C			15°C		
species	1	6/7	T-Test	1	6/7	T-Test	1	6/7	T-Test
E. hanseni **Benguela**	48.5 ± 24.9 (4.0±0.5)	44.4 ± 23.6 (4.5±0.1)	n.s.	21.2 ± 4.3 (2.9±0.8)	26.4 ± 9.8 (3.5±0.7)	n.s.	31.3 ± 4.8 (3.6±0.7)	21.8 ± 5.4 (3.2±0.8)	**0.0095**
E. hanseni **Angola**	31.5 ± 8.2 (3.8±1.0)	27.9 ± 11.1 (2.5±0.7)	n.s.	39.2 ± 12.2 (2.9±0.5)	34.0 ± 11.4 (3.8±0.8)	n.s.	25.4 ± 16.7 (4.1±1.4)	18.6 ± 7.2 (1.4±0.7)	n.s.
T-Test	n.s.	n.s.		**0.0068**	n.s.		n.s.	n.s.	

As given in Table 18, there was mainly no difference among specimens with regard to different region. Only in one case, the starvation experiment at 10°C, specimens of *E. hanseni* Angola differed significantly from those of *E. hanseni* Benguela.

Table 19: Mean Michaelis constant of CS ± standard deviation. Values are given in K_m[µmol Acetyl-CoA L^{-1}]. *E. hanseni* of the 10°C starvation experiments were exemplary analysed. The outer right column gives significances of each region with regard to days of starvation. Bottom row presents statistical comparison of regions. (n=6)

	days of starvation		
	1	6/7	T-Test
E. hanseni **Benguela**	16.16 ± 3.15	14.62 ± 4.6	n.s.
E. hanseni **Angola**	11.75 ± 3.13	9.35 ± 2.72	n.s.
T-Test	**0.0450**	**0.0361**	

As the CS activity was decreasing over the period of starvation, the enzyme efficiency of CS was increasing. From the first to the last days of starvation, the mean Michaelis

constant of CS was decreasing in animals of both regions (Table 19; Michealis-Menten saturation curves for both regions and days of starvations are presented in appendix 9.8).

Noteworthy was the significant difference between Michaelis constants of *E. hanseni* Benguela and *E. hanseni* Angola. The significantly lower values of *E. hanseni* Angola implied significant higher enzyme efficiency of CS compared to specimens from Benguela upwelling region.

3.2.6 Elemental and proximate biochemical composition

The change in elemental composition (atomic C:N-ratio) with respect to experimental temperature and starvation period is given in Figure 22.

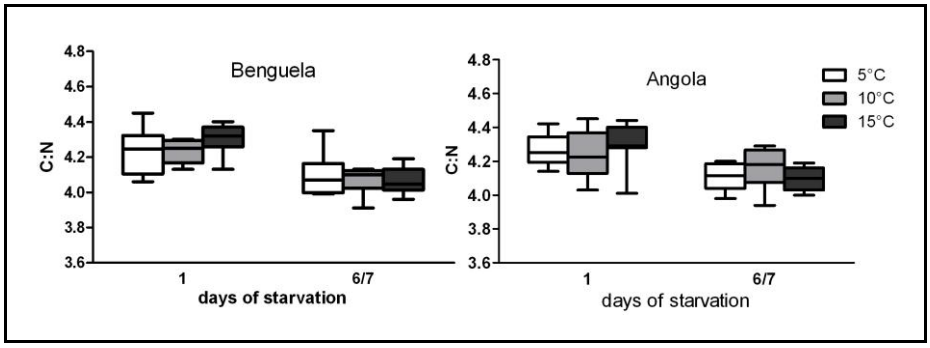

Figure 22: Atomic C:N-ratio of specimens from each region sampled on the first and the last days of starvation. Given are the means ± standard deviations. Each graph gives the comparison of the different experimental temperatures grouped by days of starvation. Single groups were tested by one-way ANOVA. Letters indicate significant differences between species in each starvation group. (n=6)

There were no significant differences between the atomic C:N-ratios of the different temperature experiments within the starvation groups in both *E. hanseni* Benguela and *E. hanseni* Angola. Noteworthy was the mostly significant decrease of atomic C:N-ratio in each experiment within the starvation period from the first to the last days of starvation (Table 20). In all experiments the atomic C:N-ratio declined by a value of 0.1 or even 0.2. The only exception was *E. hanseni* Angola in the 10°C starvation experiment. Here specimens from the first day of starvation exhibited no change in C:N-ratio compared to the last days of starvation.

Table 20: Average atomic C:N-ratio ± standard deviation for specimens of each region with respect to different experimental temperatures and starvation period. Significant differences with regard to days of starvation are given in column next to each temperature (tested by T-Test). Bottom row presents statistical comparison of regions. (n=6) Tables of body C and N are given in appendix (9.9 and 9.10)

	days of starvation								
	5°C			10°C			15°C		
species	1	6/7	T-Test	1	6/7	T-Test	1	6/7	T-Test
E. hanseni **Benguela**	4.2 ± 0.1	4.1 ± 0.1	n.s.	4.2 ± 0.1	4.1 ± 0.1	**0.0037**	4.3 ± 0.1	4.1 ± 0.1	**0.0007**
E. hanseni **Angola**	4.3 ± 0.1	4.1 ± 0.1	**0.0134**	4.2 ± 0.1	4.2 ± 0.1	n.s.	4.3 ± 0.1	4.1 ± 0.1	**0.0102**
T-Test	n.s.	n.s.		n.s.	n.s.		n.s.	n.s.	

The determination of mean total protein content in each group of animals maintained at 10°C showed no change with respect to the period of starvation. In between the regions specimens of *E. hanseni* Benguela had a slightly higher mean of total protein content compared to specimens of *E. hanseni* Angola. The difference was not significant (Table 21).

Table 21: Mean total protein content of cephalothorax and first abdominal segment ± standard deviation given in percent of dry weight. Exemplary tested on *E. hanseni* of 10°C starvation experiments. Outer right column gives significances of each region with regard to days of starvation. Bottom row presents statistical comparison of regions. (n=6)

	days of starvation		
	1	6/7	T-Test
E. hanseni **Benguela**	57.3 ± 4.8	58.5 ± 1.7	n.s.
E. hanseni **Angola**	56.1 ± 0.9	56.7 ± 2.0	n.s.
T-Test	n.s.	n.s.	

During starvation, animals of both regions showed a decrease in total lipid content on average. The differences from first to last days of starvation were visible but statistically not significant (Table 22). There was also no significant difference between both regions,

from which *E. hanseni* was caught. The total lipid content was mainly below 10% in all specimens analysed.

Table 22: Mean total lipid content of cephalothorax and first abdominal segment ± standard deviation given in percent of dry weight. Exemplary tested on *E. hanseni* of 10°C starvation experiments. Outer right column gives significances of each region with regard to days of starvation. Bottom row presents statistical comparison of regions (n=6).

	days of starvation		T-Test
	1	6/7	
E. hanseni **Benguela**	8.9 ± 2.0	7.9 ± 1.8	n.s.
E. hanseni **Angola**	8.7 ± 2.5	8.4 ± 2.3	n.s.
T-Test	n.s.	n.s.	

3.2.7 Intermoult period and growth increment

In both *E. hanseni* Benguela and *E. hanseni* Angola the different maintenance temperatures resulted in shorter periods between moults (Figure 23).

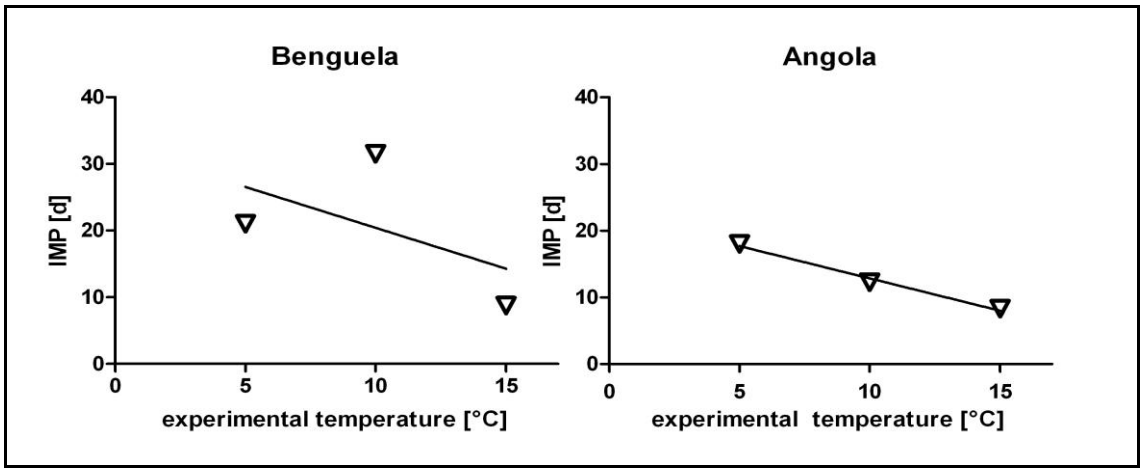

Figure 23: Intermoult period in days for each starvation experiment that was conducted at given experimental temperatures on *E. hanseni* Benguela and *E. hanseni* Angola. Linear regression lines show the relation between intermoult periods (triangles) at increasing temperature.

The decrease in intermoult period could be described by linear regression. Specimens of *E. hanseni* Benguela showed a deviation of the overall trend at the experimental

temperature of 10°C. Only few animals moulted in this experiment, which leaded to the observed high intermoult period. Nevertheless, the trend was equivalent to *E. hanseni* Angola. Parameters of regression are given in Table 23. The differences of IMP with an increase of experimental temperature from 5°C to 15°C calculated by regression factors confirmed the similar reaction with regard to duration of intermoult period. According to the calculation, the temperature increase of 10°C resulted in a reduction of intermoult period of 12 days for *E. hanseni* Benguela and of 10 days for *E. hanseni* Angola.

Table 23: Regression parameters of linear regressions given in Figure 23. The outer column gives change of the intermoult period with an increase of temperature from 5°C to 15°C (calculated from regression parameters). Both regions are compared.

species	regression parameter a	b	r^2	p	ΔIMP [d]
E. hanseni **Benguela**	32.67	-1.225	0.2894	0.6384	**12**
E. hanseni **Angola**	22.55	-0.9690	0.9890	0.0669	**10**

The measured growth increment was similar for both different maintenance temperatures and different regions (Figure 24).

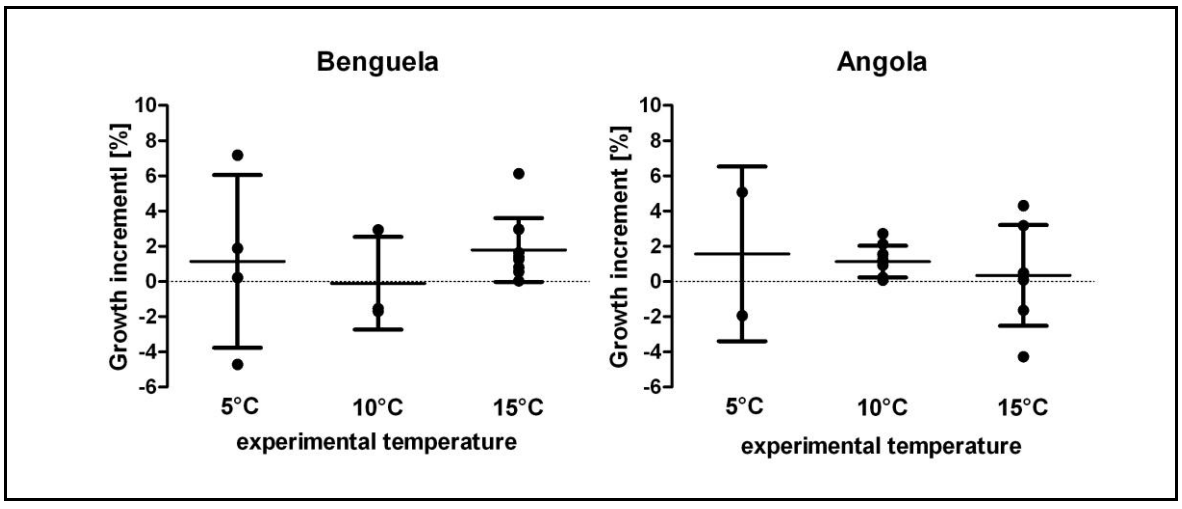

Figure 24: Growth of moulted animals out of starvation experiments. The percentaged growth of uropod length of each species maintained and moulted at given temperatures is presented. Values are given in means ± standard deviation. (n=2-9)

In both regions, the mean percentage of growth varied between zero and 1.6 % (Table 24). The scattering was relatively high as can be exemplary seen in *E. hanseni* Benguela at 5°C and 10°C (Figure 24). Regardless of maintenance temperature, the mean overall growth increment was 1.3 % for *E. hanseni* Benguela and 0.8 % for *E. hanseni* Angola.

Table 24: Mean percentaged growth of animals out of starvation experiments divided into different experimental temperatures and regions. Outer right column gives the mean percentaged growth of each region regardless of experimental temperature.

		experimental temperature	Ø GI ± SD	overall Ø GI ± SD
species	*E. hanseni* **Benguela**	**5°C**	1.2 ± 4.9	**1.3 ± 2.9**
		10°C	-0.01 ± 2.6	
		15°C	1.8 ± 1.8	
	E. hanseni **Angola**	**5°C**	1.6 ± 4.9	**0.8 ± 2.2**
		10°C	1.1 ± 0.9	
		15°C	0.4 ± 2.9	

4 Discussion

In the following chapter, the results of the presented thesis will be discussed and correlated with previous scientific data.

The difficulty in Benguela krill is that scientific knowledge is still scarce, which complicates the comparison of results with appropriate literature data. World-wide there have been several investigations on krill of temperate and polar regions discussing species' performance. Data is mostly available from the northern krill *Meganyctiphanes norvegica* and southern krill *Euphausia superba*. Investigations include data of the metabolism in *E. superba* (Ikeda and Mitchell 1982; Hirche 1983, 1984; Meyer et al. 2010) and *M. norvegica* (Saborowski et al. 2000; Saborowski et al. 2002) and enzyme activity (Meyer et al. 2002; Saborowski and Buchholz 2002) as well as starvation experiments (Meyer and Oettl 2005; Auerswald et al. 2009) in respective species. In krill of the Benguela Current system, however, scientific data are principally published on *Euphausia lucens*, like studies on abundance (Stuart and Pillar 1988; Gibbons et al. 1991), population growth (Stuart and Pillar 1988), migration (Pillar et al. 1989; Gibbons 1993), diet (Stuart 1986, 1989; Gibbons et al. 1991; Gibbons 1993) and metabolism (Stuart 1986), reproduction (Stuart and Nicol 1986) as well as chemical composition (Stuart 1986). Comparatively less is known about *Euphausia hanseni*, where vertical migration and zonation were investigated by Barange and Pillar (1992), reproduction by Stuart and Nicol (1986), larval development by Weigmann-Haass (1977) and about feeding behaviour by Barange et al. (1991). On *Nematoscelis megalops* until now only vertical migration (Barange 1990; Wiebe et al. 1982) and feeding behaviour (Barange et al. 1991) were investigated and on *Nyctiphanes capensis* only reproduction (Stuart and Nicol 1986) and zonation patterns are known (Barange and Pillar 1992).

Summarizing, for the first time, physiological data on ecologically important krill species of the Northern Benguela Current (namely *E. hanseni*, *N. megalops* and *N. capensis*) were collected and compared in this thesis presented. This work also presents first results concerning starvation capacity of krill from an upwelling system on the example of the highly abundant species of *E. hanseni*.

The chapter is divided in three parts. The first part (4.1) covers the discussion of results concerning the comparison of the species, the second part (4.2) addresses the discussion of results that were received from starvation experiments on *E. hanseni* and the third part (4.3) involves the observations on the intermoult period and growth increment of *E. hanseni*.

As some reference data from previous investigations relate to dry weight (DW) of each specimen, respective data were recalculated by taking own measurements into account that the dry weight equals to about 20% of the specimens' fresh weight (FW).

4.1 Inter-species comparison

The three Euphausiid species *E. hanseni*, *N. megalops* and *N. capensis* showed no overall differences in physiological parameters oxygen consumption, specific citrate synthase activity and elemental composition. However, strong distinctions with regard to each species' excretion rates and atomic O:N-ratios were noted.

The respiration rates of all investigated species were not significantly different over all experimental temperatures, although the species *E. hanseni*, *N. megalops* and *N. capensis* strongly differed in body length and weight. Therefore, the commonly emphasised relation of size to oxygen consumption of krill and many other zooplankton species (Ikeda and Mitchell 1982; Hirche 1983) could not be found. According to Hirche (1983) the respiration rates increase with decreasing size (weight) of specimens. However, it is questionable whether an allometric effect could have been found between the three species as such a result was observed on a wide range of size classes (Hirche 1983). Furthermore, the species differed in their gender distribution as *N. capensis* specimens, for example primary consisted of juveniles that generally could have reacted metabolically different, which could have distorted the results for comparison. In conclusion, a possible relationship of size and oxygen consumption within each species cannot be excluded, because it was not measured in sufficient detail in this thesis.

Concerning the relationship of respiration rates and temperature, in both *E. hanseni* Benguela and *E. hanseni* Angola as well as in *N. megalops* oxygen consumption rates increased from an average of 7 µmol O_2 gFW^{-1} h^{-1} at a temperature of 5°C to 10 µmol O_2 gFW^{-1} h^{-1} at 10°C to an average of 16 µmol O_2 gFW^{-1} h^{-1} at 15°C. However, *E. hanseni* Benguela was not studied at 20°C experimental temperature, whereas *N. capensis* was only studied at 18°C. The average respiration rates of *E. hanseni* Angola, *N. megalops* and *N. capensis* ranged from 22.9 to 27.2 µmol O_2 gFW^{-1} h^{-1} with a common mean of about 25 µmol O_2 gFW^{-1} h^{-1}. For comparison with previous investigations, it is believed that respiration data collected on the polar Euphausiid *E. superba* are not well suited, because experimental temperatures for krill of respective environments naturally ranged from only between 1 to -1.1°C (Ikeda and Mitchell 1982; Hirche 1983, 1984; Auerswald et al. 2009; Meyer et al. 2009). However, it turned out that the species investigated at 5°C in

this study had only little higher respiration rates compared to *E. superba* studied at about 1°C. In Hirche (1984) oxygen consumption for the just mentioned experimental temperature on *E. superba* is given in 0.64 ml O_2 gDW^{-1} h^{-1}. The approximated value (for calculation the ideal gas law was taken into account, which states that 1 µmol equals 22.4 µl of gas; also used for further transformation of data for comparison) for Benguela krill analysed at 5°C in contrast is about 0.8 ml O_2 gDW^{-1} h^{-1}. This might indicate that krill species regardless of climatic zone have a similar level of metabolism with regard to their oxygen consumption rates. However, more detailed experiments would have been necessary to strengthen this possible explanation.

For further inter-specific comparison with respect to the experimental temperature and oxygen consumption rates, the three Benguela krill species can be best compared to studies on *M. norvegica*. According to Saborowski et al. (2002) data of respiration rates are available from temperatures of 4°C to 16°C. *M. norvegica* was studied at three different locations and showed rates of 19.9 to 33.3 µmol O_2 gDW^{-1} h^{-1} at an experimental temperature of 4°C and 73.8 to 89.9 µmol O_2 gDW^{-1} h^{-1} at 16°C. The values of Benguela krill also lie in this range after having been converted to dry weight. For the experimental temperature of 5°C the average respiration rates ranges at 35 µmol O_2 gDW^{-1} h^{-1} and for 15°C at 80 µmol O_2 gDW^{-1} h^{-1}.

Similar to experiments of Saborowski et al. (2002), the oxygen consumption rates indicated an increasing trend with increasing temperature in experiments in *E. hanseni* and *N. megalops*. This reaction corresponds to the Q_{10}-rule (van't Hoff 1884), which states that an increase of 10°C in temperature results in a doubling of metabolic parameters. In previous studies, this effect was especially assumed for metabolic parameters like respiration by indicating an exponential temperature relationship with Q_{10}-values between 2 and 3 (Hirche 1984; Montagnes et al. 2003). The same applies to the Benguela krill species of this study, as Q_{10}-values of respiration rates (generated from a 10°C temperature increase from 5°C to 15°C) ranged from 2.29 for *N. megalops* to 2.44 in *E. hanseni* Angola and 2.7 in *E. hanseni* Benguela. These values also correspond for *E. superba* (Q_{10}= 2.62 and 2.8; Hirche 1984) and *M. norvegica* (Q_{10}=2.53-2.62; Hirche 1984 and Q_{10}= 1.87-2.12; Saborowski et al. 2002). Accordingly, the Q_{10}-values of the three species lie within the range of previous measurements on other krill species and within the common biological range between 2 and 3 (Hirche 1984).

The ammonium excretion rates showed strong distinctions between *E. hanseni* Benguela and *N. megalops*. Significant differences were measured at all experimental temperatures with *N. megalops* having much lower excretion rates at 15°C at an average of 0.6 µmol NH_4-N gFW^{-1} h^{-1} compared to 2.3 µmol NH_4-N gFW^{-1} h^{-1} in *E. hanseni* Benguela.

Ammonium excretion of *N. capensis* and *E. hanseni* Angola were equal at experimental temperatures of 18°C and 20°C at 1.3 µmol NH_4-N gFW^{-1} h^{-1} (*N. capensis*) and 1.2 µmol NH_4-N gFW^{-1} h^{-1} (*E. hanseni* Angola). Altogether, the average excretion rates of *E. hanseni* Angola were different from the same species of the Benguela Current while being more similar to the low rates of *N. megalops*. This indicates a possible difference in food availability between both regions, where *E. hanseni* was caught. Euphausiids generally are opportunistic omnivorous feeders but most of them, like *E. hanseni*, show predominant herbivorous feeding if conditions are optimal (Barange et al. 1991). Excretion rates closely relate to the ingested food source as it was observed in copepods (Corner et al. 1965) as well as in *M. norvegica* (Saborowski et al. 2002) namely that high phytoplankton concentrations were correlated to high ammonium excretion rates, whereas the absence of food leads to a decrease in excretion. This could explain the difference in ammonium excretion of *E. hanseni*. According to Barange et al. (1991) *N. megalops* is believed to be an active hunter, i.e. depending on a more carnivorous diet. The similarity of excretion rates between *N. megalops* and *E. hanseni* Angola could explain a shift from a herbivorous to a zooplankton dominated diet in *E. hanseni*, due to lack of sufficient phytoplankton concentration in the region that was at this time highly influenced by the warm Angola Current. In Figure 6 (see foldout map in appendix 9.11) the chlorophyll-a concentrations are given as an indication of primary production and phytoplankton concentration respectively. The highest chlorophyll-a concentrations are found in the area south of 20°S, which corresponds to the habitat of *E. hanseni* Benguela. This would confirm the relationship between a better nutritional situation and higher excretion rates in *E. hanseni* Benguela in comparison to *E. hanseni* Angola. The reason why *N. capensis* did not differ from *E. hanseni* Angola might also be explained by the trophic situation as the station T6-4 (see map in back cover) directly lies on the transition of the two regions differently influenced by the Benguela and Angola currents at 20°S. However, the data set of *N. capensis* might be inappropriate for a more precise comparison, because most of the specimens were still juvenile and therefore could have had a different food ingestion and ammonium excretion compared to adult *E. hanseni* and *N. megalops*.

An additional indication, how the species differ with respect to metabolised diet, is given by the atomic O:N-ratios of each species. The ratio is derived from the measurements of respiration and excretion rates. As mentioned before, the respiration rates did not differ while there was a significant difference between species regarding ammonium excretion. This leads to strong distinctions in O:N-ratios, especially between *E. hanseni* Benguela and *N. megalops*.

According to Ikeda (1974) an O:N-ratio of 24 determines the value of equal protein and lipid metabolism. A ratio below is considered to indicate the preferred use of protein. Preferential use of lipids is suggested accordingly above an atomic O:N-ratio of 24 (Ikeda 1974). The results of this work showed the lowest ratios in *E. hanseni* Benguela (from 10 to 18) and the highest values in *N. megalops* (from 49 to 71). Accordingly, *E. hanseni* Benguela is characterised by a protein dominated metabolism (even pure protein metabolism as reported by Mayzaud and Conover (1988)) whereas *N. megalops* is characterized by a lipid dominated metabolism. O:N-ratios of *E. hanseni* Angola range between both species. With values of 28-37, *E. hanseni* Angola shows a lipid-orientated metabolism like *N. megalops*, which again emphasizes the assumption, that *E. hanseni* specimens where found in different nutritional situations concerning the region of catch. Furthermore, the lipid-orientated metabolic character of *N. megalops* supports the presumption that this species is an active hunter feeding preferentially on zooplankton (Barange et al. 1991).

The specific enzyme activity of citrate synthase (CS) from abdominal muscle showed no difference in all species investigated. Citrate synthase plays a key role in the function of the tricarboxylic acid cycle for gaining energy in aerobic metabolism. In previous studies the activity of CS was described as a proxy for metabolic activity, which is highly correlated with oxygen consumption rates of specimens (Torres and Somero 1988b, 1988a; Meyer et al. 2009). This would agree with results of this study as it was shown that both respiration rates as well as CS activity indicated no distinctions between species. Although respiration increased with experimental temperature, no increase of CS activity was found. This might be explained by the shortness of incubation times, during which the respective animals were measured in respiration measurements (1.5 h - 6 h). In such a short time it is probably not possible to adjust specific enzymes. The specific enzyme activity per milligram of protein content averaged at about 30 mU $mgProtein^{-1}$ in all species studied. Values in the literature were mostly given related to fresh weight. By comparison, examined values for activity of CS ranged from 2.9 U gFW^{-1} in *E. hanseni* Benguela to 5.5 U gFW^{-1} in *N. megalops*, whereas krill from polar and temperate regions indicated slightly higher activities of about 7 U gFW^{-1} to 10.9 U gFW^{-1} in *E. superba* (Meyer et al. 2002) and 8 U gFW^{-1} to 14 U gFW^{-1} in *M. norvegica* (Saborowski and Buchholz 2002). However, Saborowski and Buchholz (2002) investigated differences of enzyme activity with regard to single abdominal segments, which indicated higher activities of the fifth and fourth abdominal segments compared to the remaining abdomen. Lower CS activity could therefore be explained because of having measured the specific enzyme activity of the second abdominal segment, which is believed to have generally

lower activity. Meyer et al. (2002) as well as Saborowski and Buchholz (2002) used the fourth and fifth abdominal segment respectively.

With regard to specific enzyme activity it was noticed that there was a generally indication that *N. megalops* (about 5 U gFW^{-1}) showed no distinction with respect to activity per mg of protein but suggested elevated activity concerning g fresh weight compared to *E. hanseni* specimens (about 3-4 U gFW^{-1}). This might be an indication of different enzyme concentrations of abdominal muscle between both species. Further study will be needed to test this hypothesis.

Analysis of elemental composition in terms of atomic relation of carbon and nitrogen is primarily used for estimation of the specimens' proximate chemical composition (Meyer and Oettl 2005), whereby lipid and glycogen would be believed to be indirectly described by carbon content and proteins by atomic nitrogen content, respectively. The atomic C:N-ratio found between 4.1 and 4.3 indicates that *E. hanseni* as well as *N. megalops* and *N. capensis* did not differ with respect to chemical composition. These values also approximately correlate with C:N-ratios that were found in Antarctic krill *E. superba* (3.6-5.0 according to Meyer and Oettl 2005 and Auerswald et al. 2009) and Benguela krill *E. lucens* (3.73 according to Stuart 1986). Accordingly, the atomic C:N-ratios of the here studied species resemble the values of previous investigations on other krill species. Nevertheless, it should be noted that the measurements of *E. superba* are given for larval krill (Meyer and Oettl 2005) and for krill that was caught at the beginning of Antarctic summer i.e. at the beginning of the feeding season (Auerswald et al. 2009). According to Auerswald et al. (2009) the measured C:N-ratio was only half of that values of previous studies suggesting that *E. superba* specimens were still recovering from the starvation period during winter. Therefore these values may not have been suited for comparison with *E. hanseni*, *N. megalops* and *N. capensis* that were caught under late summer conditions of the Benguela Current. Further investigation would be necessary.

4.2 Short-term starvation of *E. hanseni*

The results of this investigation revealed that only seven days of starvation caused an effect on the metabolism and elemental composition of *E. hanseni* from both regions, Benguela and Angola. It was also observed that the strength of the starvation effect on specimens differed between both regions with regard to oxygen uptake, excretion, atomic O:N-ratio and enzyme efficiency of citrate synthase. In both regions the short-term

starvation indicated no change in proximate biochemical composition concerning total lipid and total protein content.

All *E. hanseni* specimens responded to the period of starvation with a more or less continuous decrease in oxygen consumption. The only exceptions were animals from the 5°C experiment, which was conducted on *E. hanseni* Benguela. Here the low temperature seems to compensate the effect of starvation among the individual specimens. In all other experiments the respiration rates decreased by more or less 50% within the first and last days of starvation while showing no indication of an additional effect of temperature.

The decline of metabolic activity concerning oxygen consumption during starvation in order to adapt to lack of food and to save energy reserves was also described by Meyer and Oettl (2005) and Auerswald et al. (2009). In these studies larval and adult *E. superba* were investigated during a short-term period of food depletion. According to Meyer and Oettl (2005), respiration rates of larval krill decreased from 1.3 µl O_2 $mgDW^{-1}$ h^{-1} to 0.7 µl O_2 $mgDW^{-1}$ h^{-1} during only six days of starvation. In adult *E. superba*, according to Auerswald et al. (2009), the respiration rate fell by 53% during a period of two weeks starvation.

From analysis of regression it was found that *E. hanseni* Angola reacted more sensitive to food deprivation. During the course of starvation the oxygen consumption fell to up to 63% in the 5°C experiment. It could not be clarified if this property was due to a lower energy storage potential. However, ammonium excretion pointed to the same direction: The rate of ammonium excretion in *E. hanseni* Benguela shows a continuous decrease within the period of starvation whereas the rates of *E. hanseni* Angola in general fluctuated around one level in each experiment and remained constant throughout the whole starvation period. This observation could be explained by the different nutritional situations, to which the specimens were exposed to before catch. This was already found out and discussed in chapter 4.1 as it was realized that animals from the Angola region had a different or worse basis of food. The observations in this case revealed that specimens of *E. hanseni* Angola might have already been exposed to food depletion before the experiments, which would explain that excretion rates are not changing with period of starvation. A decreasing trend of ammonium excretion, observed on *E. hanseni* Benguela, was also detected by Meyer and Oettl (2005) during a starvation experiment on larval krill of *E. superba*. In this experiment the excretion rates decreased by 50% in the first six days. After that time, however, they rose to initial values of about 0.04 µg NH_4 $mgDW^{-1}$ h^{-1}. According to Auerswald et al. (2009) excretion rates of adult *E. superba* even increased along the entire experiment of two weeks of starvation. Neither, the increase after six days of starvation nor a generally increase in ammonium excretion could not be confirmed by the

measurements of this work. This might also be explained by a different nutritional condition, in which *E. hanseni* was found compared to Antarctic krill. Nevertheless, a possible return to an increasing tendency of excretion rates cannot be excluded, because the experiments were only run for seven days instead of 12 (Meyer and Oettl 2005) and 20 days (Auerswald et al. 2009) respectively.

In summary, compared to *E. hanseni* Angola, the impact of food deprivation had a stronger effect on the ammonium excretion rates of *E. hanseni* Benguela. The different reaction to starvation is most probably due to the fact that *E. hanseni* Benguela was initially exposed to more favourable food conditions and therefore had the necessary basis to show a measurable response to starvation with respect to ammonium excretion.

Furthermore, the different experimental temperatures resulted in different amounts of ammonium excretion (highest excretion rates in the 15°C experiment), but showed no additional influence or difference on the reaction of *E. hanseni* specimens due to lack of food. This implies that the changes shown in the excretion rates could be mainly due to the effect of short-term starvation.

Changes in the atomic O:N-ratios correspond to the species' oxygen consumption rates and ammonium excretion rates. As both the respiration rates and the ammonium excretion decreased over the starvation period, the atomic O:N-ratio of *E. hanseni* Benguela revealed no difference between the first and the last days of starvation at the 10°C and 15°C experiments. The ratio is fluctuating around a value below 24 and therefore indicated a protein dominated metabolism (Ikeda 1974). At 5°C of experimental temperature it was suggested that respiration rates of *E. hanseni* Benguela were not influenced by starvation, because of a compensating temperature effect. Because the deprivation of food had an effect on the excretion, however, the respective atomic O:N-ratios were slightly higher with values ranging from 10.6 to more than 24. However, there was no significant difference in O:N-ratios with regard to the period of starvation.

Regardless of temperature, specimens of *E. hanseni* Angola responded to starvation stress with a decrease in respiration rates but no change in excretion rates, which therefore lead to a decline of atomic O:N-ratio over the time of starvation. Primarily, this effect was found at 10°C and 15°C of experimental temperature, at which the initial value of about 30 (indicating lipid dominated metabolism according to Ikeda (1974)) decreased after seven days of starvation to an atomic ratio of about 10, which corresponds to a pure protein catabolism (Mayzaud and Conover 1988). The initial value of about 30 confirms again the suggestion that *E. hanseni* Angola was exposed to different nutritional conditions and also validates the presumption of a shift from herbivorous to zooplankton

dominated diet in *E. hanseni* Angola due to lack of sufficient phytoplankton concentration in the respective region, which was influenced by the warm Angola Current (see chapter 4.1).

The effect of short-term starvation on both *E. hanseni* Benguela and *E. hanseni* Angola had no significant impact with regard to the specific enzyme activity and efficiency of citrate synthase. Nevertheless, there was a trend, which indicated a decrease in activity as well as a decrease in Michaelis-Menten constants between the first and the last days of starvation. This observation might be explained by a kind of compensation effect: the specific activity is slowed down (indicating a reduction of metabolism) but at the same time the efficiency of the respective enzyme is increased. This would comply with the investigations of Buchholz and Saborowski (2000, 2002) on *M. norvegica*, who suggested that during unfavourable food conditions an increase of substrate affinity may compensate for the metabolic reduction, which again is expressed by the lowering of specific citrate synthase activity.

Noteworthy in comparison of *E. hanseni* within both regions is the significantly lower Michaelis-Menten constant in specimens of *E. hanseni* Angola. Considering the statements of Buchholz and Saborowski (2000, 2002) this would again indicate the less favourable food conditions for specimens from the warm Angola Current in comparison to *E. hanseni* Benguela and confirm the assumption that animals from Angola might have been under food deprivation.

The analysis of elemental composition in terms of atomic relation of carbon and nitrogen revealed mostly a significant decrease during the period of starvation in all specimens of both regions and all experimental temperatures. This might indicate the preferential usage of carbon containing food for fulfilment of energy requirements during the initial times of food depletion as it was also described during short-term starvation in *E. superba* (Meyer and Oettl 2005; Auerswald et al. 2009). According to Auerswald et al. (2009) the proximate biochemical composition declined significantly with starvation time with respect to total lipid content and total glycogen content in percentage per gram dry weight. However, significances were firstly observed after 15 days of starvation. On the other hand the percentage content of total protein remained stable over the whole period.

In the study presented it was observed that both *E. hanseni* Benguela as well as *E. hanseni* Angola indicated no difference in percentage total lipid and protein content per gram dry weight. This might be explained by the shortness of experiment. Nevertheless, there was a decreasing trend of total body lipids as well as percentage total carbon content (see appendix 9.9) with regard to days of starvation. The negative impact of food

deprivation on elemental composition could be additionally explained by the possible usage of glycogen as energy source that contributed to the loss of carbon. In this thesis carbohydrates and glycogen were not analysed although it is believed to contribute as energy source (Sánchez-Paz et al. 2006).

4.3 Intermoult period and growth increment of *E. hanseni*

The investigations of intermoult period and growth increment were integrated in the starvation experiments and conducted during the first three days of maintenance.

The studies of intermoult period revealed a strong temperature dependency as the moult interval decreased with increasing temperature in all experiments that were conducted on *E. hanseni*. Only exceptions were specimens of *E. hanseni* Benguela, which were subjected to 10°C of experimental temperature. The observation that temperature has a strong influence on the intermoult period is already known from many previous studies, mostly conducted on *E. superba* (Buchholz et al. 1989; Buchholz 1991; Buchholz 2003). According to Buchholz (1991) it was observed that favourable feeding conditions immediately initiate growth, which leads to synchronicity of moulting within krill swarms (Buchholz 2003). Buchholz (2003) gives one hypothesis of this phenomenon that phytoplankton or rather exploitable food patches indirectly accelerate moult by accumulation of reserves. Concerning synchronicity, the variation of *E. hanseni* Benguela from the 10°C experiment (see subchapter 3.2.7) could be thereby explained. Specimens of these experiments were only caught on station T7-2 (see Table 12 and map in appendix 9.11), which is located very close to the area of highest phytoplankton concentration (as shown in the chlorophyll-a concentration; see map in appendix 9.11). One reason that these specimens did not moult during the experiment as it was expected might have been due to the just mentioned synchronism as they immediately took advantage of the nutritional conditions. Accordingly, *E. hanseni* Benguela might have already completed their moult cycle synchronously shortly before they were employed in the experiment.

Linear regression analyses indicated a decrease of intermoult period of 12 days for *E. hanseni* Benguela to 10 days for *E. hanseni* Angola, not considering the difference in the just discussed group of 10°C *E. hanseni* Benguela. Compared to other Benguela krill species, Stuart and Pillar (1988) found intermoult periods of adult *E. lucens* that ranged from four to six days at 13°C. Therefore the values could be compared to the results of 10°C and 15°C experiments of this work, which in turn resulted in a strong difference. *E.*

hanseni of this study showed intermoult periods of about 8 days, which is double the time of the 4 days that were measured by Stuart and Pillar (1988). Noteworthy is the fact that the experiments of Stuart and Pillar (1988) lasted for up to two months in the laboratory. Possibly, the results could have been maintenance artefacts caused by the long incubation period as it was emphasized by Buchholz (2003) that short-term experiments are indispensable to determine moult behaviour more close to field conditions which is essential to avoid such maintenance artefacts.

The growth increment in each of the experiments showed high variances. Growth ranged from shrinkage to a growth increment at moult of about 8%. Nevertheless, the investigations revealed positive growth in most of the specimens. According to Buchholz (1991) abnormal growth rates could also be caused by inadequate maintenance conditions that even could lead to shrinkage. This could also be applied to the results of this work. On average (over all temperatures), specimens of *E. hanseni* Benguela indicated a growth of 1.3 ± 2.9 % and *E. hanseni* Angola showed a growth increment of 0.8 ± 2.9 %. Previous studies of the growth increment of Antarctic krill *E. superba* showed comparatively higher growth. Values ranged from an average of 3% (Buchholz 1991) and 3.8% (Buchholz 1989) to 7.7% (Meyer et al. 2010) depending on the season of investigation. The deviation of *E. hanseni* from these results could be explained by effect of external factors but might also lead to the assumption of being more sensitive to laboratory and maintenance artefacts that Antarctic *E. superba*. Further studies to test this hypothesis are needed.

5 Conclusion and perspectives

For the first time, a larger and consistent set of physiological data on three ecologically important krill species of the Northern Benguela Current were collected.

The comparison of *Euphausia hanseni*, *Nematoscelis megalops* and *Nyctiphanes capensis* revealed no difference between the oxygen consumption rates, specific of citrate synthase activities as well as atomic C:N-ratios.

It has been shown that krill of the Benguela upwelling adjust their overall metabolic rates to a prevailing temperature gradient by approximately following the Q_{10}-rule.

Strong distinctions between species were found with respect to ammonium excretion. Average ammonium excretion was also different within the species of *E. hanseni* where *E. hanseni* from the "Angola" region was compared to "Benguela"-krill. The former is more similar to *N. megalops*, which was explained by each species' nutritional situation. *E. hanseni* from the Angola-region may have been confronted to unfavourable food conditions due to lack of sufficient phytoplankton concentrations, which may have resulted in shifts from a herbivorous to a zooplankton dominated diet. The same was indicated by atomic O:N-ratios. The assumption of a more carnivorous feeding behaviour of *N. megalops* could be strengthened as the atomic O:N-ratio also indicated a lipid-orientated metabolism.

Further studies would be interesting regarding the composition of the food ingested, namely stomach analyses, analysis of fatty acids as well as determination of digestive enzymes. The ecophysiological data collected will be useful for ecosystem modelling purposes and will contribute to the GENUS-project in order to determine each species' trophic position within the plankton community of the Northern Benguela Current system.

A major focus of this thesis was to present observations concerning the starvation capacity of krill from an upwelling system on the example of the highly abundant species of *E. hanseni*. *E. hanseni* specimens continuously adjusted their overall metabolic rates with regard to the period of food deprivation in order to adapt the organisms' metabolism to the new situation. Under starvation conditions oxygen consumption rates as well as ammonium excretion rates were therefore generally down regulated. Furthermore, the species' specific activity of citrate synthase showed a decreasing tendency whereas the enzyme efficiency tended to increase over the period of starvation. This was assumed to be a compensatory effect to the situation of starvation. It was suggested, however, that a longer starvation period would have been necessary for verification of such an effect. The same applies to the changes in proximate biochemical composition with special regard to

total lipid and protein contents. Accordingly, previous and the own studies indicated that a starvation period of seven days might have been too short.

There was basically no indication of an additional temperature influence with regard to the stress of food absence. In one case the lowest temperature showed a kind of compensation effect to the adjustment of metabolic parameters with regard to oxygen consumption rates.

Furthermore, it can be concluded that *E. hanseni* specimens differed in the extent of physiological reactions to short-term starvation with regard to the region, in which they were caught. Specimens from this part of the Northern Benguela Current, which was more strongly influenced by the warm Angola water masses, reacted more sensitive to food deprivation with respect to the down regulation of oxygen consumption and showed no reaction regarding ammonium excretion. It was again assumed that these animals were exposed to unfavourable trophic conditions and therefore might have already been under starvation conditions before they were subjected to the experiments. These assumptions were also strengthened by significant differences in enzyme activity and atomic O:N-ratio.

In future experiments, it would be necessary to apply longer experimental periods and additional analyses in order to determine the trophic conditions of species. These analyses could range from simple visual analysis of stomach fullness to observations on stomach content and composition and activity of digestive enzymes.

The third part of this thesis provided information about the intermoult period and growth increment in *E. hanseni* of the Northern Benguela Current. The intermoult period was highly correlated to experimental temperature and decreased with increasing temperature. The growth increment at moult showed high variances and, compared to krill from other climate zones, relatively low values. Further investigations would be necessary to validate these first observations with respect to possible inadequate maintenance and other external, which may have distort the results.

In summary, this thesis aimed at collecting data on the physiology and proximate elemental composition in *E. hanseni*, *N. megalops* and *N. capensis* as well as concerning the starvation capacity and growth increment with intermoult period in *E. hanseni*. Data and conclusions may serve as a data basis for future investigations and comparisons of zooplankton communities in upwelling systems as well as for the comparison of krill species world wide to extend the applicability of krill as a biological indicator in ecosystem analysis and modelling.

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7 List of figures

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9 Appendix

9.1 List of chemicals

Acetyl-Coenzyme A (trilithium salt)	Roche, Mannheim, Germany
Ammonium chloride (NH_4Cl)	Sigma-Aldrich, Steinheim, Germany
BSA (Albumin bovine fraction V, pH 7)	Serva, Heidelberg, Germany
Dichlorisocyanuric acid sodium salt dihydrate	Merck, Darmstadt, Germany
Dichloromethane	Merck, Darmstadt, Germany
DTNB (2,2'-Dinitro-5,5'-dithiodibenzoic acid)	AppliChem, Darmstadt, Germany
EDTA (Ethylenediamine-Tetraacetic acid)	Sigma-Aldrich, Steinheim, Germany
Ethanol (96%)	AppliChem, Darmstadt, Germany
HCL (Hydrochloric acid)	AppliChem, Darmstadt, Germany
KCl (Potassium chloride)	Merck, Darmstadt, Germany
Methanol	Merck, Darmstadt, Germany
NaCl (Sodium chloride; molecular biology grade)	AppliChem, Darmstadt, Germany
NaOH (Sodium hydroxide pellets)	AppliChem, Darmstadt, Germany
Oxaloacetic acid (≥97%)	Sigma-Aldrich, Steinheim, Germany
Phenol	Sigma-Aldrich, Steinheim, Germany
Protein Assay (500-0006)	Bio-Rad, München, Germany
Reagent A (D_c Protein Assay; 500-0113)	Bio-Rad, München, Germany
Reagent B (D_c Protein Assay; 500-0114)	Bio-Rad, München, Germany
Sodium nitroprusside dihydrate	Merck, Darmstadt, Germany
Sodium sulfite (Na_2SO_3)	Sigma-Aldrich, Steinheim, Germany
Tris ultrapure	AppliChem, Darmstadt, Germany
tri- Sodium citrate dihydrate	Merck, Darmstadt, Germany

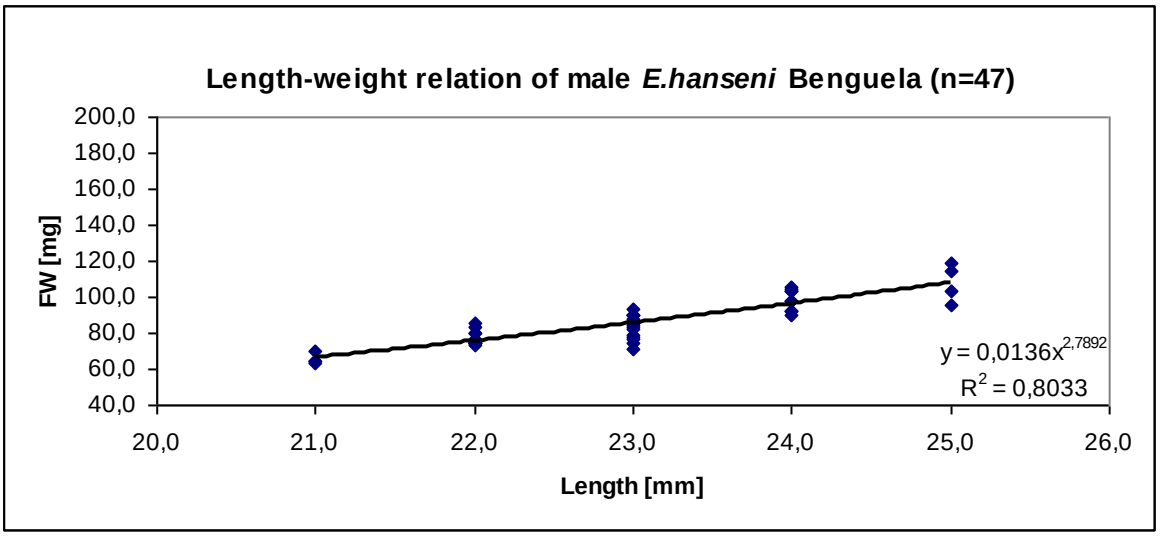
9.2 Table A: Dilution series for ammonium determination according to Solorzano (1969)

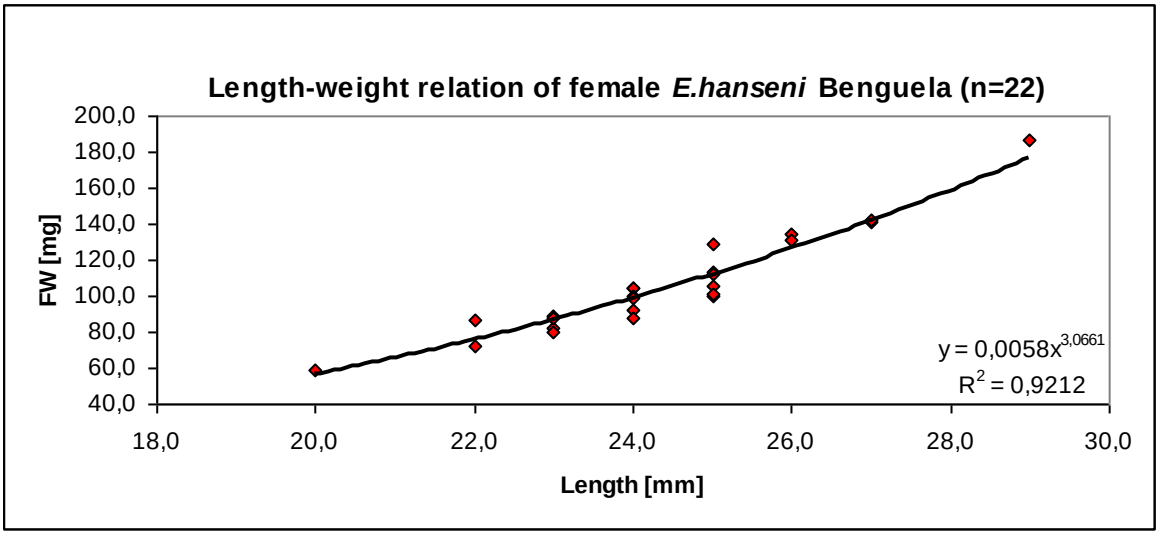
0 µl	working solution	+	500 µl NaCl-solution	= c (NH_4^+)	**0 µg/L**
5 µl	working solution	+	495 µl NaCl-solution	= c (NH_4^+)	**20 µg/L**
25 µl	working solution	+	475 µl NaCl-solution	= c (NH_4^+)	**100 µg/L**
50 µl	working solution	+	450 µl NaCl-solution	= c (NH_4^+)	**200 µg/L**
75 µl	working solution	+	450 µl NaCl-solution	= c (NH_4^+)	**300 µg/L**
100 µl	working solution	+	400 µl NaCl-solution	= c (NH_4^+)	**400 µg/L**
125 µl	working solution	+	450 µl NaCl-solution	= c (NH_4^+)	**500 µg/L**
150 µl	working solution	+	350 µl NaCl-solution	= c (NH_4^+)	**600 µg/L**

9.3 Table B: Detailed morphometric data of compared species additionally divided into experimental temperatures

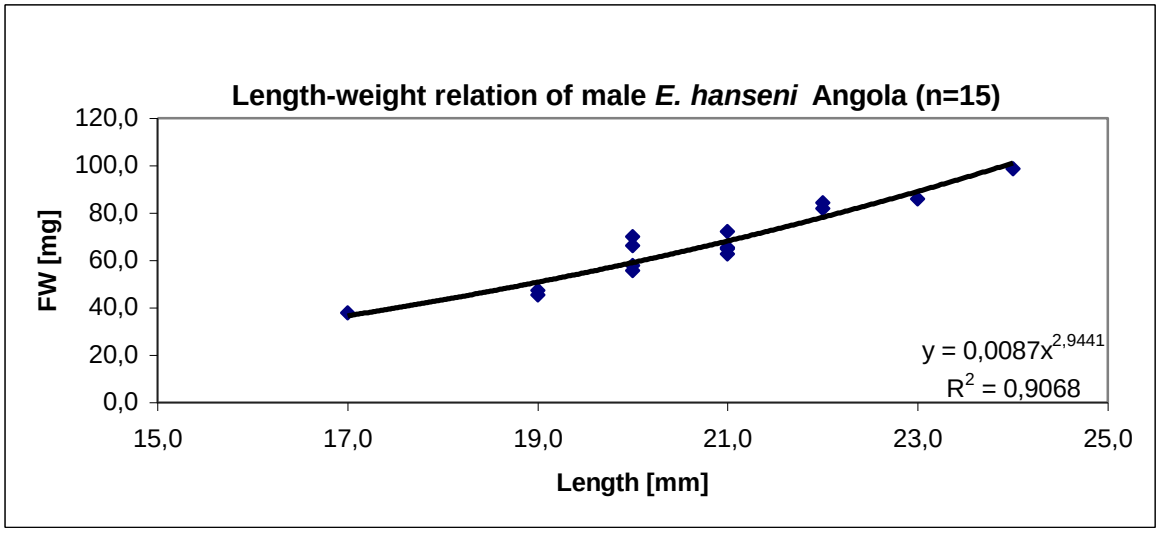
Species	exp. T.°C	Station	Length [mm]	Fresh weight [mg]	Sex [%] j/m/f	n
E. hanseni Benguela	5	T8-1d	25.4 ± 1.8	119.1 ± 28.2	0/33/67	6
	10	T7-2	25.6 ± 1.2	119.7 ± 18.2	0/33/67	6
	15	T7-1,T8-1d	24.8 ± 1.0	108.6 ± 14.7	0/33/67	6
E. hanseni Angola	5	WKT2b/d	22.4 ± 1.2	79.0 ± 12.1	0/0/100	6
	10	WKT2b/d	23.3 ± 1.0	89.9 ± 11.3	0/17/83	6
	15	T1-3a	20.6 ± 1.6	62.7 ± 14.6	0/17/83	6
	20	T1-3a	19.3 ± 2.5	52.9 ± 22.4	0/50/50	6
N. megalops	5	T1-3	16.5 ± 1.7	31.8 ± 9.3	0/17/83	6
	10	T1-3, T5-2	15.5 ± 1.8	25.3 ± 8.6	17/17/66	12
	15	T1-3, T5-2	16.2 ± 1.9	29.9 ± 9.3	8/33/59	12
	20	T1-3a	16.0 ± 0.6	27.7 ± 8.2	0/33/67	6
N. capensis	18	T6-4	12.5 ± 0.9	10.2 ± 1.9	86/14/0	7

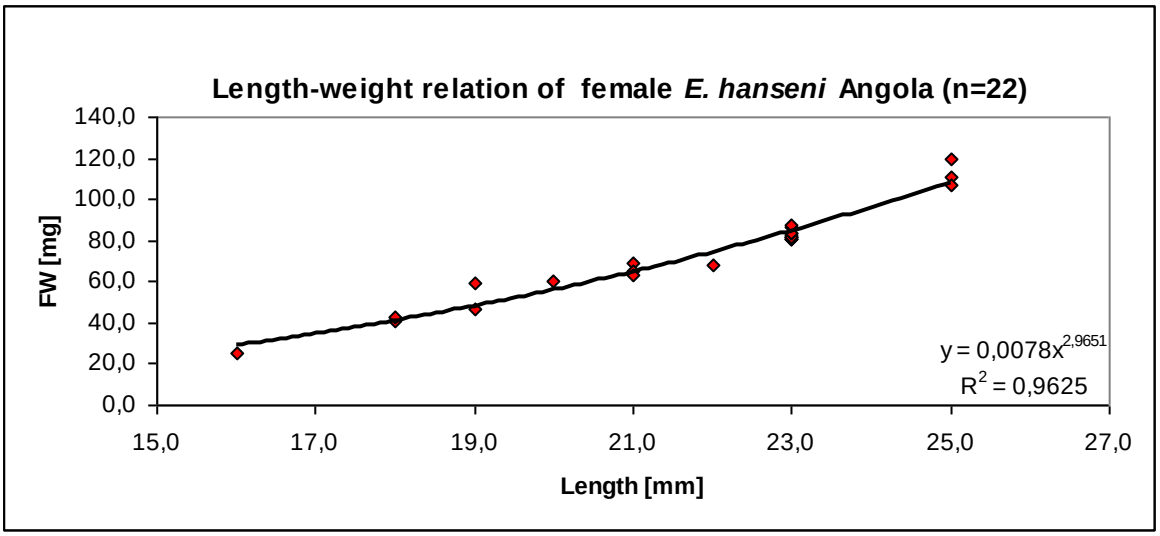
9.4 Graph A: Length-weight relations *E. hanseni* Benguela



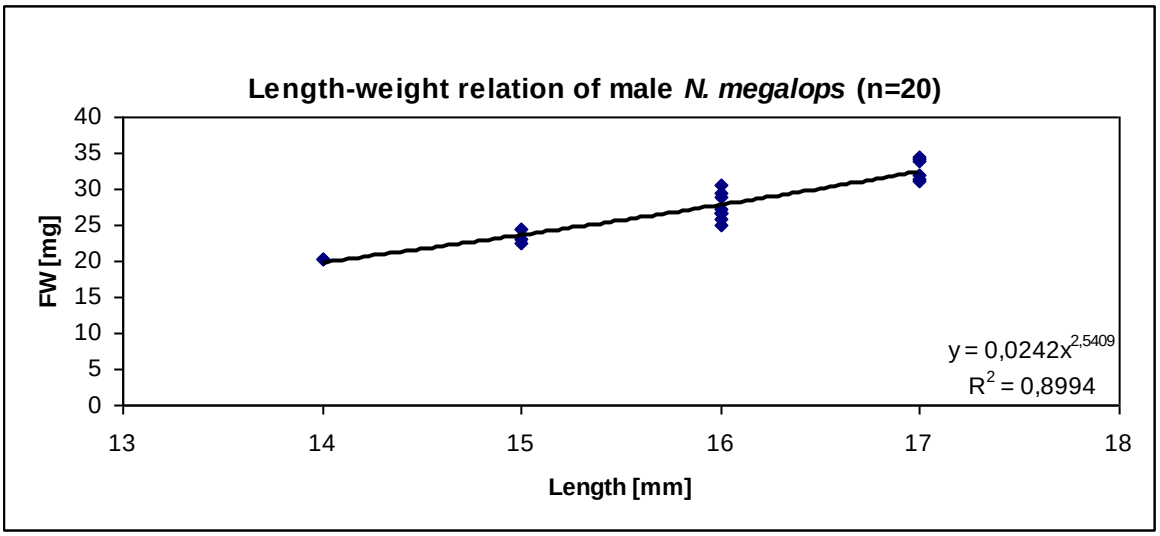


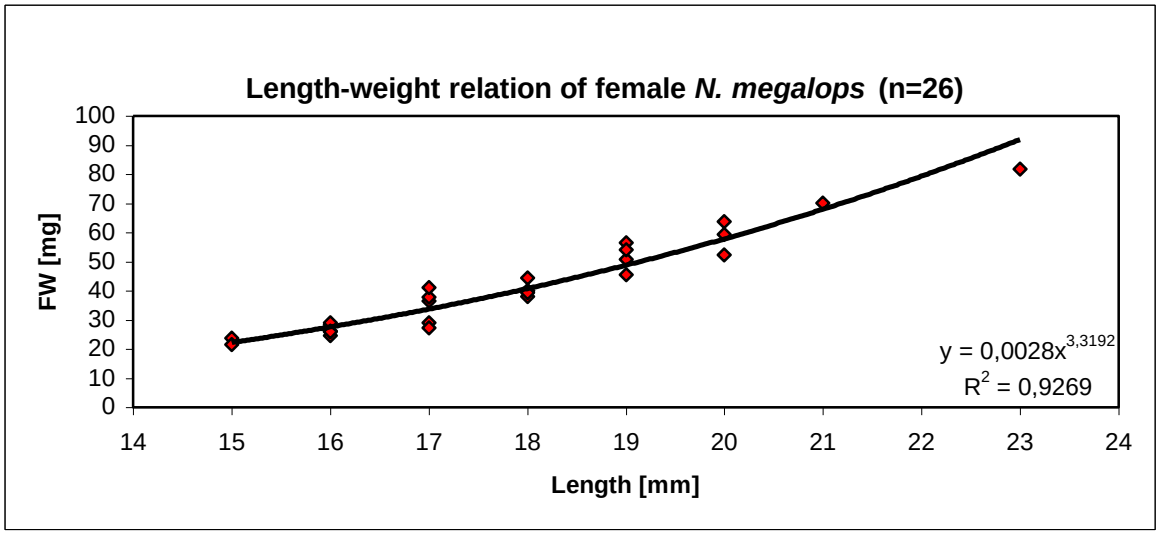
9.5 Graph B: Length-weight relations *E. hanseni* Angola



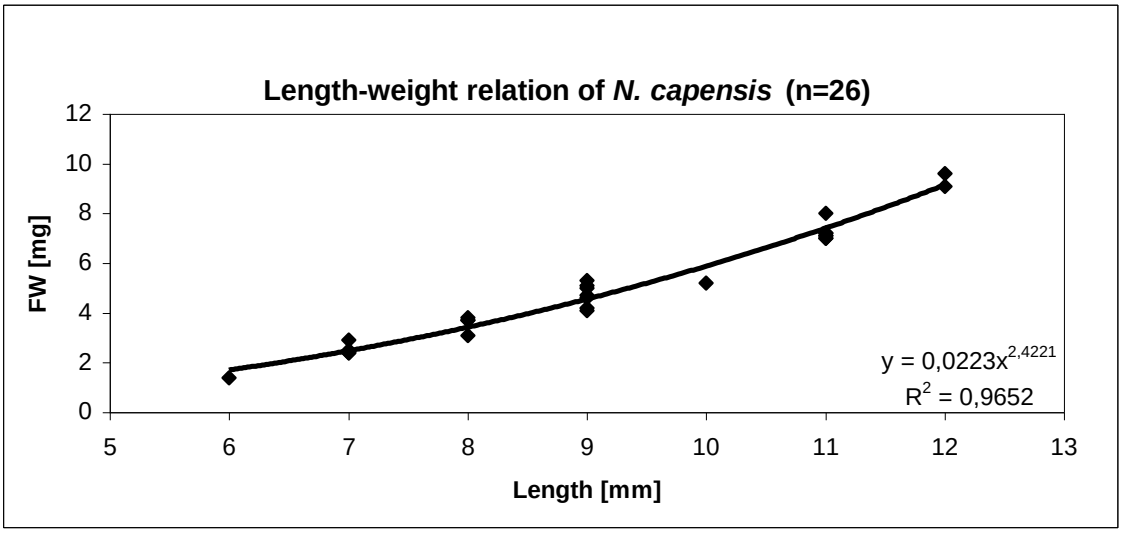


9.6 Graph C: Length-weight relations *N. megalops*

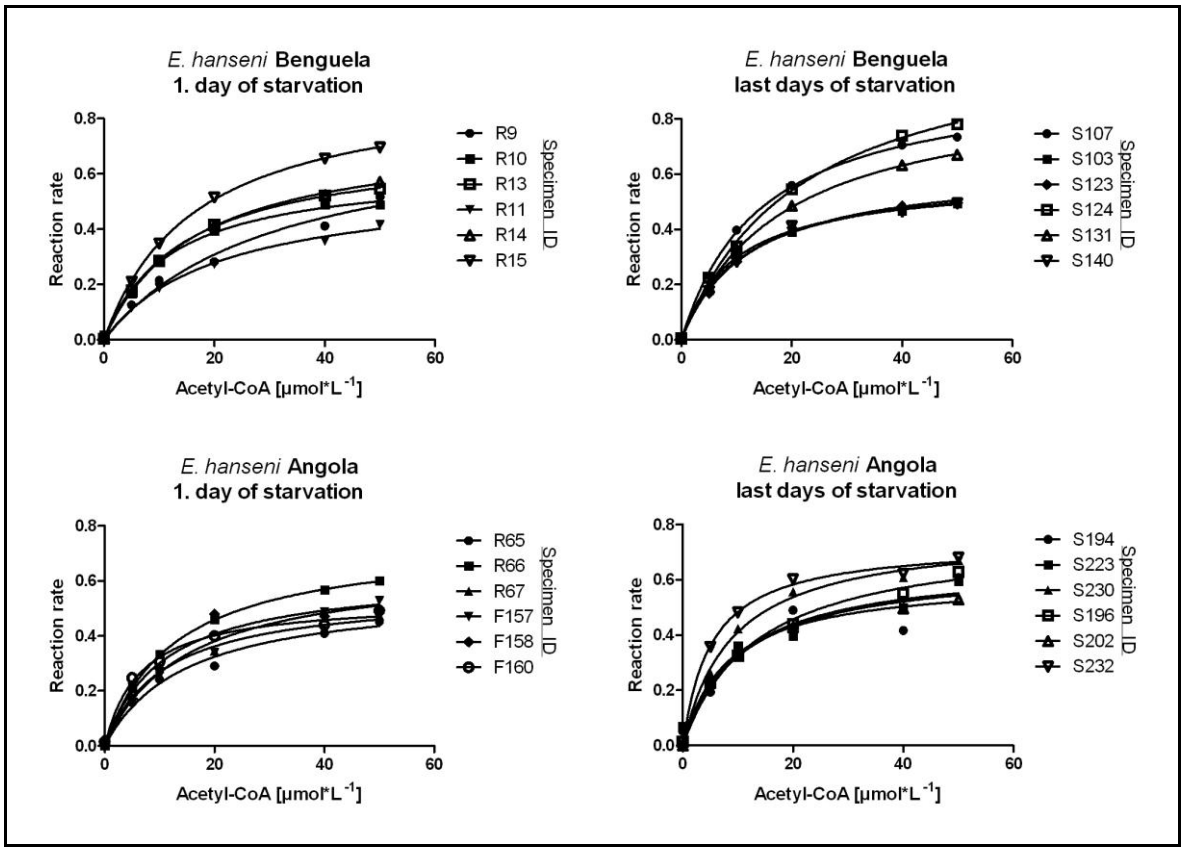




9.7 Graph D: Length-weight relation *N. capensis*



9.8 Graph E: Michaelis-Menten saturation curves for CS-kinetics in 10°C starvation experiments conducted on *E. hanseni* Benguela and *E. hanseni* Angola



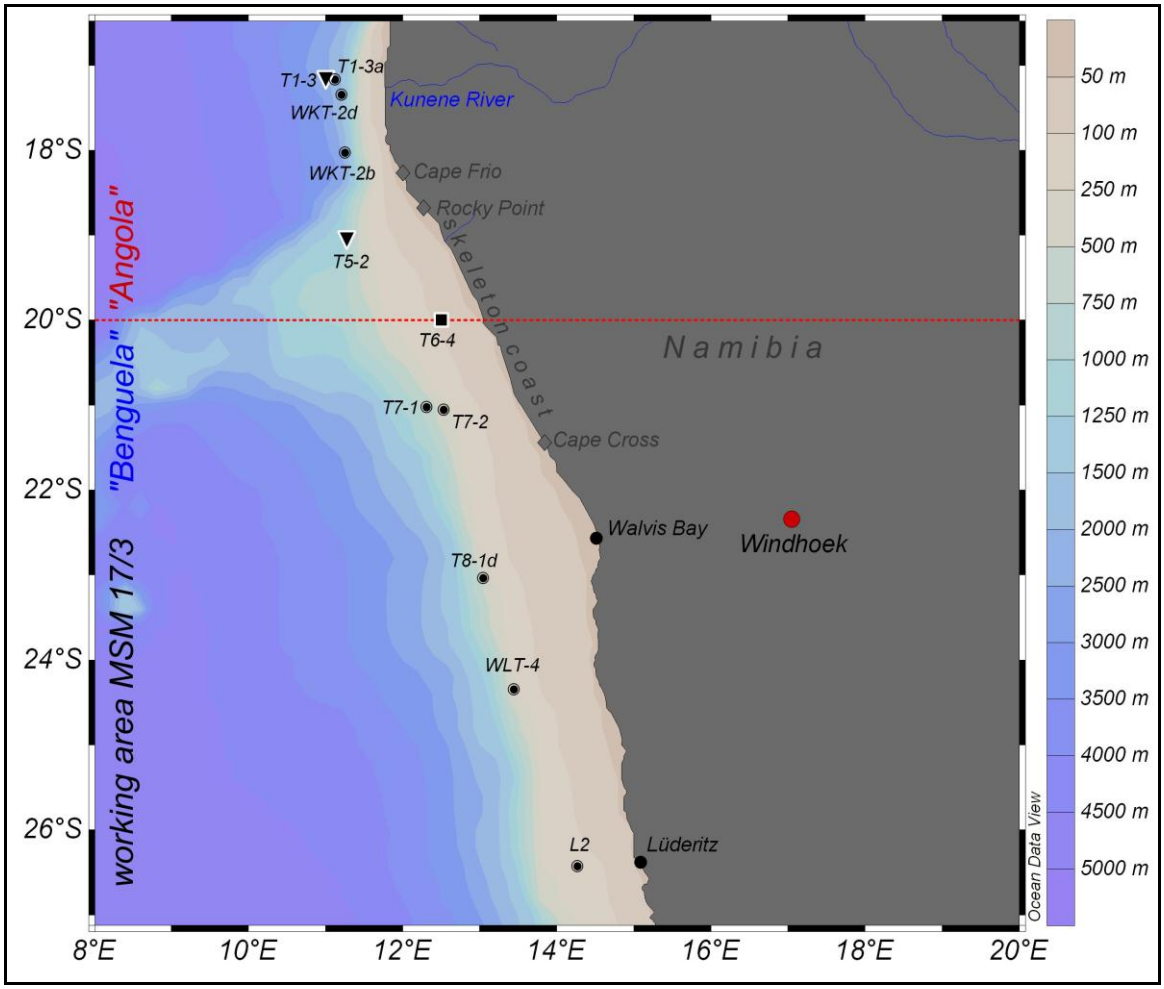
9.9 Table C: Total body carbon (C) in percentage of dry mass. Given is the change during starvation of *E. hanseni* specimens.

	days of starvation								
	5°C			10°C			15°C		
species	1	6/7	T-Test	1	6/7	T-Test	1	6/7	T-Test
E. hanseni Benguela	42.6 ± 0.7	41.1 ± 1.2	**0.0260**	39.4 ± 0.8	39.3 ± 1.0	n.s.	40 ± 1.0	39.1 ± 0.9	n.s.
E. hanseni Angola	41.4 ± 0.6	40.8 ± 0.9	n.s.	39.2 ± 0.7	38.9 ± 0.7	n.s.	39.6 ± 0.9	38.7 ± 2.0	n.s.
T-Test	n.s.	n.s.		n.s.	n.s.		n.s.	n.s.	

9.10 Table C: Total body nitrogen (N) in percentage of dry mass. Given is the change during starvation of *E. hanseni* specimens.

	days of starvation								
	5°C			10°C			15°C		
species	1	6/7	T-Test	1	6/7	T-Test	1	6/7	T-Test
E. hanseni Benguela	11.8 ± 0.5	11.7 ± 0.6	n.s.	10.8 ± 0.3	11.3 ± 0.3	n.s.	10.8 ± 0.5	11.2 ± 0.5	n.s.
E. hanseni Angola	11.3 ± 0.4	11.6 ± 0.3	n.s.	10.8 ± 0.3	10.9 ± 0.3	n.s.	10.8 ± 0.4	11.0 ± 0.8	n.s.
T-Test	n.s.	n.s.		n.s.	n.s.		n.s.	n.s.	

9.11 FIGURE A: Map overview of working area MSM 17/3 – Stations and hydrography



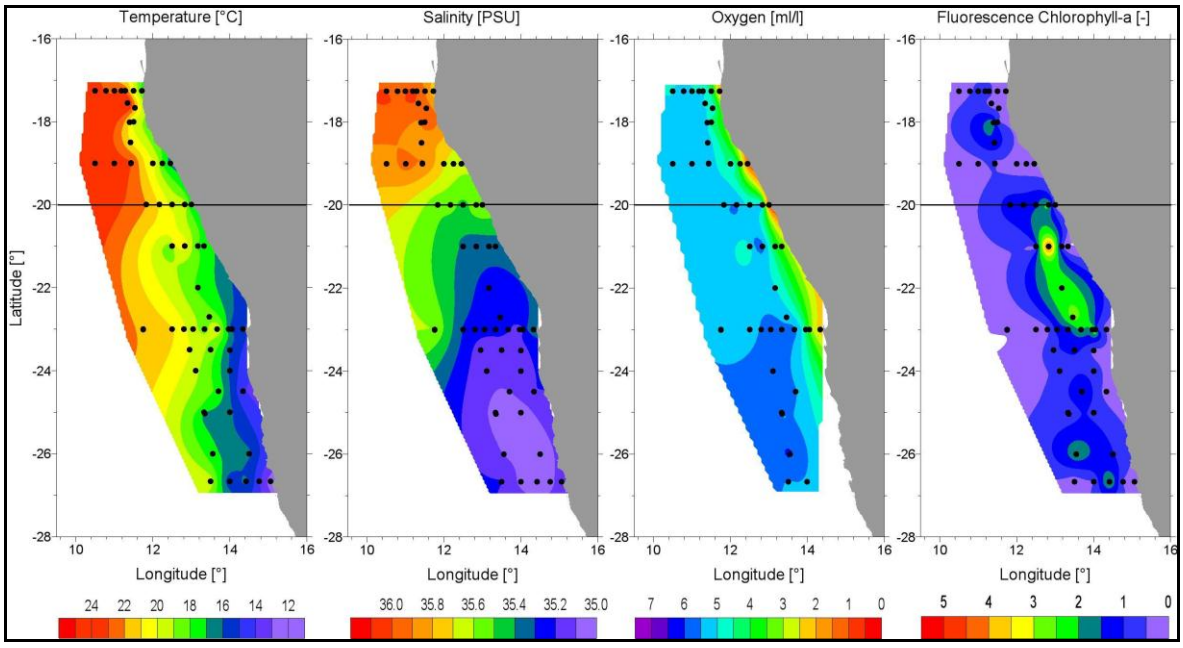


Figure was taken from hydrographic cruise report (Mohrholz et al. 2011)

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Statutory Declaration

I hereby declare that I have independently authored this thesis and have not used other than the named references or resources. I have marked and have explicitly stated all materials and informations, which were derived from other sources.

Ich versichere hiermit, dass die vorliegende Arbeit von mir selbstständig angefertigt wurde und ich keine anderen als die angegebenen Quellen und Hilfsmittel verwendet habe. Alle Stellen, die wörtlich oder sinngemäß anderen Werken, veröffentlicht oder unveröffentlicht, entnommen sind, wurden von mir unter Angabe der Quelle kenntlich gemacht.

Bremen, July 2011 _____

Lara Kim Hünerlage