Universität Bremen

Tolerance mechanisms and responses of krill species of different latitudes to oxygen minimum zones

Dissertation zur Erlangung des Grades eines
Doktors der Naturwissenschaften
-Dr. rer. nat.-
Fachbereich 2 Biologie/Chemie
vorgelegt von

Nelly Tremblay
B.Sc., Université du Québec à Rimouski, 2006
M.Sc., Instituto Politécnico Nacional, 2008

Universität Bremen
April 30th 2014

ALFRED-WEGENER-INSTITUT
HELMHOLTZ-ZENTRUM FÜR POLAR-
UND MEERESFORSCHUNG

Prüfungsausschuss:

1. Gutachter: Prof. Dr. Wilhelm Hagen (Marine zoology, Universität Bremen)
2. Gutachter: PD Dr. Doris Abele (Funktionelle Ökologie, Alfred-Wegener-Institut, Helmholtz-Zentrum für Polar- und Meeresforschung, Bremerhaven)

1. Prüfer: Dr. Werner Ekau (Department of Ecology, Leibniz Center for Tropical Marine Ecology, Bremen)
2. Prüfer: Dr. Hauke Flores (Iceflux - Ice-ecosystem carbon flux in polar oceans, Polar Biological Oceanography, Alfred-Wegener-Institut, Helmholtz-Zentrum für Polar- und Meeresforschung, Bremerhaven)

Contents

List of Figures

List of Tables

Frequently used abbreviations

ANN: artificial neural network
CAT: catalase
CS: citrate synthase
cDNA: complementary deoxyribonucleic acid
chl *a*: chlorophyll *a*
CTD: "conductivity, temperature, depth" system
CCS: California Current System
D: depth
DLh: daylight hours
DoY: Day of Year
DNA: deoxyribonucleic acid
DVM: diel vertical migration
ETP: Eastern Tropical Pacific
GAM: general additive model
GPx: glutathione peroxidase
GR: glutathione reductase
GSH: reduced glutathione
GSSG: oxidized glutathione
GST: glutathione-S-transferase
H_2O_2: hydrogen peroxide
HCS: Humboldt Current System
HPLC: high-performance liquid chromatography
HSP: heat-shock protein
HSR: heat-shock response
LAT: latitude
LON: longitude
M: body mass
MDA: malondialdehyde
MLR: multiple regression model
MNE: mean normalized expression
MODIS: moderate-resolution imaging spectroradiometer
N_2: nitrogen
NCCS: northern California Current System
O_2: oxygen
$O_2^{\bullet-}$: superoxide anion
$OH^{\bullet}$: hydroxyl radical
OMZ: oxygen minimum zone
***p*c**: critic partial pressure of oxygen
pO_2: partial pressure of oxygen
RNA: ribonucleic acid
ROS: reactive oxygen species
RR: respiration rate
RT-qPCR: reverse transcription quantitative polymerase chain reaction
SMR: standard metabolic rate
SOD: superoxide dismutase
SST: sea surface temperature
T: temperature
U: activity unit

Thesis summary

Euphausiids (krill) constitute a major part of the macrozooplankton community in terms of total biomass and play a key role in the food webs of the most productive marine ecosystems of the world. From the species found along the Eastern Pacific coastline many do not tolerate hypoxia and they do not distribute where shallow oxygen minimum zones (OMZs) prevail. Only very few species are endemic of OMZs. In my thesis, I investigated the physiological strategies and OMZ tolerance mechanisms of euphausiids on a global-scale to explain the current zoogeographical pattern of major species and project it in the future.

In a first step, the basal respiration rate of the species investigated was measured. This simple measurement is one of the best proxy to identify the optimal environmental window and the metabolic requirement scale wherein the organism is. A global euphausiid respiration ANN (Artificial Neural Network) model was built with 2479 data sets enclosing 23 of the total 86 species. The model included the effect of latitude (LAT), the day of the year (DoY), and the number of daylight hours (DLh), in addition to the basal variables that determine ectothermal oxygen consumption (temperature, body mass and depth). The ANN model indicated a decrease in respiration with increasing LAT and decreasing DLh. For seasonality, a General Additive model (GAM) successfully integrated DLh and DoY effects on respiration rates of the Antarctic krill, *Euphausia superba*, yielding the minimum metabolic activity in mid-June and the maximum at the end of December. For the North Pacific krill, *Euphausia pacifica*, we found no effect of DLh or DoY and the results for the North Atlantic krill, *Meganyctiphanes norvegica* were not meaningful, because the seasonal data were insufficient. The activity of the citrate synthase, Krebs cycle enzyme, also seems to be a promising tool for euphausiid respiration prediction and should be further analysed in pair with respiration measurements to develop a model in the future. The results emphasize that respiration measurements of Euphausiid key species should considered all seasons to improve the comparative physiological and ecological models.

Respiratory measurements and experiments combining hypoxia/reoxygenation exposure coupled with warming were conducted to understand adaptation of species to OMZs. Experimental krill species had their distribution from the Antarctic to the Humboldt Current system (HCS, Chilean coast), and the Northern California Current system (NCCS, Oregon). *Euphausia*

mucronata from the HCS starts metabolic suppression below 80% oxygen (O_2) saturation (18 kPa) showing adaptation to OMZ conditions. The two species investigated in the NCCS showed different energetic strategies. *Thysanoessa spinifera* had a lower standard metabolic rate than *Euphausia pacifica*, and a respiration pattern more close to oxyconformity. Lactate accumulation, measured when the lowest oxygen partial pressure (pO_2) was reached during respiratory experiment, was higher in *T. spinifera*, showing higher utilization of the anaerobic pathway. The NCCS krill, *E. pacifica*, and the Antarctic krill, *E. superba* were characterized as oxyregulators and maintain respiration rates constant down to 30% (6 kPa) and 55% O_2 (10 kPa) saturation, respectively.

E. mucronata and *E. pacifica* had higher SOD (superoxide dismutase) values in winter than in summer, which relate to higher winter metabolic rate (in *E. pacifica*). In both species, antioxidant enzyme activities remained constant during hypoxic exposure at habitat temperature. The normoxic subsurface oxygenation in the HCS during winter already poses a "high oxygen stress" for *E. mucronata*. Warming by 7°C above habitat temperature in summer increased SOD activities and glutathione (GSH) levels in *E. mucronata* (HCS), but no oxidative damage occurred. In winter, when temperature is homogenous and the OMZ absent, a +4°C warming combined with hypoxia represents a lethal condition for *E. pacifica*. In summer, when the OMZ expands upwards (100 m subsurface), antioxidant defences counteracted hypoxia and reoxygenation effects in *E. pacifica*, but only at mildly elevated temperature (+2°C). Experimental warming by +4°C reduced antioxidant activities and caused mortality of exposed specimens during the winter. Climate change scenario combining warming and hypoxia thus represents a serious threat to *E. pacifica* and, as a consequence, NCCS food webs.

Antarctic krill had the lowest antioxidant enzyme activities, but the highest concentrations of the molecular antioxidant glutathione (GSH) and was not lethally affected by 6 h exposure to moderate hypoxia. Gene expression related to aerobic metabolism, antioxidant defence, and heat-shock response under severe (2.5% O_2 saturation or 0.6 kPa) and threshold (20% O_2 saturation or 4 kPa) hypoxia exposure was investigated to detect aspects of the molecular stress response. Expression levels of the genes citrate synthase (CS), mitochondrial manganese superoxide dismutase (SODMn-m) and the heat-shock protein isoform (E) were higher in euphausiids incubated 6 h at 20% O_2 saturation than in animals exposed to normoxic conditions. The transcription is likely to prepare the krill for eventual reoxygenation, which connects with the swarming behaviour of this species. This cold-adapted species thus possesses the cellular tools from its sub-polar ancestor to tolerate levels of hypoxia severer than the oxygen concentration of its habitat, indicating a good plasticity to confront future stressful conditions of other types.

Zusammenfassung

Euphausiden (Krill) machen einen Großteil der Biomasse in Makrozooplanktongemeinschaften aus und spielen eine Schlüsselrolle in den Nahrungsnetzen von produktiven marinen Ökosystemen in der ganzen Welt. Viele der Arten, die entlang der ostpazifischen Küste vorkommen, sind intolerant gegenüber Hypoxie und sie verbreiten sich nicht in seichten Sauerstoff-Minimum-Zonen (OMZs). Nur sehr wenige Arten sind endemisch für die OMZs. In meiner Dissertation habe ich auf globaler Ebene die physiologischen Strategien und die Hypoxietoleranz von Euphausiden untersucht, um die momentanen Verbreitungsmuster der wichtigsten Arten zu erklären und auf die Zukunft zu projizieren.

Als erstes wurde die basale Respirationsrate der untersuchten Arten gemessen. Diese einfache Messung ist eine der besten Methoden, die optimalen Umweltbedingungen und metabolischen Anforderungen der Organismen zu ermitteln. Ein globales Euphausiden-Respirations-ANN (Artificial Neural Network)-Modell wurde aus 2479 Datensätzen, die 23 der weltweit 86 vorkommenden Arten umfassten, entwickelt. Neben den basalen Variablen, die den ektothermalen Sauerstoffverbrauch bestimmen (Temperatur, Körpergröße und Tiefe), berücksichtigte das Modell auch den Effekt des Breitengrades (LAT), den Tag des Jahres (DoY) und die Tageslichtdauer (DLh). Die neu implementierten Parameter verknüpfen Raum und Zeit hinsichtlich Jahreszeit und Photoperiode mit der Respiration von Krill. Das ANN Modell deutete auf eine Abnahme der Respiration mit zunehmender LAT und abnehmender DLh hin. Die Respirationsrate der verbreitetsten Arten wurde mit einer Multiple Linear Regression (MLR) oder einem General Additive Model (GAM) getestet. GAM integrierte erfolgreich die Effekte von DLh und DoY auf die Respirationsrate des antarktischen Krills, *Euphausia superba*, und ergab für die metabolische Aktivität ein Minimum Mitte Juni und ein Maximum Ende Dezember. Für den nordpazifischen Krill, *Euphausia pacifica*, konnten wir keinen Effekt von DLh oder DoY feststellen und die Ergebnisse für den nordatlantischen Krill, *Meganyctiphanes norvegica*, waren nicht aussagekräftig, da die saisonalen Daten nicht ausreichten. Die Aktivität der Citratsynthase (Citratzyklus-Enzym) scheint auch ein viel versprechendes Werkzeug zu sein, um die Respirationsrate von Euphausiden vorherzusagen, und sollte in Zukunft zusammen mit Respirationsmessungen analysiert werden, um ein Modell für die Zukunft zu entwickeln. Die Ergebnisse unterstreichen, dass

bei Respirationsmessungen von globalen Euphausiden Schlüsselarten alle Jahreszeiten berücksichtigt werden sollten, um die vergleichenden physiologischen und ökologischen Modelle zu verbessern.

Als nächstes wurde die respiratorische Reaktion gemessen und Experimente durchgeführt, die Hypoxie/Reoxigenierung mit Erwärmung kombinierten, um die Adaptation von Arten an Sauerstoff-Minimum-Zonen (OMZs) zu verstehen. Die im Experiment verwendeten Krillarten stammten aus der Antarktis, dem Humboldt Strom System (HCS, chilenische Küste) und dem nordkalifornischen Strom System (NCCS, Oregon). *Euphausia mucronata* aus dem HCS begann mit der metabolischen Suppression bei einer Sauerstoffsättigung (pO_2) von unter 80% (18 kPa), was auf eine Adaptation an OMZ Verhältnisse hinweist. Die beiden untersuchten Arten aus dem NCCS zeigten verschiedene energetische Strategien. *Thysanoessa spinifera* wies eine niedrigere metabolische Standardrate als *E. pacifica* und einen Atmung näher zu Konformität Muster. Die Laktatakkumulation, welche beim Erreichen des niedrigsten Sauerstoffpartialdrucks (pO_2) während des Respirationsexperimentes gemessen wurde, war höher in *T. spinifera*, was auf einen höheren Gebrauch des anaeroben Stoffwechselweges hindeutet. Der Krill aus dem NCCS, *E. pacifica*, und der antarktische Krill, *E. superba*, zeichneten sich als Sauerstoffregulatoren aus und behielten konstante Respirationsraten bis zu einer Sauerstoffsättigung von 30% (6 kPa) bzw. 55% (10 kPa).

E. mucronata und *E. pacifica* wiesen im Winter höhere SOD (Superoxid Dismutase) Werte als im Sommer auf, was auf eine höhere metabolische Aktivität im Winter zurückzuführen ist (*E. pacifica*). Bei beiden Arten blieben die antioxidantischen Enzymaktivitäten während Hypoxie bei Habitattemperatur konstant. Der normale Sauerstoffggehalt unter der Oberfläche im HCS während des Winters bedeutet bereits einen hohen Sauerstoffstress für *E. mucronata*. Eine Erwärmung von 7°C über die Habitattemperatur im Sommer erhöhte die SOD Aktivitäten und den Gluthation-(GSH)-Gehalt bei *E. mucronata* (HCS), verursachte aber keine oxidativen Schädigungen. Im Winter, wenn die Temperaturen gleichmäßig sind und keine OMZ vorliegt, wirkt eine Erwärmung um 4°C kombiniert mit Hypoxie für *E. pacifica* tödlich. Im Sommer, wenn sich die OMZ nach oben ausbreitet (100 m unter der Oberfläche), wirkten bei *E. pacifica* antioxidantische Reaktionen der Hypoxie und Reoxigenierung entgegen, dies aber nur bei leicht erhöhten Temperaturen (+2°C). Eine Erwärmung um 4°C in den Experimenten reduzierte die antioxidantische Aktivität und verursachte ein Sterben der Organismen, wie im Winter. Ein Klimawandel-Szenario, welches Erwärmung und Hypoxie kombiniert, stellt daher eine starke Bedrohung für *E. pacifica* und konsequenterweise für das NCCS Nahrungsnetz dar.

Der antarktische Krill zeigte die niedrigste antioxidantische Enzymaktivität, wies aber die höchsten Konzentrationen an molekularem antioxidantischem Glutathione (GSH) auf und zeigte keine letalen Effekte bei einer sechsstündigen Aussetzung bei moderater Hypoxie. Eine

Genexpressionsanalyse hinsichtlich des aeroben Metabolismus, der Antioxidantien-Abwehr und der Hitzeschock-Antwort unter schwerer (2.5% O_2 oder 0.6 kPa) und grenzwertiger (20% O_2 oder 4 kPa) Hypoxie wurde durchgeführt, um Aspekte der molekularen Stressreaktion zu detektieren. Die Expressionsniveaus der Gene für die Citratsynthase (CS), der mitochondrialen Mangan Superoxid Dismutase (SODMn-m) und eines Hitzeschock-Proteins Isoform (E) waren höher in den Euphausiden, die sechs Stunden einer Saurstoffsättigung von 20% ausgesetzt waren, als bei den Tieren unter Normalbedingungen. Die Transkription könnte den Krill auf eventuelle Reoxigenierung vorbereiten, was zum Schwarm-verhalten dieser Art passt. Diese an Kälte angepasste Art besitzt somit auf zellulärer Ebene Werkzeuge ihrer subpolaren Vorfahren, um Hypoxie, die weit unter den Sauerstoffkonzentrationen ihres Habitats liegt, zu tolerieren, was auf eine gute Anpassungsfähigkeit an zukünftige Bedingungen deutet.

Chapter 1

General introduction

Euphausiids constitute a major part of the macrozooplankton community in terms of total biomass and play a key role in the food webs of the world most productive marine ecosystems. They act as intermediates between primary and secondary production, and larger predators, and contribute to the vertical carbon flux by undertaking diel vertical migrations (DVMs). A typical euphausiid DVM pattern consists of an upward migration at dusk to feed in the productive layers of the oceans, and a downward movement at dawn to avoid visual predators (Zaret and Suffern, 1976; Ohman, 1984), decreasing at the same time their metabolic rates due to the lower water temperature and O_2 concentrations (McLaren, 1963; Enright, 1977). It was showed that their excretion of fecal pellets in deeper layers stimulates bacteria recycling processes in the Southern Ocean (Arístegui *et al.*, 2014), which underline their importance at all ecosystem levels. During their DVM, many krill species cross pronounced gradients of temperature, salinity, and oxygen indicating that these species must be of a broad ecophysiological plasticity.

Two modes of reproduction have been observed in euphausiids: sac-spawners (26 species) and broadcast-spawners (60 species; Brinton *et al.*, 2003, updated 2008; Gómez-Gutiérrez, 2002, 2003). Species with broadcast-spawning inhabit all latitudes (from tropical to polar ecosystems) while sac-spawning species are exclusively found in tropical and temperate regions (Gómez-Gutiérrez *et al.*, 2010). The latter species are expected to have lower fecundity and shorter life span (6-8 months) than broadcast-spawners (>6 yrs; Siegel, 2000; Gómez-Gutiérrez *et al.*, 2010). Euphausiids are able to adapt their reproductive cycles (Tarling and Cuzin-Roudy, 2003), and quickly adjust growth and moulting rhythms as trophic conditions change (Buchholz, 2003; Shaw *et al.*, 2010). This capacity of doing fast modifications in their energy allocation when favourable or unfavourable situations dominate makes euphausiids excellent indicators for abrupt changes or cyclic climatic oscillations (Pacific Decadal Oscillation, El Niño-Southern Oscillation, etc.; Brinton and Townsend, 2003; Richardson, 2008; Lavaniegos and Ambriz-Arreola, 2012), as they cannot swim away like fish from adverses conditions.

Informations about speciation processes of the 86 species remain scarce, and research has mainly focussed on the large genetic divergence between the Antarctic and sub-Antarctic species (Patarnello *et al.*, 1996; Jarman *et al.*, 2000; Zane and Patarnello, 2000). All works agree that a vicariant separation[1] of polar species from sub-polar species happened, most likely after the formation of the Antarctic Polar Frontal Zone (Patarnello *et al.*, 1996). The strong circum-Antarctic currents likely prevented population diversification of the Antarctic krill *Euphausia superba* (Zane and Patarnello, 2000).

Seven global species of the genus *Euphausia* from the sub-antarctic and the southern hemisphere showed sympatric[2] and potentially parapatric[3] speciation and were clearly separated from the non-circumglobal north hemisphere species *Euphausia pacifica* (Jarman *et al.*, 2000). Another phylogenetic paper analyzing the four euphausiid species of the neritic[4] genus *Nyctiphanes* pointed towards a dispersion speciation to explain their current anti-tropical bi-hemisphere distribution (D'Amato *et al.*, 2008). Indeed, *Nyctiphanes simplex* distributed north and south of the Eastern Tropical Pacific, *Nyctiphanes australis* is mostly south of Australia continent, *Nyctiphanes couchi* all over the northeastern Atlantic, and *Nyctiphanes capensis* is off South African coastal region (Brinton *et al.*, 2003, updated 2008). This genus may have appeared when the south hemisphere water became temperate after the formation of the circum-Antarctic current, and may have spread towards the northern hemisphere when contraction of the tropical regions occurred following a period of glacial cooling (D'Amato *et al.*, 2008).

The same pattern of dispersion was observed in different populations of a single species, the widely distributed north Atlantic species *Meganyctiphanes norvegica*, for which three different genetic pools were characterized and probably maintained by the major currents and gyres that limit gene flow (Zane and Patarnello, 2000; Patarnello *et al.*, 2010). Future dispersion of tropical euphausiid species toward higher latitudes is likely to occur as Letessier *et al.* (2011) predict higher euphausiids abundance and diversity in both Atlantic and Pacific oceans between latitudes 30° and 60° under future warming scenario. Species or populations of species confined to one hemisphere (anti-tropical) or one part of the ocean (neritic *vs.* oceanic) become often specialists, neither widely distributed nor physiologically versatile, and can be predicted to suffer from the effects of ocean warming and oxygen minimum zones expansion.

[1] when the distribution of a taxon is split by the formation of a physical barrier to gene flow
[2] evolution from a single ancestor while inhabiting the same geographic region
[3] species biogeographically distinct sharing a minimal contact zone
[4] shallow marine environment reaching approx. 200 m, often corresponding to the continental shelf

1.1 Oxygen minimum zones: implications for zooplankton distribution

The natural oxygen minimum zones or OMZs differ from the "dead zones" phenomena caused by anthropogenic coastal eutrophication found *e.g.* in the Gulf of Mexico (Rabalais *et al.*, 2002; Diaz and Rosenberg, 2008). OMZs are permanent midwater features occurring at intermediate depth (between 300 to 2 500 m) in most of the oceans (Emelyanov, 2005), and represent approx. 8% of the total oceanic area (Paulmier and Ruiz-Pino, 2009). Global ocean warming is among the causes of OMZ expansion, which can occur horizontally into areas previously not experiencing hypoxic conditions, or consists in vertical expansion of an existing OMZ. The largest (horizontally and vertically), most pronounced (abrupt oxycline), and shallowest (upper boundary) OMZs are located in the Northern Indian Ocean, the Eastern Atlantic off northwest Africa, and the Eastern Tropical Pacific (ETP; Wyrtki, 1962; Kamykowski and Zentara, 1990; Olson *et al.*, 1993). Notably, the OMZ of the ETP and the Eastern Atlantic off northwest Africa have expanded to higher latitudes during the past 50 years (Stramma *et al.*, 2008), suggesting changes in zoogeographic distribution patterns, compression of habitats, and restricted zones of biomass production (Prince and Goodyear, 2006; Koslow *et al.*, 2011; Stramma *et al.*, 2011; Gilly *et al.*, 2013).

The **California Current System** (CCS; 32-52°N, 117-130°W; temperate climate; sea surface temperature (SST) range from 10 to 14°C) and the **Humboldt Current System** (HCS; 12-45°S, 75-77°W; temperate and subtropical climates; SST from 0 to 21°C) are part of the four major eastern boundary upwelling ecosystems in the world's oceans, highly productive all year-round and crucially important for fisheries (Chavez and Messié, 2009). Both CCS and HCS are generated by equatorward winds blowing of the subtropical gyres of the Pacific, which reinforce the trade winds and drive the boundary California and Humboldt currents. Interaction with earth's rotation (Coriolis) along the shoreline and the continent topography produce a surface wind-driven offshore Ekman flow which is replaced by cool, nutrient-rich and oxygen-poor waters from below. The permanent shallow OMZ of the HCS concentrates the vertical distribution of commercial fishes to the surface layers (like anchovies), explaining the much higher amount of fisheries catch in this area compared to the CCS (Chavez and Messié, 2009). Euphausiids are the most abundant zooplankton in terms of biomass in these ecosystems (Gómez-Gutiérrez *et al.*, 1996; Mackas *et al.*, 1997; Antezana, 2010).

From the 47 euphausiid species found along the Eastern Pacific coastline, 21 do not tolerate hypoxia or distribute where shallow OMZ prevail, while only 6 species are endemic of these particular zones (Brinton *et al.*, 2003, updated 2008). Warming and OMZ expansion can cause smaller or less densely packed swarms (Brierley and Cox, 2010), restrict horizontal distribution and DVM patterns (Tremblay *et al.*, 2010; Wishner *et al.*, 2013), decrease production (growth

and egg) rates (Gómez-Gutiérrez *et al.*, 2012), and change predator-prey interactions (Abraham and Sydeman, 2006). This thesis is intended as a step forward to predict how warming with concurrent expansion of OMZs toward higher latitude is physiologically affecting the productive euphausiid species from the CCS and HCS.

1.2 Study areas and species

1.2.1 Permanent OMZs

The OMZ in the ETP is due to the poor lateral ventilation of surface waters (Reid, 1965; Luyten *et al.*, 1983) and the formation of a strong thermocline, which limits O_2 diffusion into the deeper layers of the ocean (Lavín *et al.*, 2006). Very high temperatures at the surface result in strong stratification, at which the zooplankton aggregates and increases locally the oxygen consumption (Bianchi *et al.*, 2013). Oxygen at this depth is consumed faster than replaced by the horizontal mixing of the water mass (Wyrtki, 1962; Fiedler and Talley, 2006; Karstensen *et al.*, 2008), creating the shallow OMZ. The oxygen utilization is particularly enhanced during El Niño-Southern Oscillation and inter-annual changes in upwelling conditions, thus partly explaining the vertical OMZ expansion of the ETP since the 1980s (Ito and Deutsch, 2013). ***Euphausia lamelligera*** (Adults: 7-11 mm length; Brinton *et al.*, 2003, updated 2008; Fig. 1.1a, 1.3) and ***Euphausia distinguenda*** (Adults: 10-15 mm length; Brinton *et al.*, 2003, updated 2008; Fig. 1.1b, 1.3) are two relatively small euphausiid species, endemic of the OMZ of the ETP. In the region adjacent to the Gulf of Tehuantepec (Mexico) up to the entrance of the Gulf of California (Mexico), *E. lamelligera* dominates the neritic space while *E. distinguenda* distributes more in oceanic waters (Brinton, 1962, 1979; Färber-Lorda *et al.*, 1994, 2004, 2010). Because of its neritic preference, *E. lamelligera* does not migrate as much as *E. distinguenda*, which remains in the OMZ during the day and migrates to oxygenated waters during the night.

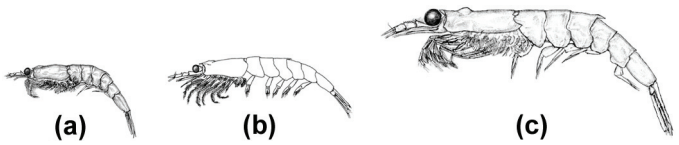

Figure 1.1: OMZ adapted species: (a) *Euphausia lamelligera* (ETP), (b) *Euphausia distinguenda* (ETP), (c) *Euphausia mucronata* (HCS). The illustrations are reflecting the adults scale size of the species (2:1) and were modified from the Marine Species Identification Portal (`http://species-identification.org/index.php`).

In the **HCS**, the OMZ have been associated with lateral mixing of the Equatorial Subsurface Water mass, which is a frequent source of upwelling water in the region. This water mass

produces a large area with reduced oxygen concentration in surface waters (Copin-Montégut and Raimbault, 1994; Charpentier *et al.*, 2012). The upper boundary of the OMZ off Chile is represented by a sharp oxycline, which is usually located close to the surface (approx. 50 m depth in the neritic area; Judkins, 1980; Morales *et al.*, 1999). Over the continental shelf of Concepción (Chile), the OMZ is shallower and more steep during the austral summer compared to winter (Paulmier *et al.*, 2006). In this area, seasonal variability of dissolved oxygen concentrations was found to be mainly influenced by the biological net oxygen production and the alternation of upwelling and downwelling oceanographic processes (Charpentier *et al.*, 2012). This seasonal variability or intensification of hypoxia might affect the species that remain at great depth to avoid visual predation in the euphotic layers. ***Euphausia mucronata*** (Adults: 17-22 mm length; Brinton *et al.*, 2003, updated 2008; Fig. 1.1c, 1.3) is endemic to the temperate HCS and plays a keystone role in the food web as principal prey of jack mackerel and anchovy (Antezana, 2010). Highest abundance and biomass of *E. mucronata* coincide with the transition from upwelling (austral autumn) to downwelling (austral winter) conditions (Riquelme-Bugueño *et al.*, 2013). The species performs extensive DVM down to 250 m into the OMZ in all seasons (Escribano *et al.*, 2000; Antezana, 2002b), but the highest abundances occur in areas where the upper boundary of the OMZ is deeper (Escribano *et al.*, 2000). Fast and continuous swimming movements observed in this species help to keep O_2 uptake constant in hypoxic conditions (Antezana, 2002a), so that *E. mucronata* maintains the same rate whether exposed to surface pO_2 (70% saturation or 17 kPa), or to pO_2 typical for OMZ layers (20% saturation or 4 kPa) in the HCS, also during the warm season (Teal and Carey, 1967; Antezana, 2002a; Donoso and Escribano, 2014).

E. distinguenda is also reported in the OMZ of the HCS (Antezana, 2002a, 2009). Both *E. distinguenda* and *E. mucronata* possess larger gills relative to their body size (Antezana, 2002a), increasing contact surface for O_2 diffusion from the hypoxic environment. Antezana (2009) also observed that both were among the last OMZ species to begin their ascent to the surface at dusk in the HCS, thus extending the deep hypoxic residence time to a maximum. Habitat segregation was suggested to explain this behaviour, which consists in avoiding spatial and temporal co-occurrence with other species within the same area. This finding was based on body and gills size analysis, feeding appendages, and HCS food resources.

1.2.2 Non-permanent or less severe OMZs

In the northern CCS (**NCCS**), a critical expansion of severe hypoxia (as low as <0.5 mL L^{-1}) was described for the first time in 2006. This unusual level of hypoxia with a spatial horizontal extension of 3000 km^2 between the shelf break and the inner shelf persisted all summer into autumn (Chan *et al.*, 2008). According to the World Ocean Atlas of 2009, the severe hypoxia seems to come back every year, and is most pronounced in the months of August and September.

Patterns and occurrence of severe and seasonal hypoxia were recently analyzed using a 15 yr data set (1998-2012) of hydrographic measurements at 40-48.5°N latitude (Peterson *et al.*, 2013). The greatest expansion and severity of hypoxia was observed in years with the lowest concentration of dissolved oxygen in upwelled waters. Large-scale circulation processes are involved in the severity of hypoxia in the NCCS as a connection between the variability in dissolved oxygen concentration of upwelled waters and the North Pacific Gyre Oscillation, a wind related index, was established by the same authors. ***Euphausia pacifica*** (Adults: 11-25 mm length; Brinton *et al.*, 2003, updated 2008; Fig. 1.2a, 1.3) is the most abundant euphausiid species in the coastal waters of the NCCS and important prey for many fish species (Brodeur and Pearcy, 1992; Tanasichuk, 1999), specifically herring (Mangel and Nicol, 2000). All along the Pacific coast of the United-States of America, juveniles and adults of this oceanic species perform DVM between the surface and depths of at least 250 m (Brinton, 1967). In fjords and bays their downward migration is often reduced (Bollens *et al.*, 1992), sometimes limited by seasonal hypoxic or anoxic conditions in bottom water layers (Kunze *et al.*, 2006). Recently, Fisheries and Oceans Canada Ministry (2013) has expressed concern regarding the future of this important commercial species. They observed unprecedented year-to-year fluctuations of krill biomass and hypothesized that climate change and enhanced predation may be responsible for the high mortalities in the low krill years.

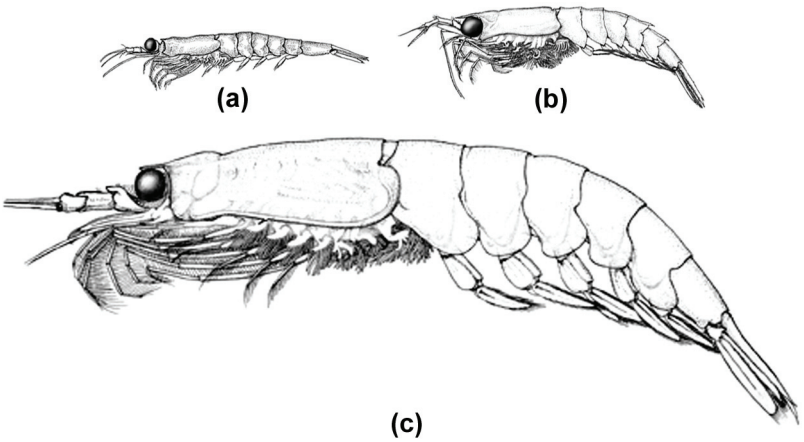

Figure 1.2: Non-adapted OMZ species: (a) *Euphausia pacifica* (NCCS), (b) *Thysanoessa spinifera* (NCCS), (c) *Euphausia superba* (South Georgia, Antarctic). The illustrations are reflecting the adults scale size of the species (2:1) and were modified from the Marine Species Identification Portal (`http://species-identification.org/index.php`).

Thysanoessa spinifera (Adults: 16-25 mm length; Brinton *et al.*, 2003, updated 2008; Fig. 1.2b, 1.3) is another euphausiid species found in the neritic area of the NCCS, strongly associated with upwelling events and cold water (Smith and Adams, 1988; Lavaniegos and Ambriz-Arreola, 2012). It was shown that seabirds and salmons abundance vary in accordance with *T. spinifera*

biomass in the shelf waters of the Gulf of the Farallones (off California; Sydeman *et al.*, 2013). No acoustic or direct observation related to OMZ or hypoxia has been reported for this species, but an unusual massive stranding event on approx. 400 km coastline in June 2013 (Tyburczy *et al.*, 2013) could be the consequence of an intolerance of severely hypoxic conditions.

Polar waters do not count to the OMZ regions, but represent a great point of comparison as severe hypoxia below 20% O_2 saturation (4 kPa) does not exist. Nonetheless, mild-hypoxia (50% O_2 saturation) was reported in the Indian sector of the Southern Ocean at depth greater than 500 m (Dehairs *et al.*, 1990). Deoxygenation in the Southern Ocean is currently taking place in this sector at 200-400 m depth between 50 and 60° of latitude (Matear *et al.*, 2000; Aoki, 2005). The principal concern in the Antarctic is not deoxygenation, but the decline of sea-ice extent as a consequence of water warming (Meredith *et al.*, 2008; Pritchard *et al.*, 2012; Rignot *et al.*, 2013). The Antarctic krill ***Euphausia superba*** (Adults: 42-65 mm length; Brinton *et al.*, 2003, updated 2008; Fig. 1.2c, 1.3) is a central constituent of Antarctic food webs and forms large biomasses in the Southern Ocean (Atkinson *et al.*, 2004; Murphy *et al.*, 2007). Cumulative impacts of sea ice decline and ocean warming have negatively modified the abundance, distribution and life cycle of this species (Flores *et al.*, 2012). Recent experimental works also showed negative impact of higher pCO_2 concentrations on embryonic and larval development (Kawaguchi *et al.*, 2010, 2013), and on adult feeding and excretion rates together with metabolic key enzyme activities (Saba *et al.*, 2012). This euphausiid species is the most studied of all, because of its importance in the Antarctic ecosystem.

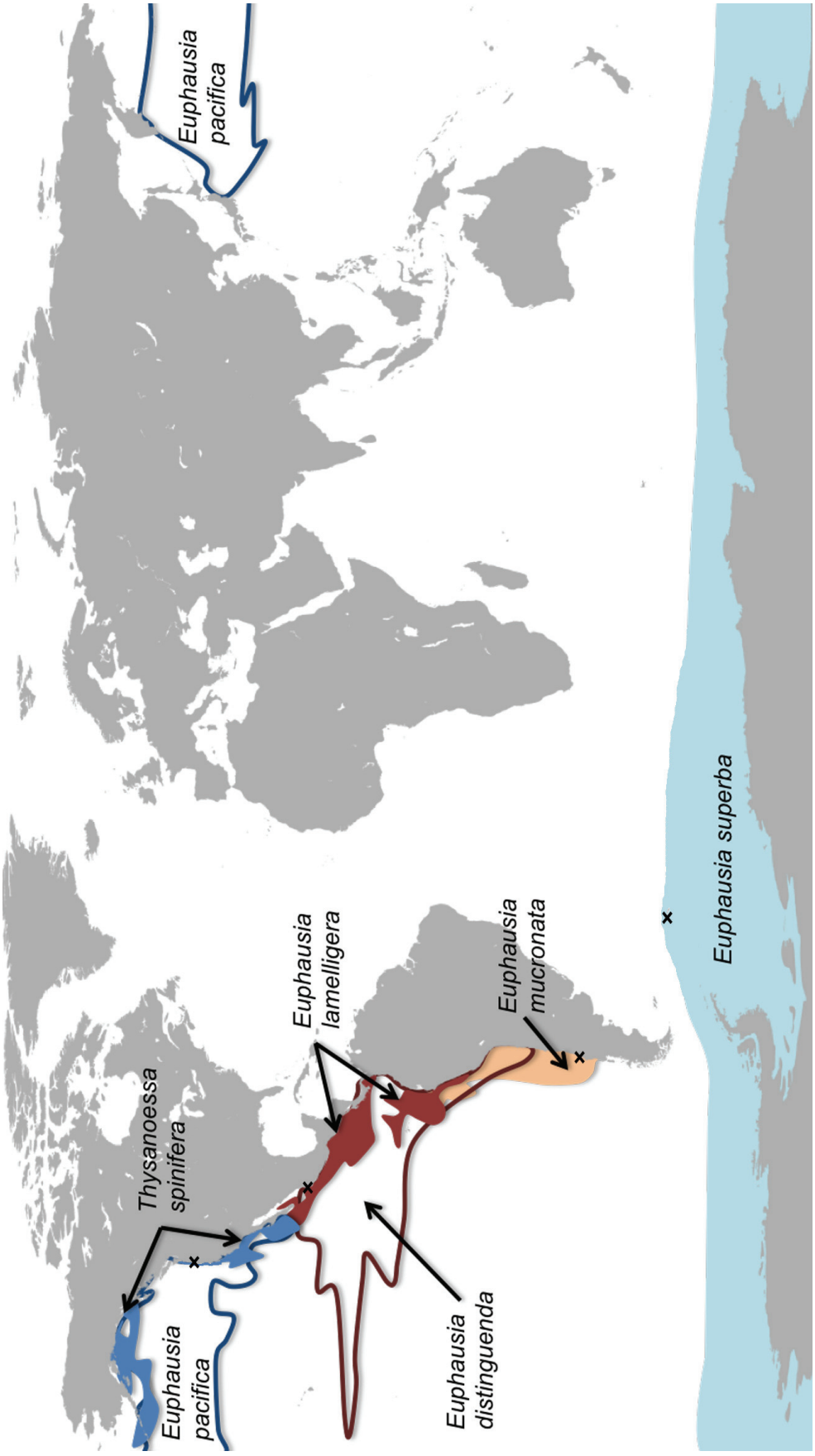

Figure 1.3: Spatial distribution of the species analyzed with black crosses marking the sampling sites. The black arrows show the distribution of each species (except *Euphausia superba*), which was built from the Marine Species Identification Portal (`http://species-identification.org/index.php`)

1.3 Energy metabolism in euphausiids

Aerobic organisms rely mainly on O_2 for their energy production. When partial pressure of oxygen (pO_2) decreases, they can adopt two oxystrategies, oxyconformity or oxyregulation. Oxyconformity is observed when the respiration rate is decreasing in accordance with the ambient pO_2 in a linear manner, while oxyregulation is a maintenance of the same oxygen uptake independently of the decreasing pO_2 (Bishop, 1973). Oxyregulators can keep their respiration rate until a certain point called the critical pO_2 (pc), below which they switch to oxyconformity.

Many organisms with larval stages change oxystrategies in response to decreasing pO_2 throughout their life cycle. The best observations representing pO_2 ontogenic strategies in euphausiids from egg to post-larva form were reported for the Antarctic krill *E. superba* and are summarized in three phases (Quetin and Ross, 1989): 1) During the embryonic stage until just before the hatching of eggs, oxygen uptake is mainly through diffusion which qualifies respiration of embryos as pO_2-dependent or 100% oxyconforming. As embryos rely on lipid reserves, they spend only <5% of the metabolic costs of the other non-feeding stages. Eggs are generally released in mid-water layers and sink to deeper layers where temperature is often colder and sometimes less oxygenated. 2) After hatching, the larvae still breathe by diffusion, but their metabolic rate is significantly less O_2-dependent. Even if the pre-stages do not have gills, the area available for O_2 diffusion increases as different appendices are developped, and oxygen uptake is facilitated by swimming movements. The first ascent to upper ocean layers begins to reach the feeding areas. 3) During the postlarval (feeding) stages, diffusive oxygen uptake decreased as the larva grows and as the exoskeleton becomes thicker. At this stage, euphausiids have higher energy requirements and possess external gills (from Furcilia I stage) to increase significantly the respiratory surfaces and the uptake of O_2. Although all adult euphausiids possess external and developed gills, they are not all strictly oxyregulating when pO_2 is decreasing.

In addition to environmental pO_2, the respiration rates of euphausiids depend on temperature (Small and Hebard, 1967; Gilfillan, 1972) and salinity (Gilfillan, 1972). When oxyregulators reach pc and switch to oxyconformity, the aerobic scope decreases and the anaerobic pathway is normally initiated to maintain a minimum energy production and fullfil the essential metabolic requirements. This pc is normally shifted upward if the organism is exposed to higher temperatures, as metabolic rate increases (Seibel, 2011; Fig. 1.4).

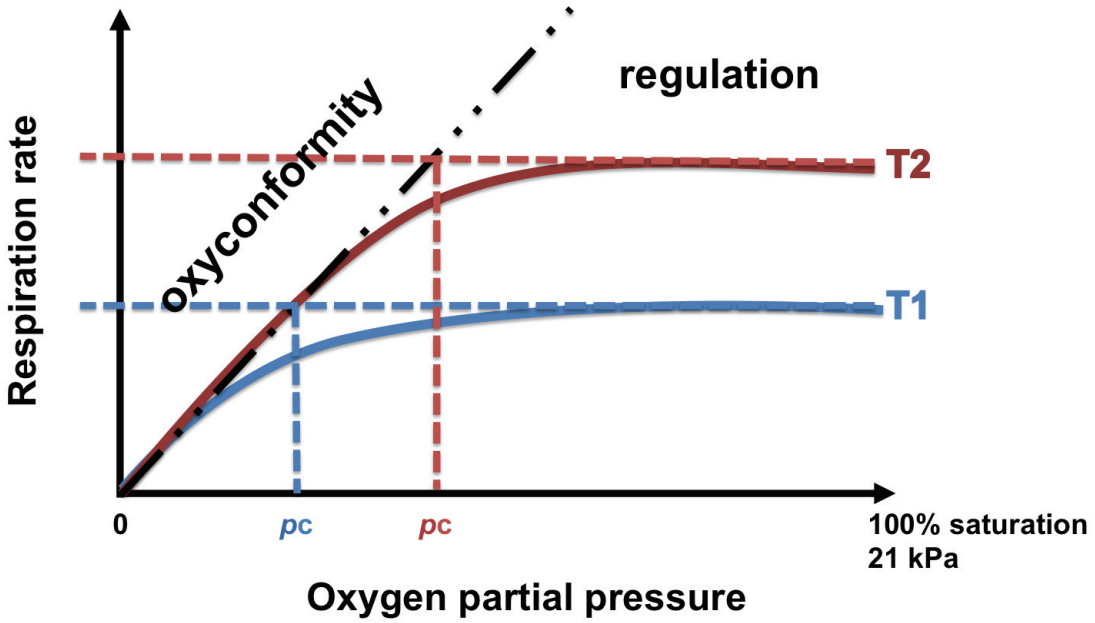

Figure 1.4: Schematic representation of the temperature effect on critical oxygen partial pressure (*pc*). Blue and red colours refers to the respiration rates at lower (T1) and higher temperature (T2), respectively. The *pc* is determined at the intersection of the oxyconformity (black dash and dot) and the oxyregulation (blue and red dash) tangent lines. The *pc* value is higher at T2.

The induction of the anaerobic pathway can be corroborated with the measurement of an end-products, *i.e* lactate in the case of euphausiids. If pO_2 remains below *pc* and energy reserves (glycogen) are exhausted by anaerobic respiration, the organisms are bound to die. Glycogen reserves were never assessed in euphausiids, but are fast depleted in other crustacea under anaerobic conditions (Taylor and Spicer, 1987; Hill *et al.*, 1991). For example, in the north Pacific krill *Euphausia pacifica*, *pc* was detected at 20% oxygen saturation (4 kPa pO_2,), which manifested in a dramatic reduction of swimming activity, and in high mortalities below this critical pO_2 (Childress, 1975; Ikeda, 1977; *pc* of 18 mm Hg at 10°C and 20% O_2 saturation at 13°C, respectively). This was also observed in a "natural" experiment caused by an event of unusual hypoxic deep water in the Gullmarsfjord (Sweden), inhabited by the migrating northern Atlantic krill *M. norvegica* (Spicer *et al.*, 1999; Spicer, 2013). In this work, lactate measurements of krill maintained in the hypoxic bottom waters of the fjord was run in parallel with laboratory metabolic measurements (respiration rates and *pc*), and showed insufficient anaerobic capacity for prolonged exposure to the hypoxic conditions prevailing in the fjord (6.1 kPa; 70 m depth; 6.5°C). These examples show how basal O_2 consumption rate, oxystrategy and *pc* value are particularly relevant to explain hypoxia intolerance in marine organisms.

1.4 Indicators of stress tolerance mechanisms

Oxidative stress parameters can be helpful to determine the level of cellular stress an organism experiences and mechanisms involved in the response. The term *oxidative stress* refers to a state of respiratory imbalance in which animals cannot maintain constant tissue oxygenation and instead experience rapid shifts between over and under-oxygenation. In this case, especially when animals are re-oxygenated after hypoxic exposure, like euphausiids crossing an OMZ during DVM, reactive oxygen species (ROS)[5] are formed which, if not neutralized by the organism's antioxidant defences, cause oxidative damage and eventually cellular disorders and death (Halliwell and Gutteridge, 2007).

Each cell of an organism produces a basal rate of approx. 0.1% ROS from the oxygen it consumes under aerobic conditions (Fridovich, 2004). ROS formation can change as a function of animal O_2 consumption, although there is no strict one to one relationship between both parameters (see Buttemer *et al.*, 2010), and it remains extremely controversial during hypoxia and anoxia (Murphy, 2009). Mitochondria isolated from rat liver exposed to low O_2 concentration (5 µmol of O_2 to anoxia) decreased their ROS production in a respiration dependent manner (Hoffman *et al.*, 2007), which fits well with the respiration rates ROS production theory. Lower ROS production during hypoxia was observed as well in two bivalves and in a marine worm (Strahl *et al.*, 2011; Rivera-Ingraham *et al.*, 2013a,b). The opposite was altough observed in different classes of animals (Abele and Oeschger, 1995; Bickler and Buck, 2007; Clanton, 2007), in which ROS formation increased even if O_2 consumption was decreasing. In these cases, ROS potentially act as cell messengers to induce cell recovering or maintenance responses if hypoxia is prolonged or if oxygen returns during reoxygenation (Hochachka and Lutz, 2001; Guzy and Schumacker, 2006; Hamanaka and Chandel, 2009). As some euphausiids species cross important gradients of oxygen (sometimes OMZ) during their DVMs, thus continuously changing from hypoxia to well-oxygenated waters, they are supposed to have developed physiological defence mechanisms to compensate for these disturbances. Thus we would expected some anti-oxidative strategies in species from permanent OMZ regions (ETP and HCS) compared to species normally not experiencing strong oxygen variations (NCCS and Antarctic).

In this thesis, oxidative stress defence systems were investigated in different krill species *in situ* and exposed experimentally to hypoxia. The antioxidant defence system refers to molecular compounds (reduced glutathione, vitamins C and E, transferrins, haptoglobin) and enzymes that detoxify cells either by converting ROS to less toxic oxygen derivatives or by their elimination (Fig. 1.5). The glutathione exists in both reduced (GSH) and oxidized (GSSG) states. The reduced state possesses the thiol group of cysteine, which is able to donate a reducing equivalent (H^+ or

[5] reactive molecules derived from oxygen, such as the superoxide anion ($O_2^{\bullet -}$), hydroxyl radical ($OH^{\bullet}$), and hydrogen peroxide (H_2O_2)

e^-) to other unstable molecules, like xenobiotics or ROS. By doing so, glutathione itself becomes highly reactive and immediately combines with other glutathione anion (GS^-) to form glutathione disulfide (GSSG), or oxidized glutathione (Fig. 1.5). GSSG is converted back to GSH by the enzyme glutathione reductase (GR; Fig. 1.5). Thus, fluctuation of the GSSG: GSH redox ratio could indicate either ROS overproduction or inhibition of the GR. The main antioxidant enzymes that eliminate or change the configuration of the ROS are the superoxide dismutase (SOD; Fig. 1.5), catalase and glutathione peroxidase (CAT; GPx; Fig. 1.5), and the glutathione-S-transferase (GST; Fig. 1.5). Both GPx and GST enzymes rely on GSH, which connect the enzymatic pathway with the GSSG: GSH redox ratio and GSH availability in cells (Fig. 1.5). GR uses nicotinamide adenine dinucleotide phosphate (NADPH) as substrate, which is supplied by the action of the glucose-6-phosphate dehydrogenase (G6PDH; Fig. 1.5). GR and G6PDH activities were not analyzed in this thesis. Depending of the organ, organism, type, and duration of stress exposure, antioxidant levels (or activity in the case of the enzymes) can either increase or decrease. When the antioxidant system fails to detoxify ROS, $O_2^{\bullet -}$ and H_2O_2 can react with a transition metal (like Fe) that is not securely bound to a protein by Haber-Weiss or Fenton reactions and form $OH^\bullet$, the most reactive and damaging ROS (Fig. 1.5).

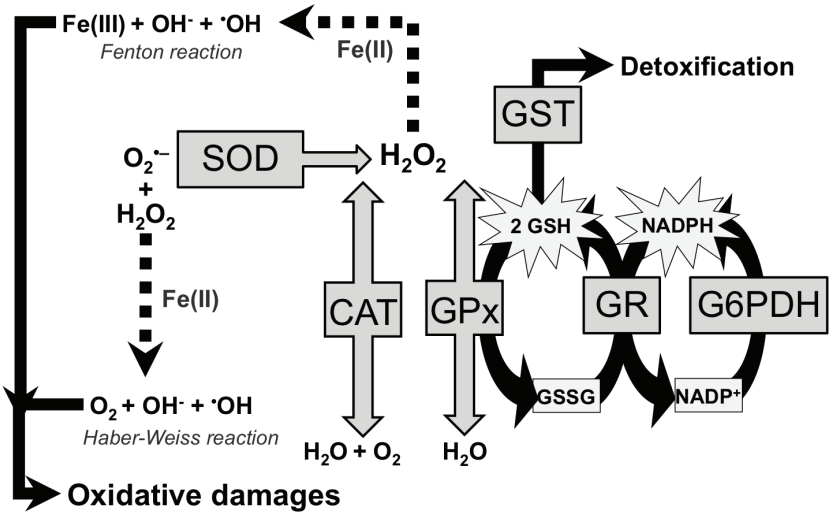

Figure 1.5: Enzymatic and glutathione antioxidant defence systems (modified from Hermes-Lima, 2004). When a superoxide anion ($O_2^{\bullet -}$) is formed, the superoxide dismutase (SOD) converts it to hydrogen peroxide (H_2O_2). The latter is removed by the action of the catalase (CAT) without substrate or by the glutathione peroxidase (GPx) using reduced glutathione (GSH) as substrate. Once used by the GPx, the oxidized glutathione (GSSG) is reconverted to GSH by the action of the glutathione reductase (GR), which needs nicotinamide adenine dinucleotide phosphate (NADPH) to achieve this task. The NADPH is furnished by the action of the glucose-6-phosphate dehydrogenase (G6PDH). The last enzyme of this system, the glutathione S-transferase (GST), transforms xenobiotics into other conjugates as part of a detoxification route from GSH. If $O_2^{\bullet -}$ or H_2O_2 are not eliminated by the antioxidant system, they can react with a transition metal (like Fe), not securely bound to a protein, by Haber-Weiss or Fenton reactions and form the hydroxyl radical ($OH^\bullet$). The $OH^\bullet$ is the most reactive oxygen species and will probably causes oxidative damages.

Lipid and protein oxidation by $OH^{\bullet}$ are two of the most important processes that cause cell damages (Halliwell and Gutteridge, 2007). In proteins, ROS modify the amino acid specific site or the peptide chain fragment, alter electrical charge, and increase degradation (Catalgol *et al.*, 2011). ROS generation can also induce lesions in deoxyribonucleic acid (DNA), particularly in mitochondrial DNA (Addabbo *et al.*, 2009), causing deletions, mutations, and other lethal genetic injuries (Cooke, 2003). The oxidative stress state is determined by measuring an increase of oxidative cell damage to lipids, proteins or DNA, or when the tissue reduced glutathione (GSH) is depleted by oxidation.

An experimental approach in the white shrimp *Litopenaeus vannamei* confirmed oxidative stress after exposure to hypoxia-reoxygenation (Zenteno-Savín *et al.*, 2006). The antioxidant capacity of hepatopancreas and muscle were mainly affected during the first hours of reoxygenation, but started to recover two hours after return to normoxia. This type of recuperation has also been observed in the crab *Chasmagnathus granulata* (de Oliveira *et al.*, 2005). Thus, some crustaceans used to hypoxic exposure have the capacity to restore their normoxic physiological homeostasis after a readjustment process during reoxygenation.

By analyzing *in situ* oxidative stress indicators on three euphausiid species in the Gulf of California (Mexico), Tremblay *et al.* (2010) found distinct oxidative stress indicator levels between species that were crossing the OMZ and species that do not. *Nematoscelis difficilis*, which remains inside or slightly above the OMZ, had higher GPx activity than the species that always stays above OMZ, *Nyctiphanes simplex*. The latter has its lipids severely oxidized in warmer conditions, when OMZ was shallower. By possessing higher GPx activity, *N. difficilis* is tolerating hypoxic water and can avoid the damaging warmer strata. Referring to *Euphausia eximia*, which is also present in the HCS, higher $O_2^{\bullet -}$ production[6] caused by its DVM through the OMZ was balanced by its antioxidant enzyme activities, as smaller lipid peroxidation was detected. Higher $O_2^{\bullet -}$ production as well as SOD and GR activities in *N. simplex* and *N. difficilis* in deeper strata were interpreted as a preparation for the reoxygenation derived from their posterior migrations to upper oceanic layers (Tremblay *et al.*, 2011). Higher SOD activity and lipid peroxidation levels of *E. eximia* at the surface compared to animals from deeper water layers were interpreted as a reoxygenation consequence after daytime residence in hypoxic conditions (16% O_2 saturation or 3.3 kPa; Tremblay *et al.*, 2011). These *in situ* physiologic measurements give some idea about how the OMZ adapted and non-adapted species can react to hypoxia-reoxygenation (Tremblay *et al.*, 2010, 2011), but remains speculative as no experiment was achieved at that time to really test the effect of both factors.

[6]measured as a potential production rate in the tissue after Drossos *et al.* (1995)

Another stress defence system is the heat-shock response (HSR), which involves induction of heat shock protein (HSP) expression. HSPs are molecular chaperones, which prevent the formation of nonspecific protein aggregates and assist with re-folding of partially denatured proteins damaged under stress (Lindquist, 1986; Morimoto, 1993; Sørensen *et al.*, 2003; Kültz, 2005). Contrary to what its name may suggest, the HSR response is not only expressed during stressful heating (Moran *et al.*, 1978; Piano *et al.*, 2002), but also when facing cold (Rinehart *et al.*, 2007; Sinclair *et al.*, 2007), anoxia (Ramaglia and Buck, 2004), hyperoxia (Wong *et al.*, 1998), hypoxia (Baird *et al.*, 2006), ultraviolet radiation (Bonaventura *et al.*, 2006; Niu *et al.*, 2006), and pollution (Pyza *et al.*, 1997; Mukhopadhyay *et al.*, 2003). Thus, the HSR represents an complemental tool for physiological studies, as basal expression level of HSPs differs among species depending on their resistance to stress and according to their adaptive history. HSP expression was already analyzed to understand climate change stressors in several studies (Hofmann, 1999; Tomanek, 2010; Pöhlmann *et al.*, 2011; Clark *et al.*, 2013).

1.5 Aims of the thesis

In my thesis, I investigated the oxystrategies and OMZ tolerance mechanisms of euphausiids on a global-scale with the aim to explain the current zoogeographical patterns of major species and project it into the future. Three main questions have arisen from this overall aim:

Question 1: Is there a seasonal influence on euphausiids metabolic rate visual at global scale? (Chapters 3 and 4)

Background

The aerobic metabolic rate is a proxy for energy turnover in ecological studies. In a recent global model, euphausiid metabolic rates were shown to significantly depend on body mass, habitat temperature, and water depth of sampling (Ikeda, 2012). In addition to these variables, the estimation of respiration rates should take into consideration the season changes in oceanographic conditions (*e.g.* upwelling/downwelling). Indeed, increasing metabolic rates are expected when higher primary production occurs, which would increase the carbon flux to the deeper layers. Important differences in the diel vertical migration (DVM) pattern (downward limit) have been reported in euphausiid species from diverse climatic areas which are link to season and carbon transit to the deeper layers (Brinton, 1979; Gaten *et al.*, 2008; Taki, 2008; Tremblay *et al.*, 2010; Sato *et al.*, 2013; Werner and Buchholz, 2013; Haraldsson and Siegel, 2014). A global euphausiids respiration model including the effect of latitude, the day of the year, and the number of daylight hours, in addition to the basal variables that determine ectothermal

oxygen consumption (temperature, body mass and depth) was attempted to link space and time in terms of season and photoperiod to krill respiration.

Strategy

Compilation of euphausiid respiration data sets (2550 respiration data sets; 31 species; 52 sources) and integration of three extra parameters (latitude, day of the year, and daylight hours) in a global euphausiid respiration artificial neural network model. Test for seasonality (multiple linear regression or general additive model) in the respiration rate of single species: the Antarctic krill *Euphausia superba*, the North Pacific krill *Euphausia pacifica*, and the North Atlantic krill *Meganyctiphanes norvegica*. The selection of single species was based on the number of data sets available, the multi-seasonal coverage, and the geographical distribution of the measurements. Further, relationship between the citrate synthase activity, the first enzyme of the Krebs cycle from which energy intermediates are produced during aerobic respiration, and respiration measurements was tested globally at *in situ* temperature to see if this enzyme could be used as a predictor for respiration rate in euphausiids.

Question 2: How do ocean warming and widening of the OMZ affect euphausiid species in different seasons? (Chapters 5 and 6)

Background

One of the most important consequences of climatic change at tropical and temperate latitudes is the expansion of oxygen minimum zones (OMZ), especially in coastal and shelf regions (Helly and Levin, 2004). During their diel vertical migrations (DVM), krill cross important gradients of temperature, salinity, and oxygen indicating that these species need a broad ecophysiological tolerance. Upward migration of some subtropical and temperate productive species are restricted by thermocline formation, whereas downward migration can be limited by a shallower OMZ (Tremblay *et al.*, 2010; Wishner *et al.*, 2013). Impairment of DVM can enhance visual predation at the surface (Fernández-Álamo and Färber-Lorda, 2006) and may cause mass mortality of krill when oceanographic conditions suddenly change (Tyburczy *et al.*, 2013; Oregon and northern California). The objective was to understand the seasonal effect of oxygen deficiency and higher temperature on major krill species to predict their response to OMZ exacerbation.

Strategy

Sampling different euphausiid species known to differ in terms of hypoxia tolerance and climatic environment during two different seasons in which OMZ showed variation in intensity

and/or occurrence. I used *in situ* samples and experimental approaches to understand why some species are able to rapidly migrate in and out of the hypoxic OMZ layers without suffering pronounced oxidative stress compared to other species from the same geographic or climatic background. Controlled laboratory experiments on the field were aimed to understand how hypoxia, reoxygenation and higher temperatures modulate the euphausiid's survival and physiological response. The comparison was based on the respiratory pattern (oxyconformity or oxyregulation), and the investigation of basal metabolic and oxidative stress indicators *in situ* and under experimental exposure.

Question 3: Is the hypoxia stress response lost in the cold adapted Antarctic krill *Euphausia superba*? (Chapter 7)

Background

Once gene flow was restricted by the Antartic Polar Front Zone (APFZ), speciation of cold adapted organisms took place to sustain normal metabolic activity against the slowing effect of constant low environmental temperatures. In the last decades, sea ice decline and ocean warming have jointly reduced the abundance, distribution and impacted life cycle of the key stone krill species *Euphausia superba* (Atkinson *et al.*, 2004; Murphy *et al.*, 2007; Flores *et al.*, 2012). *E. superba* stocks located in the border of the APFZ at South Georgia (54°17'S; 36°30'W) are likely vulnerable to the environmental stresses projected for the Antarctic. Severe hypoxic conditions do not occur in the Southern Ocean, but OMZs have been expanding in the Indian sector between 200 and 400 m (Matear *et al.*, 2000; Aoki, 2005). The effect of hypoxia, consequence of global change scenario, has been studied in some polar fish and molluscs (Wells *et al.*, 1989; Woods *et al.*, 2009; Clark *et al.*, 2013), but remains unknown for *E. superba*. It is an open question whether or not *E. superba* maintains some hypoxia tolerance in spite of its long standing evolution in cold and well oxygenated waters, which would help to adapt to worse environmental stressors in future scenarios.

Strategy

Exposure experiments with *E. superba* to severe hypoxia (2.5% O_2 saturation or 0.6 kPa) for 1 h, and threshold hypoxia level (20% O_2 saturation or 4 kPa) for 6 h. Measurements of basal metabolic and oxidative stress indicators complemented by studies of stress and metabolic gene expression related to aerobic metabolism, antioxidant defences, and heat-shock response.

Chapter 2

Materials and methods: overview

2.1 Data acquisition for the euphausiids global respiration model: Chapter 3

Euphausiid respiration data were searched in the literature and recent unpublished data were provided by several colleagues. Each data set included the following parameters:

- Sampling site latitude *LAT* and longitude *LON*;
- Sampling water depth *D*;
- Day of the year *DoY*;
- Number of daylight hours *DLh* at the *DoY* calculated from the latitude with the sunrise-sunset calculator (`aa.usno.navy.mil/data/docs/RS_OneDay.php`);
- Measurement temperature *T*;
- Body mass *M* (J), converted from original body mass units;
- Specific respiration rate *RR* (J J^{-1} day^{-1});
- Taxonomic informations (species, genus, family);

After quality control and data transformations, fully factorial multiple regression models (MLR) as well as Artificial Neural Network (ANN) were applied for the general respiration model. Then, euphausiid species for which a considerable number of data set is distributed throughout the year were selected, *i.e.*, *Euphausia superba*, *Euphausia pacifica* and *Meganyctiphanes norvegica* for the analysis of seasonality in respiration rate with MLR. If a linear relationship between *DLh* and *RR* and a corresponding sinusoidal relationship between *DoY* and *RR* were present, a General Additive Models (GAM) was used.

2.2 Comparison of stress responses to hypoxia and warming: Chapters 5, 6, 7

All laboratory analyses were conducted at the Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research in Bremerhaven (Germany) and RNA extraction, primers design and reverse transcription quantitative polymerase chain reaction (RT-qPCR) methods learning at the "Station Biologique de Roscoff" (France). During the field trips and sampling cruises, respiration rate measurements, metabolic response to warming and hypoxia/reoxygenation experiments were assessed using live euphausiids. For comparison, *Euphausia pacifica* and *Thysanoessa spinifera* were selected from the northern California Current system (NCCS; temperate region with seasonal hypoxia; Tab. 2.1), *Euphausia distinguenda* and *Euphausia lameligera* from the Eastern Tropical Pacific (ETP; tropical region with permanent hypoxia; Tab. 2.1), *Euphausia mucronata* from the Humboldt current system (HCS; temperate region with permanent hypoxia; Tab. 2.1), and *Euphausia superba* from South Georgia in Antarctica (polar region without hypoxia; Tab. 2.1).

Table 2.1: Sampling areas, Latitude/Longitude (Lat/Lon), periods, sampling gears, research vessel (R/V), off board facilities, and cold room temperature in Antarctica (South Georgia), in the Humboldt current system (HCS), in the Eastern Tropical Pacific (ETP), and in the northern California current system (NCCS).

Area	Lat/Lon	Period (season)	Water depth (m)	Sampling gear	R/V; off board facilities (if applied)	Cold room T°C
Antarctica (South Georgia)	53-55°S 37-41°W	3-10th Jan 2012 (summer)	<400	pelagic net, rectangular midwater trawl (RMT), 8 m^2 mouth area	James Clark Ross	4.0
HCS (Chile)	36.5°S 73.1°W	23th Aug- 13th Sep 2011 (winter) 24th Jan-3rd Feb 2012 (summer)	80	zooplankton net, 1 m diameter, 5 m long, 300 µm black mesh with nonfiltering cod end (0.22 m diameter and 0.70 m long)	Kay-Kay II; Universidad de Concepción, Marine biology laboratory (Dichiato, Región del Biobío)	8.0
ETP (Mexico)	15.4°N 94.5°W	16-24th Jun 2011 (summer)	245	bongo net, 0.6 m diameter, 333 µm mesh with filtering cod end	BIP XII	-
	19.2°N 104.7°W	22th Feb- 12th Mar 2012 (winter)	80	vertical zooplankton net 333 µm mesh	Universidad de Guadalajara, Centro de Ecología Costera (San Patricio Melaque, Jalisco)	20.0
NCCS (United-States of America)	44.7°N 124.7°W	14-30th Sep 2011 (summer) 7-14th Apr 2012 (winter)	275	bongo net, 0.6 m diameter, 333 µm black mesh with nonfiltering cod end	Elakha; Oregon State University, Hatfield Marine Science Center (Newport, Oregon)	10.0

Each area was visited during cold (winter) and warm (summer) seasons, except South Georgia in Antarctica, which was sampled only during the warm season (Tab. 2.1). To reduce sampling stress, krill fishing was conducted at night when the krill are near the surface. After heaving the sampling gear on deck, the collected zooplankton was immediately transferred to 20 L buckets with seawater. Live adult euphausiids, showing a lot of movement and with no visible damage, were manually sorted into bins (colman boxes, or tanks of 100 L in Antarctica) filled with filtered seawater and transferred to a cold room (see Tab. 2.1 for temperature). Directly after catch, some specimens were snap frozen in liquid nitrogen (N_2; ETP, HCS) or at -80°C (NCCS, SG) for biochemical analysis of *in situ* values (Tab. 2.2). Others were preserved in RNA*later*® (ETP) for gene expression analysis of *in situ* values (Tab. 2.2).

Table 2.2: Number of samples preserved frozen (n_{frozen}) and in RNA*later*® (n_{RNA}) directly after catch in South Georgia (SG), the Humboldt current system (HCS), the Eastern Tropical Pacific (ETP), and the northern California current system (NCCS).

Area	Period	Sea surface* T°C	Water column‡ T°C	n	n_{frozen}	n_{RNA}
SG	Jan 2012	3.2	1.5	290	290	0
HCS	Aug 2011	11.9	11.7	81	81	0
	Feb 2012	12.9	11.0	71	71	0
ETP	Jun 2011	30.1	14.4	148	0	148
	Mar 2012	21.3	16.7	100	50	50
NCCS	Sep 2011	11.9	8.1	60	60	0
	Apr 2012	9.9	7.7	90	90	0

*from 0 to 20 m depth; ‡from 20 m to maximum depth

The remaining animals were left to recover for at least 6 h before respirometry and experiments were started in the cold room. No liquid N_2 or -80°C ultra-freezer was available in the ETP in June 2011. This is why all samples were preserved in RNA*later*® .

2.2.1 Environmental data and euphausiid collection

Temperature, oxygen and salinity profiles were recorded with a Seabird SB09 "conductivity, temperature, depth" (CTD) system in all sampling areas (Tab. 2.1). Each profile was plotted to detect the upper boundary of the OMZ and the depth of the thermocline, if present. As ecosystems with different salinity and temperature profiles were compared, the oxygen solubility was calculated after Garcia and Gordon (1992). The upper boundary of the OMZ was defined as the depth where the oxygen solubility was 20% of the maximum solubility according to *in situ* temperature and salinity. The level of 20% O_2 saturation has generally been agreed as

representing severe hypoxic conditions (Burnett and Stickle, 2001). Sea surface temperature (SST; °C) and chlorophyll *a* concentration (mg m^{-3}) visualizations averaged monthly from the Moderate-resolution Imaging Spectroradiometer (MODIS) Aqua Global Level 3 (11 µm thermal infrared; 4 km spatial resolution) were produced with the Giovanni online data system[1] for each sampling area and season.

2.2.2 Euphausiid respiratory measurements: Chapters 3, 4, and 5

The standard metabolic rate (SMR) and oxystrategy of all investigated species was measured at the available temperature closest to *in situ* temperature (Tab. 2.3). I used an OXY-4 channel Oxygen Ingress Measurement system (Presens, Regensburg, Germany) with optical sensor spots. The system was equipped with 4 chambers for simultaneous measurement of three animals and a blank (for seawater bacterial oxygen demand). Cylindrical chambers of 20 mL inner volume were used except for the Antarctic krill *E. superba*. For this species, chamber volume was 250 mL to account for the larger size of the animals. The measurements were conducted on individual specimens and the calculation of respiration rate was adjusted according to the volume of the chamber and the dry mass (DM) of the individual. Chambers were equipped with a magnetic stirrer (bottom) to achieve homogeneity of the oxygen concentration, and a 30-µm mesh gauze separated the stirrer from the euphausiids. All chambers were filled with filtered seawater at 100% O_2 saturation (21 kPa) and the oxygen concentration in each chamber was measured every 15 s in mBar (or hPa). The duration of the measurements varied between 4 and 12 h, lasting until the critical oxygen partial pressure (*pc*) was reached in at least two of the three chambers (Fig. 2.1). The SMR was calculated as the respiration rate of each individual between 80 and 60% O_2 saturation (17 to 13 kPa) and expressed in µmol O_2 h^{-1} g DM^{-1}, and converted to J J^{-1} day^{-1} for the general euphausiids respiration model.

Table 2.3: Number (n) of respiration measurements in South Georgia (SG), the Humboldt current system (HCS), the Eastern Tropical Pacific (ETP), and the northern California current system (NCCS) per period and species.

Area	Species	Period	T°C	n
SG	*E. superba*	Jan 2012	4	31
HCS	*E. mucronata*	Aug 2011	8	12
ETP	*E. lamelligera*	Mar 2012	20	11
	E. distinguenda	Mar 2012	20	20
NCCS	*E. pacifica*	Sep 11	10	31
	T. spinifera	Sep 11	10	22
	E. pacifica	Apr 12	10	12

[1]developed and maintained by the NASA GES DISC; `http://disc.sci.gsfc.nasa.gov/giovanni`

The *p*c was visually detected by the maximal change of slope of the respiration curve (Fig. 2.1). The onset and intensity of anaerobiosis was analysed by measuring lactate level (in mmol L^{-1}) in the hemolymph of each individual sacrificed after respirometry using an Accutrend R Lactate system (Roche Diagnostics, Germany).

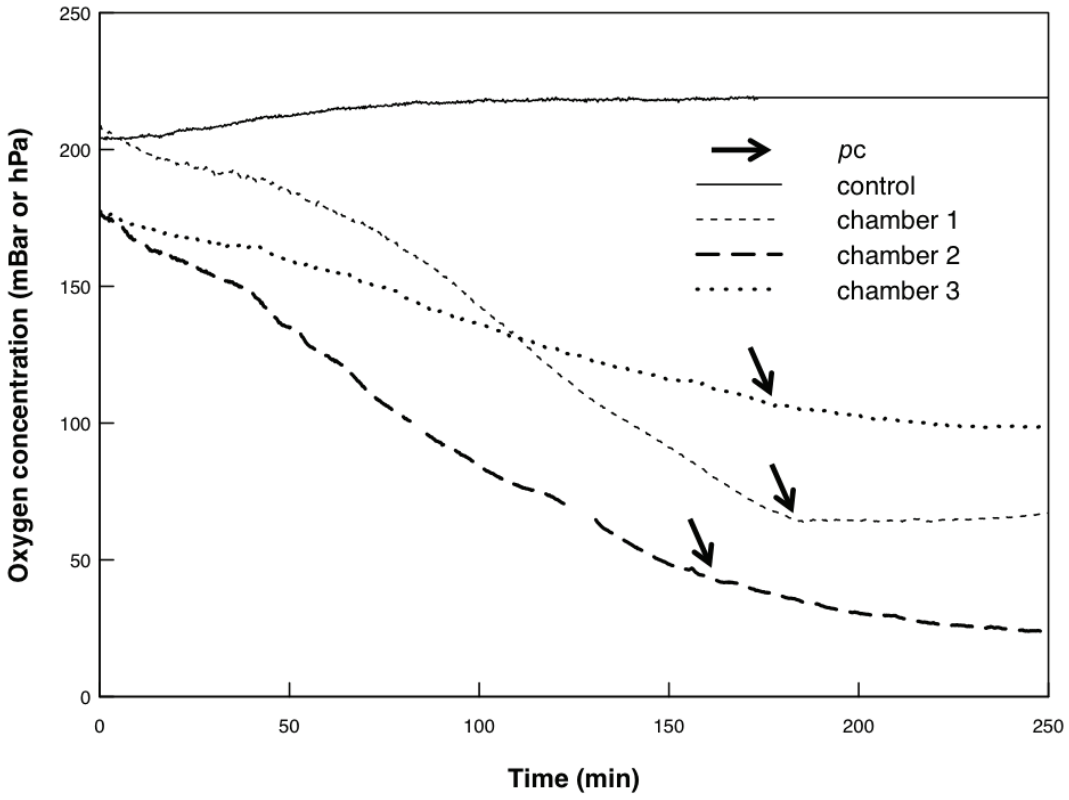

Figure 2.1: Raw data of a respiration measurement. The duration of the measurements varied between 4 and 12 h, lasting until the critical oxygen partial pressure (*p*c) was reached in at least two of the three chambers.

2.2.3 Stress experiments: Chapters 5, 6, 7

Krill were divided into eight replicates of 10 to 30 animals, according to the number of krill available and their body size (Tab. 2.4). When possible (HCS and NCCS), two experimental temperatures were simultaneously applied at 100% O_2 saturation (21 kPa; 2 replicates for each temperature at normoxic conditions), and at threshold hypoxia level of 20% O_2 saturation (4 kPa; 2 replicates for each temperature at hypoxic conditions) in which the animals were exposed over 6 h. The hypoxic treatment was made with pure gaseous N_2 injected in the water aquaria. The oxygen level was monitored with the OXY-4 channel Oxygen Ingress Measurement system (Presens, Regensburg, Germany) and maintained at 20% O_2 saturation over all the 6 h of the treatment.

Table 2.4: Conditions for experiments in South Georgia (SG), the Humboldt current system (HCS), the Eastern Tropical Pacific (ETP), and the northern California current system (NCCS): temperature (T°C), treatments (C=normoxia 100% O_2; H_S=severe hypoxia 2.5% O_2; H_T=threshold hypoxia 20% O_2; R=reoxygenation 100% O_2 after H_T), exposure time (t), number of replicate experiments (# exp), individuals at the beginning (n_i), at the end (n_f), % of mortality, sample frozen (n_{frozen}), and preserved in RNA*later*® (n_{RNA}).

Area	Period	T°C	Treatment	t (h)	# exp	n_i	n_f	% mortality	n_{frozen}	n_{RNA}
SG	Jan 12	3.5	C	1	4	60	60	0	34	26
			H_S	1	4	80	80	0	40	40
			C	6	4	60	56	7	39	17
			H_T	6	4	80	78	3	51	27
HCS	Aug 11	8.0	C	6	1	20	20	0	20	0
		15.0	C	6	1	6	6	0	6	0
	Feb 12	8.0	C	6	1	20	20	0	10	10
			H_T	6	1	40	40	0	20	20
			C	7	1	20	20	0	10	10
			R	1	1	40	40	0	20	20
		15.0	C	6	1	20	20	0	10	10
			H_T	6	1	20	20	0	10	10
			C	7	1	20	20	0	10	10
			R	1	1	20	20	0	10	10
ETP	Mar 12	20.0	C	6	3	200	197	2	99	98
			H_T	6	3	180	86	52	43	43
		26.0	C	6	1	40	37	8	19	18
			H_T	6	1	20	11	45	6	5
NCCS	Sep 11	10.0	C	6	2	30	30	0	25	5
			H_T	6	2	37	34	8	17	0
			C	7	2	30	30	0	25	5
			R	1	2	17	17	0	17	0
		12.0	C	6	1	20	20	0	16	4
			H_T	6	1	30	25	17	13	0
			C	7	1	20	20	0	16	4
			R	1	1	12	12	0	12	0
		14.0	C	6	1	20	20	0	10	10
			H_T	6	1	40	19	48	11	3
			C	7	1	20	20	0	11	10
			R	1	1	5	5	0	2	3
	Apr 12	10.0	C	6	2	50	49	2	30	20
			H_T	6	2	140	106	24	36	21
			C	7	2	50	50	0	30	20
			R	1	2	50	50	0	26	24
		12.0	C	6	1	20	20	0	10	10
			H_T	6	1	30	21	30	6	5
			C	7	1	20	19	5	10	9
			R	1	1	10	10	0	5	5
		14.0	C	6	1	20	20	0	20	0
			H_T	6	1	20	1	95	1	0

After 6 h of threshold hypoxia exposure, surviving krill from the hypoxia treatments was sampled and immediately snap frozen in liquid N_2 (HCS, ETP) or at -80°C (NCCS and Antarctica), or preserved in RNA*later*® for gene transcription analysis. Control krill (maintained in normoxia) were sampled and preserved in the same way for each replicate. A sub fraction (approx. 50%) of the individuals from the hypoxia treatment was reoxygenated for 1 h by purging the aquarium with air, and then sampled along with a second control group maintained in normoxia over the entire time span. Euphausiids that did not survive the experiments were kept in 100% ethanol for counting and identification (species). Frozen samples from all sampling locations were transported on dry ice to the Alfred Wegener Institute for biochemical and gene transcription analysis.

Experimental constraints

In Antarctica: Since security legislations do not allow gaseous N_2 handling in the cold room of the research vessel James Clark Ross, hypoxia exposure experiments were conducted on deck. The aquaria were placed in boxes with an entry and an exit to allow a seawater flow from the research vessel water supply system to keep a constant low temperature (between 3 and 3.5°C) during the experimental exposure, similar to SST at South Georgia (3.2°C). By working with this setting, the material used was limited and it was not possible to expose the Antarctic krill to warming and reoxygenation treatments. However, we were able to test tolerance of *E. superba* at severe (2.5% O_2 saturation) and threshold (20% O_2 saturation) levels of hypoxia (Chapter 7) using N_2 gas on deck of the ship.

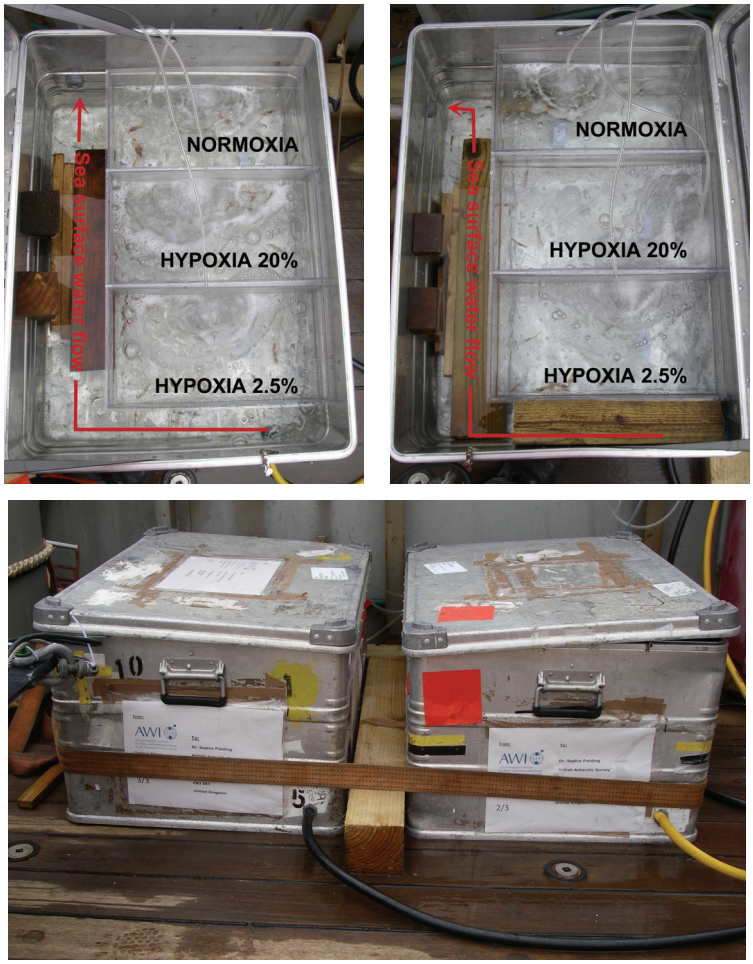

Figure 2.2: Hypoxia experiment set-up in the Antarctic. The aquaria were placed in boxes with an entry and an exit to allow a seawater flow from the research vessel water supply system to keep a constant and cold temperature (between 3 and 3.5°C during the experimental exposure), similar to sea surface temperature at South Georgia.

In the HCS and ETP: In the HCS off Chile, no hypoxic exposures were conducted in the cold season because N_2 gas was not available. In the ETP during the warm season, the research vessel BIP XII had no cold room to conduct most of the experiments. As ambient temperature was too high (>25.0°C), euphausiids were not living enough time for accurate respiration measurements and to test for the synergic effect of warming-hypoxia/reoxygenation. Also in the ETP , but during the cold season, warming-hypoxia experiments were conducted without the reoxygenation treatment. The species were very small in size (*E. lamelligera* and *E. distinguenda*), and as a minimal body mass was needed to analyze the different parameters in the laboratory, I decided to focus on hypoxia response and use a large enough number of replicates. At the end, logistic transportation of samples on dry ice to the Alfred Wegener Institute failed from Mexico (samples arrived thawed, but cold) with the sad consequences that not all biochemical assays (mostly the oxidative stress indicators) could be conducted with these samples.

2.2.4 Biochemical assays: Chapters 4, 5, 6, and 7

Mitochondrial marker

Citrate synthase (CS) is the first enzyme of the Krebs cycle, which produces reduced intermediate for aerobic respiration. Thus it is frequently measured as indicator of mitochondrial density in a tissue or organism. CS produces citrate from oxaloacetate and acetyl-coenzyme A (acetyl-CoA) and was analyzed following the method of Sidell *et al.* (1987). This test optically records the catalytic turnover of acetyl-CoA-SH by measuring the transfer of the sulfydryl groups to 5',5'-dithio-bis(2-nitro)benzoic acid (DTNB) at 412 ηm. Tissues (whole organism or abdomen) were homogenized in 1:20 (w/v) ice-cold Trizma® hydrochloride (Tris-HCl) buffer. CS activity was measured in 13 µL supernatant in a total working solution of 333 µL, at room temperature (20°C) using a micro-plate reader (Berthold Technologies Multimode reader TriStar LB 941, France). Data were calculated as activity units (U) per mg proteins^{-1} and gWM^{-1}. Soluble protein content was measured after Bradford (1976).

Oxidative stress parameters

For the oxidative stress assays, each individual was cut into two pieces separating the cephalothorax from the abdomen. The front part (cephalothorax) was used for the antioxidant enzymes superoxide dismutase (SOD; Suzuki, 2000), catalase (CAT; Aebi, 1984), and glutathione-S-transferase (GST; Habig and Jakoby, 1981) analysis. The larger cephalothorax of *E. superba* allowed the additional analysis of glutathione peroxidase (GPx) activity (Ahmad and Pardini, 1988). The cephalothorax was ground in liquid N_2 and homogenized on ice with a micropistill after adding a 6-fold volume (w/v) of phosphate buffer solution[2], and centrifuged at 23 897 g velocity for 3 min at 4°C. All antioxidant enzyme activities were measured at room temperature (20°C) using a spectrophotometer (Beckman-Coulter DU 800 UV/Vis, USA). Soluble proteins were measured using Bradford (1976) method in all supernatants to get enzyme activities expressed in activity units (U) mg proteins^{-1}. A small selection of abdominal tissue was analysed for reduced and oxidized glutathione (GSH, GSSG) concentration by high-performance liquid chromatography (HPLC) after de Almeida *et al.* (2011) with some adjustments (Chapter 5 and 7). The total pool of reduced and oxidized glutathione was quantified as glutathione equivalents (GSH-eq= $GSH + 2GSSG$) and expressed as ηmol g WM^{-1}, and the ratio GSSG: GSH was determined by GSSG and GSH concentrations. Abdominal samples were further used for the quantification of malondialdehyde (MDA) formation (lipid damages), and protein carbonyl content (protein damages). MDA concentration was assessed according to Uchiyama and Mihara

[2]50 mmol L^{-1} potassium phosphate dibasic and monobasic mixture (K_2HPO_4/KH_2PO_4), 50 mmol L^{-1} EDTA, 1 mmol L^{-1} phenylmethanesulfonyl fluoride (PMSF), pH 7.5

(1978) and expressed as ηmol MDA g WM^{-1}. Protein carbonyl content was measured using the OxiSelect Protein Carbonyl ELISA Kit (Cell Biolabs Inc., San Diego, CA) according to the manufacturer's instructions, and expressed as ηmol mg $proteins^{-1}$.

2.2.5 Gene expression analysis: Chapter 7

Total ribonucleic acids (RNAs) were extracted from the abdominal samples using the QIAGEN RNeasy® Kit. Subsequently, 1 µg of total RNA was reverse transcripted into single-stranded complementary DNA (cDNA) using oligo dT and RT-MMLV reverse transcriptase kit (Promega, USA), according to the manufacturer's instructions. Reverse transcription qPCR was then performed in a Rotor-Gene Q (Qiagen, Gemany) using Eva Green Type-it HRM PCR kit (Qiagen, Germany) to measure the expression of four reference candidate genes and seven genes potentially involved in hypoxic stress responses (see Chapter 7). Primers were designed from the transcripts of two polar krill species *E. superba* (Clark *et al.*, 2011) and *Euphausia crystallorophias* (Toullec *et al.*, 2013) using CLC Main Workbench (Version 7.0, USA) and double checked for their quality with the PerlPrimer software (Marshall, 2004). Primers were synthesized by Sigma-Aldrich (Germany). Sequencing of reverse transcription qPCR products was done to confirm the amplification of the reference and targeted genes. The reference gene chosen for the mean normalized expression (MNE) ratio (Muller *et al.*, 2002) calculation was selected with the softwares Normfinder (Andersen *et al.*, 2004) and gNorm (Vandesompele *et al.*, 2002). Mean normalized expression was calculated with the software qgene (Joehanes and Nelson, 2008) and relative expression with the Roche Applied Science E-method (Tellmann, 2006). Primers for target genes were tested with all investigated krill species, but were successful only for the Antarctic species *E. superba* with efficiencies ranging between 80 and 100%. As no annotated sequences of other species were available, genetic expression in response to hypoxia exposure was only measured in the polar species of the study.

Publications and manuscripts

The general concept of this study was developed by me and Doris Abele. I had a doctoral scholarship from the "Fonds de recherche sur la Nature et les Technologies du Québec (Canada)" and the project travel and consumables was funded by the Alfred Wegener Institute (AWI), Helmholtz Centre for Polar and Marine Research (1. PACES 2.2: Integrating evolutionary ecology into coastal and shelf processes). The Euromarine Mobility Fellowship 2012 sponsored a research stay at the "Station Biologique de Roscoff (France)" to learn RNA extraction, primers design and reverse transcription qPCR methods under the supervision of Dr. Jean-Yves Toullec.

The sampling, experiments and most of laboratory analysis, as well as method development was carried out by myself using the equipment and laboratories (Doris Abele and Christoph Held) of the Functional Ecology section, AWI, Helmholtz Centre for Polar and Marine Research, Bremerhaven. Main publications and entities of unpublished research results from this study with my declaration of contribution towards them is following:

- **Tremblay N, Werner T, Huenerlage K, Buchholz F, Abele D, Meyer B, Brey T. Euphausiid respiration model revamped: latitudinal and seasonal shaping effect on krill respiration rates. Chapter 3.**

  T. Brey developed the scientific concept of this study in discussions with me and D. Abele. Data sets used for models were built by me and statistically analyzed by T. Brey. Figures were also prepared by T. Brey. The authors T. Werner, K. Huenerlage and B. Meyer provided essential unpublished data to build the different models. I wrote the manuscript with T. Brey (methods and results), which was improved in cooperation with all co-authors.

  Submitted to "Ecological Modelling".

- **Tremblay N. Relationship between citrate synthase activity, temperature and respiration in euphausiids. Chapter 4.**

  The scientific concept of this manuscript was developed by me. Sampling of euphausiids, experiments, analytical work, evaluation and literature compilation of the data, and the writing of the research results was performed by me.

  Additional results of the thesis.

- **Tremblay N, Abele D. Response of three krill species to hypoxia and warming: An experimental approach to oxygen minimum zones expansion. Chapter 5.**

  The scientific concept of this manuscript was developed by me and D. Abele. Sampling of euphausiids, experimental and analytical work as well as the evaluation of the data was performed by me. I wrote the manuscript, which was commented and improved by the second author.

  Submitted to "Marine Ecology".

- **Tremblay N. Warming response comparison of two euphausiids species from the northern California Current System. Chapter 6.**

  The scientific concept of this manuscript was developed by me. Sampling of euphausiids, experimental and analytical work, the evaluation of the data and the writing of the research results was performed by me.

  Additional results of the thesis.

- **Tremblay N, Cascella K, Toullec JY, Held C, Fielding S, Tarling, GA, Abele D. Gene expression and physiological changes of the Antarctic krill *Euphausia superba* under different hypoxia intensities. Chapter 7.**

  I developed the scientific concept of this study in discussions with D. Abele, JY. Toullec and C. Held. Sampling, experiments, the main part of the laboratory work and the data analysis was performed by me. K. Cascella designed primers for reverse transcription qPCR analysis used in this work and helped with data interpretation. I wrote the manuscript, which was improved in cooperation with all co-authors.

  To be submitted to "Polar Biology"

Chapter 3

Euphausiid respiration model revamped: latitudinal and seasonal shaping effect on krill respiration rates

Nelly Tremblay[1], Thorsten Werner[1], Kim Huenerlage[1], Friedrich Buchholz[1], Doris Abele[1], Bettina Meyer[2], Thomas Brey[1]

Functional Ecology[1] and Polar Biological Oceanography[2], Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany

submitted to Ecological Modelling ECOMOD-14-280 (11.04.2014)

Abstract

Euphausiids constitute a major biomass component in shelf ecosystems and play a fundamental role in the rapid vertical transport of carbon from the ocean surface to the deeper layers during their daily vertical migration (DVM). DVM depth and migration patterns depend on oceanographic conditions with respect to temperature, light and oxygen availability at depth, factors that are highly dependent on season in most marine regions. Here we introduce a global krill respiration ANN (Artificial Neural Network) model including the effect of latitude (LAT), the day of the year (DoY), and the number of daylight hours (DLh), in addition to the basal variables that determine ectothermal oxygen consumption (temperature, body mass and depth). The newly implemented parameters link space and time in terms of season and

photoperiod to krill respiration. The ANN model showed a better fit ($r^2 = 0.780$) when DLh and LAT were included, indicating a decrease in respiration with increasing LAT and decreasing DLh. We therefore propose DLh as a potential variable to consider when building physiological models for both hemispheres. For seasonality, we also tested the standard respiration rate of the most common species that were investigated in a large range of DLh and DoY with Multiple Linear Regression (MLR) or General Additive model (GAM). GAM successfully integrated DLh ($r^2 = 0.563$) and DoY ($r^2 = 0.572$) effects on respiration rates of the Antarctic krill, *Euphausia superba*, yielding the minimum metabolic activity in mid-June and the maximum at the end of December. We could not detect DLh or DoY effects in the North Pacific krill *Euphausia pacifica*, and our findings for the North Atlantic krill *Meganyctiphanes norvegica* remained inconclusive because of insufficient seasonal data coverage. We strongly encourage comparative respiration measurements of worldwide Euphausiid key species at different seasons to improve accuracy in ecosystem modelling.

3.1 Introduction

Knowledge of metabolic rates under different environmental conditions and from latitudinal and seasonally differing scenarios is central information in comparative modelling of trophic carbon transport and ecosystem energetic cycling. Euphausiids constitute a significant component in many marine ecosystems and often several or even a single krill species connect primary production to apex predator trophic levels. Data on respiration rates of krill species have been collected since the 1960's as indicators for aerobic energy turnover. Recently Ikeda (2012) presented a stepwise multiple regression model (based on 39 sources of data sets composed of 24 species from various types of ecosystems) describing a significant dependence of krill respiration rates on body mass, habitat temperature, and water sampling depth. This first attempt to include water depth in a general Euphausiids respiration model indicated respiration rates to decline with water depth. The negative depth effect on krill metabolic rates was attributed to lower temperatures and diminishing oxygen concentrations at depth, affecting the Euphausiids when they migrate down at dusk (McLaren, 1963; Enright, 1977). Further, Ikeda (2012) attributed the metabolic slowdown to a reduction of the energetic costs of swimming in the absence of visual predators in deep and dark oceanic layers. Identification of “depth” as a factor modulating respiration rates raises the need to understand which environmental factors determine the vertical distribution range of krill species and the time span when they remain in the deep water layers. Indeed, important differences in timing and depth range of diel vertical migration (DVM) among seasons or under different oceanographic regimes (upwelling/downwelling) have been reported for Euphausiid species from different areas (Brinton, 1979; Gaten *et al.*, 2008; Taki, 2008;

Tremblay *et al.*, 2010; Sato *et al.*, 2013; Werner and Buchholz, 2013; Haraldsson and Siegel, 2014). Hence we presume that, next to water depth, other factors related to season and photoperiod will affect Euphausiid respiration on a global scale and most likely at the species level, too.

Here we analyse a global respiration data compilation comprising 2479 respiration data sets from 23 species that includes the factors "latitude", the "day of the year", and the "number of daylight hours" as proxies for season and photoperiod. We intend to establish a corresponding general Euphausiid respiration model and to analyse seasonal patterns of respiration within single Euphausiid species.

3.2 Materials and methods

3.2.1 Initial data

We searched the literature for Euphausiid respiration data and added recent unpublished data provided by several colleagues. The data base consists of 2550 respiration data sets referring to 31 species collected from 52 different sources (Tremblay *et al.*, 2014; Fig. 3.1). For statistical reasons, some data sets were excluded from further analysis (refer to subsection 3.2.2), leaving us with 2479 data sets relating to 23 species (Fig. 3.2, 3.3). In some cases, the public domain software ImageJ (`http://rsbweb.nih.gov/ij/`) was used to extract respiration data from figures. Each data set included the following parameters:

- Sampling site latitude LAT and longitude LON;
- Sampling water depth D (m; in 261 cases the reported depth was <5 m, these numbers were set to $D = 5$ m in order to avoid disproportionate effects of very small depth values. In 311 cases with unknown sampling depth we set $D = 80$ m, *i.e.* average depth in all data sets; in a further 14 cases where divers sampled the animals we set $D = 5$ m);
- Day of the year DoY (day of year between 1 and 365); if a range of time was provided by the original source, we set DoY =midday of this range. When DLh was set to 12h (see below), DoY was set to 264 (which correspond to equinox of September 21th when the sun spends equal amount of time above and below the horizon at every location on the Earth, so night and day are about the same length), accordingly;
- Number of daylight hours DLh, calculated from LAT and DoY by the sunrise-sunset calculator (`aa.usno.navy.mil/data/docs/RS_OneDay.php`). A few publications summarized data over a time period of more than one year; here we set DLh to 12h;

- Measurement temperature T (K); Body mass M (J), converted from original body mass units using factors provided as for Brey *et al.* (2010), and other sources when necessary;
- Specific respiration rate RR (J J^{-1} day^{-1});
- Taxonomic information (species, genus, family);

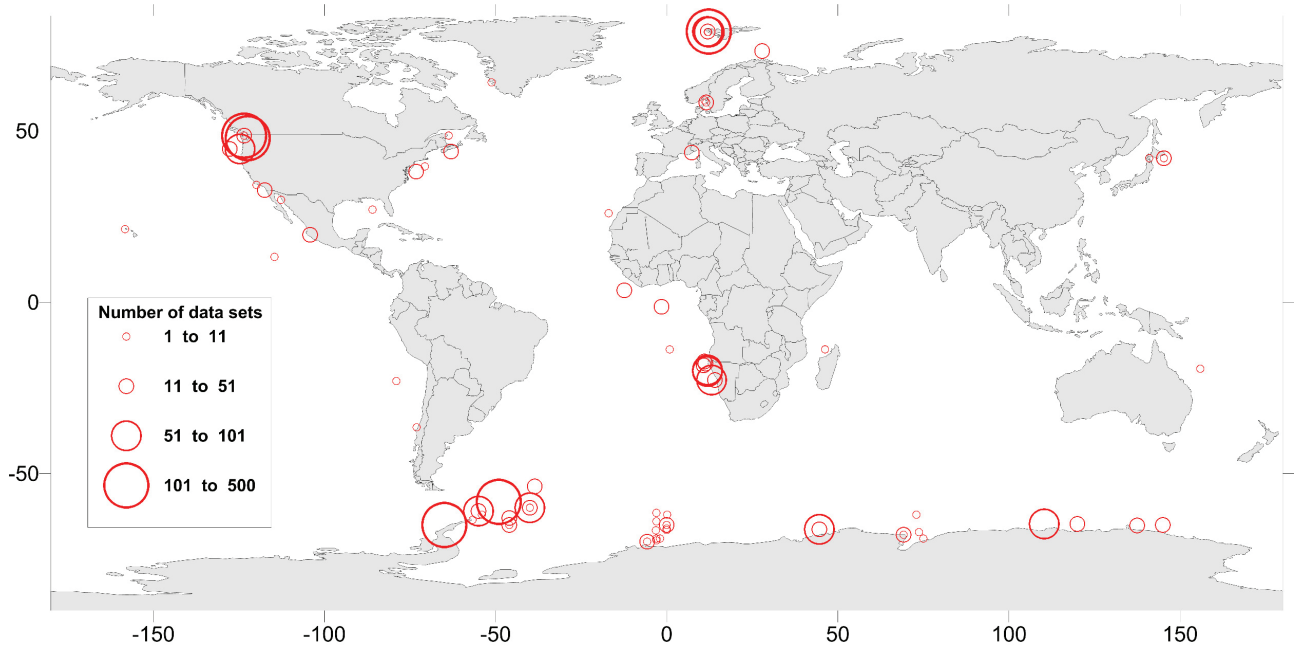

Figure 3.1: Geographical visualization of Euphausiid data used for respiration model. The data base consists of 2550 respiration data sets referring to 31 species collected from 52 different sources (Tremblay *et al.*, 2014).

3.2.2 Data transformation and pre-analysis

We decided to eliminate *a priori* four data sets with extreme water depth below 700 m. Subsequently, specific respiration rate RR, body mass M, temperature T and water depth D were transformed by approximating linear relationships between independent variables and RR according to theoretical considerations (*e.g.*, Schmidt-Nielsen, 1984; Brown *et al.*, 2004) and to empirical evidence (*e.g.* Seibel and Drazen, 2007; Brey, 2010) regarding the scaling of metabolic activity (see Brey, 2010 for a full discussion of this issue). These transformations - $log(RR)$, $log(M)$, $1/T$, $log(D)$ - also facilitate a more even distribution of data and variance in the [M, T, D] space, too. Multivariate outliers in the sample space [$log(RR)$, $log(M)$, $1/T$, $log(D)$] were identified by Hotelling's T^2 statistic (the square of the Mahalanobis distance; Barnett and Lewis, 1994; Prokhorov, 2001). Data sets with T^2 above the 97.5% percentile were excluded from further analysis, thus providing 2479 data sets referring to 23 species for statistical analysis (Fig. 3.2, 3.3).

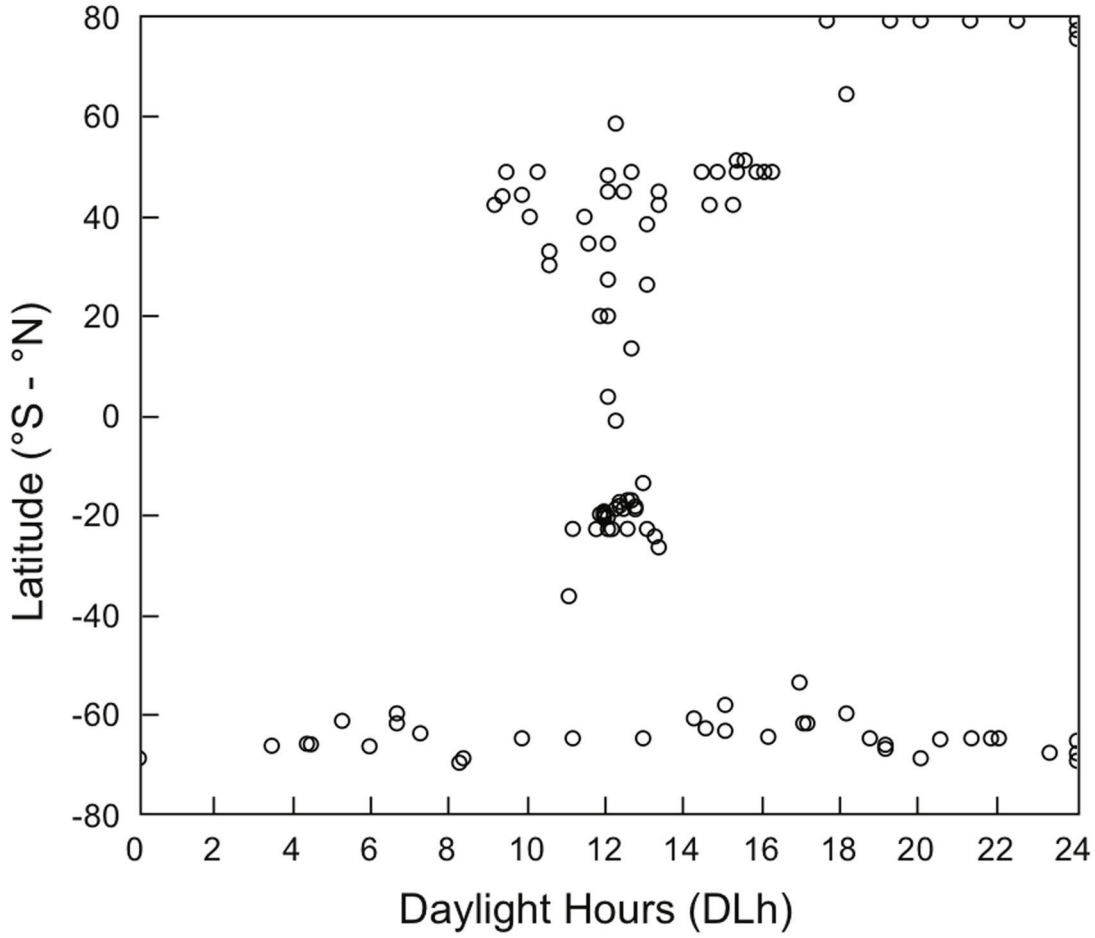

Figure 3.2: Distribution of the 2479 respiration data sets with respect to daylight hours and geographical latitude.

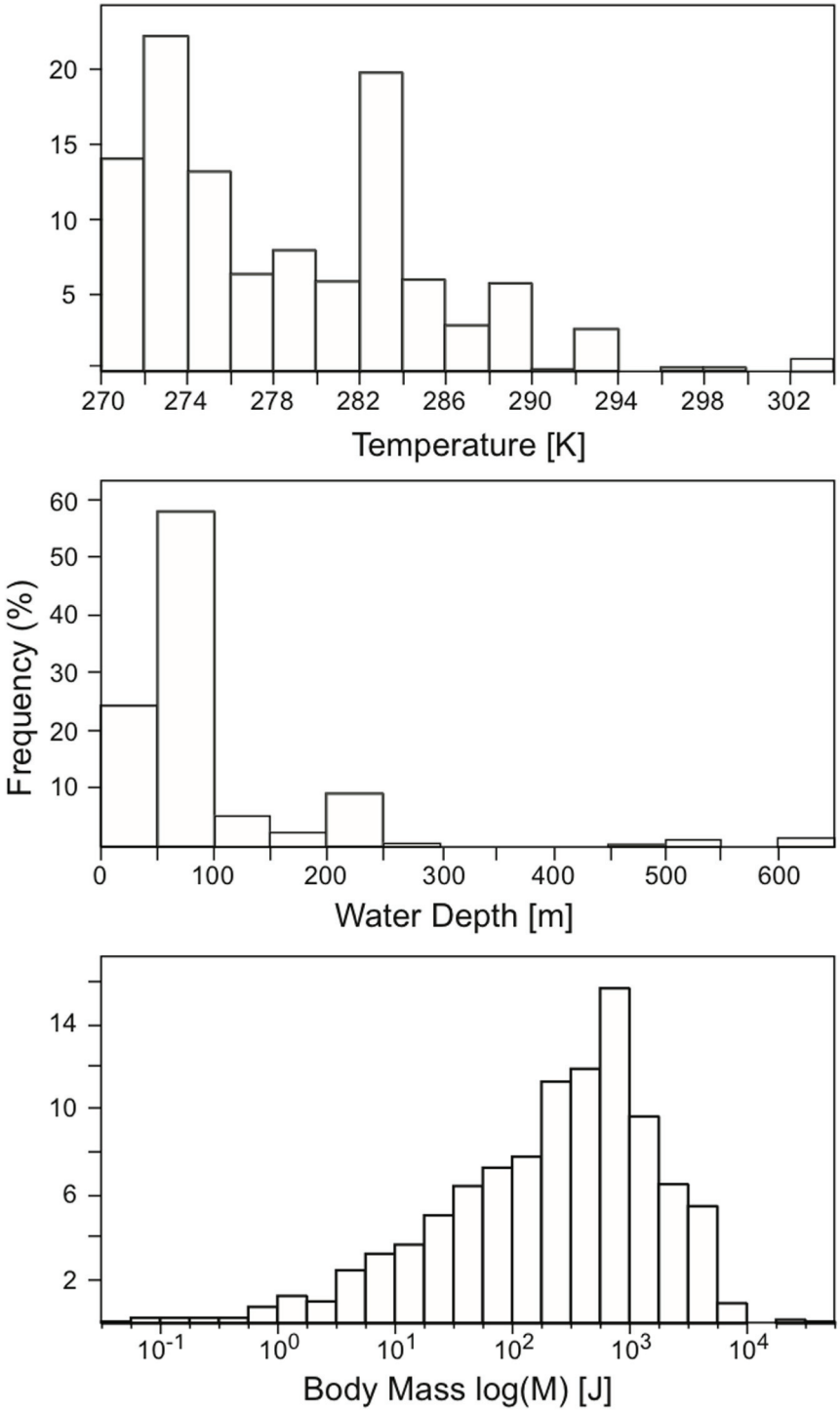

Figure 3.3: Distribution of the 2479 respiration data sets with respect to water temperature (Kelvin), water depth (meters), and mean body mass (Joule).

3.2.3 General Euphausiid respiration model

We applied fully factorial multiple regression models (MLR) as well as Artificial Neural Network (ANN). MLRs may not appropriately describe the existing relationships despite linearizing transformations (see above) and are quite sensitive to intercorrelation between independent parameters (Draper and Smith, 1998). This is the reason why we applied ANN of the back-propagation type (Hagan *et al.*, 1996). ANN "learned" the relationship between dependent and independent variables from training data and was tested for its generalization capacity by comparing prediction accuracy with training (2/3) and test (1/3) data as measured by the correlation between measured RR_m and predicted RR_{ann}. Five ANN, each trained on a different random subsample, were pooled into a composite prediction model (see Brey, 2010, 2012 for further details). Trial-runs with different sets of parameters indicated significant effects of DoY, DLh and $abs(LAT)$. We preferred DLh over DoY for model building as both parameters are strongly correlated, but DLh showed distinctly better performance. Taxonomic effects on RR were evident at the genus level and were covered by three groups, (A) *Euphausia*, (B) *Nyctiphanes* and *Thysanopoda*, (C) remaining genera (*Meganyctiphanes*, *Nematoscelis*, *Thysanoessa*). Accordingly, the MLR model had seven input parameters:

$$log(RR) = a + b_1 \times 1/T + b_2 \times log(D) + b_3 \times log(M) + b_4 \times DLh + b_5 \times abs(LAT) + b_6 \times genus.A + b_7 \times genus.C + interaction.terms$$

The interaction terms parameters were adjusted to mean = zero in order to render the test for the main effects independent of the test for interactions ("centred polynomials"). The ANN consisted of 8 input nodes, three hidden nodes (H), and one output node (Fig. 3.4). The network was parameterized as follows:

$$log(RR) = a_0 + a_1 \times H_1 + a_2 \times H_2 + a_3 \times H_3$$

with

$$H_1 = tanH(b_0 + b_1 \times 1/T + b_2 \times log(D) + b_3 \times log(M) + b_8 \times genus.C)$$
$$H_2 = tanH(c_0 + c_1 \times 1/T + c_2 \times log(D) + c_3 \times log(M) + c_8 \times genus.C)$$
$$H_3 = tanH(d_0 + d_1 \times 1/T + d_2 \times log(D) + d_3 \times log(M) + d_8 \times genus.C)$$

Note that internally the input data are normalized (mean=0, SD=1) and that the network parameter values are adjusted accordingly.

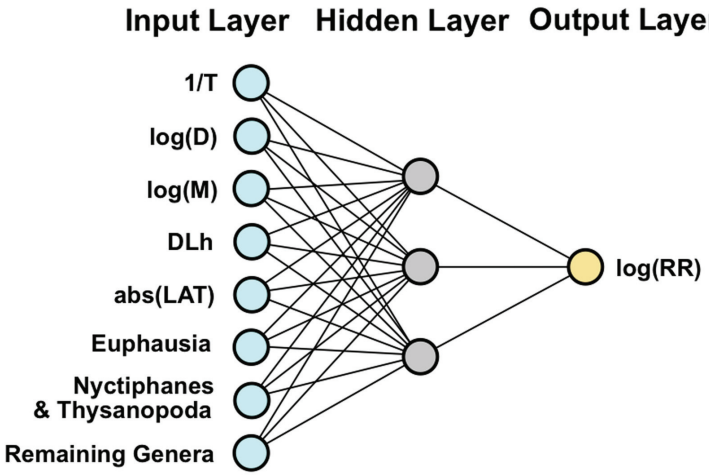

Figure 3.4: Scheme of the artificial neural network (ANN) used to predict mass specific respiration rate *RR* in euphausiids from five continuous parameters (temperature, water depth, body mass, daylight hours, latitude) and three taxonomic categories.

3.2.4 Seasonal respiration model for single Euphausiid species

We selected those euphausiid species with a considerable number of data sets distributed throughout the year, *i.e.*, *E. superba* (N=875), *E. pacifica* (N=500) and *M. norvegica* (N=132) for the analysis of seasonality in respiration rate. Other species showed great data sets, like *Euphausia hanseni* and *Thysanoessa inermis*, but their *RR* were not covering a large range of *DLh* or *DoY*. In a first step, we used a fully factorial MLR to describe the effects of *T*, *D*, and *M* on *RR* (see above):

$$log(RR) = a + b_1 \times 1/T + b_2 \times log(D) + b_3 \times log(M) + interaction.terms$$

Subsequently we checked the residuals of the MLR for effects of *DoY* and *DLh* on *RR*. We presumed that seasonal effects should manifest in a linear relationship between *DLh* and *RR*, and in a corresponding sinusoidal relationship between *DoY* and *RR*. When those relationships were present, we used General Additive Models (GAM; Hastie and Tibshirani, 1990) to gain a better understanding of the seasonal patterns in respiration rate. We added a term $f(x)$ to the MLR above that described the relationship between *RR* and *DLh* or *DoY*, respectively. The GAM equation takes the general form (MLR interaction terms neglected for clarity in this display):

$$log(RR) = a_1 + b_1 \times 1/T + b_2 \times log(D) + b_3 \times log(M) + b_4 \times f(x) \text{ with}$$

$$f(x) = a_2 + b_5 \times DLh \text{ or } f(x) = a_2 + b_6 \times sin(2\pi \times (DoY/365 - a_3))$$

3.3 Results

3.3.1 General Euphausiid respiration model

The MLR approach resulted in a very complex model with seven interaction terms (r^2=0.680, all terms significant at p<0.05, model not shown). The corresponding ANN model showed a distinctly better fit (r^2=0.780, Fig. 3.5, Tab. 3.1). In order to see whether or not certain parameters enhanced ANN's predictive power, we compared goodness of fit of differently sized ANN by means of ANOVA of the correlation coefficients r^2 of individual ANN test and training subsets. Model performance increased significantly (p<0.05) with increasing number of input parameters from three ($1/T$, $log(D)$, $log(M)$), to five (DLh and LAT included) to eight parameters (three genus terms included). The corresponding overall correlation between mean ANN prediction RR_{ann} and measured RR_m was r^2=0.732, 0.760, and 0.780, respectively. The ANOVA further indicated that there were no differences in goodness of fit between test and training data sets. The contour plot in Fig. 3.6 demonstrates the effect of DLh and of LAT on RR_{ann}.

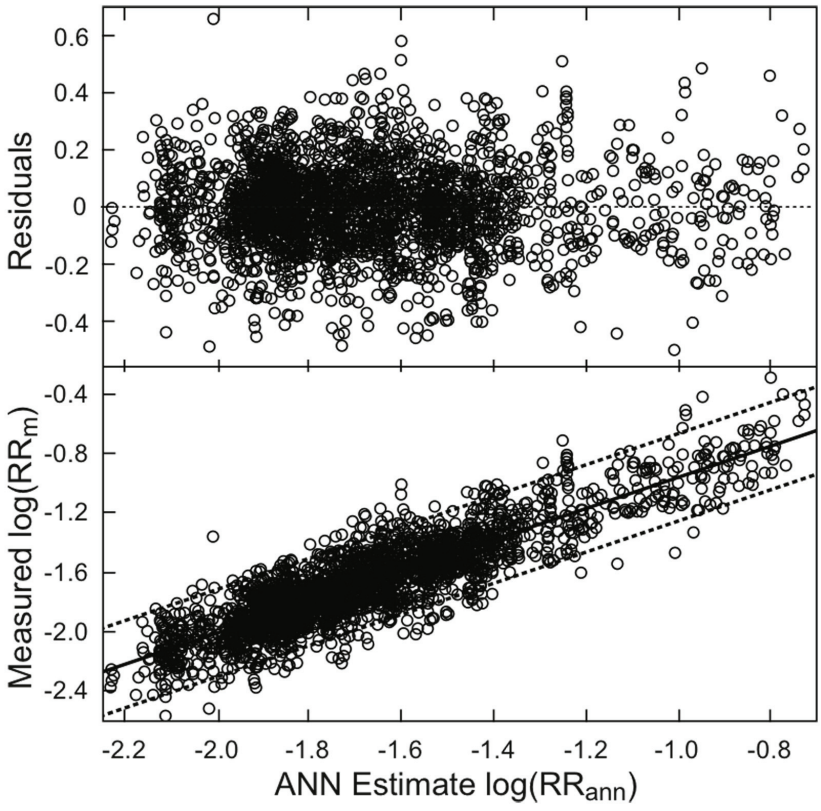

Figure 3.5: Relationship between measured RR_m and ANN predicted RR_{ann} (below) and corresponding residual plot (above). See Tab. 3.1 for ANN model parameters. Stippled lines indicate 95% confidence range of predictions.

Table 3.1: Euphausiid global respiration model. genus.A: *Euphausia*, genus.B: *Nyctiphanes* & *Thysanopoda*, genus.C: remaining genera. r^2train, r^2test, r^2ann: correlation between measured and predicted RR in training (N=1652) and test data (N=826); r^2ann: correlation between measured RR and average prediction of the 5 ANN.

$log(RR) = a_0 + a_1 \times H_1 + a_2 \times H_2 + a_3 \times H_3$
with
$H_1 = tanH(b_0 + b_1 \times 1/T + b_2 \times log(D) + b_3 \times log(M) + b_8 \times genus.C)$
$H_2 = tanH(c_0 + c_1 \times 1/T + c_2 \times log(D) + c_3 \times log(M) + c_8 \times genus.C)$
$H_3 = tanH(d_0 + d_1 \times 1/T + d_2 \times log(D) + d_3 \times log(M) + d_8 \times genus.C)$

	ANN1	ANN2	ANN3	ANN4	ANN5
$a_0 =$	-1.57197	-1.51099	-1.57066	-1.64152	-1.57065
$a_1 =$	0.38857	-0.21050	0.17855	0.38984	-0.45136
$a_2 =$	-1.37002	0.38061	-1.04624	-0.47103	0.21583
$a_3 =$	-0.42258	-0.19251	0.42496	-1.01710	-0.13727
$b_0 =$	-86.77930	-194.63700	-125.32500	33.20542	-47.94690
$b_1 =$	27854.45	57230.88	14617.81	-9652.23	16404.96
$b_2 =$	2.59290	-0.12465	-18.67730	2.36937	-1.00100
$b_3 =$	1.04828	-0.49462	9.78115	0.15848	0.96578
$b_4 =$	-0.39650	-0.02417	2.91508	0.19465	-0.05447
$b_5 =$	-0.12200	-0.14740	0.62981	-0.07905	-0.04532
$b_6 =$	-0.67903	0.75253	-2.30198	-3.28122	0.94072
$b_7 =$	-5.14599	-0.37181	10.78545	-0.46730	-8.10712
$b_8 =$	1.10279	-1.23824	2.61575	-2.55901	-0.08386
$c_0 =$	-9.85757	2.09214	-18.65530	35.89489	95.61789
$c_1 =$	2298.77	2022.25	4485.97	-4279.08	-12644.40
$c_2 =$	0.82025	-1.20340	0.13205	-4.02182	-0.43695
$c_3 =$	0.36519	-1.70364	0.68528	-2.99786	-5.58249
$c_4 =$	0.00655	-0.03503	0.11844	0.01114	-1.05142
$c_5 =$	0.00417	-0.09131	-0.00873	-0.15218	-0.29912
$c_6 =$	-0.25620	0.32410	0.57758	-1.74016	5.36206
$c_7 =$	-0.41454	1.35509	0.36973	0.78609	-9.49179
$c_8 =$	0.32634	-2.09254	0.73716	-2.36783	-5.76894
$d_0 =$	-92.03570	-84.04100	32.26541	-22.47070	-110.62100
$d_1 =$	28677.77	25377.93	-7718.31	4158.86	-23008.00
$d_2 =$	-2.10915	-0.20977	0.56556	2.17255	128.19910
$d_3 =$	0.12831	3.43577	0.76223	1.27308	0.39724
$d_4 =$	-0.18612	-0.35817	0.26208	0.07651	0.22187
$d_5 =$	-0.11352	-0.22685	-0.14171	0.02428	0.23487
$d_6 =$	1.00402	4.27923	-0.75113	-0.72823	-4.39197
$d_7 =$	0.95282	5.95089	-0.63204	-1.12687	-54.49430
$d_8 =$	-1.12534	-3.35537	-0.38697	-0.34093	5.77147
$r^2train =$	0.756	0.746	0.740	0.744	0.746
$r^2test =$	0.751	0.746	0.741	0.740	0.760
$r^2ann =$	0.780				
$N =$	2479				

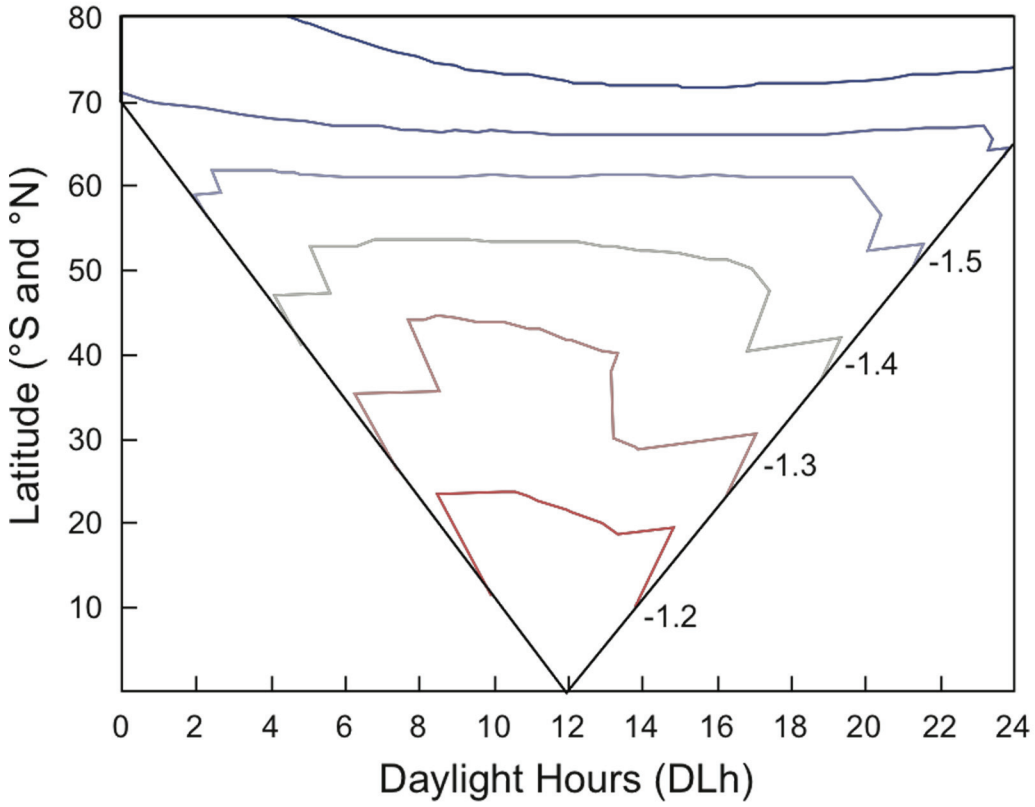

Figure 3.6: Contour plot of RR_{ann} predicted by the Euphausiid global respiration model (ANN) in the daylight hours *DLh* versus geographical latitude *LAT* (north and south combined) space. *DLh* (as well as temperature) has been restricted to the range defined by geographical latitude. RR_{ann} represents an average for body mass 0.1, 1, 10, 100, and 1000 J.

3.3.2 Seasonal respiration model for single Euphausiid species

Euphausia superba

Of the total 2479 data sets, 875 sets collected from 20 sources referred to *E. superba* (Fig. 3.7). We detected significant effects ($p<0.001$) of *DLh* and *DoY* on *RR* (Fig. 3.8). The corresponding GAM (Tab. 3.2; Fig. 3.9) fitted the data distinctly better that the basic MLR (r^2=0.561 and 0.572 compared to 0.440). Furthermore, depth *D* did not contribute significantly to GAM predictive power and was therefore removed from the GAM equations. Fig. 3.9 indicates that the GAM term fully accounted for seasonal effects in *RR*. These effects were visualized in the contour plots in Fig. 3.10.

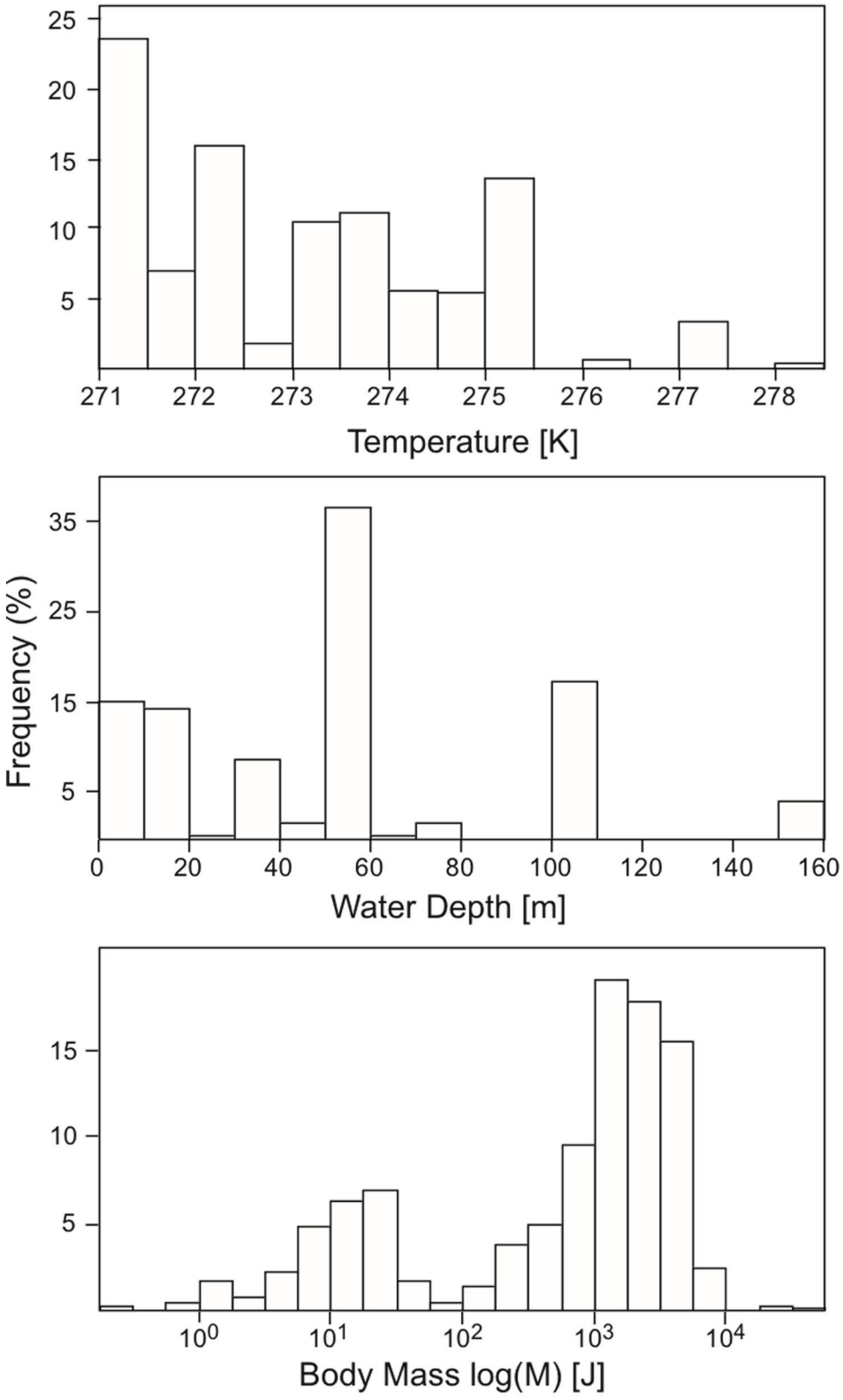

Figure 3.7: *Euphausia superba*: Distribution of the 875 data sets used for model building with respect to water temperature (Kelvin), water depth (meters), and mean body mass (Joule).

Table 3.2: *Euphausia superba* respiration models. Only significant terms (p<0.05) are shown. Note the adjustment to mean=zero for $log(M)$, $1/T$ and DLh.

Multiple Linear Regression (MLR)	General Additive Model (GAM) with DLh	General Additive Model (GAM) with DoY
$log(RR) = a+$	$log(RR) = a_1+$	$log(RR) = a_1+$
$b_1 \times 1/T+$	$b_1 \times 1/T+$	$b_1 \times 1/T+$
$b_2 \times log(D)+$	$b_2 \times log(M)+$	$b_2 \times log(M)+$
$b_3 \times log(M)+$	$b_3 \times (1/T - 0.00366)\times$	$b_3 \times (1/T - 0.00366)\times$
$b_4 \times (1/T - 0.00366)\times$	$log(M - 2.6409)+$	$log(M - 2.6409)+$
$log(M - 2.6409)+$	$b_4 \times f(DLh)$	$b_4 \times f(DoY)$
$b_5 \times (1/T - 0.00366)\times$	$f(DLh) = a_2+$	$f(DoY) = a_2+$
$log(D - 1.4751)$	$b_5 \times (DLh - 14.1929)$	$b_5 \times sin(2\pi \times (DoY/365 - b_6))$
$a = 14.4498$	$a_1 = 14.9328$	$a_1 = 11.0246$
b_1 = -4301.6310	$a_2 = 257.2753$	$a_2 = 91.2073$
b_2 = -0.1298	b_1 = -4501.6350	b_1 = -3387.1049
b_3 = -0.1196	b_2 = -0.1688	b_2 = -0.1684
b_4 = -1105.8590	b_3 = -835.8796	b_3 = -1300.6526
$b_5 = 2804.0944$	$b_4 = 0.00068$	b_4 = -0.000084
	$b_5 = 33.4871$	$b_5 = 185.3023$
		$b_6 = 0.2650$
$N = 875$	$N = 875$	$N = 875$
$r^2 = 0.440$	$r^2 = 0.563$	$r^2 = 0.572$

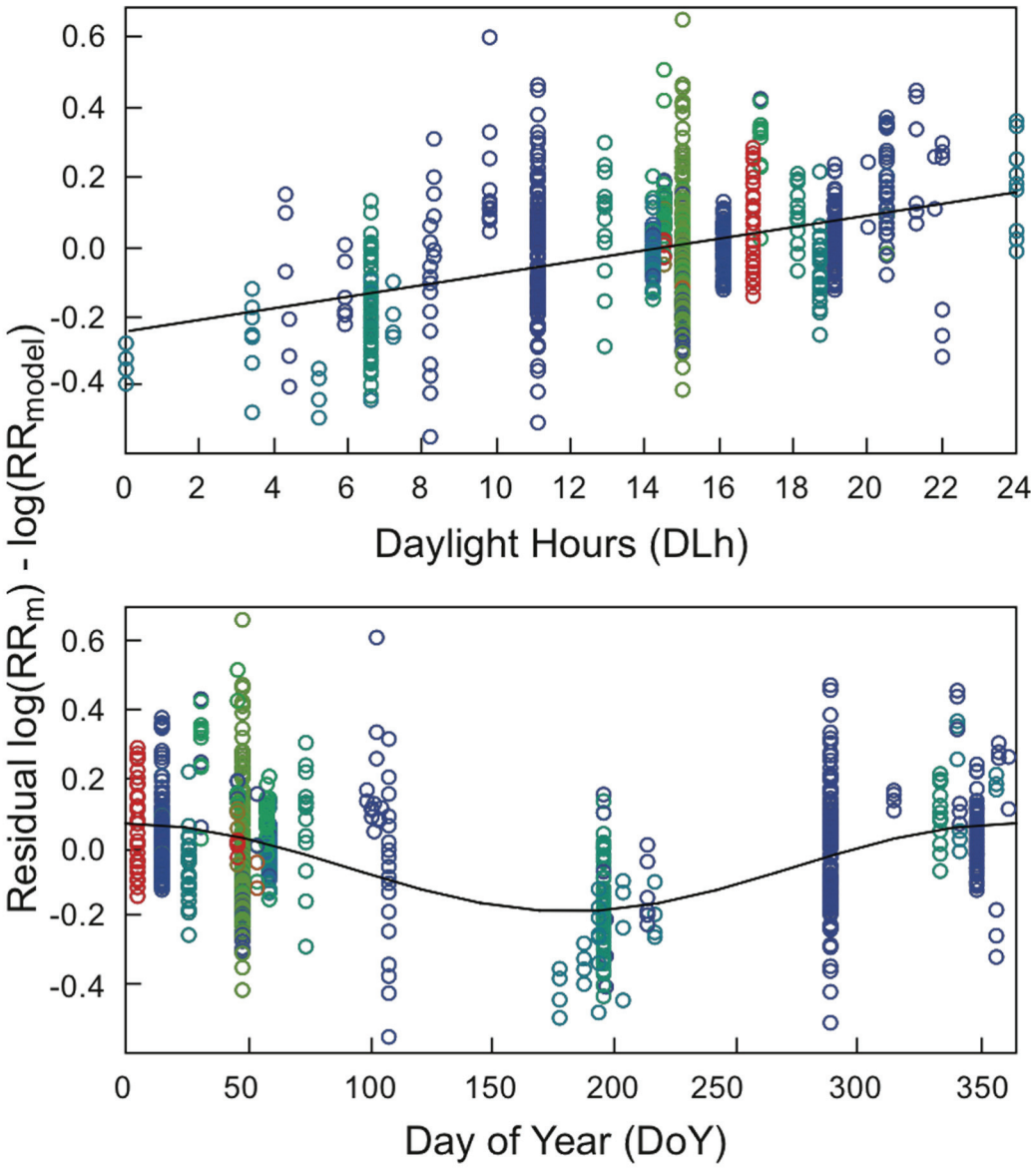

Figure 3.8: *Euphausia superba*: Residuals of Multiple Linear Regression (MLR) (see Tab. 3.2 for model parameters) plotted versus Daylight hours *DLh* and Day of Year *DoY*. There is a significant linear relationship between residuals and *DLh* (r^2= 0.179, p<0.001) and a significant sinusoidal relationship between residuals and *DoY* (r^2= 0.176, p<0.001). Colors indicate temperature at measurement ranging from 271 K (blue) to 278 K (red).

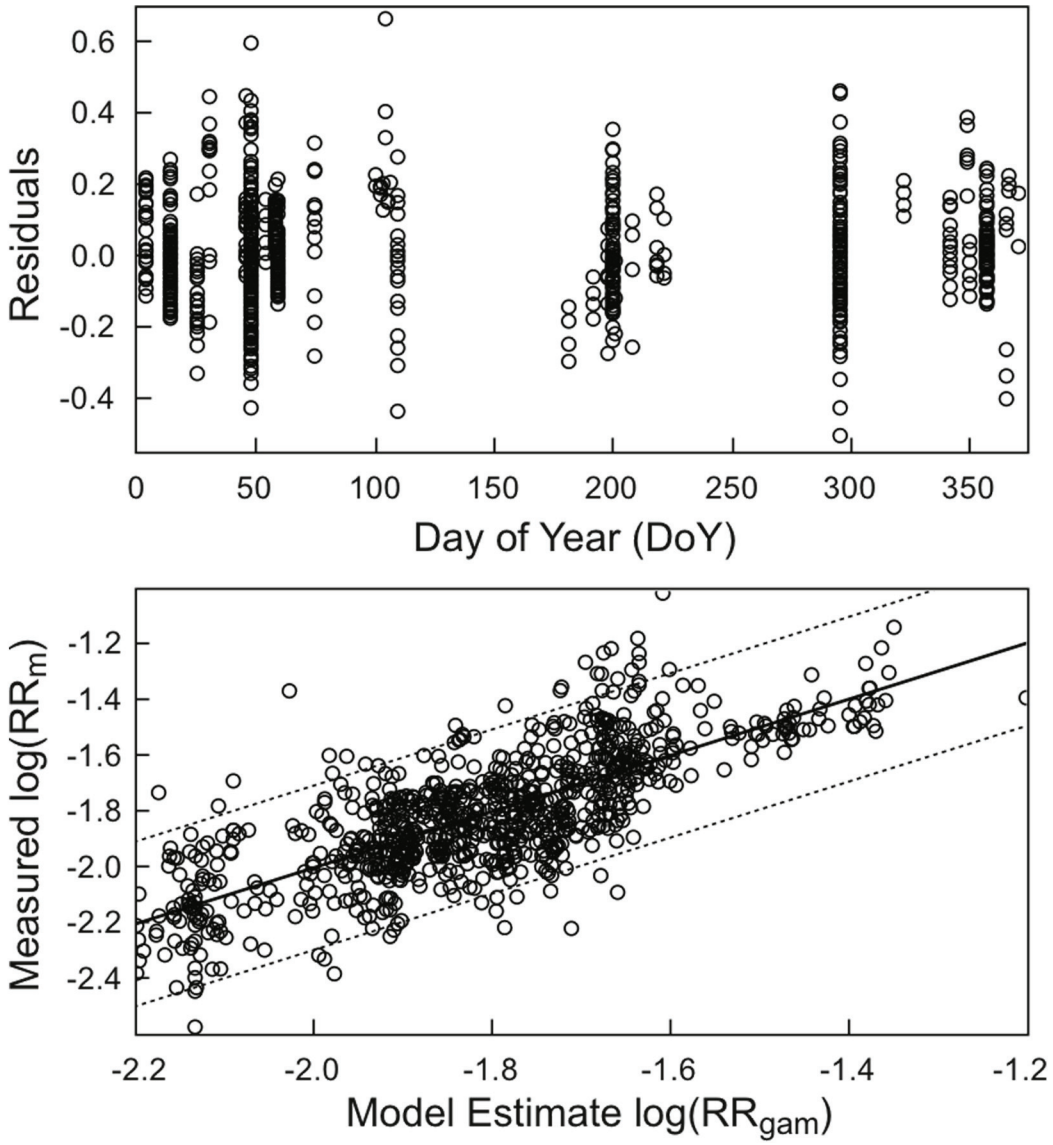

Figure 3.9: *Euphausia superba*: Goodness of fit of the General Additive model (GAM) with Day of Year *DoY* term (see Tab. 3.2 for model parameters). Plot of residuals versus *DoY* indicates no significant relationship (p>0.1).

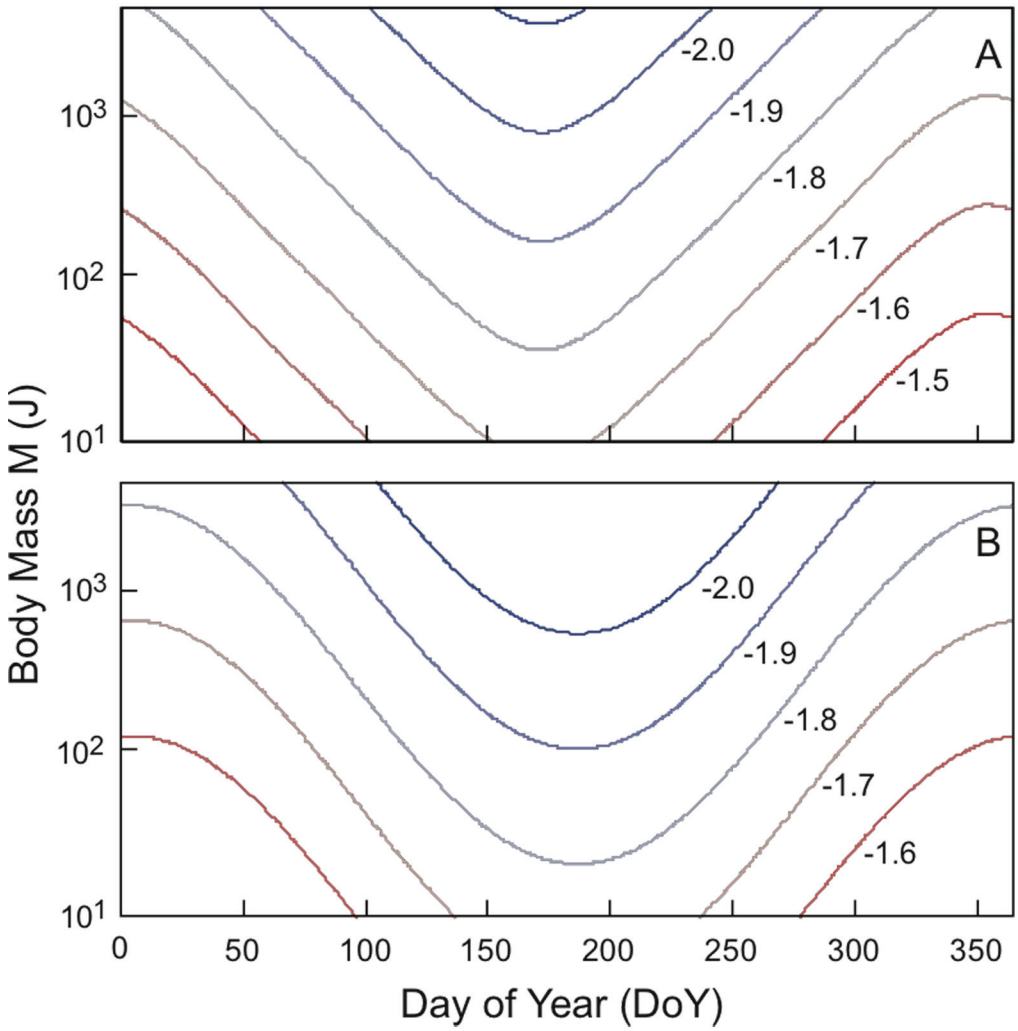

Figure 3.10: *Euphausia superba*: Contour plot of RR_gam predicted by the General Additive (GAM) models in the day of Year DoY versus bodymass $log(M)$ space. Model with DLh term (A) and with DoY term (B), see Tab. 3.2 for model parameters. The relationship between DoY and DLh used in (A) refers to 62°S, *i.e.* the average latitude in all 875 data sets.

Euphausia pacifica

Of the 498 *E. pacifica* data sets (11 sources), one proved to be a consistent and distinct outlier in all models and was therefore excluded from further analysis. A fully factorial MLR analysis indicated significant effects of T, D, and M on RR as well as significant interactions between independent parameters (Tab. 3.3). There was a weak albeit significant sinusoidal relationship between the residuals of the MLR model and DoY (r^2=0.099, p<0.001), and a significant negative relationship between MLR residuals and DLh (r^2=0.137, p<0.001). We checked whether or not these relationships were artificially caused by one single source by means of excluding one source (with $\geq$10 data sets) in turn from the residual analysis. The removal of the data published

by Paranjape (1967) rendered the effects of *DoY* and *DLh* insignificant (see Fig. 3.11). Hence the available data do not provide sufficient evidence for a clear effect of seasonality on *RR* in *E. pacifica*.

Meganyctiphanes norvegica

A fully factorial MLR analysis of the 132 *M. norvegica* data sets (7 sources) indicated significant effects of *T*, *D*, and *M* on *RR* (Tab. 3.3). There was no significant sinusoideal relationship between the residuals of the MLR model and *DoY* (p=0.941). However, MLR residuals correlated negatively with *DLh* (slope=-0.012, r^2=0.186, p<0.001, Fig. 3.12). As there were no data available for *DLh* <8h, the seasonal pattern in *M. norvegica* metabolic activity remains inconclusive.

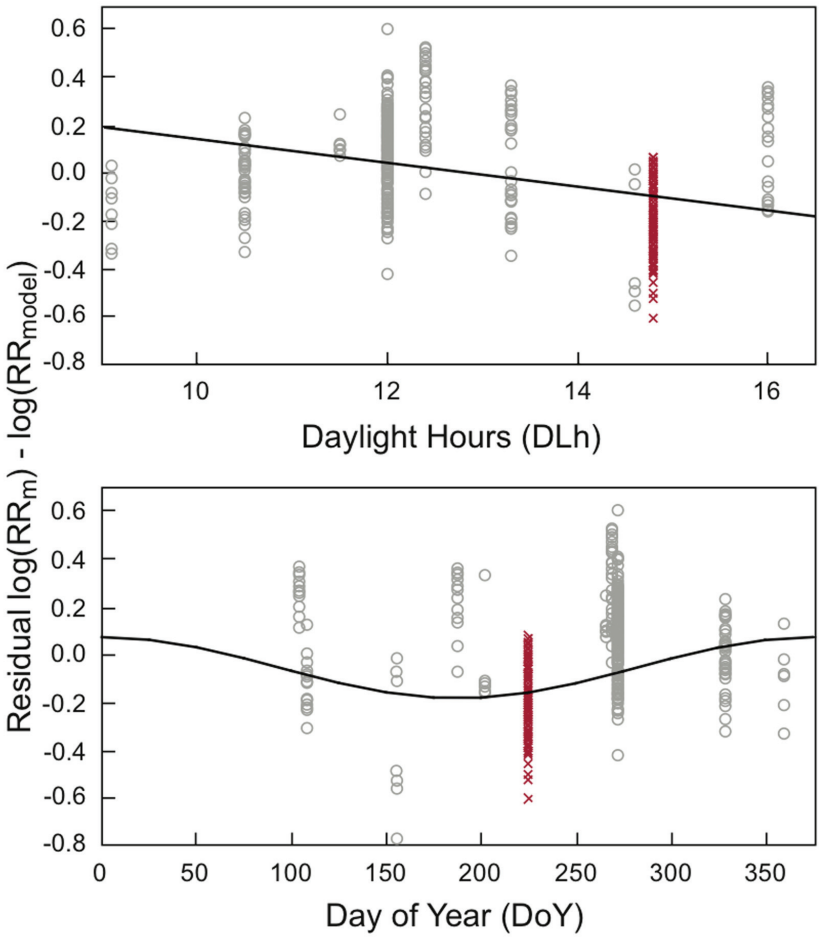

Figure 3.11: *Euphausia pacifica*: Residuals of Multiple Linear Regression (MLR) (see Tab. 3.3 for model parameters) plotted versus Daylight Hours *DLh* and Day of Year *DoY*. The significant linear negative relationship with *DLh* (slope= -0.048, r^2= 0.137, p<0.001) as well as the sinusoidal relationship with *DoY* (r^2= 0.099, p<0.001) become insignificant when the data of Paranjape (1967; cross symbols) are excluded

.

Table 3.3: Multiple Linear Regression (MLR) of *Euphausia pacifica* and *Meganyctiphanes norvegica* respiration. Only significant terms (P < 0.05) are shown. For *E. pacifica*, MLR model uses temperature only in the interaction term $1/T \times log(D)$, the single temperature term was not significant. Mean = zero for $log(M)$, $1/T$, DoY, and DLh. For *M. norvegica*, MLR model uses depth only in the interaction term $log(D) \times log(M)$, the single depth term was not significant.

E. pacifica
$log(RR) = a + b_2 \times log(D) + b_3 \times log(M) + b_4 \times (1/T - 0.00353) \times log(D - 1.8037)$
$+b_5 \times log(D - 1.8037) \times log(M - 1.80367)$
a = -0.3437 b_2 = -0.4294 b_3 = -0.1664 b_4 = -5019.8520 b_5 = 0.3757
$N = 497$ $r^2 = 0.494$
M. norvegica
$log(RR) = a + b_1 \times 1/T + b_2 \times log(M) + b_3 \times log(D - 1.9689) \times log(M - 2.70036)$
a = 8.4833 b_1 = -2763.9620 b_2 = -0.1103 b_3 = -0.5963
$N = 132$ $r^2 = 0.526$

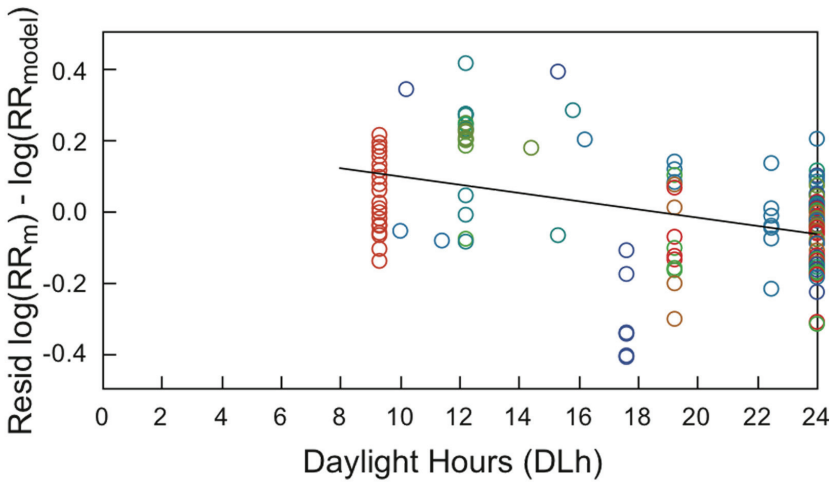

Figure 3.12: *Meganyctiphanes norvegica*: Residuals of Multiple Linear Regression (MLR) (see Tab. 3.3 for model parameters) plotted versus Daylight Hours DLh. Significant linear negative relationship with slope= -0.012, r^2= 0.186, p<0.001. Colors indicate temperature at measurement ranging from 273 K (blue) to 289 K (red).

3.4 Discussion

3.4.1 General Euphausiid respiration model

The ANN model confirms effects of *DLh*, *LAT* and genus adding geographical, seasonal and taxonomic consideration parameters to the Euphausiid respiration global-bathymetric model presented by Ikeda (2012). The good model fit (r^2=0.780) is confirmed by an acceptable residual variance, that is narrower than in a previous aquatic invertebrate respiration ANN in which Euphausiids represented only 3% of the data sets (Brey, 2010). The three taxonomic groups identified may, to some extent, relate to the geographical distribution of the corresponding genera. *Meganyctiphanes* and *Thysanoessa* are mainly present beyond 50°N, while *Nematoscelis* are found around 40° in both hemispheres. *Nyctiphanes* and *Thysanopoda* species predominate around 30° latitude in the data sources.

According to the present ANN model, Euphausiid specific respiration rate *RR* decreases with higher latitude and decreasing *DLh*. The latitudinal influence is related to both body mass and temperature changes and follows the pattern observed by Ikeda (1985) from net zooplankton community respiration. The *DLh* or photoperiod length correlates with high productivity events (spring bloom) at higher latitudes, which probably cause enhanced feeding activities and higher metabolic rates. However, the influence of *DLh*, *LAT* and genus should not be over-interpreted. We cannot be sure whether we see a truly generalizable pattern of respiration, or whether this pattern represents an empirical best fit of the data, forced by the uneven geographical and seasonal distribution of species and data sources. The only latitude at which almost all day lengths (light hours) occur throughout the year is at 60°S, where measurements are available for only one species, *Euphausia superba*.

3.4.2 Seasonal respiration model for single Euphausiid species

Euphausia superba

E. superba is the most extensively studied species both in terms of seasonal differences as well as geographically, rendering a large and comprehensive data set available for our GAM approach. The GAM indicates *DLh* and *DoY* to be explanatory variables for *RR* whereas it excluded *D*, presumably because sampling occurred almost exclusively within the upper 80 m of the water column and therewith in a narrow depth range. Including the *DLh* term in the model revealed minimum metabolic activity in mid-June as opposed to a metabolic maximum at the end of December. A linear dependency of *RR* on photoperiod (*DLh*) and the seasonal sinusoidal trend with *DoY* was found by Meyer (2011) who reviewed investigations on seasonal metabolic activity of krill in different regions of the Southern Ocean. Our study confirms those earlier findings, but

on a broad base of data from different studies looking at animals from regions across the whole Antarctic Ocean. This pattern shows evidence for a general metabolic strategy in *E. superba*, which has been investigated from the molecular (Seear *et al.*, 2009; Teschke *et al.*, 2011) to the organism level (Atkinson *et al.*, 2002; Teschke *et al.*, 2007; Gaten *et al.*, 2008; Pape *et al.*, 2008; Meyer *et al.*, 2010; Brown *et al.*, 2013).

Although, the signaling cascade that links the photoperiod cue to the target response still remains unknown, the photoperiodic cycle clearly seems to act as a major *Zeitgeber* for the seasonal cycle of *RR*, suggesting that krill has evolved an endogenous time keeping system that perceives seasonal variations in photoperiod (Meyer, 2011). Teschke *et al.* (2011) identified an endogenous circadian timing system in Antarctic krill and found evidence for its link to metabolic key processes on a 24 h basis, which could also be involved in the control of seasonal events. Thus, the seasonal cycle of *RR* in krill could be linked to an endogenous timing system, synchronized with the seasonal course of photoperiod in the environment. In a long-term experimental study lasting several years, Brown *et al.* (2013) maintained *E. superba* first under simulated natural photoperiod, before they exposed part of the group to complete darkness and variable food availability and temperature over several months. These experiments showed that *E. superba* maintained similar *RR* patterns under constant darkness as under a simulated natural light regime. The authors suggested an endogenous rhythm of *RR* that was naturally "imprinted" and sustained during the one-year experimental acclimatization period under the natural light cycle. The sinusoidal pattern shown by the GAM therefore represents an applicable tool for the investigation of deviations from the "internal clock" mechanism (Kawaguchi *et al.*, 2007; Seear *et al.*, 2009; Brown *et al.*, 2011; Meyer, 2011; Teschke *et al.*, 2011) by revealing conditions that cause divergence from the theoretical annual pattern of synchronized respiration.

Euphausia pacifica and *Meganyctiphanes norvegica*

Unfortunately much less data sets are available for *E. pacifica* and *M. norvegica* than for *E. superba*. These two species are widely distributed over the north Pacific and Atlantic (from 27 to 66°N and 30 to 80°N, respectively; Brinton *et al.*, 2003, updated 2008), and the data sets are geographically wide spread, accordingly, making difficult to detect significant seasonal patterns. In *E. pacifica*, detection of *DoY* or *DLh* effects depended exclusively on the data set of Paranjape (1967), data which were treated as outlier also in earlier studies, as the reported *RR* is conspicuously low (Ikeda *et al.*, 2000). This is thought to reflect the permanent anoxic conditions in the deep waters of Saanich Inlet (Canada; Ikeda *et al.*, 2000).

In *M. norvegica*, the available data indicate a negative correlation between the MLR residuals and *DLh* (Fig. 3.12), *i.e.* just the opposite of the relationship found in the Antarctic *E. superba*. However, our data base does neither represent the full range of *DLh* nor the natural temperature

range experienced by *M. norvegica*. There is some evidence for seasonal patterns in respiration of this species at lower latitudes (43°N; Saborowski *et al.*, 2002), but more data covering a wider range of the natural conditions experienced by *M. norvegica* are required for the establishment of a reliable model.

3.5 Conclusion

The present work confirms the effect of latitude, the day of the year of measurement, and the number of daylight hours on the respiration of Euphausiids. With this model we display the current global state of knowledge with respect to metabolic measurements available for some of the major Euphausiids, indicating where (degree of latitude) and when (time of the year) data are available or missing. Many existing data gaps with respect to both, degree of latitude and timing, call for better coverage to improve future modelling attempts. The highest data coverage for the GAM model was available for the Antarctic krill *Euphausia superba*, which helped to simulate and put numbers to the strong seasonal metabolic adjustments observed in this species.

Acknowledgements

This study is based on the careful respiration measurements of many euphausiid and zooplankton experts of the world. Special thanks to Joseph J. Torres and Tsutomu Ikeda for sharing their complete data sets.

Chapter 4

Relationship between citrate synthase activity, temperature and respiration in euphausiids

Additional results of the thesis

4.1 Introduction

Zooplankton respiration and relative enzymatic activity measurements, like citrate synthase, were reviewed in Ikeda *et al.* (2000) to standardize the methods and allow a better comparison among species and studied areas. Citrate synthase (*CS*) is the first enzyme of the Krebs cycle, from which reduced intermediate of energy is produced during aerobic respiration. This enzyme is frequently measured as indicator of mitochondrial density in different tissues for physiological comparative studies. In euphausiids, attempts to link organismal respiration with one of the biochemical parameters involved in a respiratory function have so far remained limited to the Antarctic region, where *CS* activity was strongly correlated with the respiration rate in juveniles of the species *Thysanoessa macrura* (Donnelly *et al.*, 2004). The measurements of *CS* activity of the variety of species conducted in my thesis research were tested for relationship with the respiration rates and with the *in situ* temperature to detect climatic pattern of the enzyme.

4.2 Materials and methods

CS activity was analyzed in adults of all species covered by the present study, except the northern California Current System species *Thysanoessa spinifera*, which we failed to catch in high enough quantities. Samples were preserved in liquid N_2 or at -80°C immediately after catch. Whole organisms were homogenized in 1:20 (w/v) ice-cold Trizma® hydrochloride (Tris-HCl) buffer. CS activity was measured in 13 µL supernatant diluted in a total work solution of 333 µL containing the substrate acetyl-Coenzyme A and 5',5'-dithio-bis(2-nitro)benzoic acid (DTNB) at 412 ηm according to Sidell *et al.* (1987), at room temperature (20°C) using a micro-plate reader (Berthold Technologies Multimode reader TriStar LB 941, France). Data were calculated as activity units (U) per mg protein^{-1} and g of wet mass (WM)$^{-1}$. Soluble proteins content was measured after Bradford (1976).

To test if *CS* measurements can be used as a respiration marker in euphausiids, I searched the literature for respiration data of adult euphausiid at a specified habitat or experimental temperature (T) and corresponding *CS* activity per mg protein^{-1} ($CS_{protein}$) and/or gWM^{-1} (CS_{WM}) for which a WM was provided. In some cases, the public domain software ImageJ (`http://rsbweb.nih.gov/ij/`) was used to extract respiration or citrate synthase data from figures. Unpublished measurements recorded by Dr. Thorsten Werner were also added. The standard metabolic rate was converted to specific respiration rate (RR; J J^{-1} day^{-1}) using body mass calculated after Brey *et al.* (2010) to standardize the data sets. According to Meyer *et al.* (2010), the method of Lowry *et al.* (1951) used for protein determination in her study is more suitable for krill, as Bradford (1976) underestimates the protein concentration in krill tissues by up to 3-fold. This aspect was unfortunately not considered in many studies (including mine), thus I divided the $CS_{proteins}$ calculated with Bradford (1976) method by 3 to correct this error. All original data and converted respiration are compiled in Tab 4.1.

Statistic analysis and figures were done with R (R Core Team, 2012). Normality (Shapiro test) of RR, T, CS_{WM}, and $CS_{protein}$ were test to perform Pearson (or Spearman if non-parametric) correlation between RR and CS_{WM}, RR and $CS_{protein}$, CS_{WM} and T, and $CS_{proteins}$ and T with the package "Hmisc" (Harrell, 2014). When Pearson correlation was significant, the relationship between both variables was tested by linear regression. The level of significance was assumed as 95% (p=0.05).

Table 4.1: Oxygen consumption, calculated specific respiration rate *RR* and citrate synthase (CS) activity of adult krill species from polar, temperate, subtropical and tropical regions. *Body mass conversion after Brey *et al.* (2010); Season: a or b (austral or boreal), S, A, W, or Sp (Summer, Autumn, Winter or Spring); WAP: Western Antarctic Peninsula; ETP: Eastern Tropical Pacific; CCS: California Current System; BCS: Benguela Current System; HCS: Humboldt Current System; Ab: abdomens; WO: whole organism; mean ±SD.

Species	Season	Areas	*in situ* T°C	O_2 consumption	Calculated O_2 consumption ($J\ J^{-1}\ day^{-1}$)*	CS activity (U mg $proteins^{-1}$)	CS activity (U g WM^{-1})	Tissue	References
Polar regions (Antarctica)									
Euphausia superba	aSp & aA	Scotia & Weddell Sea	0.5	0.15 ±0.04 µL O_2 $mgWM^{-1}$ h^{-1}	0.016 ±0.004	-	1.6 ±0.4	WO	Torres *et al.*, 1994
	aW		0.5	0.11±0.07 µL O_2 $mgWM^{-1}$ h^{-1}	0.012 ±0.005	-	0.27 ±0.22	WO	
	aS	Adelaide Island & WAP	-0.1	0.58±0.03 µL O_2 $mgDM^{-1}$ h^{-1}	0.016 ±0.001	-	10.9 ±2.7	Ab	Meyer *et al.*, 2002
	aA	Lazarev Sea	-0.9	0.20±0.01 µL O_2 $mgDM^{-1}$ h^{-1}	0.0056 ±0.0003	-	7.0 ±0.7	Ab	
	aSp & AS	Weddell Sea	0.5	0.129 ±0.010 µL O_2 $mgWM^{-1}$ h^{-1}	0.020 ±0.003	-	2.2 ±0.7	WO	Donnelly *et al.*, 2004
	aSp	Lazarev Sea	-1.5	0.71±0.16 µL O_2 $mgDM^{-1}$ h^{-1}	0.021 ±0.004	0.10 ±0.03	-	Ab	Meyer *et al.*, 2010
	aA		-1.4	0.37±0.04 µL O_2 $mgDM^{-1}$ h^{-1}	0.010 ±0.003	0.05 ±0.01	-	Ab	
	aW		-1.8	0.19±0.04 µL O_2 $mgDM^{-1}$ h^{-1}	0.005 ±0.003	0.04 ±0.01	-	Ab	
	aS	South Georgia	3.2	32±8 µmol O_2 gDM^{-1} h^{-1}	0.021 ±0.005	0.44 ±0.16	2.2 ±0.6	WO	this study
Temperate regions									
Meganyctiphanes norvegica	bS	Kattegat, Denmark	5.0	34 ±6 µmol O_2 gDM^{-1} h^{-1}	0.017 ±0.003	-	14.5 ±1.5	Ab	Saborowski *et al.*, 2002[1], Saborowski and Buchholz, 2002[2]
	bW		5.0	34 ±6 µmol O_2 gDM^{-1} h^{-1}	0.017 ±0.003	-	13.7 ±1.7	Ab	
	bA	Clyde Sea, Scotland	9.0	33 ±4 µmol O_2 gDM^{-1} h^{-1}	0.016 ±0.002	-	11.0 ±1.7	Ab	
	bSp		9.0	33 ±4 µmol O_2 gDM^{-1} h^{-1}	0.016 ±0.002	-	8.6 ±1.0	Ab	
Euphausia pacifica	bS	Northern CCS	10.0	93 ±28 µmol O_2 gDM^{-1} h^{-1}	0.07 ±0.02	0.13 ±0.11	20 ±12	WO	this study
	bSp		10.0	119 ±22 µmol O_2 gDM^{-1} h^{-1}	0.08 ±0.02	0.08 ±0.04	34 ±23	WO	
Euphausia mucronata	aW	HCS	8.0	100 ±85 µmol O_2 gDM^{-1} h^{-1}	0.04 ±0.02	0.06 ±0.02	17 ±8	WO	

continued on following page...

Table 4.1 – Continued; Season: a or b (austral or boreal), S, A, W, or Sp (Summer, Autumn, Winter or Spring); WAP: Western Antarctic Peninsula; ETP: Eastern Tropical Pacific; CCS: California Current System; BCS: Benguela Current System; HCS: Humboldt Current System; Ab: abdomens; WO: whole organism.

Species	Season	Areas	*in situ* T°C	O_2 consumption	Calculated O_2 consumption ($J\ J^{-1}\ day^{-1}$)*	CS activity (U mg $proteins^{-1}$)	CS activity (U g WM^{-1})	Tissue	References
Subtropical regions									
M. norvegica	bS	Ligurian Sea, France	12.0	37 ±16 µmol O_2 gDM^{-1} h^{-1}	0.036 ±0.007	-	8.5 ±1.1	Ab	Saborowski *et al.*, 2002[1], Saborowski and Buchholz, 2002[2]
	bW		12.0	68 ±13 µmol O_2 gDM^{-1} h^{-1}	0.019 ±0.010	-	12.4 ±1.1	Ab	
Nyctiphanes capensis	aSp	Northern BCS	15.0	20±7 µmol O_2 gWM^{-1} h^{-1}	0.031±0.010	-	5±1	Ab	Werner, unpubl.
Nematoscelis megalops	aS	Northern BCS	15.0	15±4 µmol O_2 gWM^{-1} h^{-1}	0.036±0.010	-	6.6±0.5	Ab	Werner *et al.*, 2012[1]; Werner, unpubl.[2]
Euphausia hanseni	aS	Northern BCS	15.0	14±3 µmol O_2 gWM^{-1} h^{-1}	0.015±0.003	-	6.4±0.6	Ab	Werner *et al.*, 2012[1]; Werner, unpubl.[2]
	aS	Northern BCS	15.0	18±4 µmol O_2 gWM^{-1} h^{-1}	0.0130 ±0.004	0.03 ±0.01	3.6 ±0.7	Ab	Hünerlage and Buchholz, 2013
Tropical regions									
Euphausia lamelligera	bW	ETP, Mexico	20.0	3625±197 µmol O_2 gDM^{-1} h^{-1}	3 ±2	0.13±0.11	438±304	WO	this study
Euphausia distinguenda	bW	ETP, Mexico	20.0	680±38 µmol O_2 gDM^{-1} h^{-1}	0.6 ±0.3	0.14 ±0.09	143 ±101	WO	

[1]Respiration data; [2]Citrate synthase data;

4.3 Results and discussion

The tropical species *Euphausia lamelligera* and *Euphausia distinguenda* were outlier values for all the variables tested for correlations. Smaller size and high *in situ* temperature of the tropical species probably explain the higher respiration rate and CS_{WM} activity compared to all other species. High metabolic rates were also recorded for other tropical euphausiid species in the past (Ikeda, 1974; Ivleva, 1980; Ikeda and McKinnon, 2012). *CS* activity was for the fist time recorded for tropical species in the frame of this study.

Pearson correlation run between logarithmic values of CS_{WM} and T indicated a strong positive relationship between both parameters (r=0.770, N=19, p<0.001). However, no significant linear regression was established between both variables (Fig. 4.1). This correlation demonstrates *CS* activity to be a promising indicator for the thermal range of the organisms, but more measurements are required in order to reinforce this statement.

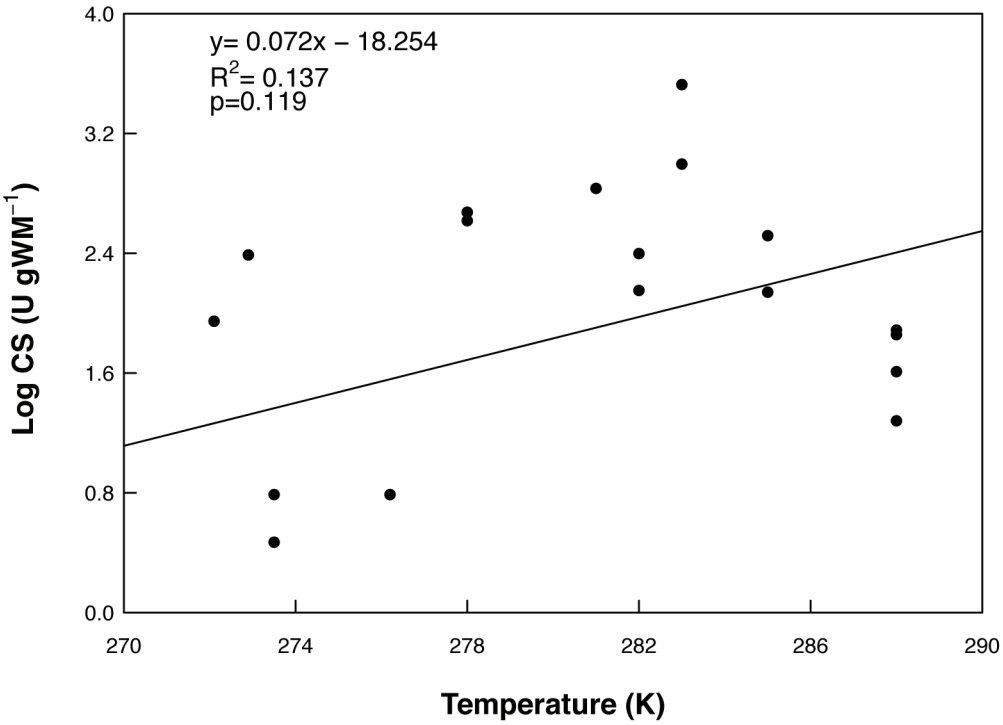

Figure 4.1: Linear relationship between logarithm transformed citrate synthase (CS) activity in unit (U) g WM^{-1} of several euphausiid species with *in situ* temperature (K) of sampling areas.

The $CS_{protein}$ data were scarce, but still present differential slopes among *Euphausia superba* measurements and the rest of the data sets (temperate and subtropical species; Fig. 4.2). The low number of data did not allowed correlation analysis, and both linear regressions do not significantly describe the relation between the two variables. However, the relationship between both parameters seems to integrate some thermal aspect, as $CS_{protein}$ activity in *E. superba* is very sensitive to slight increase of *RR*, while the opposite is observed among the temperate and subtropical species. Calculating enzymatic activity per mg proteins allows a better comparison among all species analyzed as total protein composition per g of tissues can varies among season in a same species (Falk-Petersen, 1981).

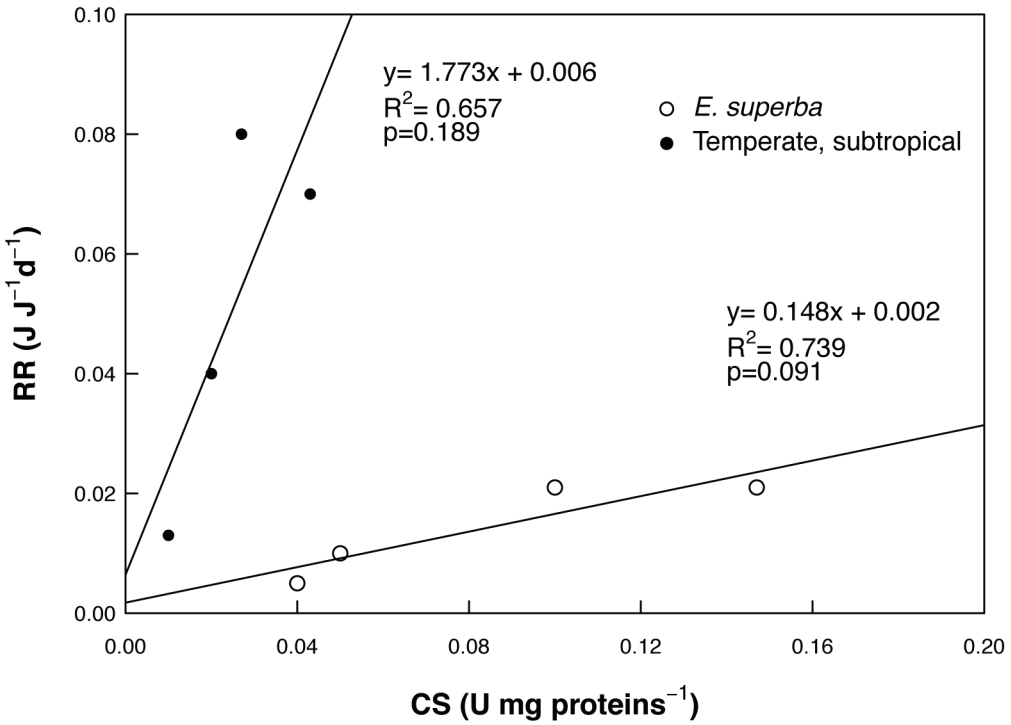

Figure 4.2: Relationship between citrate synthase (CS) activity in unit (U) mg proteins^{-1}of several euphausiid species with converted *in situ* specific respiration rate (*RR*) in J J^{-1} d^{-1} (after Brey *et al.*, 2010).

A positive and significant Spearman correlation was determined for CS_{WM} and RR (r=0.480, N=19, p=0.036; Fig. 4.3). The same observation as for $logCS_{WM}$ and T applies for this comparison, as one third of the measurements come from the cold adapted *E. superba*. To predict globally the euphausiids RR from an enzyme activity, it is thus crucial to include more measurements of species from other latitudes.

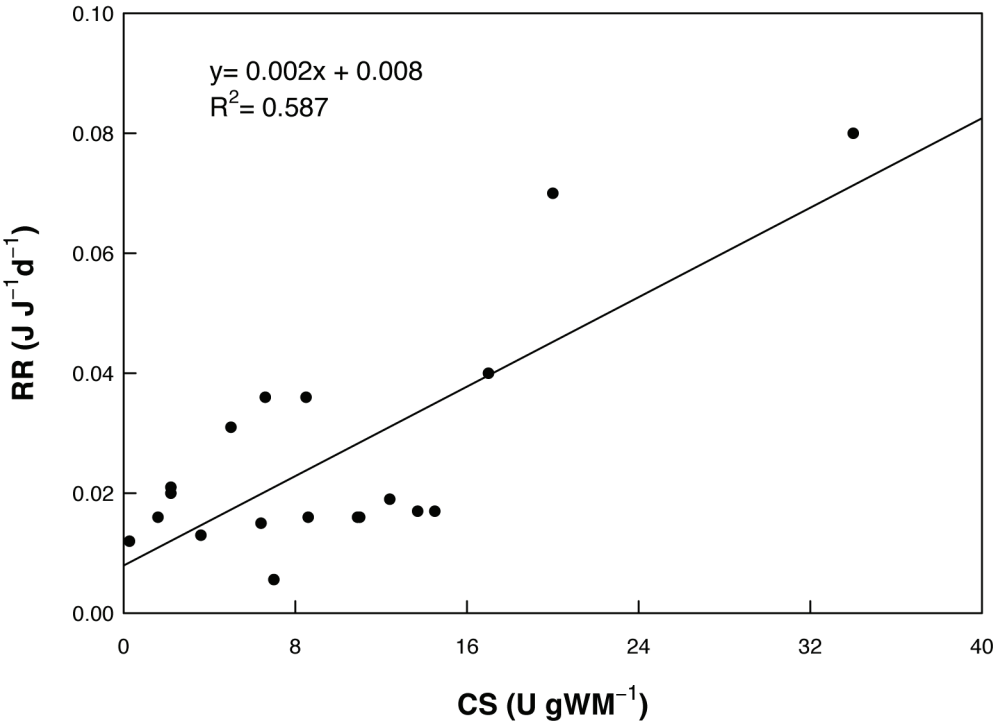

Figure 4.3: Relationship between citrate synthase (CS) activity in unit (U) g WM^{-1} of several euphausiid species with calculated specific respiration rate (RR) in J J^{-1} d^{-1} (after Brey *et al.*, 2010) at *in situ* temperature.

4.4 Conclusion

We are still far from predicting euphausiids respiration rates with a biochemical marker such as the citrate synthase activity. The relationship shown the links between this Krebs cycle marker enzyme, temperature and the process of aerobic respiration. More factors would have to be considered in the future for their potential influence on the respiration, *e.g.* the activity level of the species as showed for copepods (Bode *et al.*, 2013), the maturity stage in which the O_2-dependance may vary (Quetin and Ross, 1989), and the seasonality (like shown in Chapter 3 of this thesis).

Chapter 5

Response of three krill species to hypoxia and warming: An experimental approach to oxygen minimum zones expansion in coastal ecosystems

Nelly Tremblay and Doris Abele

Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Functional Ecology, Am Handelshafen 12, 27570 Bremerhaven, Germany

submitted to Marine Ecology MAE-2034 (20.02.2014)

Abstract

To understand adaptation of euphausiid (krill) species to oxygen minimum zones (OMZ), respiratory response and stress experiments combining hypoxia/reoxygenation exposure coupled with warming were conducted. Experimental krill species spanned from the Antarctic to Humboldt Current system (HCS, Chilean coast), and the Northern California Current system (NCCS, Oregon). *Euphausia mucronata* from the HCS is perfectly adapted to OMZ conditions and starts metabolic suppression below 80% oxygen (O_2) saturation (18 kPa). Contrary, normoxic subsur-

face oxygenation in winter may already pose a "high oxygen stress" for this species. The NCCS krill, *Euphausia pacifica*, and the Antarctic krill, *Euphausia superba* are oxyregulating and maintain respiration rates constant down to 30% (6 kPa) and 55% O_2 (10 kPa) saturation, respectively. Antarctic krill had the lowest antioxidant enzyme activities, but the highest concentrations of the molecular antioxidant glutathione (GSH) and was not affected by 6 h exposure to moderate hypoxia. Both temperate krill species had higher SOD (superoxide dismutase) values in winter than in summer, which relate to higher winter metabolic rate (*E. pacifica*). In both species, antioxidant enzyme activities remained constant during hypoxic exposure at habitat temperature. Warming by 7°C in summer increased SOD activities and GSH levels in *E. mucronata* (HCS), but no oxidative damage occurred. In winter, when temperature is homogenous and the OMZ absent, a +4°C warming combined with hypoxia represents a lethal condition for *E. pacifica*. In summer, when the OMZ expands upwards (100 m subsurface), antioxidant defences counteracted hypoxia and reoxygenation effects in *E. pacifica*, but only at mildly elevated temperature (+2°). Experimental warming by +4° reduced antioxidant activities and again caused mortality of exposed specimens. We conclude that a climate change scenario combining warming and hypoxia represents a serious threat to *E. pacifica* and, as a consequence, NCCS food webs.

5.1 Introduction

One of the most important effects of climatic change at tropical and temperate latitudes is the expansion of oxygen minimum zones (OMZ), especially in coastal and shelf regions (Helly and Levin, 2004). The expansion can be regional into areas previously not experiencing hypoxic conditions, or it can consist in vertical expansion or intensification of a normally existing OMZ. Notably the OMZ of the Eastern Tropical Pacific and the Eastern Atlantic off northwest Africa have expanded to higher latitudes during the past 50 years (Stramma *et al.*, 2008), suggesting changes in zoogeographic distribution patterns and regionalization of biomass production (Stramma *et al.*, 2011; Gilly *et al.*, 2013). Global ocean warming is among the causes of OMZ expansion, and combined effects of warming, hypoxia and ocean acidification endanger many sensitive marine species (Rosa and Seibel, 2008; Stramma *et al.*, 2011). Euphausiids (krill) are important marine biomass producers and link primary production and larger carnivorous secondary producers in marine food webs. They undertake daily vertical migrations (DVM), upward at dusk to feed in the productive surface layers and downward at dawn to avoid visual predators and digest the food and, in doing so, contribute strongly to the vertical biomass flux. During their daily migrations, krill cross gradients of temperature, salinity, and oxygen indicating that some species need a broad ecophysiological plasticity. Indeed, out of the total 86 krill species known worldwide, 54 occur in at least two different oceans (Brinton *et al.*,

2003, updated 2008). However, the most productive species are locally confined specialists, neither widely distributed nor physiologically versatile, and can be predicted to suffer from the effects of ocean warming and OMZ expansion. Hence, upward migration of subtropical and temperate productive species may be restricted by thermocline formation, whereas downward migration is limited by an OMZ (Tremblay *et al.*, 2010). Impairment of DVM can enhance visual predation (Fernández-Álamo and Färber-Lorda, 2006) or also cause mass mortality of krill under physiological stress (Tyburczy *et al.*, 2013; Oregon and Northern California).

One way to detect physiological disturbance is by measuring oxidative stress parameters. The term *oxidative stress* refers to a state of respiratory imbalance in which animals cannot maintain constant tissue oxygenation and instead experience rapid shifts between over and under-oxygenation. In this case, especially when animals are re-oxygenated after hypoxic exposure, reactive oxygen species (ROS: reactive molecules derived from oxygen, such as the superoxide anion ($O_2^{\bullet -}$), hydroxyl radicals ($OH^{\bullet}$), and hydrogen peroxide (H_2O_2)) are formed which, if not neutralized by the organism's antioxidant defence, cause oxidative damage and eventually cellular disorder and death. Tremblay *et al.* (2010) showed that OMZ/hypoxia adapted krill species in the Gulf of California (Mexico) possess sufficiently high antioxidant protection, while less adapted species suffered severe oxidative stress measurable as lipid peroxidation.

Aiming at a better understanding of the threat ocean warming and widening of the OMZ presents to krill species on a global scale, metabolic and oxidative stress indicators were investigated in three krill species known to differ in the level of adaptation to OMZ conditions. Their response to hypoxia, reoxygenation, and warming were experimentally tested. The north Pacific krill, *Euphausia pacifica* (Adults: 11-25 mm length; Brinton *et al.*, 2003, updated 2008) was chosen. This species forms massive swarms in the northern California Current system (NCCS) where mid-water hypoxia is common at the end of summer since one decade (Chan *et al.*, 2008; Connolly *et al.*, 2010; Peterson *et al.*, 2013). All along the Pacific United-States of America coast, juveniles and adults of this oceanic species perform daily migrations between the surface and depths of at least 250 m (Brinton, 1967). In fjords and bays their downward migration is often reduced (Bollens *et al.*, 1992), sometimes limited by seasonal hypoxic or anoxic conditions in bottom water layers (Kunze *et al.*, 2006). Hypoxia sensitivity of *E. pacifica* has already been investigated and described by Childress (1975) and Ikeda (1977). Both authors recognized a critical limit of performance of the species between 11 and 20% oxygen saturation (2 to 4 kPa pO_2), which manifests in a dramatic reduction in swimming activity and also in high mortalities below this critical pO_2. Recently, Fisheries and Oceans Canada Ministry (2013) has expressed concern regarding the future of this important commercial species for fisheries. They observed unprecedented year-to-year fluctuations of krill biomass and hypothesized that climate change and enhanced predation may be responsible for the high mortalities in low krill years.

A perfect counterpart to the hypoxia sensitive *E. pacifica* is its southern hemisphere antipode, *Euphausia mucronata* (Adults: 17-22 mm length; Brinton *et al.*, 2003, updated 2008), endemic to the temperate Humboldt Current system (HCS) and, thus, pertaining to a similar climatic background with respect to temperature and OMZ scenario. Here, the OMZ is associated with low oxygenated Equatorial Subsurface Water, which frequently upwells in the HCS region, producing a large and stationary hypoxic layer near the surface (Copin-Montégut and Raimbault, 1994; Thiel *et al.*, 2007). Like *E. pacifica* in the NCCS, *E. mucronata* plays a keystone role in the trophic dynamics of the HCS as principal prey of jack mackerel and anchovy (Antezana, 2010). The species perform extended DVM down to 250 m into the OMZ during all seasons (Escribano *et al.*, 2000; Antezana, 2002b). Even if this migration into OMZ is normal, the highest *E. mucronata* abundances occur in areas where the upper boundary of the OMZ is deeper (Escribano *et al.*, 2000), which indicates avoidance of extreme hypoxia/anoxia. Larger gills/ cephalothorax surface ratio in *E. mucronata* is another indicator of hypoxic adaptation (Antezana, 2002a). The standard metabolic rate (SMR) of *E. mucronata* is the same in well oxygenated surface waters as in the hypoxic OMZ (Antezana, 2002a), and Antezana (2009) observed *E. mucronata* to be one of the last OMZ species to begin its ascent to the surface at dusk, thus stretching the deep hypoxic residence time to a maximum. *E. mucronata* therefore qualifies as a highly hypoxia adapted species in comparison to *E. pacifica*, and we hypothesized that its adaptive capacity involves better oxidative stress resistance.

The last species of the comparison had so far never deals with hypoxia, the Antarctic krill *Euphausia superba* (Adults: 42-65 mm length; Brinton *et al.*, 2003, updated 2008). Note that oxidative stress indicators have not previously been reported for this Antarctic key species. Since the last two decades, warming of surface waters has been observed especially in Western Antarctica (Meredith *et al.*, 2008; Bers *et al.*, 2012), which has caused massive loss of ice sheets (Pritchard *et al.*, 2012; Rignot *et al.*, 2013) and alters the coastal ecosystems (Moline *et al.*, 2004; Norkko *et al.*, 2007; Schofield *et al.*, 2010). The Antarctic krill *E. superba*, central in Antarctic food webs (Atkinson *et al.*, 2004; Murphy *et al.*, 2007), forms large biomasses in the southern ocean and migrates close to the surface (Gaten *et al.*, 2008). The hypoxia response of *E. superba* was investigated to understand the effect of metabolic rate and habitat temperature on oxidative stress parameters, but also to include another hypoxia sensitive species from a different climate.

5.2 Materials and methods

5.2.1 Ethics statement

The present study is not involving any protected or endangered species. No specific permissions are required for sampling in the NCSS and HCS. For the species *Euphausia superba*, the British Antarctic Survey received a permit for its general operations in Antarctica from the Foreign and Commonwealth Office (United-Kingdom) as a requirement of the Antarctic Act.

5.2.2 Krill collection

Krill were collected during several day trips and some longer oceanographic cruises carried out in 2011 and 2012, details are given in Table 5.1. Each area was visited during cold and warm seasons, except Antarctica, which was sampled only during the warm season. To reduce sampling stress, krill fishing was conducted at night when the krill are near the surface. After heaving the sampling gear on deck, the collected zooplankton was immediately transferred to 20 L buckets with seawater. Live adult euphausiids, in healthy condition (showing a lot of movement and with no visible damage), were manually sorted into bins (colman boxes, or tanks of 100 L in Antarctica) and transferred to a cold room (see Table 5.1 for temperature). Directly after catch, some specimens were snap frozen in liquid N_2 (HCS) or at -80°C (NCCS and Antarctica) for biochemical analysis of *in situ* values. The rest of the animals were left to recover for at least 6 h before respirometry and experiments were started.

5.2.3 Environmental data collection

Temperature, oxygen and salinity profiles were recorded with a Seabird SB09 "conductivity, temperature, depth" (CTD) system in all sampling areas. Each profile was plotted to detect the upper boundary of the OMZ and the depth of the thermocline, if present. As ecosystems with different salinity and temperature profiles were compared, the upper boundary of the OMZ was defined at the depth where the dissolved oxygen concentration was 20% of the maximal saturation. Furthermore, sea surface temperature (SST; °C) and chlorophyll *a* concentration (mg m^{-3}) visualizations averaged monthly from the Moderate-resolution Imaging Spectroradiometer (MODIS) Aqua Global Level 3 (11 µm thermal infrared; 4 km spatial resolution) were produced with the Giovanni online data system (developed and maintained by the NASA GES DISC) for each sampling area and season.

Table 5.1: Sampling areas (Latitud/Longitud), periods, sampling gears, R/V, off board localities, and temperature conditions in Antarctica (South Georgia), in the Humboldt current system (HCS) and in the northern California current system (NCCS).

Area	Lat/Lon	Period (season)	Water depth (m)	Sampling gear	R/V; off board facilities (if applied)	Sea surface* T°C	Water column‡ T°C	Cold room T°C
Antarctica (South Georgia)	53-55°S 37-41°W	3-10th Jan 2012 (summer)	<400	pelagic net, rectangular midwater trawl (RMT), 8 m^2 mouth area	James Clark Ross	3.2	1.5	4.0
HCS (Chile)	36.5°S 73.1°W	23th Aug-13th Sep 2011 (winter)	80	zooplankton net, 1 m diameter, 5 m long, 300 µm black mesh with nonfiltering cod end (0.22 m diameter and 0.70 m long)	Kay-Kay II; Universidad de Concepción, Marine biology laboratory (Dichiato, Región del Biobío)	11.9	11.7	8.0
		24th Jan-3rd Feb 2012 (summer)				12.9	11.0	8.0
NCCS (United-States of America)	44.7°N 124.7°W	14-30th Sep 2011 (summer)	275	bongo net, 0.6 m diameter, 333 µm black mesh with nonfiltering cod end	Elakha; Oregon State University, Hatfield Marine Science Center (Newport, Oregon)	11.9	8.1	10.0
		7-14th Apr 2012 (winter)				9.9	7.7	10.0

*between 0 and 20 m; ‡ from 20 m until maximum depth

5.2.4 Respiration measurements

As ROS formation can change as a function of animal O_2 consumption (although there is no strict one to one relationship between both parameters, see also Buttemer *et al.*, 2010), we measured the standard metabolic rates (SMR) of all investigated species at *in situ* temperature (see Table 5.1 for temperature details), using an OXY-4 channel PreSens Oxygen Ingress Measurement system (Germany). The system was equipped with 4 chambers for simultaneous measurement of three animals and a blank (for seawater bacterial oxygen demand). Cylindrical chambers of 20 mL volume were used, except for the Antarctic animals where chamber volume was 250 mL to account for the larger size of *E. superba*. Chambers were equipped with a magnetic stirrer (bottom) to achieve homogeneity of the oxygen concentration, and a 30-µm mesh gauze separated the stirrer from the euphausiids. All chambers were filled with filtered seawater at 100% O_2 saturation (21 kPa) and the oxygen concentration in each chamber was measured every 15 s in mBar (or hPa). The set-up was kept in the dark and the duration of the measurements varied between 4 and 12 h, lasting until the critical oxygen partial pressure (pc) was reached in at least two of the three chambers. The pc was visually detected by the maximal change in the slope of the respiration curve. Movements of the animals were visually recorded. Not all individual respiratory tracks could be analyzed over the full pO_2 range from 100% down to hypoxia, and the number of individuals reported in both extremities of pO_2 levels corresponds to approximately one third of all measurements conducted. The onset and intensity of anaerobiosis was analysed by measuring lactate levels (in mmol L^{-1}) in the hemolymph of each individual sacrificed after respirometry using an Accutrend R Lactate system (Roche Diagnostics, Germany). Subsequently, krill were frozen at -80°C, and dry mass (DM) of each krill from the respiration experiments was measured after drying specimens 48 h at 50°C. The bacterial O_2 demand in the blank chamber was subtracted from the O_2 consumption recorded in the three chambers with krill in each run. The SMR was calculated as the respiration rate of each individual between 80 and 60% O_2 saturation (17 to 13 kPa) and expressed in µmol O_2 h^{-1} g DM^{-1}.

5.2.5 Experimental study of the synergic effect of hypoxia, reoxygenation, and warming exposure

At least one experiment was conducted for each krill species in the different areas (see Tab.5.2). Krill were divided into eight replicates of 10 to 30 animals, according to the number of krill available and their size (see Tab.5.2 for the number of krill used). Two experimental temperatures were simultaneously applied at 100% O_2 saturation (21 kPa; 2 replicates for each temperature at normoxic conditions), and at 20% O_2 saturation (4 kPa; 2 replicates for each temperature at hypoxic conditions) in which the animals were exposed to hypoxia over 6 h. The colder

experimental temperature was always the one of the available cold room (Tab.5.1), which was set closest possible to the *in situ* temperature at the sampling site. Higher temperature exposures were conducted by placing the aquaria in two boxes of water warmed with an aquarium heater (EHEIM, Germany). One control replicate (100% O_2 saturation) and one hypoxic treatment replicate (20% O_2 saturation) were incubated per box, which were covered with a lid to keep O_2 conditions and T°C constant. After transfer to the experimental aquaria, krill were allowed 1 h to acclimatize to the respective temperature. Then, water in the control aquarium was gently purged with air to achieve full saturation in the control group, while pure nitrogen (N_2) was supplied in the treatment set-up to lower the oxygen level to 20% saturation. Oxygen concentrations in hypoxic treatment aquaria were monitored using the OXY-4 channel PreSens Oxygen Ingress Measurement system at 30 min measuring intervals. After 6 h of hypoxia exposure, half of the surviving krill from the hypoxia treatments was sampled and immediately snap frozen in liquid N_2 (HCS) or at -80°C (NCCS and Antarctica). Half of control krill were sampled and preserved in the same way for each replicate. Krill left in the hypoxia treatment aquaria were reoxygenated for 1 h, by supplying the aquaria with air, and then sampled along with a second control group maintained constantly oxygenated. Frozen samples from all sampling locations were transported in dry ice to the Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research for biochemical analysis.

Deviations from this experimental set-up: Since security legislations do not allow N_2 handling in ship laboratories in the Antarctic, we conducted hypoxia exposure experiments on deck at ambient temperature. Here, we used a tank with a low certified O_2/N_2 mixture (4% O_2 air saturation which corresponded to 20% O_2 saturation in seawater) to constantly supply water in the hypoxic treatment aquaria. The aquaria were placed in boxes with an entry and an exit to allow a seawater flow from the research vessel water supply system to keep a constant and cold temperature (between 3 and 3.5°C during the experimental exposure, similar to SST at South Georgia (Tab.5.2). Working on the deck involved material limitations, and consequently it was not possible to expose the Antarctic krill to warming and reoxygenation. In the HCS off the Chilean coast, no hypoxic exposures were conducted in the cold season because N_2 was not available.

5.2.6 Biochemical analysis

Citrate synthase (CS) is a mitochondrial matrix enzyme, pacemaker of the Krebs cycle, which produces citrate from oxaloacetate, and acetyl-coenzyme A (acetyl-CoA). It is frequently measured as indicator of mitochondrial capacity in a tissue. Here, CS activity was measured in complete organisms immediately frozen after catch. Each organism was weighed into a Precellys homogenization tube (Sartorius, LA230S, Germany) and diluted 1:20 (w/v) with ice-cold Trizma®

Table 5.2: Experimental temperatures (Exp T°C), experiments (Exp), number of experiments conducted (n exp), replicates (n rep), individuals at the beginning (n_i ind), at the end (n_f ind), and % of mortality in South Georgia (SG), the Humboldt current system (HCS) and the northern California current system (NCCS). *Resp.=respiration measurements; C=control (100% O_2 saturation for 6 h); H=hypoxia (20% O_2 saturation for 6 h); C=control (100% O_2 saturation for 7 h); R=reoxygenation (100% O_2 saturation after 1 h after H treatment).

Area	Period	Exp. T°C	Exp*	n exp	n rep	n_i ind	n_f ind	% mortality
SG	Jan 12	4.0	Resp.	7	21	21	20	5
		3.5	C	4	8	60	56	7
			H	4	8	80	78	3
HCS	Aug 11	8.0	Resp.	4	12	12	12	0
			C	1	20	20	20	0
		15.0	C	1	6	6	6	0
	Feb 12	8.0	C	1	2	20	20	0
			H	1	4	40	40	0
			C	1	2	20	20	0
			R	1	4	40	40	0
		15.0	C	1	2	20	20	0
			H	1	2	20	20	0
			C	1	2	20	20	0
			R	1	2	20	20	0
NCCS	Sep 11	10.0	Resp.	11	33	33	31	6
			C	2	4	30	30	0
			H	2	4	37	34	8
			C	2	4	30	30	0
			R	2	4	17	17	0
		12.0	C	1	2	20	20	0
			H	1	2	30	25	17
			C	1	2	20	20	0
			R	1	2	12	12	0
		14.0	C	1	2	20	20	0
			H	1	2	40	19	48
			C	1	2	20	20	0
			R	1	2	5	5	0
	Apr 12	10.0	Resp.	4	12	12	11	8
			C	2	4	50	49	2
			H	2	6	140	106	24
			C	2	4	50	50	0
			R	2	6	50	50	0
		12.0	C	1	2	20	20	0
			H	1	2	30	21	30
			C	1	2	20	19	5
			R	1	2	10	10	0
		14.0	C	1	2	20	20	0
			H	1	2	20	1	95

hydrochloride (Tris-HCl) buffer (20 mM Tris-HCL, 1 mM ethylenediaminetetraacetic acid (EDTA), 0.1% (v/v) Tween‡ 20, pH 7.4). Subsequently, tubes were placed in a homogenizer (Bertin Technologies Precellys 24 Dual, Germany) at 4°C with the following cycle: 2 x 20 sec, 5000 rotations, 15 sec pause. After centrifugation at 7400 g for 5 min, the supernatant of the homogenate was used for the measurement. The test optically records the catalytic turnover of acetyl-CoA-SH by measuring the transfer of the sulfydryl groups to 5',5'-dithio-bis(2-nitro)benzoic acid (DTNB) as absorbance increase at 412 ηm (Sidell *et al.*, 1987). CS activity was measured at room temperature (20°C) using a micro-plate reader (Berthold Technologies Multimode reader TriStar LB 941, France). Soluble protein content was measured after Bradford (1976) in all supernatants. Data were calculated as activity units (U) mg proteins^{-1}.

For the oxidative stress assays, each individual was cut into two pieces at the end of the cephalothorax. The front part (cephalothorax) was ground in liquid N_2 and homogenized on ice with a micropistill after adding a 6-fold volume (w/v) of phosphate buffer solution (50 mmol L^{-1} potassium phosphate dibasic and monobasic mixture (K_2HPO_4/KH_2PO_4), 50 mmol L^{-1} EDTA, 1 mmol L^{-1} phenylmethanesulfonyl fluoride (PMSF), pH 7.5), and centrifuged at 23 897 g velocity for 3 min at 4°C. The supernatant of this extraction was analysed in triplicates for the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione-S-transferase (GST). If not enough supernatant was available, CAT and GST measurements were prioritized over SOD, as less volume is required for these assays. SOD converts $O_2^{\bullet-}$ to H_2O_2, and was measured using the xanthine-xanthine oxidase as a superoxide radical generating system and nitroblue tetrazolium as a detector (Suzuki, 2000). CAT takes away H_2O_2 preventing its increase in cells and tissues, which decrease was measured at 240 ηm (Aebi, 1984). GST transforms xenobiotics into other conjugates using reduced glutathione (GSH) as substrate, and was estimated by detecting the formation of the thioether product from the reaction between GSH and 1-chloro, 2, 4-dinitrobenzene (CNDB; Habig and Jakoby, 1981). The larger cephalothorax of *E. superba* allowed the additional analysis of glutathione peroxidase (GPx) activity, which required a minimum of 150 µL per triplicate. Like CAT, GPx removes H_2O_2 using nicotinamide adenine dinucleotide phosphate (NADPH) as substrate, and was measured by monitoring the continuous decrease in the concentration of NADPH upon addition of H_2O_2 to the assay mixture (Ahmad and Pardini, 1988). All antioxidant enzyme activities were measured at room temperature (20°C) using a spectrophotometer (Beckman-Coulter DU 800 UV/Vis, USA). Soluble protein was also measured in all supernatants to get enzyme activities expressed in activity units (U) mg proteins^{-1}.

A small selection of abdominal tissue from experimental and *in situ* samplings (*E. mucronata* in cold season) was analysed for reduced and oxidized glutathione (GSH, GSSG) concentration by high-performance liquid chromatography (HPLC) after de Almeida *et al.* (2011) with some

adjustments. For the GSSH assay, 200 µL of 40 mmol N-ethylmaleimine was added to 200 µL supernatant, and incubated for 25 min at room temperature in the dark. Then, 700 µL of 0.1 mol NaOH was added, and 50 µL of this mixture transferred to a fresh reaction vial. After the addition of 1000 µL 0.1 mol NaOH, 20 µL aliquots of this mixture were separated as replicates. To each replicate, 300 µL of 0.1 mol NaOH were added together with 20 µL of 0.1% *orto*-phthaldialdehyde (OPA) in methanol. The following steps were according to the GSH assay described by de Almeida *et al.* (2011). The replicates (three for GSH and five for GSSG) were kept at -20°C, and thawed four hours before analysis in the HPLC system (LaChrom Elite®, Hitachi High Technologies America, USA). Five replicates were necessary for the GSSG measurement because of the marginal amount of oxidized glutathione in the samples (SD among replicates was >10% when only three were analysed). Separation was achieved on a silica based C18 Hydro Reverse Phase column (250×4.6mm, 4µm particles, Phenomenex, USA) at room temperature (20°C), using isocratic elution with a solvent composed of 15% methanol in 25 mmol NaH_2PO_4 (pH 6.0) at 100%. Flow rate was 0.7 mL min^{-1}, and the peak area was recorded at a retention time of 9.8 min with excitation of 350 ηm, and emission of 420 ηm. This measurement of the actual redox state in the tissue is conducted to corroborate the measurements of antioxidant enzymes for a better comparison of the different species. The total pool of glutathione, reduced and oxidized forms, was quantified as glutathione equivalents (GSH-eq$= GSH + 2GSSG$) and expressed as ηmol g WM^{-1}. The ratio GSSG: GSH was calculated from the determined GSSG and GSH concentrations.

Abdominal samples were further used for the detection of malondialdehyde (MDA) formation, as indicator for lipid peroxidation, and protein carbonyl content, which tells us about protein oxidative damages. MDA concentrations were assessed according to Uchiyama and Mihara (1978) and expressed as ηmol MDA g WM^{-1}. Protein carbonyl content was measured using the OxiSelect Protein Carbonyl ELISA Kit (Cell Biolabs Inc., San Diego, CA) according to the manufacturer's instructions. Because of the small size of *E. mucronata* and *E. pacifica*, all experimental (control, hypoxia, and reoxygenation) abdominal samples were used for HPLC and MDA analysis. For that reason, protein carbonyls were analysed only in abdominal samples of freshly caught *E. mucronata* and *E. pacifica*. As sufficient tissue was available for the Antarctic species, *E. superba*, protein carbonyls were analysed in the abdominal parts of experimental animals. Carbonyl results are expressed in ηmol mg $proteins^{-1}$.

5.2.7 Data analysis

Sea surface temperature and chlorophyll *a* concentration maps were elaborated with Surfer (version 11, Golden Software Inc., USA). All statistic and figures were done with R (R Core Team, 2012). For all group comparisons, normality (Shapiro test) and variance homogeneity

(Bartlett test) tests were performed. Data were transformed ($log(x)$, x^{-1}, $x^{1/2}$) if the criteria of normal distribution and homogeneity of variance were not met. If no transformation of data allowed the use of analysis of variance (ANOVA), the non-parametric test Kruskal-Wallis was conducted. If a post-hoc comparison was necessary, a Tukey test or, if non-parametric, a multiple comparison test after Kruskal-Wallis from the package "pgirmess" (Giraudoux, 2013) was applied. Significant level of all comparisons was fixed at 95% (p=0.05).

5.3 Results

5.3.1 Environmental conditions

Figure 5.1 shows the vertical profiles of mean temperature, dissolved oxygen concentration, and salinity for each sampling site and season. Near South Georgia, surface temperatures were above 3°C and decreased steadily down to a thermal minimum at 120 m water depth. Between 120 and 300 m the temperature increased again to approximately 2°C (Fig. 5.1a). There was also a steep drop in surface salinity between 0 and 10 m water depth from 35.0 to 33.8 PSU (Fig. 5.1a). Similar to the temperature profile, salinity increased linearly with depth to near surface values in 400 m (Fig. 5.1a). Only the oxygen profile in South Georgia was homogenous and fully saturated between 0 and 400 m water depth (Fig. 5.1a). Antarctic krill was mainly fished between 40 to 200 m water depths in this study (Fig. 5.1a). Within the NCCS and HCS, temperature profiles were comparable, slightly stratified in the warm season and mixed in cold season with SST ranging on average between 11 and 13°C (Fig. 5.1b-e). The upper boundary of the OMZ (at 20% O_2 saturation) was detected around 30 and 60 m in the HCS in the warm and cold season, respectively (Fig. 5.1b, c). Near anoxic conditions exist below 60 m at the HCS station in summer and winter (Fig. 5.1b, c). We caught the HCS krill species, *E. mucronata*, hauling the net from 40 m depth to the surface in both seasons (Fig. 5.1b, c). In the NCCS, hypoxia was less pronounced, never reaching as low as 20% saturation (Fig. 5.1d, e). Between the surface and 60 m, a steep decline to approximately 30% O_2 saturation occurred in the warm season (Fig. 5.1d), whereas in the cold season the decrease was less steep (Fig. 5.1e). Differences in the salinity gradient were more important during winter in the HCS and NCCS (Fig. 5.1c, e). Salinity was lower in NCCS than in the HCS in both seasons, with a more pronounced lowering of surface salinity in the cold season (Apr 2012; Fig. 5.1d, e). Krill collection in the NCCS occurred also in the first 40 m from the surface in both seasons (Fig. 5.1d, e).

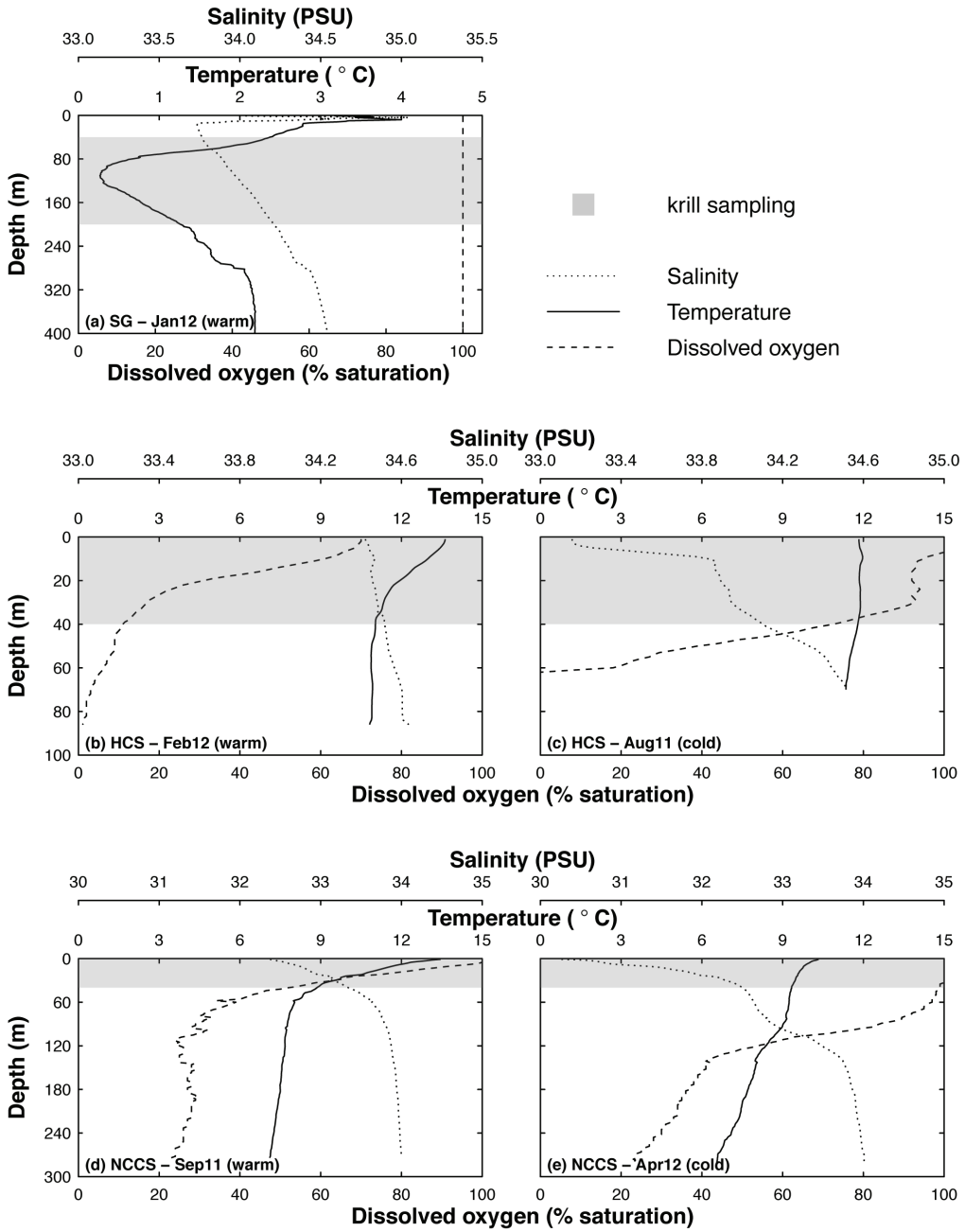

Figure 5.1: Vertical profiles of abiotic parameters and krill sampling depth in each area during sampling periods. Mean temperature (°C), dissolved oxygen concentration (% saturation), and salinity (PSU) in South Georgia (Antarctica; a), the Humboldt current system (HCS; b, c), and the northern California current system (NCCS; d, e).

Sea surface temperature (SST; °C) and chlorophyll *a* (chl *a*) concentration (mg m^{-3}) averaged monthly from MODIS-Aqua (4 km) are presented in Fig. 5.2 for each area and sampling period. At South Georgia, krill were sampled in waters with relatively poor chl *a* concentrations compared to the temperate regions (Fig. 5.2a). Strong upwelling events can easily be identified in the warm season in both temperate areas (Fig. 5.2b, d) with the SST visualisations showing a cold-warm gradient of temperature from the coastline towards the open ocean. Patches of high chl *a* concentration (>20 mg m^{-3}) confirmed nutrient enrichment in upwelling water masses (Fig. 5.2b, d). In the cold season, SST was more homogenous in both regions (Fig. 5.2c, e). Smaller patches of high chl *a* occurred in the HCS during the cold season (Fig. 5.2c), in contrast to the NCCS (Fig. 5.2e).

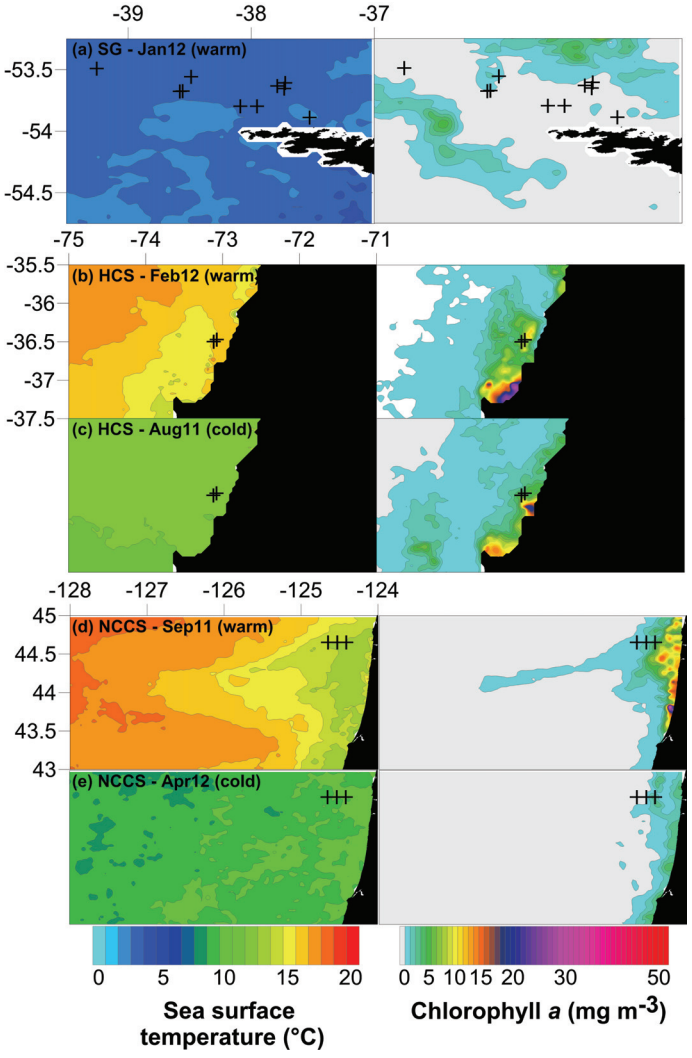

Figure 5.2: Sea surface temperature (°C; 11 µm day) and chlorophyll *a* concentration (mg m^{-3}) MODIS-Aqua (4 km) in each area. Contour maps produced with the Giovanni online data system (developed and maintained by the NASA GES DISC), during sampling periods in South Georgia (Antarctica; a), the Humboldt current system (HCS; b, c), and the northern California current system (NCCS; d, e). Euphausiid sampling stations are marked with a black cross (+).

5.3.2 Respiration measurements

Different patterns of O_2 consumption *vs.* pO_2 were observed for each species (Fig. 5.3; Tab. 5.3). The Antarctic krill *E. superba*, had by far the largest body mass (Tab. 5.3) and constant O_2 consumption down to approx. 55% O_2 saturation (10 kPa), which represents its *p*c (Fig. 5.3a). A completely different respiration pattern was observed in the HCS species *E. mucronata*. This species suppressed respiration rate by 50% between 80 to 60% O_2 saturation (17 to 13 kPa; Fig. 5.3b). Then, the O_2 saturation in the chambers did not fall much below 37% O_2 saturation (8 kPa), which was interpreted as the point where the krill switched to anaerobiosis. In the warm season, the NCCS species *E. pacifica* maintains stable respiration rates down to 40% O_2 saturation, with a transient increase of respiration between 40% (8 kPa) and *p*c at 27% (6 kPa; Fig. 5.3c). In the cold season, the same species was not able to maintain respiration rates constant as far down as in summer, and *p*c was observed already at 34% (7 kPa; Fig. 5.3d), preceding immediate death. Organisms used in the respiration measurements in winter season from the HCS and NCCS had comparable body mass (Tab. 5.3).

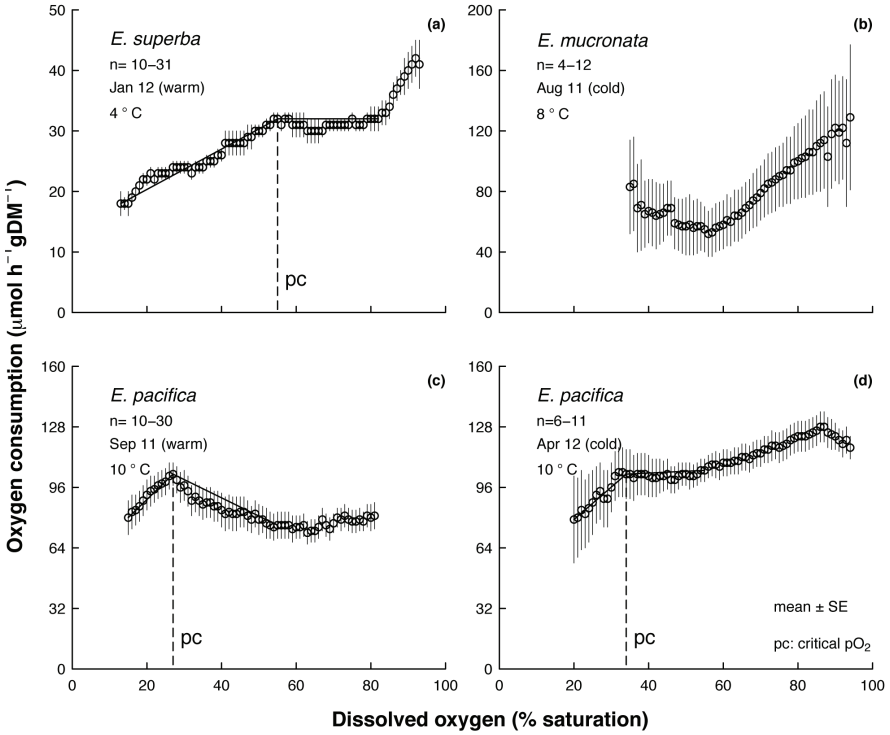

Figure 5.3: Oxygen consumption (µmol O_2 h^{-1} g DM^{-1}) associated to chamber dissolved oxygen concentration (% saturation) of *Euphausia superba* (South Georgia; a), *Euphausia mucronata* (Humboldt current system; b), and *Euphausia pacifica* (northern California current system; warm: c, cold: d); mean ±SE.

Table 5.3: Oxygen (O_2) consumption rate per mg dry mass (DM), and critical oxygen partial pressure (pc) of *Euphausia superba* (South Georgia; Jan 2012), *Euphausia mucronata* (Humboldt current system; Aug 2011), and *Euphausia pacifica* (Northern California current system; Sep 2011 and Apr 2012); data are mean ±SD (range); n: number of measurements.

Species	Period	T°C	O_2 consumption ($\mu mol\ O_2\ h^{-1}\ g\ DM^{-1}$)	n	DM (mg)	pc (O_2 saturation)
E. superba	Jan 12	4	32 ±8	31	260 ±114	55
E. mucronata	Aug 11	8	100 ±85	12	5 ±3	-
E. pacifica	Sep 11	10	93 ±28	30	14 ±3	27
	Apr 12	10	119 ±22	11	4 ±2	34

5.3.3 Interspecific comparison of lactate accumulation and CS capacities

Basal metabolic and oxidative stress parameters analysed in the different krill species from each ecosystem and season are presented in Figure 5.4. The SMR of *E. pacifica* during warm season was significantly higher (χ^2=44.42; p<0.001, Temperature of measurement (T_m)= 10°C) than the Antarctic krill *E. superba* (Fig. 5.4a, T_m= 4°C). A seasonal comparison is only possible for the NCCS species *E. pacifica*, which had significantly higher SMR in the cold season (F=7.84; p=0.008; Fig. 5.4a, T_m= 10°C in both seasons). CS activity was significantly higher (χ^2=35.60; p<0.001) in *E. superba* compared to both temperate species during the warm season and the cold season (Fig. 5.4b). Lactate accumulation was significantly higher (χ^2=6.23; p=0.013) in the hypoxia-adapted species *E. mucronata* compared to the other temperate species *E. pacifica* in the cold season (Fig. 5.4c). In *E. pacifica*, lactate accumulation differed between seasons (χ^2=8.43; p=0.004), with higher values in September 2011, at the end of summer (Fig. 5.4c). Again, the comparison is not possible for *E. mucronata* as no respiration measurements were done during the warm season. Lactate formation in *E. superba* was in the same range as in *E. mucronata* and summer *E. pacifica*.

5.3.4 Interspecific and seasonal comparison of oxidative stress parameters

Interspecific and seasonal comparisons (Fig. 5.4d-i) of antioxidant enzyme activities and glutathione concentration correspond to the values obtained as control treatments for the hypoxia-reoxygenation experiments at *in situ* temperature (*i.e.* cold room/respiration measurement temperatures, see Tab. 5.2). The only exception was that protein carbonyls of *E. mucronata* and *E. pacifica* were measured in *in situ* samples frozen directly after catch. Superoxide dismutase (SOD) activity was significantly lower (χ^2=36.61; p<0.001) in the Antarctic krill *E. superba* compared to *E. pacifica* during warm season (Fig. 5.4d). Both temperate species *E. mucronata* (χ^2=4.02; p=0.045) and *E. pacifica* (χ^2=31.46; p<0.001) had higher SOD activity in winter (Fig. 5.4d). The hypoxia

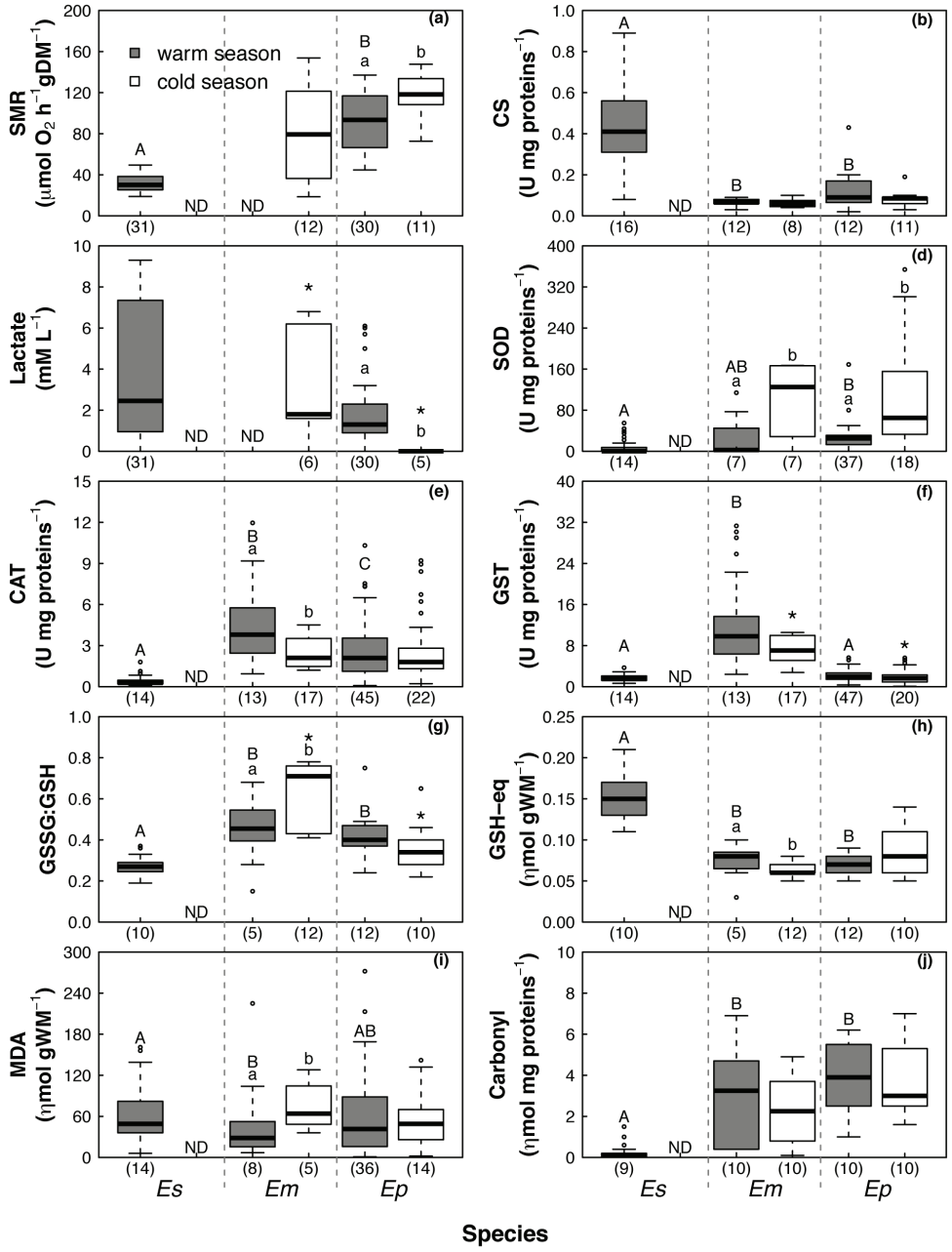

Figure 5.4: Basal metabolic and oxidative stress parameters in *Euphausia superba* (*Es*), *Euphausia mucronata* (*Em*), and *Euphausia pacifica* (*Ep*) in warm (dark gray) and cold seasons (white). (a) Standard metabolic rate (SMR), (b) CS, (c) lactate concentration at the end of respiration measurement, (d) SOD, (e) CAT, (f) GST activities, (g) oxidized/reduced glutathione (GSSG: GSH), (h) glutathione equivalents (GSH-eq), (i) malondialdehyde (MDA), (j) carbonyl concentrations. ab: intra-specific seasonal differences; AB and "*": inter-specific differences in warm and cold seasons, respectively; (n): number of samples analyzed; Dash lines separate the species; ND: no data; Horizontal bars in the box plots indicate median. Upper and lower edges of the rectangle show the 1st and 3rd quartiles, respectively. Vertical error bars extend to the lowest and highest value in a 1.5-fold inter-quartile range (R Core Team, 2012).

tolerant HCS species *E. mucronata* had the highest CAT activity in warm season, followed by the NCCS species *E. pacifica*, which ranged higher than *E. superba* (χ^2=102.94; p<0.001; Fig. 5.4e). CAT activity was significantly higher in the warm season (F=9.68; p=0.003) in *E. mucronata*, which was the opposite pattern of the SOD activity (Fig. 5.4e). The activity of the detoxifying antioxidant enzyme glutathione-S-transferase (GST) was significantly higher in *E. mucronata* during both, the warm (χ^2=104.65; p<0.001) and the cold season (χ^2=27.19; p<0.001; Fig. 5.4f), compared to any of the other two species. The Antarctic krill *E. superba* had the lowest GSSG: GSH ratio (least oxidized glutathione, χ^2=31.91; p<0.001) corresponding to the highest concentration of reduced GSH (χ^2=43.04; p<0.001) compared to both temperate species in warm season (both; Fig. 5.4g, h). In the cold season, the GSSG: GSH redox ratio of *E. mucronata* was significantly higher (F=11.31; p=0.003) than in *E. pacifica* (Fig. 5.4g). Whereas *E. pacifica* had similar glutathione ratios in both seasons, *E. mucronata* had a higher (more oxidized) GSSG: GSH ratio (F=5.84; p=0.023; Fig. 5.4g) and lower GSH-eq concentration (F=6.40; p=0.016; Fig. 5.4h) in the cold season.

The difference in malondialdehyde (MDA) concentrations were extremely subtle and the only conspicuous difference was very low MDA values in the hypoxia-adapted *E. mucronata* during summer, which had significantly less MDA per g WM than Antarctic krill *E. superba* (χ^2=7.02; p=0.030; Fig. 5.4i) and *E. mucronata* caught in winter (χ^2=8.04; p=0.005; Fig. 5.4i). The Antarctic krill *E. superba* had negligible protein carbonyl levels (χ^2=22.23; p<0.001; Fig. 5.4j) compared to the temperate species in both seasons. Both temperate species had similar protein damage levels independently of the season.

5.3.5 Intraspecific comparison of warming, hypoxia and reoxygenation responses

For the hypoxia/reoxygenation treatments, no significant differences were detected between control groups maintained during the 6 h hypoxia exposure experiment and the one maintained during the 1 h reoxygenation phase, parallel to the experimental groups. The results were therefore pooled together to form the control group for both hypoxia and reoxygenation treatments. The oxidative stress parameters analysed in the Antarctic krill *E. superba* at 4°C after 6 h of hypoxia exposure were not different from normoxic control values (Fig. 5.5). Only MDA concentration (F=5.33; p=0.025; Fig. 5.5g) increased after 6 h of hypoxia, showing signs of oxidative stress. Minor mortality or loss of individuals occurred during exposure (Tab. 5.2), but this was more related to the instability of experimental set-up on deck when heavy sea conditions started.

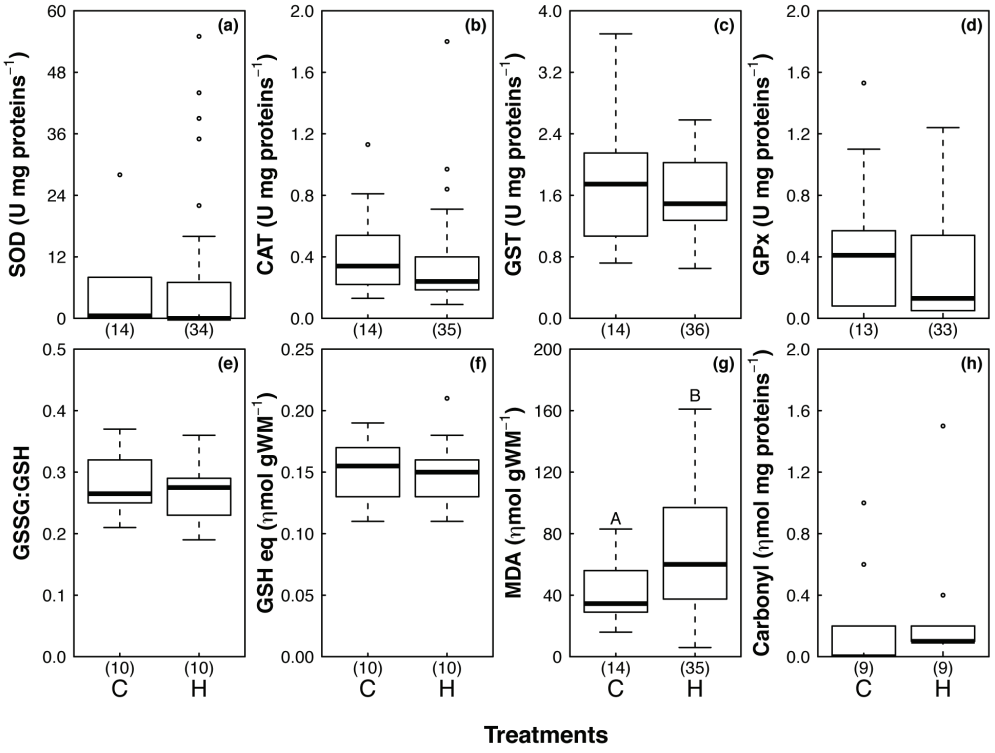

Figure 5.5: Oxidative stress parameters in the Antarctic krill *Euphausia superba* at 4°C during warm season (South Georgia, Jan 2012). C: control (6 h 100% O_2 saturation, normoxic); H: hypoxia (6 h 20% O_2 saturation); (a) SOD, (b) CAT, (c) GST, (d) GPx activities, (e) oxidized/reduced glutathione (GSSG: GSH), (g) glutathione equivalents (GSH-eq), (g) malondialdehyde (MDA), (h) carbonyl concentrations. (n): number of samples analyzed; Horizontal bars in the box plots indicate median. Upper and lower edges of the rectangle show the 1st and 3rd quartiles, respectively. Vertical error bars extend to the lowest and highest value in a 1.5-fold inter-quartile range (R Core Team, 2012).

In the HCS during the cold season, warming exposure (+7°C) reduced the activity of all antioxidant enzymes analysed, but this was only significant for CAT (F=7.80; p=0.013; Fig. 5.6b). All 15°C abdomen samples were used for GSH and GSSG HPLC measurements, which is why we could not compare MDA concentrations with experimental warming in the cold season.

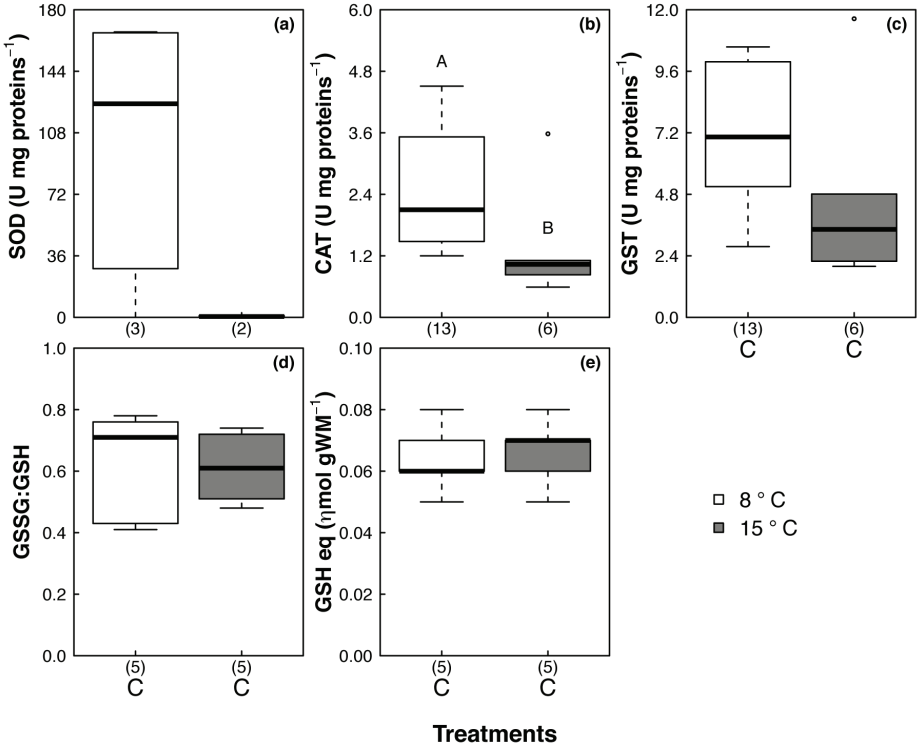

Figure 5.6: Oxidative stress parameters in *Euphausia mucronata* during the cold season. White: 8°C; Dark grey: 15°C; C: control (6 h 100% O_2 saturation). (a) SOD, (b) CAT, (c) GST activities, (d) oxidized/reduced glutathione (GSSG: GSH), (e) glutathione equivalents (GSH-eq). AB: differences between warming treatment; (n): number of samples analyzed; Horizontal bars in the box plots indicate median. Upper and lower edges of the rectangle show the 1st and 3rd quartiles, respectively. Vertical error bars extend to the lowest and highest value in a 1.5-fold inter-quartile range (R Core Team, 2012).

During the warm season, no interaction between hypoxia/reoxygenation treatments and elevated temperature (from 8 to 15°C) on oxidative stress parameters was observed (Fig. 5.7). The only effect recorded was +7°C warming on SOD with higher activity (χ^2=11.20; p=0.001; Fig. 5.7a), and a reduction of the GSSG: GSH ratio (F=7.53; p=0.009; Fig. 5.7d). There was also no significant effect of the hypoxia/reoxygenation treatments when the temperature effect was considered separately from hypoxia. No mortalities were recorded in experiments with this species in either season (Tab. 5.2).

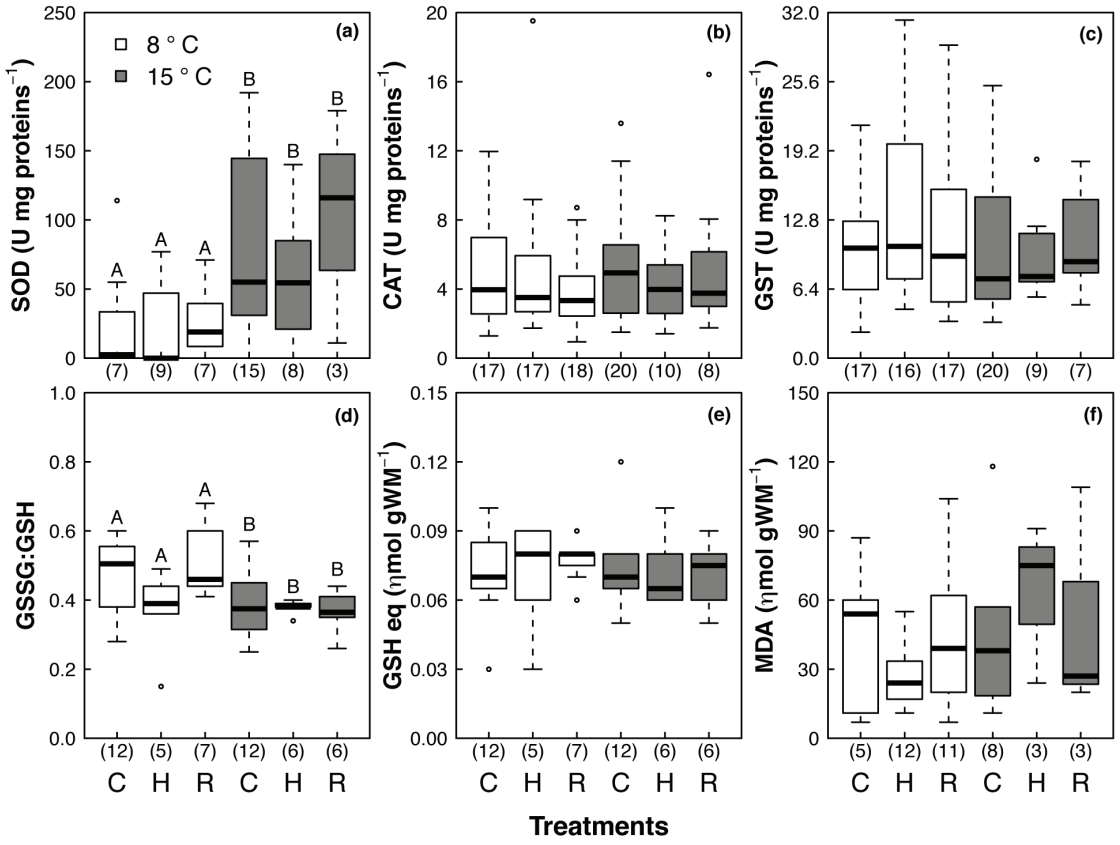

Figure 5.7: Oxidative stress parameters in *Euphausia mucronata* during the warm season. White: 8°C; Dark grey: 15°C; C: control (6 and 7 h 100% O_2 saturation); H: hypoxia (6 h 20% O_2 saturation); R: reoxygenation (1 h 100% O_2 saturation after H); (a) SOD, (b) CAT, (c) GST activities, (d) oxidized/reduced glutathione (GSSG: GSH), (e) glutathione equivalents (GSH-eq), (f) malondialdehyde (MDA). AB: differences between combined oxygen and warming treatments; (n): number of samples analyzed; Horizontal bars in the box plots indicate median. Upper and lower edges of the rectangle show the 1st and 3rd quartiles, respectively. Vertical error bars extend to the lowest and highest value in a 1.5-fold inter-quartile range (R Core Team, 2012).

The NCCS species *E. pacifica* was the only one exposed to warming and hypoxia-reoxygenation in both seasons. Mortality in the 6 h hypoxia experiments increased with higher exposure temperature with 24% of exposed krill died at 10°C, 30% at 12°C, and 95% at 14°C in the cold season (Tab. 5.2). Better survival was recorded in the warm season with 8% mortality at 10°C, 17% at 12°C, and 48% at 14°C (Tab. 5.2). No significant changes in any of the oxidative stress indicators as effect of hypoxia/reoxygenation treatments and/or warming were observed in individuals used in the experiments in the cold season (Fig. 5.8). However, it may be important to emphasize the small but consistent changes occurring during hypoxia treatment at 10°C exposure in four of the six parameters (SOD, CAT, GSH-eq, and MDA), with lower values in hypoxia than in normoxia (control) and reoxygenation treatments (Fig. 5.8a, b, e, f).

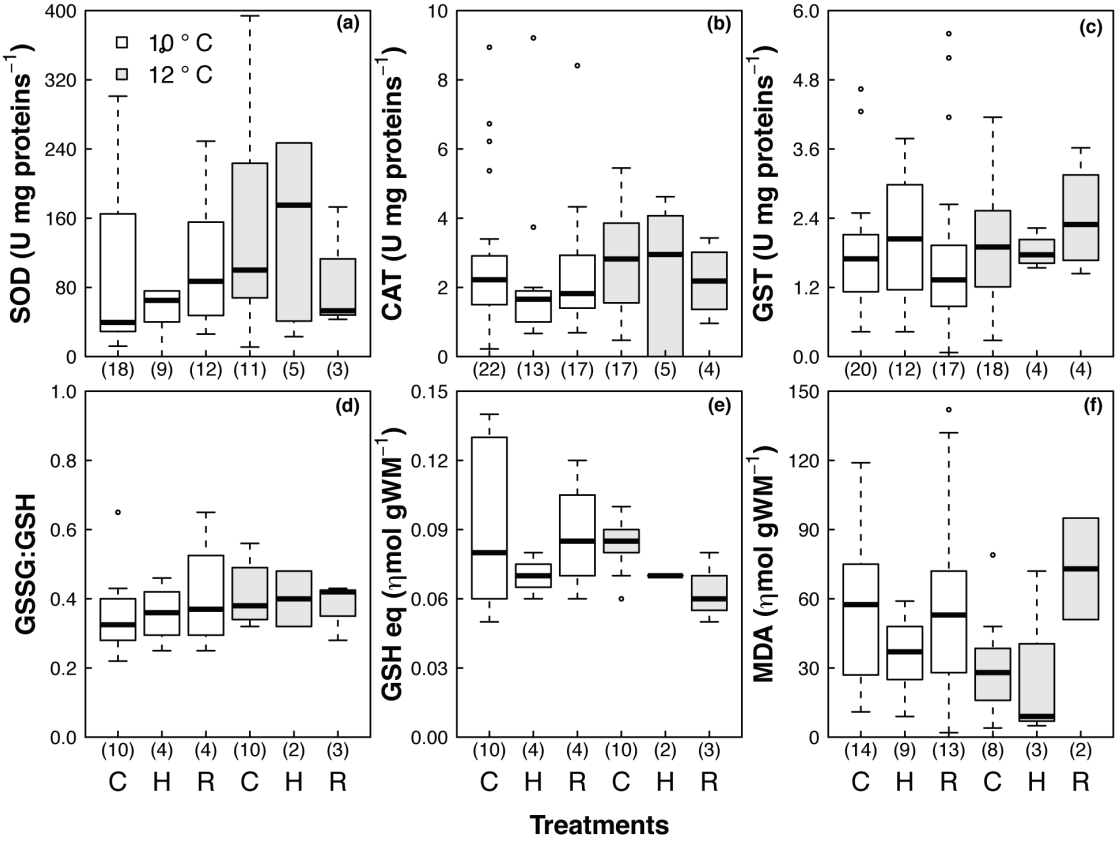

Figure 5.8: Oxidative stress parameters in *E. pacifica* during the cold season (Apr 2012). White: 10°C; Light grey: 12°C; C: control (6 and 7 h 100% O_2 saturation; normoxic); H: hypoxia (6 h 20% O_2 saturation); R: reoxygenation (1 h 100% O_2 saturation after H); (a) SOD, (b) CAT, (c) GST activities, (d) oxidized/reduced glutathione (GSSG: GSH), (e) glutathione equivalents (GSH-eq), (f) malondialdehyde (MDA). (n): number of samples analyzed; Horizontal bars in the box plots indicate median. Upper and lower edges of the rectangle show the 1st and 3rd quartiles, respectively. Vertical error bars extend to the lowest and highest value in a 1.5-fold inter-quartile range (R Core Team, 2012).

Similar to *E. mucronata*, there was no combined effect of oxygen treatments and increased temperature (from 10 to 14°C) on the oxidative stress parameters in *E. pacifica* in the warm season (Fig. 5.9). Again, temperature *per see* seems to have the major influence on almost all analysed oxidative stress parameters (Fig. 5.9). Indeed, at 14°C, SOD was significantly lower in all hypoxia/reoxygenation treatments compared to 12°C (χ^2=8.19; p=0.017; Fig. 5.9a), as CAT activity compared to 10 and 12°C (χ^2=19.08; p<0.001; Fig. 5.9b). Reduced GSH-eq followed the same pattern and was significantly depleted at 12°C compared to 10°C (χ^2=5.75; p=0.017; Fig. 5.9e). Because of the high mortality in these experiments, no samples were available for the GSH and GSSG at 14°C. Oxidative damage of lipids (MDA) was less intensive at 12°C than at colder (10°C) or warmer (14°C) temperature (χ^2=20.46; p<0.001; Fig. 5.9f). At 10°C MDA values were elevated in the reoxygenation group over normoxic (control) and hypoxic samples. Further, two-way ANOVA also revealed increased GST activity under reoxygenation treatment at all tested temperatures (F=5.57; p=0.005; Fig. 5.9c) compared to normoxic (control) and hypoxic incubations.

A comparison between the oxygen treatments performed for each tested temperature allowed a more detailed interpretation of the conspicuous trends observed with the interaction approach. At 10°C, SOD activity (χ^2=6.05; p=0.049; Fig. 5.9a) and GSSG:GSH ratios (χ^2=6.36; p=0.042; Fig. 5.9d) decreased in both hypoxia and reoxygenation treatments, while MDA levels only increased significantly after 1 h reoxygenation compared to the controls (F=4.67; p=0.018; Fig. 5.9c). GST activity increased during reoxygenation at 12°C (F=3.95; p=0.025; Fig. 5.9c) and during hypoxia at 14°C compared to normoxic controls (F=4.43; p=0.020; Fig. 5.9c).

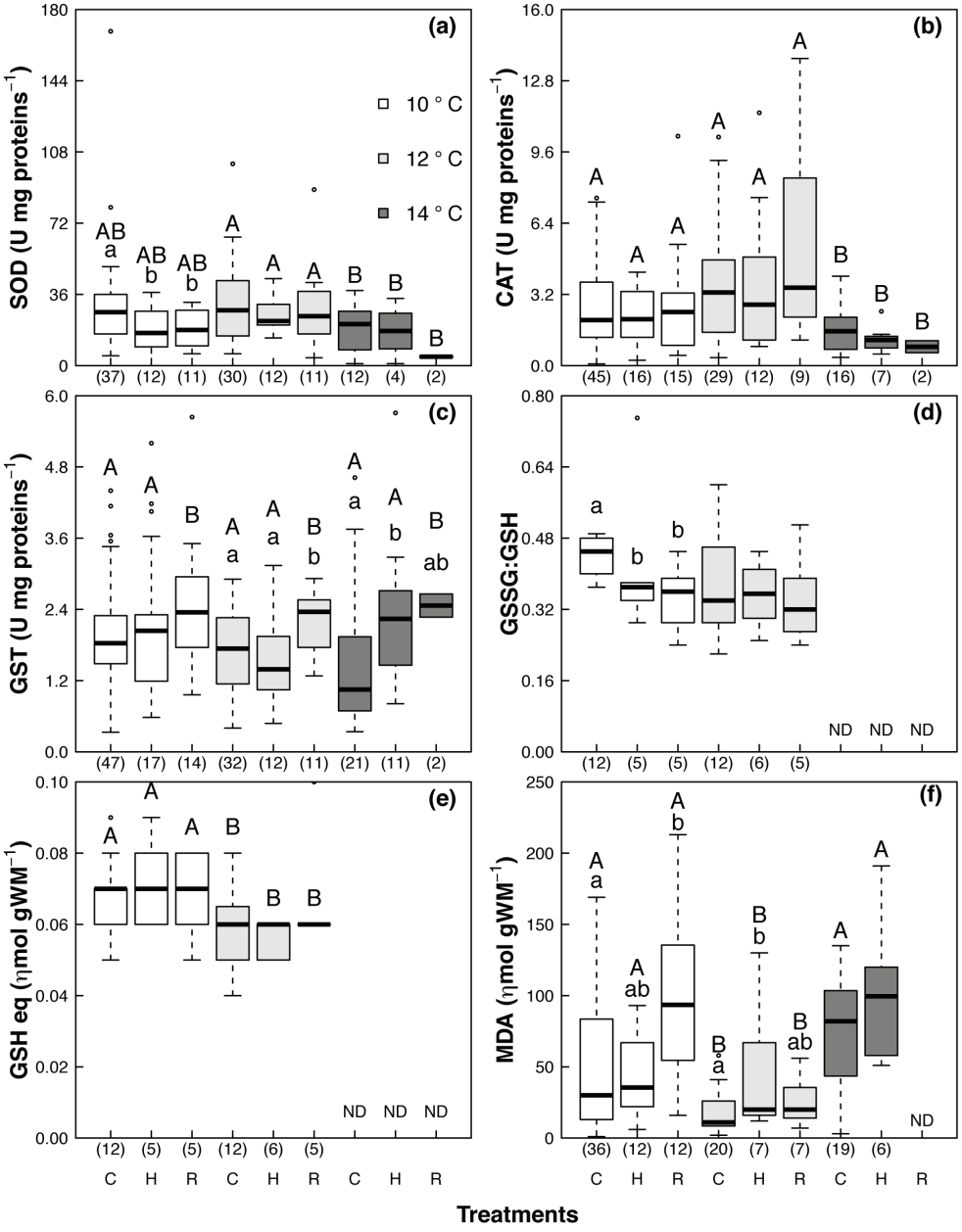

Figure 5.9: Oxidative stress parameters in *E. pacifica* during the cold season (Sep 2011). White: 10°C; Light grey: 12°C; Dark grey: 14°C; C: control (6 and 7 h 100% O_2 saturation; normoxic); H: hypoxia (6 h 20% O_2 saturation); R: reoxygenation (1 h 100% O_2 saturation after H); (a) SOD, (b) CAT, (c) GST activities, (d) oxidized/reduced glutathione (GSSG: GSH), (e) glutathione equivalents (GSH-eq), and (f) malondialdehyde (MDA). AB: differences between combined oxygen and warming treatments; AB: differences between combined oxygen and warming treatments; ab: differences among oxygen treatments for each temperature; (n): number of samples analyzed; ND: no data; Horizontal bars in the box plots indicate median. Upper and lower edges of the rectangle show the 1st and 3rd quartiles, respectively. Vertical error bars extend to the lowest and highest value in a 1.5-fold inter-quartile range (R Core Team, 2012).

5.4 Discussion

5.4.1 Effects of climatic adaptation on metabolism and oxidative stress parameters: polar *vs.* temperate regions

Antarctic marine ectotherms are often characterized as especially sensitive to oxidative stress and damage without a clear causal explanation. Their high sensitivity is associated with less saturated membrane lipids, which are more susceptible to ROS damage. In our comparison, the polar species *E. superba* clearly sticks out among the three investigated species in terms of *in situ* metabolic parameters SMR, CS activity, antioxidant enzyme activities of SOD and CAT, and the cellular oxidative stress and damage parameters (GSSG: GSH ratio, GSH-eq, and carbonyl concentrations). Although strongly hypoxic OMZ conditions are not expected in Antarctic shelf regions in the near future, Antarctic krill at South Georgia is able to regulate SMR down to 55% O_2 saturation (pc) and, moreover, to switch to anaerobic energy production (lactate) below pc, as seen at the end of the respiration measurements. *E. superba* from our study had a pc above the one reported by Torres *et al.* (1994; pc between 30 and 52 mm Hg corresponding to 19-33% O_2 saturation at 0.5°C) for the Scotia Sea. Upward shift of pc is caused by a higher SMR of krill at South Georgia water temperatures, as was observed in other krill species when respiration was measured at maximal habitat temperature (Strömberg and Spicer, 2000; Werner, 2012). Note that moderate hypoxia (50% O_2 saturation) is observed in the Indian sector of the Southern Ocean at depths below 500 m (Dehairs *et al.*, 1990). This may be problematic for the krill and perhaps explains why biomass in this Antarctic sector is 10-fold lower than in the well-oxygenated regions of the Atlantic and West Peninsula sectors (Nicol *et al.*, 2000).

The lower standard metabolic rate (SMR) recorded for the Antarctic compared to the temperate krill species *E. pacifica* can be explained by its larger body mass and the lower habitat and experimental temperatures (Ikeda, 2012). Even when normalizing mean SMR of *E. superba* to 10°C[1], SMR remains significantly lower compared with the north Pacific krill. The slow routine activity of the Antarctic krill goes hand in hand with low hemocyanin O_2 affinity (Bridges *et al.*, 1983) and high citrate synthase (CS) activity, indicating enhanced mitochondrial densities to compensate for the permanently low temperatures in Antarctic waters (Johnston *et al.*, 1998; Guderley, 2004; Morley *et al.*, 2009). Metabolic cold adaptation of mitochondrial densities at South Georgia indicates this trait to be either genetically based, or that the residence time of the krill in warmer sub-Antarctic waters is not sufficiently long to allow for an adaptive change.

SOD and CAT activities align with SMR for the three krill species. In fact, activities of both enzymes were almost not detectable in polar krill, which speaks for lower mitochondrial

[1]with $Q_{10} = 2.68$ calculated from McWhinnie (1964) which gives a SMR_n=58 µmol O_2 h^{-1} g DM^{-1}

ROS generation. High mitochondrial densities are characteristic for many Antarctic marine invertebrates and often associated with higher mitochondrial cristae densities and higher proton leak than related temperate species (Philipp *et al.*, 2005b; comparing marine clams). A higher proton leak, not yet investigated in Antarctic or other krill species, can almost certainly mitigate metabolic ROS formation (Brand, 2000) and could be instrumental in further restraining oxidative stress in *E. superba*. Higher oxidative damages to lipids was observed, but without reaching an extreme level like it was expected for this species that permanently inhabit well-oxygenated waters. The unchanged levels of oxidative stress indicators and negligible mortality rate of *E. superba* after 6 h of hypoxia treatment further supports the relatively unstressed state of the Antarctic krill when facing hypoxia at habitat temperature.

To compensate the low SOD and CAT activities, polar krill has considerable amounts of low molecular antioxidants such as vitamin E and GSH. In fact, vitamin E concentration in *E. superba* was in the range of Antarctic fish (Dunlap *et al.*, 2002), and glutathione concentration was twice as high as in temperate euphausiids. The high GSH concentration, and the very low GSSG: GSH ratio, match the idea of overall low basal oxidative stress in *E. superba*, and indicate that glutathione may be involved in buffering ROS induced by environmental insult as ultra-violet radiation or warming. Low GSSG: GSH ratios (below 0.3) were earlier detected in the Antarctic clam *Laternula elliptica* (Philipp *et al.*, 2005a) compared to temperate species, and in the Antarctic limpet *Nacella concinna* (Weihe *et al.*, 2010). It indeed may be characteristic of permanently cold adapted marine ectotherms to rely on chemical rather than enzymatic mechanisms for ROS quenching. Note that *E. superba* further had the lowest levels of protein damage, supporting our hypothesis of low ROS formation and sufficient protection. The comparison of the three krill species clearly argues against elevated oxidative stress as an inherent attribute of life in cold environments such as Arctic and Antarctic, and instead may support the concept that low metabolic ROS promote long lifespan in many polar species (Philipp and Abele, 2010; Clark *et al.*, 2013).

5.4.2 OMZ adaptation and "normoxic stress" in the hypoxia-adapted species *Euphausia mucronata*

The outstanding GST activity in the hypoxia tolerant *E. mucronata*, which was at least 4-fold higher than GST in *E. pacifica* in both seasons, might explain its success in the OMZ. A high GST activity means extra removal of GSH for detoxification purposes, which also explains the conspicuously higher GSSG: GSH ratio in this species. Such a high GSSG: GSH ratio in tissues (as a rule: GSSG: GSH should be around 0.1) often indicates oxidative stress, as it reflects oxidation of reduced GSH to its oxidized form. However, neither lipid nor protein oxidative

damage indicators were higher in *E. mucronata* than in *E. pacifica*, which argues against enhanced oxidative stress in the hypoxia tolerant *E. mucronata*. The power of glutathione has earlier been noticed in two OMZ species from the Gulf of California (Mexico), *Nematoscelis difficilis* and *Euphausia eximia* (Tremblay *et al.*, 2010). As the thermocline forms in this region and the OMZ extends upwards during the warm summer season, higher superoxide radical ($O_2^{\bullet -}$) production was counteracted by substantial use of GSH by GST and GPx activities, which prevented lipid oxidative damage.

The hypoxia-adapted *E. mucronata* does not regulate the respiration rate and, instead, suppresses metabolism in hypoxic and even in normoxia during the respiration measurements. This pattern is frequently observed in organisms that survive hypoxic or anoxic conditions on a short-time scale (hours or days), such as the squid *Dosidicus gigas* (Gilly *et al.*, 2006) and other hypoxia-adapted euphausiid species such as *Euphausia eximia* (Seibel, 2011). Metabolic suppression has been reported earlier for *E. mucronata* in different areas along the Chilean coast (Teal and Carey, 1967, 20°C; Antezana, 2002a, 12°C; Donoso and Escribano, 2014, 12°C). In a state of metabolic suppression the reduced energy demand is partly covered by less energy efficient anaerobic glycolysis, as seen in the relatively high lactate accumulation rate in *E. mucronata* compared to *E. pacifica* at the end of experiment.

A seasonal difference in SOD activity with lower values in summer and higher in winter was observed in *E. mucronata*. Whether or not this reflects higher winter SMR (as in *E. pacifica*) remains unclear, as summer respiration rates were not measured. However, higher GSSG: GSH ratio and lipid oxidation levels, together with lower CAT activity and GSH-eq in the cold season, corroborate a state of comparatively higher oxidative stress during winter. Hypoxia is a permanent condition in the Chilean stretch of the HCS and, as we stated before, the temperature and food conditions did not vary much between seasons in this upwelling ecosystem. As *E. mucronata* is physiologically and morphologically adapted to extremely hypoxic OMZ conditions, oxygenation conditions above the hypoxic range down to 60 m water depth in the cold season may already represent a scenario of "stress due to over-oxygenation". Examples of fish and scallops in which environmental hyper-oxygenation induces oxidative stress were recently compiled by Lushchak (2011) and observed in the infaunal polychaete *Heteromastus filiformis* (Abele *et al.*, 1998), the freshwater clam *Sphaerium sp.* (Joyner-Matos *et al.*, 2007), and in marine sedimentary meiofauna (Rivera-Ingraham *et al.*, 2013a).

Hypoxia-reoxygenation treatments alone were not *per se* stressful to *E. mucronata*. However the cross effect of warming was crucial, and even damaging in the cold season (Aug 2011), much more than in the warm season (Feb 2012). Warming of the habitat especially during winter would reduce antioxidant defences and support oxidative stress and damage in *E. mucronata*, compromising survival. Paradoxically, the same temperature increment applied in summer

conditions (Feb 2012) enhanced SOD activity, especially in control and reoxygenation treatments. This combined with a more reduced GSSG: GSH ratio, indicates better control of oxidative stress in summer, possibly preventing additional lipid peroxidation. Indeed, no mortality was recorded in the oxygen treatments with or without warming, although +7°C represents extreme warming stress not currently predicted for this part of the HCS region. Thus, in spite of being extremely well adapted to life in the OMZ, *E. mucronata* can suffer oxidative stress when moving upwards to normoxic environmental conditions in the cold season. This also explains in part their reluctance to surface water layers and instead to remain longer in their hypoxic OMZ niche than more oxygen tolerant species.

5.4.3 Hypoxia-reoxygenation stress is accentuated by warming in the north Pacific species *Euphausia pacifica*

A warming and deoxygenated scenario would be challenging for the NCCS species *E. pacifica*. As in Antarctic krill, *E. pacifica* is mostly an oxyregulator and switch to oxyconformity at 27% and 34% O_2 saturation, during the warm and the cold season, respectively. In both seasons, the *p*c we measured was higher than the *p*c reported in the southern part of the California current system for the same temperature (Childress, 1975; *p*c of 18mm Hg which corresponds approximately to 11% O_2 saturation at 10°C). Nevertheless, our findings are similar to values obtained by Ikeda (1977) at Saanich Inlet (Canada), a fjord located north of the NCCS, where deep water presents anoxic conditions during most of the year (Herlinveaux, 1962).

Higher SMR in April 2012 reflects slower growth of krill in spring seasons with weak upwelling (e.g. Shaw *et al.*, 2010), because the adult individuals from the spring cohort in our catches were comparatively small. As mitochondrial capacities (CS activities) remained unchanged between Sep 2011 (warm) and Apr 2012 (cold), higher spring SMR effectively means more oxygen reduction and faster electron transport in each mitochondrion. This was kept in balance by enhanced SOD activity in April 2012, whereas none of the other oxidative stress parameters and damage indicators changed, matching the view that a non-stressful increase in metabolic rate rarely causes oxidative stress. However, absence of significant lactate concentration in hypoxia, and the higher *p*c recorded during the cold season, point to a lower capacity to deal with hypoxia in cold adapted winter animals (or better hypoxia tolerance in late summer-collected animals), and thus seasonal adaptation to the shallower OMZ conditions in summer.

The same seasonal pattern of adjustments was observed in the hypoxia reoxygenation plus warming experiments, with no visible effect on either oxidative stress or metabolic indicators in the cold season. At first sight, this seems encouraging, but note that biochemical analyses

were only performed on survivors. At control temperature (10°C), the reduction of SOD and CAT activities during hypoxia was balanced by GST activity, which depleted the GSH-eq, and reduced MDA concentrations compared to control and reoxygenation treatments. Things got worse with warming, as no congruent patterns were observed at +2°C, and as practically no krill survived at +4°C. Yet, due to the small number of organisms surviving warming treatments our capacity to interpret what happened at the cellular level is quite limited.

In the warm season, when mild OMZ conditions occur already at 100 m depth, low oxygen and warming conditions need to be handled by *E. pacifica*. At *in situ* temperature of 10°C the species deal well with hypoxia. Still, reoxygenation seems to be a challenge since MDA levels increased and SOD activity had not recovered after 1 h of reoxygenation. The oxidative damage could be the result of the increased metabolic rates during reoxygenation, as the organisms make up for the hypoxic oxygen deficit (Welker *et al.*, 2013). de Oliveira *et al.* (2005) observed a decrease in SOD activity during anoxia in the gills of the crab *Chasmagnathus granulata* from Rio Grande do Sul (Brazil). Further, as in the krill, GST activities in *C. granulata* also increased at all times of reoxygenation, indicating strong detoxification requirements.

The antioxidant system of *E. pacifica* bore the weight of the oxidative stress arising in the +2°C exposure to control lipid peroxidation. Depletion of GSH-eq at 12°C backed-up the activation of non-enzymatic antioxidants relative to the 10°C experiments. Another 2°C of warming (14°C) was already lethal for almost half of the specimens and was accompanied by a reduction in antioxidant enzyme activities. Clearly, *E. pacifica* has a narrow thermal windows (see Pörtner, 2010), and warming of +4°C brings the species to its upper lethal temperature (14°C) where stress is exacerbated by hypoxia-reoxygenation exposure (which might occurred when undertaking their DVM).

5.5 Conclusion

The consequences of OMZ expansion in a warming world

The order Euphausiacea encompasses many different ecotypes, and krill tolerance to warming and OMZ expansion is species specific with metabolic and antioxidant strategies shaped strongly by species evolutionary background. This is bound to cause habitat shifts of krill species, mass stranding and mortality of sensitive species as climate change progresses. In spite of their pelagic swarm swimming and thus energy consuming lifestyle, all krill species compared could tolerate hypoxia to some extent, even the Antarctic species *E. superba*. This underlines the general tolerance of krill to survive at least moderate hypoxia. As seen before for *Meganyctiphanes norvegica* (Spicer *et al.*, 1999), lactate accumulation is not a true benefit for an active swimmer as it exhausts

energy reserves and causes tissue acidification which impinges on swimming and, therewith, the capacities to escape predation. Anaerobic capacities are not always showing up as seen for *E. pacifica*, in which seasonal cold adaptation increased its sensitivity to hypoxia treatment in winter. Both, the latitudinal and seasonal comparison indicates that enzymatic antioxidants are not the prime mechanisms of ROS protection and at least need to be complemented by high GSH levels in the cold. Major complications arise also in *E. pacifica* when warming and OMZ stress during DVM come together, as they more strongly rely on the antioxidant enzymes which lose activity during warming. The combined stress conditions cause mortality, and oxidative damage in the survivors indicates that unbalanced oxidative stress plays a role at cellular level. Oxidative stress probably occurs when more ROS are produced than the antioxidant system can handle, to activate signalling molecules for the need of protective responses (e.g. stress gene transcription, membrane pore opening and metabolic down regulation). At mid-stressing conditions (12°C), this worked out, but higher temperature stress broke down this fragile balance.

In the hypoxia adapted krill from the HCS, oxidative stress is compromising the wellbeing of the species when it eventually has to move up to the surface in the cold season, entering a well-mixed upper water layer with >70% (15 kPa) oxygenation. Here oxidative stress manifested in depletion and oxidation of the redox buffer glutathione and oxidation of membrane lipids, while catalase activity was suppressed. Under warm-hypoxic conditions, the enzymatic antioxidant system of the Chilean species was more versatile and inducible. To this end, evolutionary adaptation to hypoxia, which has shaped species morphology in *E. mucronata* (greater gill surface area Antezana, 2002a), can act as a double-edged sword under full oxygenation. Prospectively, although present habitat temperature ranges are very similar for both, *E. mucronata* will deal much better with OMZ expansion and warming than its northern relative. Better understanding of the physiological mechanisms underlying the response of krill to climate driven changes of the seasonal OMZ and thermocline formation, by the analysis of biomarkers for sublethal effects, may help us to explain why and predict when years of poor survival may occur. This will be of major importance in future risk assessment and life stocks management for these ecosystems.

5.6 Acknowledgments

This main part of the thesis would not have been possible without the support of colleagues from several institutions. We thank the crew of the R/V Kay-Kay (Chile), R/V Elahka (USA), and R/V James Clark Ross (UK) as well as the graduate students, technicians and researchers at the Pelagic and Mesozooplankton Laboratory from the Centro de Investigación Oceanográfica del Pacífico Sur-Oriental de la Universidad de Concepción (Chile), the Hatfield Marine Science Center of Oregon State University (USA), and the British Antarctic Survey (UK) for recording

environmental information, and support in collecting zooplankton samples. In particular, Rubén Escribano, Pamela Hidalgo, Ramiro Riquelme-Bugueño, Jocelyn Silva Aburto (Chile), Tracy Shaw, William T. Peterson, Jay Peterson (USA), Sophie Fielding, and Geraint A. Tarling (UK). We thank Karim Zanaty, Imke Lüdeke, and especially Stefanie Meyer for their excellence and technical help in the laboratory of AWI. We also thank Kai-Uwe Ludwichowski from the ecological chemistry group of AWI for his advices in the HPLC analysis.

Chapter 6

Warming response comparison of two euphausiid species from the northern California Current System

Additional results of the thesis

6.1 Introduction

Off Oregon (USA) two euphausiids species dominate the macrozooplankton community, the oceanic *Euphausia pacifica* (Brinton, 1962) and the neritic cold upwelling-associated *Thysanoessa spinifera* (Brinton, 1962; Smith and Adams, 1988; Lavaniegos and Ambriz-Arreola, 2012). Because of its neritic lifestyle, *T. spinifera* does not migrate as deep as *E. pacifica*, but, instead, remain within the upper 100 m during day and night (Brinton, 1962) and swarm in summer at surface for reproduction (Smith and Adams, 1988). *T. spinifera* is also known for its narrow plasticity when facing changes in the physical oceanographic conditions (Brinton, 1979). Indeed, *T. spinifera* is strongly influenced by the recently described North Pacific Gyre Oscillation (Di Lorenzo *et al.*, 2008; Sydeman *et al.*, 2013). This oscillation is connected with the winds and upwelling responses (Chenillat *et al.*, 2012) and corroborates the upwelling preference of this species. So far, no acoustic or direct observations of hypoxia and warming effects on *T. spinifera* have been reported. However, massive stranding events in several bays on the US West Coast over an area of approx. 400 km between Oregon and California were observed in summer of 2013 and related to the strongly hypoxic conditions prevailing regionally (Tyburczy *et al.*, 2013). This hypoxic zone was extending into the upper 50-100 m of water column.

In the frame of my thesis a warming experiment was carried out with *T. spinifera* from the Oregon neritic NCCS region, which is not included into the paper on stress response of different Pacific and Antarctic krill species to warming and OMZ conditions (Tremblay and Abele, in revision; Chapter 5). As *T. spinifera* was caught at low abundance, it allowed for limited measurements during the warm season (September 2011), which prevented me from including it in the paper and instead present some findings here. The comparison includes the oceanic NCCS species *E. pacifica*.

6.2 Materials and Methods

Sampling was achieved aboard the research vessel Elakha on September 14th 2011 off Newport (Oregon, USA). Krill were collected during night with a bongo net (0.6 m diameter, 333 µm black mesh with non-filtering cod end) at approximately 40 m depth. After heaving the sampling gear on deck, the cod end content was immediately transferred to 20 L buckets with seawater. Live adult krill showing a lot of movements were manually sorted into cooler boxes and transferred to a 10°C cold room of Hatfield Marine Sciences Center (Newport, Oregon). The animals were allowed to recover for at least 6 h before respiration measurements and warming experiments were started.

For the respiratory and lactate measurements we proceeded the same way as for our biogeographical comparison (Tremblay and Abele, in revision; Chapter 5). The warming treatments were conducted with both krill species jointly exposed in the same aquaria. *T. spinifera* was distinguished by the presence of spines on each segment of the abdomen. Krill were divided into two replicates of 10 animals per temperature (10 and 14°C). Higher temperature exposure was conducted by placing the aquaria in two thermostated tanks in which the water was warmed to 14°C with an aquarium heater (EHEIM, Germany). One replicate was incubated per box covered with a lid to keep the temperature constant (2 replicates at 14°C) against the room temperature of 10°C. The two other replicates were held at cold room temperature. After 6 h of treatment, surviving krill were sampled and immediately snap frozen at -80°C. Frozen samples were shipped on dry ice to the Alfred Wegener Institute for biochemical analysis. The activities of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferase (GST) were analysed in the cephalothorax part of the organisms. The detection of malondialdehyde (MDA) formation (lipid peroxidation) was conducted using the abdominal part of the euphausiids.

All statistics and figures were done with R (R Core Team, 2012). For treatment comparison, normality (Shapiro test) and variance homogeneity (Bartlett test) tests were performed for each species. Data were transformed ($log(x)$, x^{-1}, $x^{1/2}$) if the criteria of normal distribution and

homogeneity of variance were not met. If no transformation of data allowed the use of analysis of variance (ANOVA), the non-parametric Kruskal-Wallis test was conducted. Significant level of all comparisons was fixed at 95% (p=0.05).

6.3 Results

A different pattern within the respiratory response to declining pO_2 was observed between *T. spinifera* and *E. pacifica* (Fig. 6.1, 6.2a) at 10°C. Whereas *E. pacifica* maintains constant respiration rates over a wide range of pO_2 and even increases the oxygen uptake between 40 and 27% saturation in a compensatory attempt (potentially faster swimming to exit the hypoxic area), *T. spinifera* showed diminishing respiration rates below approx. 80% saturation (Fig. 6.1). The standard metabolic rate of *T. spinifera* was also significantly lower than of *E. pacifica* (SMR; F=58.83; p<0.001; Fig. 6.2a) over the whole range of pO_2. Lactate accumulation at the end of the respiration measurement in *T. spinifera* was significantly higher compared to *E. pacifica* (χ^2=8.014; p=0.005).

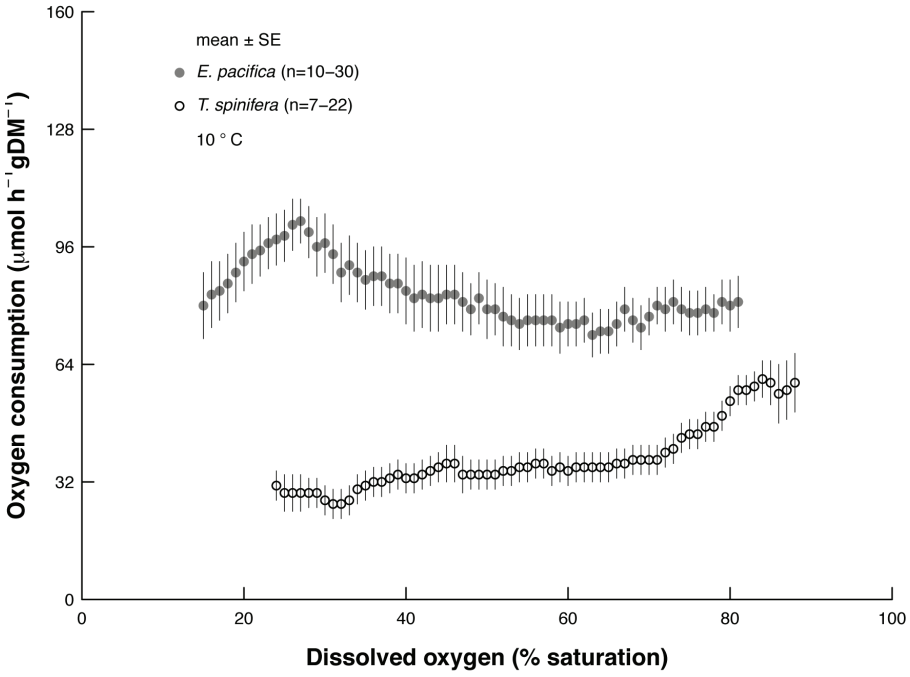

Figure 6.1: Oxygen consumption (µmol O_2 h^{-1} g DM^{-1}) associated to chamber dissolved oxygen concentration (% saturation) of *Euphausia pacifica* (gray) and *Thysanoessa spinifera* (white) from the northern California current system; mean ±SE.

No significant differences in oxidative stress parameters were detected between both species or between warming (14°C) and control temperature groups (10°C) of the same species. The GST activity was lower in *T. spinifera* compared to *E. pacifica* at control temperature, but the

low number of analyzed *T. spinifera* samples did not support a significant result. No mortality occurred during 6h of warming in either species.

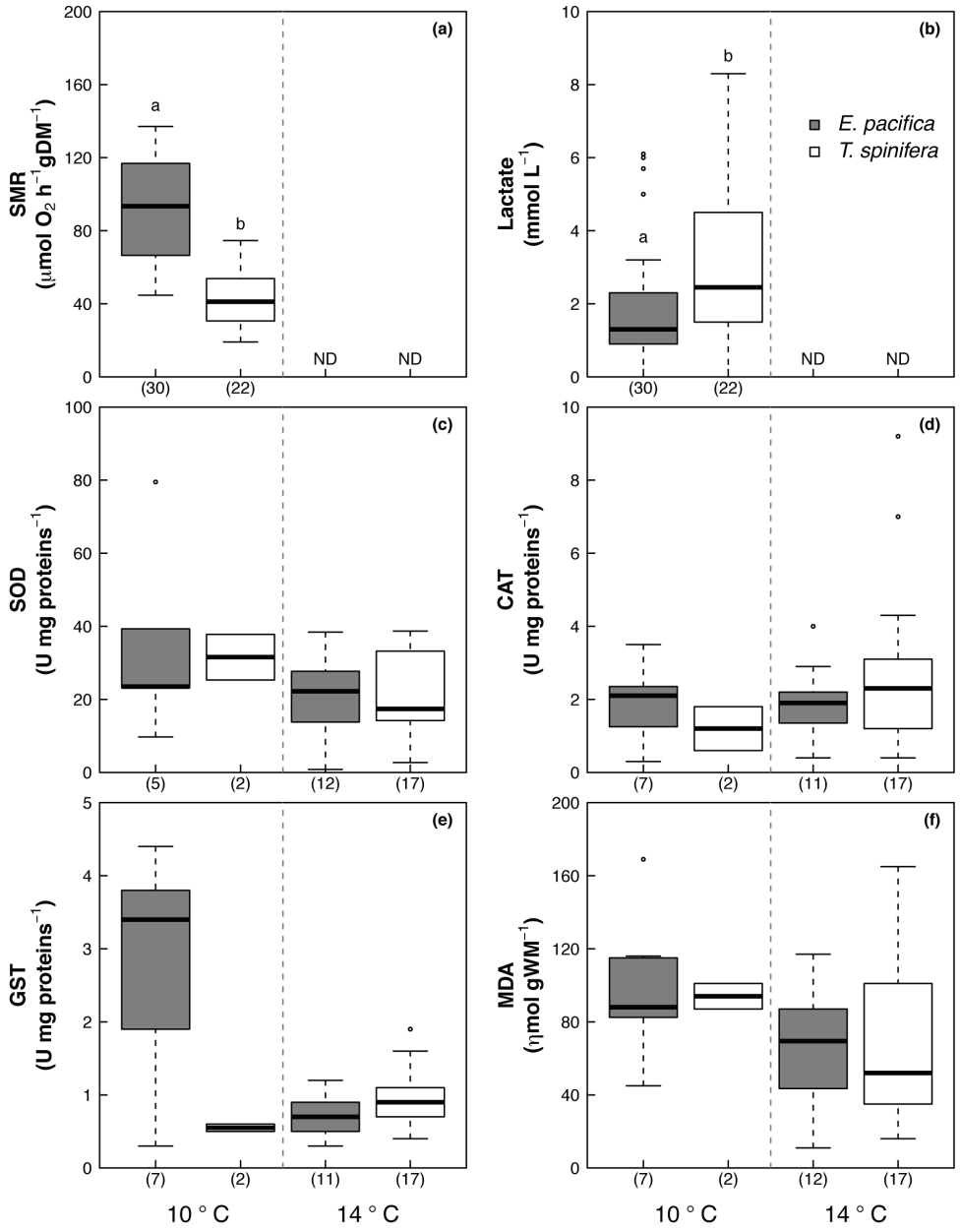

Figure 6.2: Standard metabolic rates and oxidative stress parameters in *Euphausia pacifica* (gray) and *Thysanoessa spinifera* (white) at 10 and 14°C. (a) Standard metabolic rate (SMR), (b) lactate concentration at the end of respiration measurement, (c) SOD, (d) CAT, (e) GST activities, (f) malondialdehyde (MDA). ab: inter-specific differences; Dash lines separate the temperatures; ND: no data; Horizontal bars in the box plots indicate median. Upper and lower edges of the rectangle show the 1st and 3rd quartiles, respectively. Vertical error bars extend to the lowest and highest value in a 1.5-fold inter-quartile range (R Core Team, 2012).

6.4 Discussion

The neritic lifestyle, short vertical migration distance, and strong association with upwelling areas (high nutrients, cold temperature and lower oxygen concentration) match the comparatively low metabolic rate of *T. spinifera* compared to oceanic *E. pacifica*. Oxyconforming respiration, typical for low oxygen adapted fauna, sets on as pO_2 starts decreasing in the environment (between 90 and 80% saturation). Low and oxyconforming respiration are compensated by a higher anaerobic capacity indicated by the comparatively high lactate level at the end of the respiration measurements. These results indicate that although neritic *T. spinifera* is of considerable hypoxia tolerance, the 2013 event has exceeded their resistance either by exhausting their anaerobic survival capacities or by driving them into too shallow water layers that facilitated the stranding event as suggested by Tyburczy *et al.* (2013).

Four centigrades of warming did not have a significant impact on oxidative stress parameters in *T. spinifera*. Due to the feeding conditions in shelf areas, the species has a higher tissue lipid content than *E. pacifica* (Taatjes and Cass, 2014), which could render more susceptible to lipid oxidative damage than *E. pacifica*. Our experimental results indicate similar MDA levels at both temperatures possibly caused by better protection of low molecular antioxidants such as vitamin E and glutathione that we did not measure.

6.5 Conclusion

The neritic krill species *T. spinifera* is adapted to cold and low oxygen conditions in the upwelling areas on the US West coast. Characteristic of low oxygen fauna are the comparatively low metabolic rate and the oxyconforming response to declining pO_2. The degree to which this species can tolerate hypoxia, both regarding the low pO_2 tolerance limit and the time of exposure, cannot be obtained from our experiments. The unprecedented stranding event observed in June 2013 (Tyburczy *et al.*, 2013) indicates that limits to the unwavering endurance of low oxygen conditions is challenged when the hypoxic conditions persists too long and/or extend into shallow waters, compressing *T. spinifera* vertical distribution in the upper layers.

Chapter 7

Gene expression and physiological changes of the Antarctic krill *Euphausia superba* under different hypoxia intensities

Nelly Tremblay[1], Kévin Cascella[2], Jean-Yves Toullec[2], Christoph Held[1], Sophie Fielding[3], Geraint A. Tarling[3], and Doris Abele[1]

[1]*Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Functional Ecology, Am Handelshafen 12, 27570 Bremerhaven, Germany*

[2]*UPMC University of Paris 06, UMR 7144 CNRS, Adaptation et Diversité en Milieu Marin, Station Biologique de Roscoff, Roscoff, France*

[3]*British Antarctic Survey, Natural Environment Research Council, High Cross, Cambridge, CB3 0ET, United Kingdom*

Abstract

The Antarctic krill *Euphausia superba* stock from South Georgia is one of the most northern and abundant krill populations, and will likely experience first the main effects of climate changes predicted in the West Antarctic Peninsula. We investigated gene expression related to aerobic metabolism, antioxidant defence, and heat-shock response under severe (2.5% O_2 saturation or 0.6 kPa) and threshold (20% O_2 saturation or 4 kPa) hypoxia exposure, to detect aspect of molecular stress response. Gene expression analyses were complemented with biochemical metabolic and oxidative stress indicators. Expression levels of the genes citrate synthase (CS), mitochondrial manganese superoxide dismutase (SODMn-m) and one heat-shock protein isoform

(E) were higher in euphausiids incubated 6 h at 20% O_2 saturation than in animal exposed to normoxic conditions. The transcription thought to prepare the krill for eventual reoxygenation, which connects with the swarming behaviour of this species. All biochemical antioxidant defence parameters remained unchanged among treatment and oxidative stress manifested in higher lipid peroxidation after 6 h of severe hypoxia. Physiologically, *E. superba* shows a potential adaptation and survival capacity to adverse stress conditions. The synergy of hypoxia effect with warming was however beyond the scope of this research and might negatively affects the swarms size and density, which would be detrimental for the higher trophic levels.

7.1 Introduction

Vicariant separation of euphausiid polar species and their sub-polar congeners happened approx. 20 million yrs ago (Patarnello *et al.*, 1996) following the formation of the Antarctic Polar Frontal Zone (APFZ; Kennett, 1982). Once gene flow was sufficiently restricted by the APFZ, speciation of cold adapted organisms could proceed in the Antarctic. Cold adapted species evolved to sustain basal metabolic activity against the slowing effect of constant low environmental temperatures. To this end, many actively swimming polar ectotherms display higher mitochondrial volume density, higher mitochondrial inner membrane surface area, and higher activity of respiratory chain enzymes (Guderley and St-Pierre, 2002; Guderley, 2004; Dymowska *et al.*, 2012) than temperate species if compared at the same temperature. A good indicator of mitochondrial adaptation in the Antarctic krill *Euphausia superba* is citrate synthase (CS), the Krebs cycle enzyme that uses acetyl-Coenzyme A to carboxylate oxaloacetate. The amount of CS protein in *E. superba* ranges 5-fold higher than in the North Atlantic cold-temperate and subarctic species *Meganyctiphanes norvegica* (Müller, 2003; : using Enzyme Linked Immunosorbent Assay or ELISA), illustrating the difference in population connectivity (gene flow) between both polar regions. Further, we also found 3-fold higher CS activity levels in South Georgia *E. superba* compared to temperate *Euphausia mucronata* from Southern Pacific (off Chile) and *Euphausia pacifica* from the North Pacific (off Oregon; Tremblay and Abele, in revision).

South Georgia (54°17'S; 36°30'W) is located at the Northern boundary of the APFZ, a region often considered as the physiological thermal limit for the distribution of Antarctic ectotherms (Morley *et al.*, 2010; Hogg *et al.*, 2011), including the stenothermal *E. superba* (Atkinson *et al.*, 2008). Sea surface temperature (SST) is around 4°C in summer, which is 2 to 4°C higher than other krill distribution areas of the West Antarctic Peninsula region (WAP; Barnes *et al.*, 2006). Higher water temperatures at South Georgia shift the respiration rate and critical pO_2 (pc, below which respiration becomes oxygen dependent and diffusion limited, *i.e.* oxyconforming) from 24 ±5 µmol O_2 h^{-1} g DM^{-1} and pc of 33% in the colder Scotia Sea (SST=0.5°C; Torres *et al.*, 1994) to

32 ±8 µmol O_2 h^{-1} g DM^{-1} and *p*c of 55% at South Georgia (Tremblay and Abele, in revision). Thus, temperature of 4°C at South Georgia poses limitations to *E. superba* aerobic metabolism and energy turnover, which matches the lower growth rates recorded in this area (Atkinson *et al.*, 2006; Tarling *et al.*, 2006).

In spite of the higher water temperature, the South Georgia krill stock is one of the densest around the Antarctic, mainly because young stages from different WAP populations are accumulating here. A constant primary production during all austral summer (Atkinson *et al.*, 2001; Whitehouse *et al.*, 2008) with a strong phytoplankton bloom around December (Borrione and Schlitzer, 2013) support the young krill, which, in turn, provide energy into a highly productive trophic network in the Northern Antarctic Circumpolar Current (Hogg *et al.*, 2011). Years of low sea ice in the WAP region negatively influence the recruitment of the source stocks and result in poor exportation of krill to South Georgia (Fach *et al.*, 2006). In addition to its position at the boarder of thermal tolerance, the stock of South Georgia still affords important krill fishery, totalizing more than 2.5 million tons between 1995 and 2010 (Subarea 48.3; Grant *et al.*, 2013). Although quotas issued by the Convention on the Conservation of Antarctic Marine Living Resources (CCAMLR) represents about 1% of the total estimated krill biomass, a series of years with adverse environmental conditions could seriously alter these conservative prognostics. The vulnerability of the South Georgia stock creates enhanced needs for ecophysiological studies of krill responses to global warming (see Flores *et al.*, 2012 for a recent compilation) and prompted the present research.

Studies of cellular and metabolic performance markers are useful to compare environmental stress levels in different populations of species with wide geographical distribution, or to understand the effects of environmental change by repeated measurements in a local population at the same geographical position. Initial transcriptome data are available for two polar krill species, which present promising new tools in stress research (Clark *et al.*, 2011; Toullec *et al.*, 2013) like the expression of classical "stress proteins" such as heat shock proteins of 70 and 90 kD chaperones protein folding under physiological stress conditions (heat, cold, hypoxia, hyperoxia, and pollution; Clark *et al.*, 2011). Further, several of the expressed transcripts code for proteins involved in the oxidative stress defence to prevent cellular damage produced during induction of reactive oxygen species (ROS) and oxidative burst reactions (Halliwell and Gutteridge, 2007).

In the present study we used the available genomic information on the Antarctic krill *E. superba* and *Euphausia crystallorophias* to investigate stress gene transcription in South Georgia specimens experimentally exposed to threshold (20% O_2 saturation) and severe hypoxia (2.5% O_2 saturation). The experiments were carried out as part of a broader study comparing the physiological stress response of different Pacific krill species and *E. superba* to warming and severing oxygen minimum zone conditions (Tremblay and Abele, in revision). Transcriptomic

analysis of heat shock protein and antioxidant enzyme expression compared to measurable catalytic activity was used to obtain a more complete picture of the stress response and to understand which genes are ultimately up-regulated in support of damage prevention and ROS detoxification during hypoxia. The approach was complemented with measurements of protein, lipid and glutathione oxidation levels under the applied conditions.

7.2 Materials and Methods

7.2.1 Krill collection and hypoxia exposure

Sampling and experiments were achieved aboard the research vessel James Clark Ross between January 1st and 10th 2012 northwest of South Georgia. Krill swarms were detected acoustically with a SIMRAD EK60 echo sounder connected to hull-mounted split-beam 38, 120 and 200 kHz transducers. When a dense sound-scattering layer was recorded, krill were collected during nighttime with a remotely operated opening/closing Rectangular Midwater Trawl (RMT8; 8 m^2 mouth area). After heaving the sampling gear on deck, cod end content was immediately transferred to 20 L buckets with seawater. Live adult krill showing a lot of movement were manually sorted in 100 L tanks filled with filtered seawater in a 4°C cold room. The animals were left to recover for at least 6 h before hypoxia experiments started.

Krill were divided into three groups, each with two replicates (aquaria): control (normoxia; 100% O_2 saturation), severe hypoxia (2.5% O_2 saturation or 0.6 kPa), and threshold hypoxia (20% O_2 saturation or 4 kPa). As *E. superba* was able to survive down to a pO_2 of 13% O_2 saturation in South Georgia (Tremblay and Abele, in revision), these two levels of hypoxia were especially selected to measure hypoxia effects above and below this limit. Krill were first allowed 1 h to acclimatize without any air or gas injection in their aquaria. We used pumps and dispersion stones to aerate the control group, and certified O_2/N_2 mixtures of 4% O_2 (20% O_2 saturation in seawater) and 0.5% O_2 (2.5% O_2 saturation) in both hypoxic treatments. Out of security reasons, the experiments had to be conducted outside on deck of the ship. The aquaria were placed in boxes with an entry and an exit to allow a seawater flow from the research vessel water supply system and keep a constant and cold temperature similar to SST at South Georgia in December (between 3 and 3.5°C). The number of krill per replicate varied according to the catch and the size of the krill (from 6 to 20 per aquarium). The same experiment was conducted four times, amounting to a total of eight replicates for each group. Both hypoxia exposures were intended to last for 6 h, but krill from the severe hypoxia treatment (2.5% O_2 saturation) did not show movements of the pleopods after 30 min of exposure. Therefore, they were retrieved from the experiment one hour after the beginning with half of the control group. From the total amount

sampled, half of the individuals were snap frozen at -80°C for biochemical analysis, while the abdominal muscle from the other half was dissected and preserved in RNAlater*later*® at 4°C for 12 h, and then transferred to -80°C. These RNA*later*® preserved samples were specifically for gene expression analysis. The threshold hypoxia treatment (20% O_2 saturation) last for 6 h, and the preservation of samples was completed the same way.

7.2.2 Reverse transcription quantitative polymerase chain reaction (qPCR)

Primers were designed from the transcriptomes of two krill species *E. superba* (Clark *et al.*, 2011) and *E. crystallorophias* (Toullec *et al.*, 2013) by using CLC Main Workbench (Version 7.0, USA) and the PerlPrimer software (Marshall, 2004) to double checked for their quality (Tab. 7.1). Primers were synthesized by Sigma-Aldrich (Germany). The 18S ribosomal RNA (18S), the elongation factor 1-alpha (EF1*a*), the glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and the ribosomal protein L8 (RPL8) were selected as reference gene candidates. The genes of interest (target), which were successful in reverse transcription qPCR analysis, were the citrate synthase (CS), the antioxidant defence enzyme manganese superoxide dismutase isoform in both mitochondria and cytosol (SODMn-m and SODMn-c), and four of the five isoforms of Hsp70 (Hsp70-A, -C, -D and -E). The expression for the Hsp70-B is not reported due to non-specificity of the primers. The isoforms A and E presented a carboxy-terminal tetrapeptide repeat, glycine-glycine-methionine-proline (GGMP), which is a pattern found in the subfamily of constitutive Hsp70 (Cascella *et al.*, in prep.). The form C has high similarity with inducible Hsp70 identified in other decapods, and the form D was defined as mitochondrial (Cascella *et al.*, in prep.).

Total ribonucleic acid (RNA) from the abdominal samples were extracted using the QIAGEN RNeasy® Kit, and 1 µg of total RNA was reverse transcripted into single-stranded complementary DNA (cDNA) using oligo dT and RT-MMLV reverse transcriptase kit (Promega, USA), according to the manufacturer's instructions. Reverse transcription qPCR was performed in a Rotor-Gene Q (QIAGEN, Gemany) using Eva Green Type-it HRM PCR kit (QIAGEN, Germany). Sequencing of reverse transcription qPCR products was conducted to confirm the targeted amplification. Expression levels were normalized using EF1*a*, which was selected as the best applicable (most constitutive) reference gene out of the four candidates identified using the Normfinder (Andersen *et al.*, 2004) and gNorm (Vandesompele *et al.*, 2002) algorithms. Mean normalized expression (Muller *et al.*, 2002) was calculated with the software qgene (Joehanes and Nelson, 2008) and relative expression with the Roche Applied Science E-method (Tellmann, 2006).

Table 7.1: Primer sequences used in the reverse transcription quantitative polymerase chain reaction (RT-qPCR). Reference gene candidates: 18S ribosomal RNA (18S), elongation factor 1-alpha (EF1*a*), glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and the ribosomal protein L8 (RPL8). Genes of interest (target): citrate synthase (CS), mitochondrial and cytosolic manganese superoxide dismutase (SODMn-m and SODMn-c), and HSP70 isoforms (A, B, C, D and E). The prefixes EuS and EuC used in the gene names means that the primers were designed on the species *Euphausia superba* (Clark *et al.*, 2011) or *Euphausia crystallorophias* (Toullec *et al.*, 2013), respectively.

Genes	Forward (5'-3')	Reverse (5'-3')	Fragment size (bp)	Primer efficiency
Reference genes				
EuC_18S	TTCCGTCAATTCCTTTAAGT TTCAGC	CCCTAGTTCTAACCATAAAC GATGC	94	2.04
EuC_EF1*a*	GTACAGGTAAGGAACTTGAA TCT	TCTTACATACAGCCTTGATA ACA	140	2.04
EuC_GAPDH	GGAGTAACCAAATTCGTTGT CAT	GATGTTGTTTCTACAGACTT TGT	80	2.05
EuC_RPL8	CTGAAGGTACCATTGTTTGT AACCTC	CTTCAAGATGGGCTTATCAA TACGTC	169	1.86
Target genes				
EuC_CS	GGCAGATCCAACCAAGTGG	GCAGCAACAATCTACCGAAA TC	207	2.09
EuS_SODMn-m	CCACCGTGACCCTAAGTAAC C	GGTGATGTTTGGAGTGATGC	188	2.08
EuS_SODMn-c	GGCAGACTCAAAGGACGC	GGCAGCTATCTGTGGATCAA C	211	2.10
EuC_HSP70A	AATCATTACCAAGATGTACC AGGC	CTGGGGCACTTGCGTC	148	2.19
EuC_HSP70C	AAGAAGAGAAAGGATCAATC AGTAGTCAG	AGTTATGAATCTTAGCAGCA AGTGG	147	2.16
EuC_HSP70D	TCTGCAACAATCTTCACTGA AACTC	AACTAAATGCCAATTTATTG TTTGCCAC	159	2.17
EuC_HSP70E	AAAGGTGTGCAGTCCTATCA TCAC	ATCCTAAATCAACTTATAAT TGGTCCTTTAGTC	205	2.13

7.2.3 Biochemical and data analysis

The activities of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), glutathione peroxidase (GPx), and the anaerobic accumulation of lactate concentration were analysed in the cephalothorax part of the organisms. Citrate synthase (CS) activity, the reduced and oxidized glutathione (GSH, GSSG) concentration, the detection of malondialdehyde (MDA) formation (lipid peroxidation), and protein carbonyl content (protein oxidative damages) were analysed in the abdominal part of the euphausiids. We used the same methodology as for our biogeographical comparison (Tremblay and Abele, in revision; Chapter 5).

All statistic and figures were done with R (R Core Team, 2012). The horizontal bars in the box plots indicate median and all upper and lower edges of the rectangle show the 1st and 3rd quartiles, respectively. Vertical error bars in the box plots extend to the lowest and

highest value in a 1.5-fold inter-quartile range (R Core Team, 2012). For treatment comparison, normality (Shapiro test) and variance homogeneity (Bartlett test) tests were performed for each hypoxia experiment separately. Data were transformed ($log(x)$, x^{-1}, $x^{1/2}$) if the criteria of normal distribution and homogeneity of variance were not met. If no transformation of data allowed the use of analysis of variance (ANOVA), the non-parametric test Kruskal-Wallis was conducted. Significant level of all comparisons was fixed at 95% (p=0.05).

7.3 Results

No accumulation of the anaerobic glycolysis end product (lactate) was detected in *E. superba* exposed to either the severe or the 6 h-threshold hypoxia treatment (Fig. 7.1) indicating a low contribution of anaerobic energetic pathways under the conditions applied in our experiments. Also CS activity remained unchanged in both hypoxia treatments (Fig. 7.2a), although 2-fold higher transcript levels were recorded in the 6 h threshold hypoxia treatment compared to the normoxic control (F=5.50; p=0.041; Fig. 7.2b).

Activities of the antioxidant enzymes SOD and CAT were not affected by hypoxia (Fig. 7.3a, b). However, mitochondrial Mn-SOD expression increased in both hypoxic treatments, 3-fold in the severe (F=9.99; p=0.008; Fig. 7.3c) and 2-fold in the threshold (F=13.76; p=0.004; Fig. 7.3c). Contrary, the cytosolic compartment Mn-SOD isoforms had unaltered expression levels (Fig. 7.3c, d).

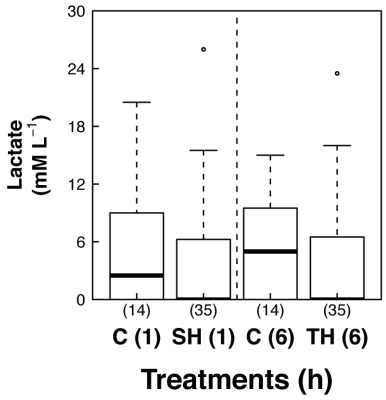

Figure 7.1: Anaerobic indicator (lactate) analysed in *Euphausia superba* exposed to different hypoxia levels. C: control (1 and 6 h 100% O_2 saturation, normoxic); SH: severe hypoxia (2.5% O_2 saturation for 1 h); TH: threshold hypoxia (20% O_2 saturation for 6 h); dashline separates experiments; (n): number of samples analysed; circle: outlier; median, 1st and 3rd quartiles (rectangle), lowest and highest value in a 1.5-fold inter-quartile range (error bars).

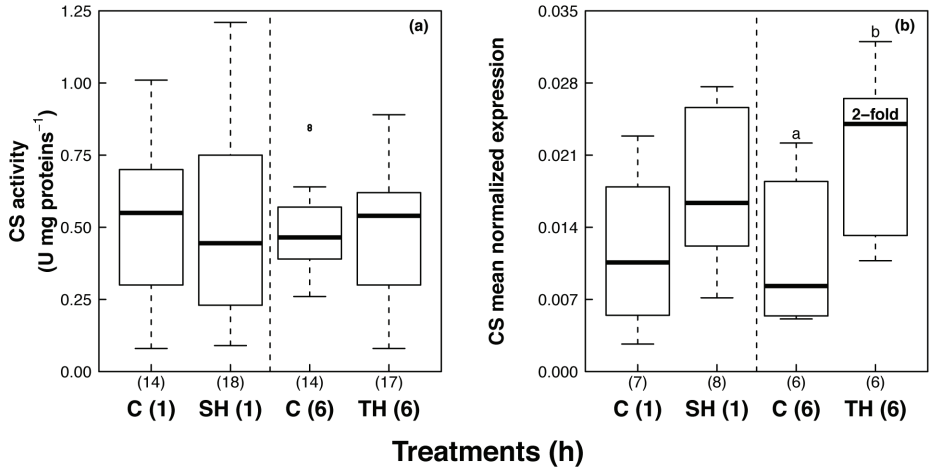

Figure 7.2: Citrate synthase (a) activity and (b) mean normalized expression analysed in *Euphausia superba* exposed to different hypoxia levels. C: control (1 and 6 h 100% O_2 saturation, normoxic); SH: severe hypoxia (2.5% O_2 saturation for 1 h); TH: threshold hypoxia (20% O_2 saturation for 6 h); "ab" marks significant difference of hypoxia treatment respective to its control; the fold (bold) is the relative gene expression; dashline separates experiments; (n): number of samples analysed; circle: outlier; median, 1st and 3rd quartiles (rectangle), lowest and highest value in a 1.5-fold inter-quartile range (error bars).

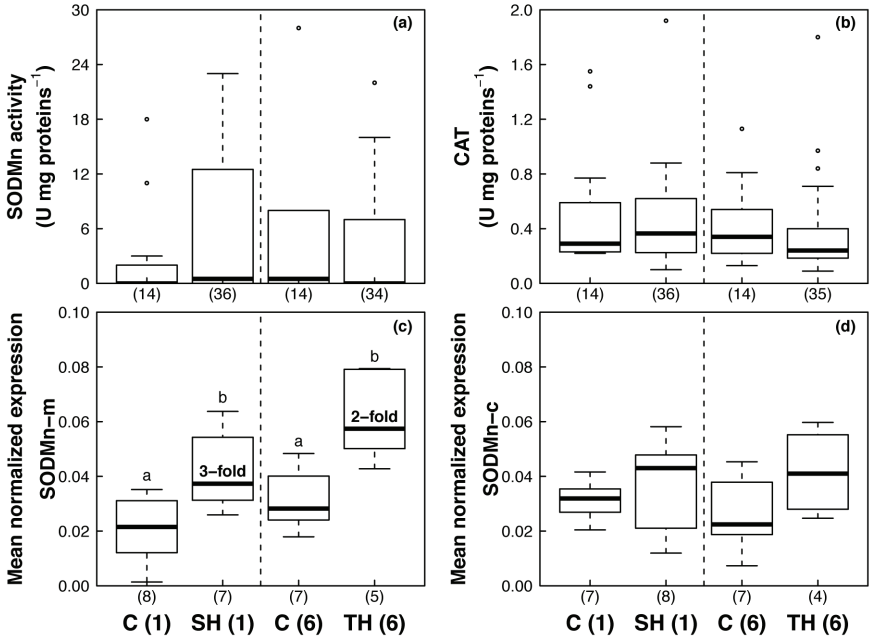

Figure 7.3: (a) Total superoxide dismutase (SOD) activity, (b) catalase (CAT) activity and mean normalized expressions of (c) mitochondrial SOD-Mn and (d) cytosolic SOD-Mn analysed in *Euphausia superba* exposed to different hypoxia levels. C: control (1 and 6 h 100% O_2 saturation, normoxic); SH: severe hypoxia (2.5% O_2 saturation for 1 h); TH: threshold hypoxia (20% O_2 saturation for 6 h); "ab" marks significant difference of hypoxia treatment respective to its control; the fold (bold) is the relative gene expression; dashline separates experiments; (n): number of samples analysed; circle: outlier; median, 1st and 3rd quartiles (rectangle), lowest and highest value in a 1.5-fold inter-quartile range (error bars).

The inducible Hsp70-E isoform (χ^2=6.56; p=0.010; Fig. 7.4d) was the only Hsp70 induced during hypoxic exposure, with a 30-fold higher expression in euphausiids incubated 6 h at 20% O_2 saturation (threshold treatment) compared to normoxically treated animals. The mitochondrial isoform Hsp70-D was the most highly expressed of all Hsp70 (Fig. 7.4c), while the inducible Hsp70-C and constitutive Hsp70-E genes were less strongly expressed (Fig. 7.4b, d).

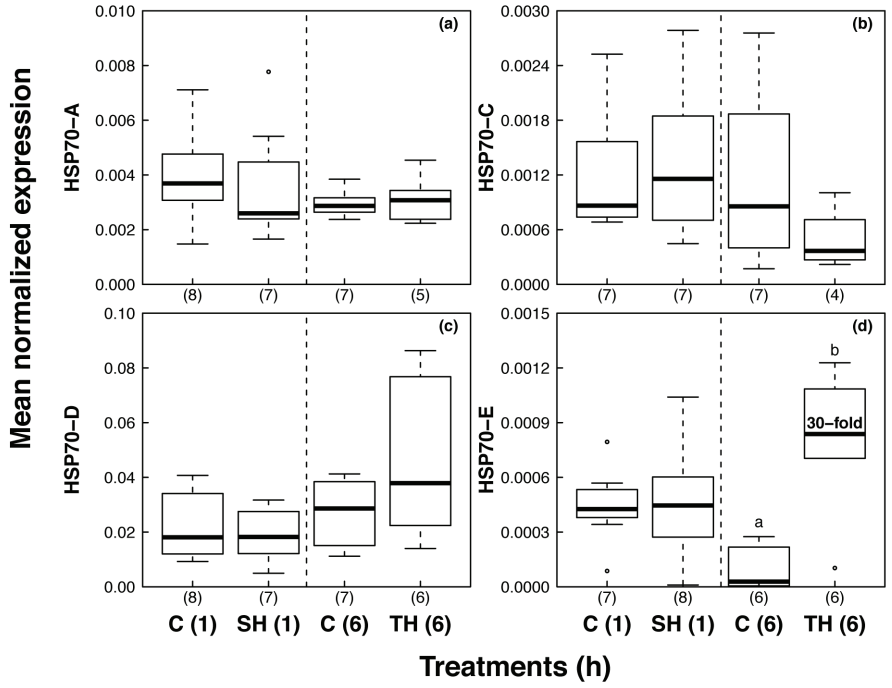

Figure 7.4: Heat-shock proteins 70 (Hsp70) mean normalized expression of the (a) A constitutive isoform, (b) C inducible isoform, (c) D mitochondrial isoform, and (d) E constitutive isoform analysed in *Euphausia superba* exposed to different hypoxia levels. C: control (1 and 6 h 100% O_2 saturation, normoxic); SH: severe hypoxia (2.5% O_2 saturation for 1 h); TH: threshold hypoxia (20% O_2 saturation for 6 h); "ab" marks significant difference of hypoxia treatment respective to its control; the fold (bold) is the relative gene expression; dashline separates experiments; (n): number of samples analysed; circle: outlier; median, 1st and 3rd quartiles (rectangle), lowest and highest value in a 1.5-fold inter-quartile range (error bars).

GST and GPx activities did not differ between normoxia and hypoxia exposures (Fig. 7.5a, b) and the same was observed with the GSSG: GSH ratio and the total glutathione content in abdominal tissues (Fig. 7.5c, d).

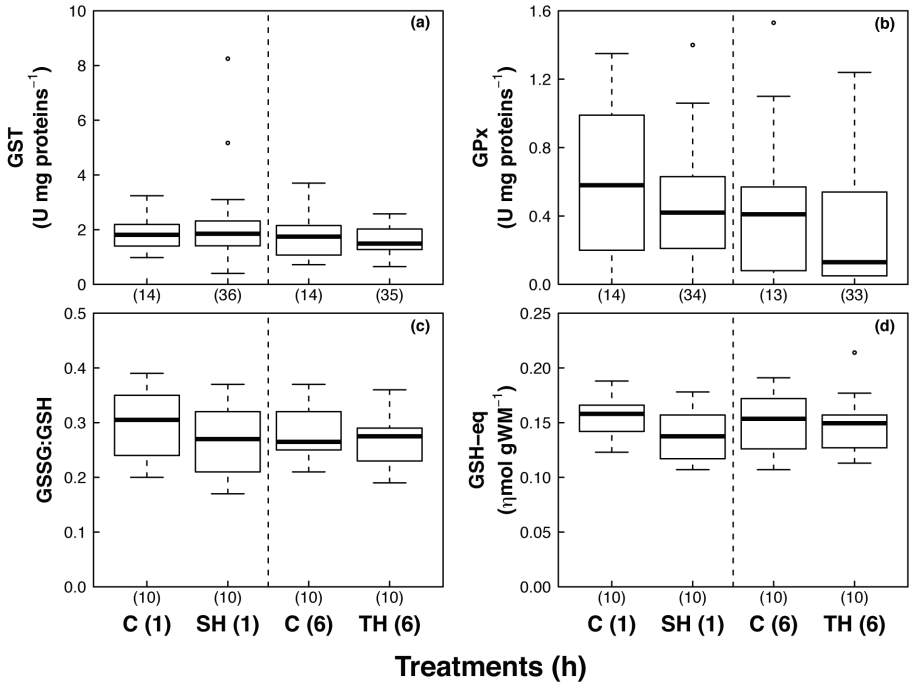

Figure 7.5: (a) Glutathione-S-transferase (GST) activity, (b) glutathione peroxidase (GPx) activity, (c) oxidized/reduced glutathione (GSSG: GSH), and (d) glutathione equivalents (GSH-eq) analysed in *Euphausia superba* exposed to different hypoxia levels. C: control (1 and 6 h 100% O_2 saturation, normoxic); SH: severe hypoxia (2.5% O_2 saturation for 1 h); TH: threshold hypoxia (20% O_2 saturation for 6 h); dashline separates experiments; (n): number of samples analysed; circle: outlier; median, 1st and 3rd quartiles (rectangle), lowest and highest value in a 1.5-fold inter-quartile range (error bars).

Evidence of oxidative stress occurrence in terms of higher lipid peroxidation rates was recorded in the euphausiids exposed to the longer and threshold hypoxia level (F=5.33; p=0.025; Fig. 7.6a). However, this treatment has no effect on the accumulation of oxidized proteins in the same tissue (Fig. 7.6b).

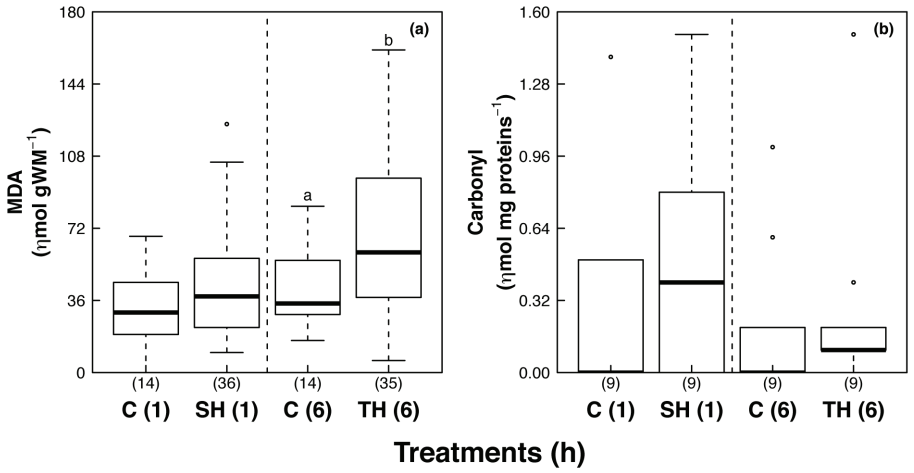

Figure 7.6: Oxidative damages in (a) lipids (malondialdehyde or MDA) and (b) proteins (carbonyl) analysed in *Euphausia superba* exposed to different hypoxia levels. C: control (1 and 6 h 100% O_2 saturation, normoxic); SH: severe hypoxia (2.5% O_2 saturation for 1 h); TH: threshold hypoxia (20% O_2 saturation for 6 h); "ab" marks significant difference of hypoxia treatment respective to its control; dashline separates experiments; (n): number of samples analysed; circle: outlier; median, 1st and 3rd quartiles (rectangle), lowest and highest value in a 1.5-fold inter-quartile range (error bars).

7.4 Discussion

7.4.1 Short-term exposure to hypoxia alters mitochondrial metabolic and antioxidant capacities of Antarctic krill, but does not induce anaerobic metabolism

Antarctic krill at South Georgia has been credited earlier for its relative tolerance to hypoxia, unexpected for a pelagic ectotherm swimmer in the Antarctic. Clarke and Morris (1983) reported oxyregulation (constant aerobic respiration rates) down to 30% oxygen saturation (in an open system at unknown experimental temperature), and we determined a critical low pO_2 at 55% oxygen saturation in the population we sampled in South Georgia (measured in a closed system at *in situ* temperature 4°C; Tremblay and Abele, in revision). Our study also produced initial data on *E. superba* hemolymph lactate levels and, while basal lactate concentrations in *E. superba* are equally high as in the hypoxia tolerant *Euphausia mucronata* from the OMZ area of the Humboldt current after reaching *pc* (Tremblay and Abele, in revision), neither severe nor threshold hypoxia

exposure caused an accumulation of lactate over normoxic level. Thus, the capacity for anaerobic glycolysis is confirmed, but not induced during the metabolically reduced state. Contrary, transcription of the mitochondrial marker CS in the Antarctic krill was induced during 6 h hypoxia exposure (and also enhanced within 1h of severe hypoxic exposure), indicating an attempt to sustain CS activities and mitochondrial aerobic capacities during short-term oxygen deficiency. This is an astonishing finding, as protein new synthesis is a costly process under hypoxic conditions. As we did not measure CS protein concentrations we can only speculate that transcriptional induction (much less energetic expenditure than protein synthesis) serves to support CS activity in this active, highly energetic swimmer (Zhou and Dorland, 2004) once the animals return to normoxia and a fully and unrepressed aerobic metabolism.

So, what should cause a need for *E. superba* to adjust metabolism on a lower level in fully oxygenated Antarctic water around South Georgia? Antarctic krill is an "obligatory schooling species" and its social behaviour within the swarm impinges on many aspects of its biology (Hamner and Hamner, 2000). Swarm density has been estimated at 25, 000 ind m^{-3} and higher (Hamner and Hamner, 2000) and can reach size of 100 km^{-2} (Nowacek *et al.*, 2011). Possibly a rapid adjustment of individual oxygen uptake is instrumental in maintaining high enough water pO_2 at varying swarm density (our CTD measurements showed no alteration in water column oxygen levels that were relatable to presence of krill swarms). If water pO_2 within the swarm is decreasing too much, trade-off between individuals might become necessary to access well-oxygenated water (Brierley and Cox, 2010). Avoidance of lactate accumulation and the resulting muscular and blood acidosis seems pertinent to maintain individual swim speed within swarms, which face a high risk of predation once they fall behind the school (Brierley and Cox, 2010). This might even be more critical in polar species, in which lactate removal by reconversion to pyruvate or through gluconeogenesis is curtailed by the low temperatures (Bushnell *et al.*, 1994).

Not only CS mRNA, but also transcription of the mitochondrial isoform of SODMn was significantly induced in both hypoxic treatments. This aligns with the "theory of preparation for reoxygenation stress" (Welker *et al.*, 2013), which seems to apply to some hypoxia tolerant animal species, in which mitochondrial SODMn up-regulation prepares the animals for the oxidative burst during reoxygenation. Like in the Antarctic krill, upregulation of the mitochondrial SODMn gene was observed after several days of cycling hypoxia in the blue crab *Callinectes sapidus* (Brown-Peterson *et al.*, 2005) and the grass shrimp *Palaemonetes pugio* (Brown-Peterson *et al.*, 2008), both from Ocean Springs, Mississippi (USA). To this end, our data indicate that hypoxic exposure of Antarctic krill induces biochemical mechanisms that stabilize aerobic capacities and antioxidant defences, rather than anaerobic energy production.

It is interesting to discuss why we were not able to detect higher SOD activities in the

hypoxic krill, given the upregulation of the transcript. First of all, as in the case of CS induction, activities may not follow the pattern of transcript levels under stressful conditions when the enzyme molecules may be less functional. In the present case, however, we were also unable to separate cytosolic and mitochondrial SOD activity which is both SODMn (and not Cu,ZnSOD as in the cytosol of most other animals). Instead the SODMn isoform is the main form in the cytosolic fraction in many hemocyanin carrying Malacostraca crustaceans (Brouwer *et al.*, 1997) like euphausiids, which made it impossible to separate both compartments in the enzymatic measurement.

7.4.2 The heat-shock response in hypoxia

In higher eukaryotes, the Hsp70 family comprises constitutive and inducible isoforms (Tavaria *et al.*, 1996). A 30-fold increase of Hsp70E upon 6 h of threshold hypoxia indicates this *E. superba* heat shock protein to act as an inducible isoform under oxygen deficiency. By analysing the same Hsp70 isoforms 4 h after a thermal shock of 3 h at 3°C, Cascella et al. (in prep.) also found an inducible behaviour of the Hsp70E with a 24-fold increase. Hypoxia-induced Hsp70 expression was also observed in the Antarctic clam *Laternula elliptica* after 16 days of hypoxia exposure and was associated to an anti-apoptotic function (Clark *et al.*, 2013). In the grass shrimp *P. pugio*, inducible Hsp70s were down regulated after 3 (Brown-Peterson *et al.*, 2008) and 7 days (Brouwer *et al.*, 2007) of cyclic-hypoxia exposure. The general slow down of protein synthesis explained this down regulation as the shrimp entered a reduced metabolic state in hypoxia. Parallel increase of Hsp70E, CS and MnSOD transcripts indicates adaptive stimulation of metabolism during hypoxic exposure in *E. superba*, to compensate for energetic deficiency and to support protein stabilization in hypoxia.

Of all Hsp isoforms, the mitochondrial Hsp70D was most highly expressed together with the high expression of both MnSOD enzymes. Mitochondrial Hsp70s are located in the mitochondrial matrix to stabilize polypeptide chains, subunits of mitochondrial enzymes, synthesized in the cytosol and entering the mitochondrion through the inner membrane (Kang *et al.*, 1990; Chacinska *et al.*, 2009). They also remove denatured proteins through the membrane into the matrix for proteolysis (Lee *et al.*, 2004; Doyle and Wickner, 2009). The fact that this mitochondrial Hsp70D was not upregulated during hypoxic exposure is a further indication for an abrogation of protein synthesis under insufficient oxygenation.

None of the other oxidative stress defences showed variations during both hypoxia exposures in *E. superba*, which further speak for a stability grant by the higher reduced glutathione buffering capacity compared to temperate congeners (Tremblay and Abele, in revision). This is support by the inexistent lipid and protein oxidative damages under the short drastic hypoxia exposure, and the not dramatically high lipid damages observed after 6 h of threshold hypoxia.

7.5 Conclusion

Our results document considerable physiological flexibility that helps *E. superba* to survive short periods (6 h) of threshold hypoxia and prevent cellular damage resulting from hypoxia and potentially from reoxygenation. As in many other Antarctic ectotherms, the stress response has not been entirely lost during 20 million yrs of stenothermal cold adaptation. When actively swimming in a dense swarm or escaping from a predator, *E. superba* deals with short period of stress and have developed cellular response to maintain or re-enter rapidly in homeostasis (preparation for reoxygenation). It is still not clear whether this stress response would helps *E. superba* to adapt and survive to the prognosticated warming in South Georgia, the main short-term threat of this region (Flores *et al.*, 2012). Swarming in a warming habitat, even if *E. superba* copes well with hypoxia, could generate major changes, *e.g*. less dense and smaller swarms (Brierley and Cox, 2010), and deeper or dispersed distribution to avoid the warmer water masses at surface (Hill *et al.*, 2013). These downside points might have considerable negative impact on their predators.

A comparison among polar and temperate species was not possible because of the lack of genomic information in the other krill species. However, a joint comparison of physiological and molecular stress response will certainly be within the scope of future work.

Acknowledgments

This study would not have been possible without the support of the R/V James Clark Ross crew. The Euromarine Mobility Fellowship 2012 sponsored a research stay at the Station Biologique de Roscoff to work with K.C. and J.Y.T. The Alfred Wegener Institute (AWI), Helmholtz Centre for Polar and Marine Research, supported this research (1. PACES 2.2: Integrating evolutionary ecology into coastal and shelf processes). We thank Imke Lüdeke, and especially Stefanie Meyer for their excellence and technical help in the laboratory of AWI. We also thank Kai-Uwe Ludwichowski from the ecological chemistry group of AWI for his advices in the HPLC analysis. N.T. had a doctoral scholarship from the Fonds de recherche sur la Nature et les Technologies du Québec (Canada).

Chapter 8

General discussion

The aim of my thesis was to investigate the oxystrategies and oxygen minimum zone (OMZ) tolerance mechanisms of euphausiids on a global-scale to explain the current zoogeographical distribution patterns of major species, and project these into the future of climate driven system change. An approach using respiratory and oxidative stress parameters was proposed and confirmed the hypothesis that stress responses to declining pO_2 and warming are highly influenced by the adaptive background in each species. A global respiration model points out strong season and latitude effects on euphausiid basal metabolic rates (Chapter 3), which underlines the importance of the present comparative study. Below, I highlight the main results from this thesis and discuss the possible consequences of ocean warming and deoxygenation for the euphausiids species according to their current physiological response spectrum.

8.1 What awaits the main species from the northern California Current System?

High tolerance to hypoxia was assumed in the past for the north Pacific species *Euphausia pacifica* because of its low critical pO_2 (pc=18 mm Hg, 2 kPa or 11% O_2 saturation at 10°C), lower than what the species experiences *in situ* at 350 m depth in its habitat (off South California; Childress, 1975). However, acoustic and stratified sampling have demonstrated that the vertical distribution of this species is restricted by hypoxic conditions in fjords off Washington state (USA) and British-Columbia (Canada; anoxia at 100 m depth in Saanich Inlet; Bollens *et al.*, 1992; Kunze *et al.*, 2006). In these environments, pc values of *E. pacifica* were higher (pc=4 kPa or 20% O_2 saturation at 10°C), showing less hypoxia tolerance (Ikeda, 1977). No such data were available for the neritic species *Thysanoessa spinifera*, but its strong association with upwelling conditions likely signifies some adaptation to tolerate low oxygen concentrations.

The seasonal analysis that I did for *E. pacifica* in the frame of the chapter 5 of my thesis shows that it is not so simple. From season to season, this species optimizes its adaptation to environmental stressors as they arise. With measurements of its basal pO_2-dependent metabolism, an increased of tolerance to hypoxia was clearly observed between the winter fully mixed cold and oxygenated conditions to summer stratified hypoxic conditions where this species has a lower *p*c value. The total absence of lactate accumulation in the cold adapted winter animals when exposed to declining pO_2 supports the view that seasonal cold adaptation reduce the capacity to deal with hypoxia.

The synergetic warming-hypoxia/reoxygenation experiments confirmed more robustly the latter. The most obvious observation was the 95% mortality of the organisms exposed to +4°C warming and threshold hypoxia during the cold season, while this rate was reduced to <50% when animals were adapted to conditions in summer. The same trend was observed at control temperature and with +2°C, *i.e.* higher mortality rates in winter compared to summer experimental groups. The stages of development present in the water column during the winter were younger adults with higher energetic requirements, which probably explains their lower tolerance and reduced capacity to react to stress.

The winter-summer comparison also highlights the strong relationship between the respiration rate of *E. pacifica* and the production of reactive oxygen species (ROS). Apparently, higher ROS amount are formed at higher respiration, as the antioxidant enzyme superoxide dismutase (SOD) activity followed the higher breathing rate of the organisms in winter conditions. In summer, the importance of SOD during normoxic respiration at control temperature was especially clear during reoxygenation as the animals resumed their normal respiration rates after hypoxia exposure of 6 h. In this case, the lack of SOD results in oxidative damage, even if the detoxifying glutathione S-transferase (GST) enzyme increased in activity. Both SOD and GST patterns were also observed in the gills of the crab *Chasmagnathus granulata* from Rio Grande do Sul (Brazil) during an anoxia/reoxygenation exposure (de Oliveira *et al.*, 2005). Supported by +2°C warming, the antioxidant system of *E. pacifica* was able to compensate the oxidative stress situation observed at control temperature (10°C). Depletion of glutathione (GSH-eq) backed-up the activation of non-enzymatic antioxidants to protect the tissues from the ROS detrimental effects. The synergic exposure to both environmental stressors (hypoxia + warming) resulted in different stress responses, which emphasizes the importance of testing the combined effect of the stressors in experiments.

No such complete experiment was run in parallel on the neritic species *T. spinifera* due to sampling limitations. Nevertheless, based on its oxystrategy, totally different results should be expected with respect to hypoxia/reoxygenation processes. As this species has comparatively more lipids than *E. pacifica* due to the food conditions of the shelf areas (Taatjes and Cass, 2014),

it could experience enhanced lipid oxidative damages when exposes to combined threshold hypoxia stress and warming. Another aspect that speaks for a different hypoxia/reoxygenation response of *T. spinifera* is it lower metabolic rate and more intensive reliance on anaerobic glycolysis when pO_2 is decreasing (chapter 6).

8.1.1 Zoogeographical dynamics

The California Cooperative Oceanic Fisheries Investigations (CalCOFI) and the "Investigaciones Mexicanas de la Corriente de California" (IMECOCAL) hold long time-series on the California Current System (CCS). Zooplankton abundances recorded in some areas since the 50s allow large spatial analysis of their distribution over climatic cycles (like Pacific Decadal Oscillation or PDO, El Niño Southern Oscillation or ENSO, the North Pacific Gyre Oscillation or NPGO, etc.) or unexpected events. Several research papers cover these zoogeographical dynamics: Peterson and Mackas (2001) analyzed 1985-2000 data sets in the northern part (off Oregon and British-Columbia), Brinton and Townsend (2003) worked on the longest time-serie (1950-2002) from the CAlCOFI grid (26-38°N), and Lavaniegos and Ambriz-Arreola (2012) searched for patterns of abundance and species distribution in the 1997-2007 data from the IMECOCAL project (24-34°N). Other studies give attention to a smaller area, between Point Sur and Point Arena (38°N off California) with approx. 30 yrs data set collected in the frame of an annual ecosystem survey. In the latter, Sydeman *et al.* (2013) searched for demographic attributes of salmon and seabirds related to the increasing variability in the composition of local food webs, using krill as a biological indicator, while García-Reyes *et al.* (2014) characterized upwelling and relaxation events to predict their effect on local krill populations.

In the northern part of the CCS, where the present study area is located, important changes in both species distribution occurred during the El Niño event of 1992-1993, after which biomass of *T. spinifera* fell by more than 70% off Oregon and British-Columbia (Tanasichuk, 1999). When *T. spinifera* was absent in the neritic area, *E. pacifica* spreads its distribution over the continental shelf with lower abundance than in normal years (Peterson and Mackas, 2001). Another El Niño event in 1997-1998 caused low krill abundance in the central CCS, but these effects were weaker north of the CCS (Gulf of Alaska). Indeed, *T. spinifera* abundance was unchanged in these shelf areas (Pinchuk *et al.*, 2008). Both species subsequently regained their neritic and oceanic habitat off Oregon and British-Columbia late 1998 and early 1999, when a return to cold conditions happened under the influence of La Niña (1998-2000; Peterson and Mackas, 2001).

The long CAlCOFI time-series analysis highlights a reduction of *T. spinifera* in the central part of the CCS, and a total absence of this species in the southern part (off Baja California) during the 80s (Brinton and Townsend, 2003). This period was dominated by negative anomalies of the PDO index, coinciding with six important La Niña events, which were favourable for the

abundance of *E. pacifica* (Brinton and Townsend, 2003).

At 38°N (off California), *T. spinifera* is strongly influenced by the NPGO (Sydeman *et al.*, 2013), an oscillation connected with the winds and upwelling responses (Chenillat *et al.*, 2012). In the same area, low upwelling conditions from both El Niño events of 1992 and 1998 were corroborated by the Nutrient Upwelling Index (NUI) and coincided with low krill years (García-Reyes *et al.*, 2014). Krill term here includes *E. pacifica*, *T. spinifera* and the subtropical neritic species *Nyctiphanes simplex*. NUI is also considering SST variability and thanks to this index, the optimal krill productivity was detected during moderate upwelling conditions.

In the southern part of the CCS (at approx. 30°N; Off Baja California), *E. pacifica* took some time to recover after the El Niño event (1997-1998), but was abundant again during summers of 2000, 2002 and 2005. These high abundances were linked to La Niña in 2000, a sub-Arctic water intrusion in 2002 and to high upwelling conditions in 2005 (Lavaniegos and Ambriz-Arreola, 2012). The southward spreading was evident by the decline in 2001 of *E. pacifica* in the oceanic waters of the Gulf of Alaska (Pinchuk *et al.*, 2008). The high nutrients that resulted from the upwelling event of 2005 also attracted *T. spinifera* and may explain the important decrease of abundance in the northern CCS sectors for the same year, as many species from different climatic affiliation (temperate, subtropical and tropical) were relatively abundant in the south to take advantage of this upwelling event (Lavaniegos and Ambriz-Arreola, 2012).

8.1.2 Predictions for the future

Timmermann *et al.* (1999) was the first to predict higher frequency of El Niño/La Niña events in a scenario of global warming under the influence of increased greenhouse-gas effect. Recently, it was however shown that this phenomenon is entirely unpredictable on a decadal scale (Wittenberg *et al.*, 2014). It is clear that El Niño brings low upwelling conditions (low food availability) and warmer deoxygenated water, which are not optimal for the temperate species of the NCCS (Fig. 8.1). The spawning season off Oregon and in Puget Sound (Washington) normally extend from March to September (Ross *et al.*, 1982; Gómez-Gutiérrez *et al.*, 2007) and was associated with high chl *a* during the upwelling events in summer (Gómez-Gutiérrez *et al.*, 2007). Brood sizes and chl *a* were high in 2003-2004 compared to the post-El Niño period of 1999-2001 (Gómez-Gutiérrez *et al.*, 2007), meaning that the species take time to recover from such perturbation. I showed in my thesis that after the overwintering period, a replacement of the cold productive upwelling conditions with warmer and more hypoxic waters could have catastrophic impact on the stock available, as the individuals from April 2012 were extremely sensible to both warming and hypoxia (Fig. 8.1).

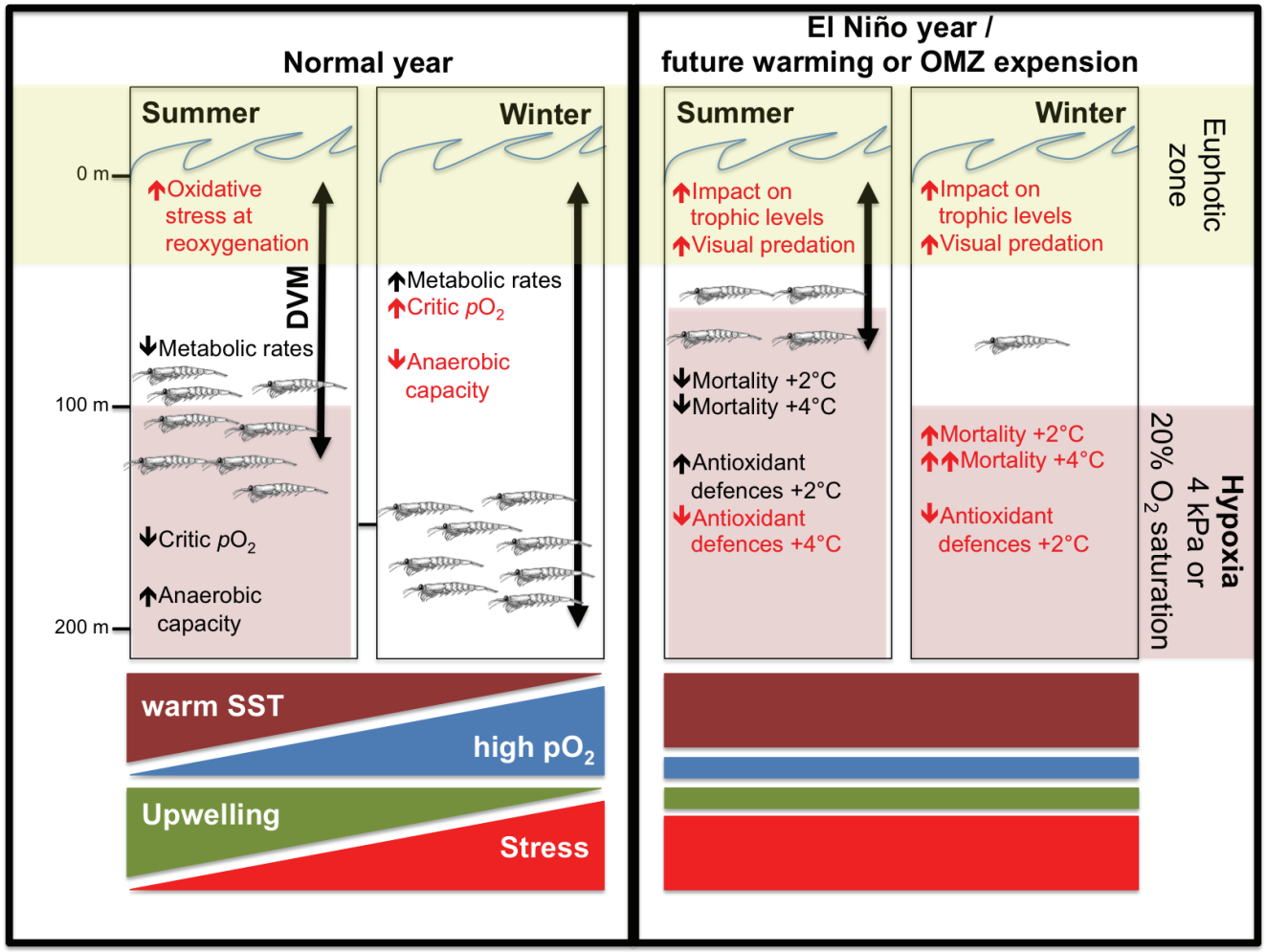

Figure 8.1: Schematic model of the seasonal (summer and winter) adaptations of *Euphausia pacifica* in the northern California Current System during a normal climatic year (left) and with the El Niño influence (right). The latter could also applies for a warming and deoxygenation global scenario. The parameters are compared for each climatic situation separately (between summer and winter). The yellow and red shade areas mark the euphotic and the oxygen minimum zones, respectively. The black arrows are delimiting the range of vertical migration. The parameters in red represent negative responses to both hypoxia and warming.

The warming-hypoxia/reoxygenation experiments on *E. pacifica* support the shift of distribution observed since the 50s along the whole CCS caused mainly by El Niño negatively effects, which might also applies for *T. spinifera*. Indeed, *T. spinifera* have more energy requirements, starts the reproduction earlier than *E. pacifica* (Gómez-Gutiérrez *et al.*, 2007), and is only abundant in area or years with strong upwelling conditions (Lavaniegos and Ambriz-Arreola, 2012; Sydeman *et al.*, 2013; García-Reyes *et al.*, 2014). Alternation between El Niño and La Niña events maintains the abundance of krill across time, but it is clear that if strong El Niño event like the one of 1997-1998 last longer or occurs more often, both *T. spinifera* and *E. pacifica* stocks would probably disappear from the CCS and continue their life cycle at higher latitudes in the Gulf of Alaska, where they are not so affected by the El Niño event. This would have strong consequences for the higher trophic levels of the CCS.

Referring to the massive stranding of *T. spinifera* in June 2013 on 400 km coast along the northern CCS (Tyburczy *et al.*, 2013), a similar situation was observed in the Gulf of California (Mexico) with the subtropical species *Nematoscelis difficilis* (López-Cortés *et al.*, 2006). The authors proposed that high unusual upwelling conditions promoted a phytoplankton bloom, which indirectly depleted the oxygen concentration with the sinking of organic matter below the compensation depth. This would have forced the mesopelagic *N. difficilis* to migrate upwards towards more oxygenated waters and then to be washed out by the surface currents. *N. difficilis* was shown to be relatively tolerant to hypoxic conditions, but less than the tropical species *Euphausia eximia* (Tremblay *et al.*, 2010). In the case of *T. spinifera*, hypoxic conditions were also present, but not caused by a phytoplankton bloom. Hypoxia tolerance would need to be experimentally investigated to state if the oxygen level recorded between 50 and 100 m depth (2 to 4 mg O_2 L^{-1} which corresponds to a range of 26-35% O_2 saturation or 6 to 8 kPa) is threatening the species and could be responsible for more stranding events.

***Euphausia pacifica* and *Thysanoessa spinifera* showed vulnerability to warming and hypoxia. Even if *Euphausia pacifica* showed a difference of its tolerance limits to both stressors between seasons, its biomass fluctuations over the past 60 yrs in the California Current System suggests little resilience to a warming and deoxygenating habitat. Both species are thus bound to decrease if more unpredictable and strong El Niño events occur.**

8.2 Can we expect *Euphausia mucronata* to spread its distribution range with OMZs expansion?

Euphausia mucronata is endemic to the Humboldt Current System (HCS) and has always been regarded as highly hypoxia tolerant (Teal and Carey, 1967; Escribano *et al.*, 2000; Antezana, 2002b). The explanation for this hypoxic tolerance was mostly based on the morphological traits (increased gills area) that support absorbance of dissolved oxygen in a low pO_2 environment (Antezana, 2002a). As shown previously (Teal and Carey, 1967; Antezana, 2002a; Donoso and Escribano, 2014) and in the chapter 5 of this thesis, *E. mucronata* does not regulate the respiration rate in any range of environmental pO_2 and, instead, suppresses metabolism even in normoxia. Oxyconforming respiration pattern is characteristic of species adapted to low oxygen concentrations that survive hypoxic and even anoxic conditions at least on a short-time scale (Gilly *et al.*, 2006; Seibel, 2011).

From the oxidative stress parameters analyzed in this species, GST activity was at an outstanding level, which might explain its success in the OMZ of the HCS (Chapter 5). Indeed, the high GST matches the higher oxidized/reduced glutathione (GSSG: GSH) ratio in this species, as GSH is the substrate of the enzyme. Another important physiological trait related to the high GSSG: GSH ratio in its tissues (>0.5) is that neither lipid nor protein oxidative damage indicators were higher in *E. mucronata* than in *E. pacifica*, which argues against enhanced oxidative stress in *E. mucronata* caused by its diel vertical migration in and out of the OMZ. However, during the austral winter, oxidative stress indicators all leaned towards an imbalance compared to summer conditions, which was seen for the first time and interpreted as a "stress due to over-oxygenation". An experimental +7°C warming caused a reduction of *E. mucronata* antioxidant defences. The same temperature increment applied in summer conditions caused enhanced SOD activity and decreased the GSSG: GSH ratio, indicating better control of oxidative stress and adequate adaptation to hypoxic and warm water conditions. Thus, like the NCCS species *E. pacifica*, *E. mucronata* reacts differently in different seasons to the same stressors and shows optimal responses during summer, when the OMZ is shallower and more intense.

8.2.1 Zoogeographical dynamics

The information about *E. mucronata* past abundance and distribution in the HCS is scarcer than for the euphausiids of the CCS area. As the HCS is closer to the equator than the CCS, especially off Peru, the impact of an El Niño event is more direct and causes important loss of the productive upwelling conditions (Nixon and Thomas, 2001; Pennington *et al.*, 2006). Taylor *et al.* (2008) investigated ecosystem bottom-up and top-down drivers in the north HCS (4-16°S) for the

1995-2004 period and suggested that a deeper thermocline and boundary of the OMZ during 1997-1998 El Niño would have increased the vulnerability of the larger euphausiids, as they were not able to find a refuge inside the OMZ from the visual predators. This was supported further south (18-30°S; off Chile), where the intrusion of warm oxygenated and low-nutritive waters into the shelfs from the same El Niño decreased the abundance of large *E. mucronata* during the core of the event, while the larval stages increased in abundance (Escribano *et al.*, 2004). The adults are likely more affected as they normally spend the whole day in the OMZ to avoid predation, while larvae and juveniles stages go unnoticed thanks to their small size and can stay in the surface layer (Antezana, 2002a,b). The larvae and juveniles probably depend more on a normoxic environmental pO_2 as their external gills are not yet fully developed (see Quetin and Ross, 1989).

Off Concepción (Chile; 30-45°S), the highest abundances of *E. mucronata* were correlated when the OMZ depth was shallower (<20 m) (Escribano *et al.*, 2007), and when the winds were characteristic of an upwelling event, just before entering in a downwelling period (transition between austral summer to winter; Riquelme-Bugueño *et al.*, 2012). During this period, biomass secondary production (reproduction) was particularly enhanced (Riquelme-Bugueño *et al.*, 2013).

8.2.2 Predictions for the future

Well oxygenated waters, caused either seasonally during winter or by cyclic El Niño events, are not optimal for *E. mucronata*. The abundance of adults, which are the main target of the important commercial fish like anchovies, is greatly reduced during this climatic event and the coldest annual conditions. In the frame of my thesis, I showed that the species is experiencing oxidative stress under downwelling conditions in the winter season. The investigation also corroborated the high abundance of *E. mucronata* in the warm-hypoxic conditions, when the enzymatic antioxidant system is more versatile and better inducible. The adverse situation experienced by the adults during winter conditions with oxygenated layers may relate to their morphologic hypoxia adaptation (greater gills area; Antezana, 2002a), which cause them to absorb more oxygen than needed.

As *E. mucronata* peaks in abundance and reproduction just before the unfavourable downwelling condition (Riquelme-Bugueño *et al.*, 2013), this may be part of a reproductive strategy as younger stages do not show lower abundances in the same adverses conditions (downwelling and El Niño). The low oxygen conditions during summer are potentially not optimal for the younger stages, which depend on a more oxygenated environment than the adults (Quetin and Ross, 1989). Thus, the adults need crucially to hide from their visual predators in the OMZ during the summer to invest their energy into reproduction and spawn when downwelling conditions take over in the winter season.

With respect to the main question, which was if *E. mucronata* can extend the range of its distribution, Riquelme-Bugueño *et al.* (2012) detected oceanic presence of this species that was not recorded in the previous survey conducted 30 years earlier. However, the individuals found in the oceanic zone were small and thus, probably advected from the shelf by the surface currents as they do not vertically migrate. In any case, as the HCS conditions (mainly OMZ depth) greatly changes among upwelling and downwelling seasonal cycles, the *E. mucronata* parent generation could easily extend spatially their distribution over a prolonged upwelling hypoxic warm event, but would hardly have the capacity to persist in the area during the next downwelling period.

***Euphausia mucronata* is an endemic species highly specialized to the Humboldt Current System. More oxygen in their environment, either caused by the reduced upwelling conditions during the austral winter season or as a consequence of an El Niño event, are unfavourable for the adults as they experience oxidative stress. This species is highly adapted to hypoxic conditions.**

8.3 Is the cold-adapted Antarctic krill *Euphausia superba* fitted for hypoxia?

Antarctic euphausiid species are separated from their congeners since at least 20 million years following the formation of the Antarctic Polar Frontal Zone (Patarnello *et al.*, 1996). It was hypothesized in the frame of this thesis that euphausiids from the Antarctic probably lost their plasticity to respond to severe hypoxia, as they adapted to a stable cold-oxygenated environment.

The investigation on hypoxia was conducted on *Euphausia superba* from the South Georgia area (54°17'S; 36°30'W), the Northern boundary of its distribution range (Atkinson *et al.*, 2008). Due to the higher temperature at South Georgia, aerobic metabolism and energy turnover is enhanced 1.5-fold (Chapter 5) compared to other colder regions like the Scotia Sea (Torres *et al.*, 1994). This species keeps the same respiration rate down to 55% O_2 saturation (pc) and switches to anaerobic energy production (lactate accumulation) below pc (Chapter 5). Lower pc values (so higher hypoxia tolerance) have been observed earlier by Clarke and Morris (1983) and Torres *et al.* (1994) in colder area. Lactate concentration in this species at the end of the respiration measurements, and thus in short term hypoxic exposure, was similar to the levels found in *E.*

mucronata (Chapter 5). However, this lactate level was recorded in individuals that were not actively swimming (confined in the respiration chamber) thus not requiring a lot of energy for mobility. Contrary, during the hypoxia experiments at 3.5°C in which the organisms had space to swim and were interacting with each others, the hemolymph lactate levels were equally high in the normoxia as in both hypoxia exposure groups. High capacity for anaerobic glycolysis exists in this species, but was not particularly induced during active state of the organism when exposed to a low pO_2, as in the hypoxia experiments.

This is particularly relevant for pelagic swarm swimming, a behaviour energetically expensive for which lactate accumulation is not a true benefit as it exhausts energy reserves and causes tissue acidification. So, the daily metabolic problem of this Antarctic species is not vertical migration in hypoxic strata like the temperate species, but could be of a similar intensity when forming dense swarms. According to Brierley and Cox (2010), the oxygen concentration in a median packed *E. superba* swarm (40 m diameter, 111 ind m^{-3}) can fall from 6.8 to 5.8 ml O_2 L^{-1} (76 to 65 % saturation or 16 to 14 kPa in South Georgia) after approx. 3 min spent in the middle. Swarm density can reach 25, 000 ind m^{-3} (Hamner and Hamner, 2000) or spread over hundreds km^{-2} (Nowacek *et al.*, 2011), so we can easily envisage that the reduction in oxygen availability in the middle of these biological features may be dramatically higher. This is probably the reason why *E. superba* has preserved unexpected high hypoxia tolerance.

To compensate for this lack of oxygen, hypoxic exposure of Antarctic krill induces biochemical mechanisms that stabilize aerobic capacities and antioxidant defences. Rapidly, upregulated transcription of the mitochondrial enzyme citrate synthase (CS) in both hypoxia exposures happened, in an attempt to sustain CS activities and mitochondrial aerobic capacities during short-term oxygen deficiency (Chapter 7). Transcription of the mitochondrial SOD manganese isoform (SODMn) was also significantly induced in both hypoxic treatments (Chapter 7), which aligns with the "theory of preparation for reoxygenation stress" (Welker *et al.*, 2013). As CS and SODMn protein concentrations were not measured, it remains elusive if this up-regulation really lead to proteins biogenesis. However, as the enzyme activities were unchanged and gene expression up-regulated in the same way in both hypoxia exposures, the cells had the common goal to maintain or re-enter rapidly the normoxic conditions.

The information provided by the heat-shock response also supported an initiation of cytosolic processes. A 30-fold increase of the heat-shock protein 70E (Hsp70E) upon 6 h of threshold hypoxia indicates this hsp to be inducible and could be associated to an anti-apoptotic function as found in the Antarctic clam *Laternula elliptica* (Clark *et al.*, 2013) exposed to hypoxia.

***Euphausia superba* is able to perform fast metabolic slow down when actively facing water depleted in oxygen without employing energetically costly anaerobic pathways. Hypoxia tolerance seems to be crucial, as oxygen concentration decreases significantly when this species is part of a dense swarm. Rapidly upregulated transcription of the mitochondrial enzyme citrate synthase and superoxide dismutase manganese isoform in hypoxia align with the theory of "preparation for reoxygenation stress".**

For a long time, it was thought that polar organisms were more exposed to oxidative stress because of high oxygen content in cold water and the constant radiation during the austral summer. It is true that ROS (H_2O_2) are released in the Antarctic polar seas via the photo-oxidation of the dissolved organic carbon (DOC) stored in the melting sea-ice during the austral spring, but the levels are negligible when compared to temperate areas with higher DOC in suspension (Regoli *et al.*, 2011). Nevertheless, the oxidative stress parameters analysed in the frame of the multi-latitudinal comparison show significant differences between *E. superba* and the other species studied, but more related to its cold rather than pro-oxidative environment. Indeed, mitochondria density in *E. superba* was higher corroborated by high CS activity (Chapters 5) to compensate for the permanently low temperatures (Johnston *et al.*, 1998; Guderley, 2004; Morley *et al.*, 2009).

Very low superoxide dismutase (SOD) and catalase (CAT) activities speak for low mitochondrial ROS endogenous generation compared to temperate congeners (Chapter 5), probably as a result of the lower metabolic rate recorded in this species. Moreover, the higher reduced glutathione (GSH) concentration, which keeps the GSSG: GSH ratios low (Chapter 5), seems to contribute to the protection of this species in order to avoid severe lipid and protein oxidative damages.

Regardless of its localization in the Southern Ocean, the effect of photoperiod (seasons) is greatly influencing the energy requirements of the Antarctic krill *E. superba* (Chapter 3). Indeed, respiration rate follows a sinusoidal pattern with minimum metabolic activity in mid-June (austral winter) and maximum at the end of December (austral summer). The photoperiodic cycle seems to be "imprinted" as a major *Zeitgeber* (Meyer, 2011; Teschke *et al.*, 2011), and is independent of light exposure, increments or decrements in temperature, and food availability (Brown *et al.*, 2013). Thus, it is important to keep in mind that the effect of environmental stressors on the metabolism in *E. superba* could have an insignificant impact or be shaded by the imposed *Zeitgeber* regime. More investigation is currently on-going to understand what regulates this biological timer in *E. superba* (Bettina Meyer, pers. communication).

The success of *Euphausia superba* in the Southern Ocean resides in significant physiological adaptations to compensate the cold environment and potentially to its *Zeitgeber* regulation.

8.4 The importance of integrative physiological comparison approaches and "natural experiments"

In the present thesis, I used integrative approaches to compare the hypoxia adaptation of different euphausiid species on a latitudinal and seasonal scale. The sampling strategy and experimental set-up of this study was thought to represent the environment conditions that were prevailing in the area. According to the view given by Spicer (2013), the interpretation issued from this thesis would be more realistic than simplistic as we combined field controlled experiments with natural variability (seasonal). The fact that similar experiments were run among different seasons and species in a short time-scale add a realistic argument to the main findings. In addition, no paradox or unexplained pattern arose from the comparison made, which reinforce the quality of my experiments and analysis.

The most completed picture for seasonal hypoxia effects in synergy with warming was achieved for the NCCS species *E. pacifica*. Ideally, the same achievement was hoped for all the species of the studies, but it was not possible due to logistic or technical limitations. The study particularly failed to understand the adaptations of the species inhabiting the core of the OMZ of the Eastern Tropical Pacific. These species are adapted to warm waters and are the ones that would mainly spread their distribution towards higher latitudes in a warming (and OMZs expansion) scenario (Letessier *et al.*, 2011). The significantly higher respiration rates measured in *Euphausia lamelligera* and *Euphausia distinguenda* (Chapter 4) and their smaller size are two strong characteristics of tropical species, and it would have been interesting to test their responses to colder water with the oxidative stress parameters approach. It can be expected from the higher metabolic rates that this species would have a greater physiological flexibility.

The used of molecular approach was particularly useful in *E. superba* to understand its fast cell adjustments when exposed to short-drastic and longer less severe hypoxia exposures (Chapter 7). For the superoxide dismutase (SOD) enzyme, as the manganese isoform (normally exclusively mitochondrial) is also the main form in the cytosolic fraction in euphausiids (Brouwer *et al.*, 1997), the gene expression analysis allowed a separation of the cytosolic and mitochondrial

manganese isoform contribution (Chapter 7). If more genomic information become available for other euphausiid species, a joint comparison of molecular stress response will certainly be within the scope of future work, including the species from the ETP (as many samples were preserved in RNA*later*® and could still give great outputs). Even if the correspondence between metabolical and physiological proxies was often established and reinforced our interpretation of the species responses to the environmental stressors, the genomic part of the puzzle is missing for almost all the species and could provide more information about the on-going or missing evolutive processes.

The integrative comparison of metabolical, physiological and genetical proxies and the field experimental approaches of this thesis allowed a realistic interpretation of the euphausiids stress responses to hypoxia and warming on a global-scale.

Conclusions and perspectives

This study provides a comprehensive picture on cellular oxidative processes and adaptations in euphausiid species from different climatic regions. The overall metabolic strategies and OMZ tolerance mechanisms of euphausiids were hard to assess as many specific adaptations take place in each studied species from the different zoogeographical regions. More information about the tolerance of three of the most productive euphausiid species confirmed some of the hypothesis derived from their abundance and distribution during warming and/or hypoxic events.

The next steps, as raised by Dam (2013), would be to combine more tightly stress physiological and gene expression indicators of euphausiids from one productive region with their biology and ecology (vertical and horizontal distribution, growth, reproduction, etc.) by realizing monthly stratified vertical sampling during the day and the night at the same locations to associate stress physiological parameters to the diel vertical migration pattern and the environmental conditions. In parallel, a complete experimental set-up to evaluate growth, reproduction stages and tolerance to the synergic effects of hypoxia/reoxygenation with warming would help to identify weaknesses of the species link to their current oceanographic conditions. Then, it would be possible to predict with accuracy the survival and distribution of the species if important hypoxia intensification or warming episode occur, and to export this model to other similar ecosystems.

Bibliography

Abele, D., and R. Oeschger. 1995. Hypoxia-induced autoxidation of haemoglobin in the benthic invertebrates *Arenicola marina* (Polychaeta) and *Astarte borealis* (Bivalvia) and the possible effects of sulphide. Journal of Experimental Marine Biology and Ecology **187**: 63–80.

Abele, D., H. Großpietsch, and H. Pörtner. 1998. Temporal fluctuations and spatial gradients of environmental pO_2, temperature, H_2O_2 and H_2S in its intertidal habitat trigger enzymatic antioxidant protection in the capitellid worm *Heteromastus filiformis*. Marine Ecology Progress Series **163**: 179–191.

Abraham, C. L., and W. J. Sydeman. 2006. Prey-switching by Cassin's auklet *Ptychoramphus aleuticus* reveals seasonal climate-related cycles of *Euphausia pacifica* and *Thysanoessa spinifera*. Marine Ecology Progress Series **313**: 271–283.

Addabbo, F., M. Montagnani, and M. S. Goligorsky. 2009. Mitochondria and Reactive Oxygen Species. Hypertension **53**: 885–892.

Aebi, H. 1984. Catalase *in vitro*. Methods in enzymology **105**: 121–126.

Ahmad, S., and R. S. Pardini. 1988. Evidence for the presence of glutathione peroxidase activity toward an organic hydroperoxide in larvae of the cabbage looper moth, *Trichoplusia ni*. Insect biochemistry **18**: 861–866.

Andersen, C. L., J. L. Jensen, and T. F. Ørntoft. 2004. Normalization of real-time quantitative reverse transcription-PCR data: a model-based variance estimation approach to identify genes suited for normalization, applied to bladder and colon cancer data sets. Cancer research **64**: 5245–5250.

Antezana, T. 2002a. Adaptive behaviour of *Euphausia mucronata* in relation to the oxygen minimum layer of the Humboldt Current, pp. 29–40. *In* J. Färber-Lorda [ed.], Oceanography of the eastern Pacific. CICESE.

Antezana, T. 2002b. Vertical distribution and diel migration of *Euphausia mucronata* in the oxygen minimum layer of the Humboldt Current, pp. 13–28. *In* J. Färber-Lorda [ed.], Oceanography of the eastern Pacific. CICESE.

Antezana, T. 2009. Species-specific patterns of diel migration into the oxygen minimum zone by euphausiids in the Humboldt current ecosystem. Progress in Oceanography **83**: 228–236.

Antezana, T. 2010. *Euphausia mucronata* A keystone herbivore and prey of the Humboldt Current System. Deep Sea Research Part II: Topical Studies in Oceanography **57**: 652–662.

Aoki, S. 2005. Interdecadal water mass changes in the Southern Ocean between 30 °E and 160 °E. Geophysical Research Letters **32**: L07607.

Arístegui, J., C. M. Duarte, I. Reche, and J. L. Gómez-Pinchetti. 2014. Krill excretion boosts microbial activity in the Southern Ocean. PLoS ONE **9**: e89391.

Atkinson, A., M. Whitehouse, J. Priddle, G. C. Cripps, P. Ward, and M. A. Brandon. 2001. South Georgia, Antarctica: a productive, cold water, pelagic ecosystem. Marine ecology progress series **216**: 279–308.

Atkinson, A., B. Meyer, D. Stübing, W. Hagen, K. Schmidt, and U. Bathmann. 2002. Feeding and energy budgets of Antarctic krill *Euphausia superba* at the onset of winter-II. Juveniles and adults. Limnology and Oceanography **47**: 953–966.

Atkinson, A., V. Siegel, E. Pakhomov, and P. Rothery. 2004. Long-term decline in krill stock and increase in salps within the Southern Ocean. Nature **432**: 100–103.

Atkinson, A., R. S. Shreeve, A. G. Hirst, P. Rothery, G. A. Tarling, D. W. Pond, R. E. Korb, E. Murphy, and J. L. Watkins. 2006. Natural growth rates in Antarctic krill (*Euphausia superba*): II. Predictive models based on food, temperature, body length, sex, and maturity stage. Limnology and Oceanography **51**: 973–987.

Atkinson, A., V. Siegel, E. Pakhomov, P. Rothery, V. Loeb, R. Ross, L. Quetin, K. Schmidt, P. Fretwell, E. Murphy, G. Tarling, and A. Fleming. 2008. Oceanic circumpolar habitats of Antarctic krill. Marine Ecology Progress Series **362**: 1–23.

Baird, N., D. Turnbull, and E. Johnson. 2006. Induction of the heat shock pathway during hypoxia requires regulation of heat shock factor by hypoxia-inducible factor-1. Journal of Biological Chemistry **281**: 38675.

Barnes, D. K. A., V. Fuentes, A. Clarke, I. R. Schloss, and M. I. Wallace. 2006. Spatial and temporal variation in shallow seawater temperatures around Antarctica. Deep Sea Research Part II: Topical Studies in Oceanography **53**: 853–865.

Barnett, V., and T. Lewis. 1994. Outliers in Statistical Data, 3rd ed. John Wiley & Sons, Inc.

Bers, A. V., F. Momo, I. R. Schloss, and D. Abele. 2012. Analysis of trends and sudden changes in long-term environmental data from King George Island (Antarctica): relationships between global climatic oscillations and local system response. Climatic Change **116**: 789–803.

Bianchi, D., E. D. Galbraith, D. A. Carozza, K. A. S. Mislan, and C. A. Stock. 2013. Intensification of open-ocean oxygen depletion by vertically migrating animals. Nature Geoscience **6**: 545–548.

Bickler, P. E., and L. T. Buck. 2007. Hypoxia Tolerance in Reptiles, Amphibians, and Fishes: Life with Variable Oxygen Availability. Annual Review of Physiology **69**: 145–170.

Bishop, D. W. 1973. Respiration and metabolism. *In* C. L. Prosser [ed.], Comparative animal physiology. W.B. Saunders Company.

Bode, M., A. Schukat, W. Hagen, and H. Auel. 2013. Predicting metabolic rates of calanoid copepods. Journal of Experimental Marine Biology and Ecology **444**: 1–7.

Bollens, S. M., B. W. Frost, and T. S. Lin. 1992. Recruitment, growth, and diel vertical migration of *Euphausia pacifica* in a temperate fjord. Marine Biology **114**: 219–228.

Bonaventura, R., V. Poma, R. Russo, F. Zito, and V. Matranga. 2006. Effects of UV-B radiation on development and hsp70 expression in sea urchin cleavage embryos. Marine Biology **149**: 79–86.

Borrione, I., and R. Schlitzer. 2013. Distribution and recurrence of phytoplankton blooms around South Georgia, Southern Ocean. Biogeosciences **10**: 217–231.

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry **72**: 248–254.

Brand, M. D. 2000. Uncoupling to survive? The role of mitochondrial inefficiency in ageing. Experimental gerontology **35**: 811–820.

Brey, T. 2010. An empirical model for estimating aquatic invertebrate respiration. Methods in Ecology and Evolution **1**: 92–101.

Brey, T. 2012. A multi-parameter artificial neural network model to estimate macrobenthic invertebrate productivity and production. Limnology and Oceanography: Methods **10**: 581–589.

Brey, T., C. Müller-Wiegmann, Z. Zittier, and W. Hagen. 2010. Body composition in aquatic organisms—A global data bank of relationships between mass, elemental composition and energy content. Journal of Sea Research **64**: 334–340.

Bridges, C. R., A. Savel, W. Stocker, J. Markl, and B. Linzen. 1983. Structure and function of krill (*Euphausia superba*) haemocyanin - Adaption to life at low temperature, pp. 353–356. *In* E. J. Wood [ed.], Structure and function of invertebrate respiratory proteins. Life Chemistry Reports.

Brierley, A., and M. Cox. 2010. Shapes of krill swarms and fish schools emerge as aggregation members avoid predators and access oxygen. Current Biology **20**: 1758–1762.

Brinton, E. 1962. The distribution of Pacific euphausiids. Bulletin of the Scripps Institution of Oceanography **8**: 51–269.

Brinton, E. 1967. Vertical migration and avoidance capability of euphausiids in the California Current. Limnology and Oceanography **12**: 451–483.

Brinton, E. 1979. Parameters relating to the distributions of planktonic organisms, especially euphausiids in the eastern tropical Pacific. Progress in Oceanography **8**: 125–168.

Brinton, E., and A. Townsend. 2003. Decadal variability in abundances of the dominant euphausiid species in southern sectors of the California Current. Deep Sea Research Part II: Topical Studies in Oceanography **50**: 2449–2472.

Brinton, E., M. D. Ohman, A. Townsend, M. D. Knight, and A. L. Bridgeman. 2003, updated 2008. Euphausiids of the World Ocean. ETI Bioinformatics.

Brodeur, R. D., and W. G. Pearcy. 1992. Effects of environmental variability on trophic interactions and food web structure in a pelagic upwelling ecosystem. Marine Ecology Progress Series **84**: 101–119.

Brouwer, M., T. H. Brouwer, W. Grater, J. J. Enghild, and I. B. Thogersen. 1997. The paradigm that all oxygen-respiring eukaryotes have cytosolic CuZn-superoxide dismutase and that Mn-superoxide dismutase is localized to the mitochondria does not apply to a large group of marine arthropods. Biochemistry **36**: 13381–13388.

Brouwer, M., N. J. Brown-Peterson, P. Larkin, V. Patel, N. Denslow, S. Manning, and T. H. Brouwer. 2007. Molecular and whole animal responses of grass shrimp, *Palaemonetes pugio*, exposed to chronic hypoxia. Journal of Experimental Marine Biology and Ecology **341**: 16–31.

Brown, J. H., J. F. Gillooly, A. P. Allen, V. M. Savage, and G. B. West. 2004. Toward a metabolic theory of ecology. Ecology **85**: 1771–1789.

Brown, M., S. Kawaguchi, R. King, P. Virtue, and S. Nicol. 2011. Flexible adaptation of the seasonal krill maturity cycle in the laboratory. Journal of Plankton Research **33**: 821–826.

Brown, M., S. Kawaguchi, S. Candy, T. Yoshida, P. Virtue, and S. Nicol. 2013. Long-term effect of photoperiod, temperature and feeding regimes on the respiration rates of Antarctic krill (*Euphausia superba*). Open Journal of Marine Science **03**: 40–51.

Brown-Peterson, N. J., P. Larkin, N. Denslow, C. King, S. Manning, and M. Brouwer. 2005. Molecular indicators of hypoxia in the blue crab *Callinectes sapidus*. Marine Ecology Progress Series **286**: 203–215.

Brown-Peterson, N. J., C. S. Manning, V. Patel, N. D. Denslow, and M. Brouwer. 2008. Effects of cyclic hypoxia on gene expression and reproduction in a grass shrimp, *Palaemonetes pugio*. The Biological Bulletin **214**: 6–16.

Buchholz, F. 2003. Experiments on the physiology of southern and northern krill, *Euphausia superba* and *Meganyctiphanes norvegica*, with emphasis on moult and growth - a review. Marine and Freshwater Behaviour and Physiology **36**: 229–247.

Burnett, L. E., and W. B. Stickle. 2001. Physiological responses to hypoxia. Coastal and Estuarine Studies **58**: 101–114.

Bushnell, P. G., J. F. Steffensen, H. Schurmann, and D. R. Jones. 1994. Exercise metabolism in two species of cod in arctic waters. Polar Biology **14**: 43–48.

Buttemer, W. A., D. Abele, and D. Costantini. 2010. From bivalves to birds: oxidative stress and longevity. Functional Ecology **24**: 971–983.

Catalgol, B., S. Grimm, and T. Grune. 2011. Protein carbonyl measurement by enzyme linked immunosorbent assay, pp. 432–439. *In* D. Abele, T. Zenteno-Savín, and J. P. Vázquez-Medina [eds.], Oxidative Stress in Aquatic Ecosystems. John Wiley & Sons, Ltd.

Chacinska, A., C. M. Koehler, D. Milenkovic, T. Lithgow, and N. Pfanner. 2009. Importing mitochondrial proteins: machineries and mechanisms. Cell **138**: 628–644.

Chan, F., J. A. Barth, J. Lubchenco, A. Kirincich, H. Weeks, W. T. Peterson, and B. A. Menge. 2008. Emergence of anoxia in the California current large marine ecosystem. Science **319**: 920–920.

Charpentier, J., D. Mediavilla, and O. Pizarro. 2012. Modeling the seasonal cycle of the oxygen minimum zone over the continental shelf off Concepción, Chile (36.5° S). Biogeosciences Discussions **9**: 7227–7256.

Chavez, F. P., and M. Messié. 2009. A comparison of Eastern Boundary Upwelling Ecosystems. Progress in Oceanography **83**: 80–96.

Chenillat, F., P. Rivière, X. Capet, E. Di Lorenzo, and B. Blanke. 2012. North Pacific Gyre Oscillation modulates seasonal timing and ecosystem functioning in the California Current upwelling system. Geophysical Research Letters **39**: L01606.

Childress, J. 1975. The respiratory rates of midwater crustaceans as a function of depth of occurrence and relation to the oxygen minimum layer off southern California. Comparative Biochemistry and Physiology Part A: Physiology **50**: 787–799.

Clanton, T. L. 2007. Hypoxia-induced reactive oxygen species formation in skeletal muscle. Journal of Applied Physiology **102**: 2379–2388.

Clark, M., M. Thorne, J. Toullec, Y. Meng, L. Peck, and S. Moore. 2011. Antarctic krill 454 pyrosequencing reveals chaperone and stress transcriptome. PLoS ONE **6**: e15919.

Clark, M. S., G. Husmann, M. A. S. Thorne, G. Burns, M. Truebano, L. S. Peck, D. Abele, and E. E. R. Philipp. 2013. Hypoxia impacts large adults first: consequences in a warming world. Global Change Biology **19**: 2251–2263.

Clarke, A., and D. Morris. 1983. Towards an energy budget for krill: the physiology and biochemistry of *Euphausia superba* Dana. Polar Biology **2**: 69–86.

Connolly, T., B. Hickey, S. Geier, and W. Cochlan. 2010. Processes influencing seasonal hypoxia in the northern California Current System. Journal of Geophysical Research **115**: C03021.

Cooke, M. 2003. Oxidative DNA damage: mechanisms, mutation, and disease. The Federation of American Societies for Experimental Biology Journal **17**: 1195–1214.

Copin-Montégut, C., and P. Raimbault. 1994. The Peruvian upwelling near 15°S in August 1986. Results of continuous measurements of physical and chemical properties between 0 and 200 m depth. Deep Sea Research Part I: Oceanographic Research Papers **41**: 439–467.

Dam, H. G. 2013. Evolutionary adaptation of marine zooplankton to global change. Annual Review of Marine Science **5**: 349–370.

D'Amato, M. E., G. W. Harkins, T. Oliveira, P. R. Teske, and M. J. Gibbons. 2008. Molecular dating and biogeography of the neritic krill *Nyctiphanes*. Marine Biology **155**: 243–247.

de Almeida, E. A., D. G. Humberto Silva, A. C. Dias Bainy, F. P. Freitas, F. D. Motta, O. F. Gomes, M. H. Genneri de Medeiros, and P. Di Mascio. 2011. Evaluation of glutathione status in aquatic organisms, pp. 381–388. *In* D. Abele, T. Zenteno-Savín, and J. P. Vázquez-Medina [eds.], Oxidative stress in aquatic ecosystems. John Wiley & Sons, Ltd.

de Oliveira, U. O., A. S. da Rosa Araújo, A. Belló-Klein, R. S. M. da Silva, and L. C. Kucharski. 2005. Effects of environmental anoxia and different periods of reoxygenation on oxidative balance in gills of the estuarine crab *Chasmagnathus granulata*. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology **140**: 51–57.

Dehairs, F., L. Goeyens, N. Stroobants, P. Bernard, C. Goyet, A. Poisson, and R. Chesselet. 1990. On suspended barite and the oxygen minimum in the Southern Ocean. Global Biogeochemical cycles **4**: 85–102.

Di Lorenzo, E., N. Schneider, K. M. Cobb, P. J. S. Franks, K. Chhak, A. J. Miller, J. C. McWilliams, S. Bograd, H. Arango, E. Curchitser, T. M. Powell, and P. Rivière. 2008. North Pacific Gyre Oscillation links ocean climate and ecosystem change. Geophysical Research Letters **35**: L08607.

Diaz, R. J., and R. Rosenberg. 2008. Spreading dead zones and consequences for marine ecosystems. Science **321**: 926–929.

Donnelly, J., H. Kawall, S. P. Geiger, and J. J. Torres. 2004. Metabolism of Antarctic micronektonic crustacea across a summer ice-edge bloom: respiration, composition, and enzymatic activity. Deep Sea Research Part II: Topical Studies in Oceanography **51**: 2225–2245.

Donoso, K., and R. Escribano. 2014. Mass-specific respiration of mesozooplankton and its role in the maintenance of an oxygen-deficient ecological barrier (BEDOX) in the upwelling zone off Chile upon presence of a shallow oxygen minimum zone. Journal of Marine Systems **129**: 166–177.

Doyle, S. M., and S. Wickner. 2009. Hsp104 and ClpB: protein disaggregating machines. Trends in Biochemical Sciences **34**: 40–48.

Draper, N. R., and H. Smith. 1998. Applied regression analysis. Wiley-Interscience.

Drossos, G., A. Lazou, P. Panagopoulos, and S. Westaby. 1995. Deferoxamine cardioplegia reduces superoxide radical production in human myocardium. The Annals of thoracic surgery **59**: 169–172.

Dunlap, W., A. Fujisawa, Y. Yamamoto, T. Moylan, and B. Sidell. 2002. Notothenioid fish, krill and phytoplankton from Antarctica contain a vitamin E constituent (α-tocomonoenol) functionally associated with cold-water adaptation. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology **133**: 299–305.

Dymowska, A. K., T. Manfredi, J. J. C. Rosenthal, and B. A Seibel. 2012. Temperature compensation of aerobic capacity and performance in the Antarctic pteropod, *Clione antarctica*, compared with its northern congener, *C. limacina*. Journal of Experimental Biology **215**: 3370–3378.

Emelyanov, E. 2005. The barrier zones in the ocean. Springer Verlag.

Enright, J. T. 1977. Diurnal vertical migration: adaptive significance and timing. Part 1. Selective advantage: a metabolic model. Limnology and Oceanography **22**: 856–872.

Escribano, R., V. Marín, and C. Irribarren. 2000. Distribution of *Euphausia mucronata* at the upwelling area of Peninsula Mejillones, northern Chile: the influence of the oxygen minimum layer. Scientia Marina **64**: 69–77.

Escribano, R., G. Daneri, L. Farías, V. A. Gallardo, H. E. González, D. Gutiérrez, C. B. Lange, C. E. Morales, O. Pizarro, and O. Ulloa. 2004. Biological and chemical consequences of the 1997–1998 El Niño in the Chilean coastal upwelling system: a synthesis. Deep Sea Research Part II: Topical Studies in Oceanography **51**: 2389–2411.

Escribano, R., P. Hidalgo, H. González, R. Giesecke, R. Riquelme-Bugueño, and K. Manríquez. 2007. Seasonal and inter-annual variation of mesozooplankton in the coastal upwelling zone off central-southern Chile. Progress in Oceanography **75**: 470–485.

Fach, B. A., E. E. Hofmann, and E. J. Murphy. 2006. Transport of Antarctic krill (*Euphausia superba*) across the Scotia Sea. Part II: Krill growth and survival. Deep Sea Research Part I: Oceanographic Research Papers **53**: 1011–1043.

Falk-Petersen, S. 1981. Ecological investigations on the zooplankton community of Balsfjorden, northern Norway: Seasonal changes in body weight and the main biochemical composition of *Thysanoessa inermis* (krøyer), *T. Raschii* (M. Sars), and *Meganyctiphanes norvegica* (M. Sars) in relation to environmental factors. Journal of Experimental Marine Biology and Ecology **49**: 103–120.

Färber-Lorda, J., M. F. Lavín, M. A. Zapatero, and J. M. Robles. 1994. Distribution and abundance of euphausiids in the Gulf of Tehuantepec during wind forcing. Deep Sea Research Part I: Oceanographic Research Papers **41**: 359–367.

Färber-Lorda, J., A. Trasviña, and P. Cortés-Verdín. 2004. Trophic conditions and zooplankton distribution in the entrance of the Sea of Cortés during summer. Deep Sea Research Part II: Topical Studies in Oceanography **51**: 615–627.

Färber-Lorda, J., A. Trasviña, and P. Cortés-Verdín. 2010. Summer distribution of euphausiids in the entrance of the Sea of Cortés in relation to hydrography. Deep Sea Research Part II: Topical Studies in Oceanography **57**: 631–641.

Fernández-Álamo, M., and J. Färber-Lorda. 2006. Zooplankton and the oceanography of the eastern tropical Pacific: a review. Progress in Oceanography **69**: 318–359.

Fiedler, P., and L. Talley. 2006. Hydrography of the eastern tropical Pacific: A review. Progress in Oceanography **69**: 143–180.

Fisheries and Oceans Canada Ministry. 2013. Pacific region - Integrated fisheries management plan - Euphausiids - January 1, 2013 to December 31, 2017. Tech. rep., Fisheries and Oceans Canada.

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

Fridovich, I. 2004. Mitochondria: are they the seat of senescence? Aging Cell **3**: 13–16.

Garcia, H., and L. Gordon. 1992. Oxygen solubility in seawater: Better fitting equations. Limnology and Oceanography **37**: 1307–1312.

García-Reyes, M., J. L. Largier, and W. J. Sydeman. 2014. Synoptic-scale upwelling indices and predictions of phyto-and zooplankton populations. Progress in Oceanography **120**: 177–188.

Gaten, E., G. Tarling, H. Dowse, C. Kyriacou, and E. Rosato. 2008. Is vertical migration in Antarctic krill (*Euphausia superba*) influenced by an underlying circadian rhythm? Journal of genetics **87**: 473–483.

Gilfillan, E. 1972. Reactions of *Euphausia pacifica* Hansen (Crustacea) from oceanic, mixed oceanic-coastal and coastal waters of British Columbia to experimental changes in temperature and salinity. Journal of Experimental Marine Biology and Ecology **10**: 29–40.

Gilly, W. F., U. Markaida, C. H. Baxter, B. Block, A. Boustany, L. Zeidberg, K. Reisenbichler, B. Robison, G. Bazzino, and C. Salinas. 2006. Vertical and horizontal migrations by the jumbo squid *Dosidicus gigas* revealed by electronic tagging. Marine Ecology Progress Series **324**: 1–17.

Gilly, W. F., J. M. Beman, S. Y. Litvin, and B. H. Robison. 2013. Oceanographic and biological effects of shoaling of the oxygen minimum zone. Annual Review of Marine Science **5**: 393–420.

Giraudoux, P. 2013. pgirmess: Data analysis in ecology.

Gómez-Gutiérrez, J. 2002. Hatching mechanism and delayed hatching of the eggs of three broadcast spawning euphausiid species under laboratory conditions. Journal of Plankton Research **24**: 1265–1276.

Gómez-Gutiérrez, J. 2003. Hatching mechanism and accelerated hatching of the eggs of a sac-spawning euphausiid *Nematoscelis difficilis*. Journal of Plankton Research **25**: 1397–1411.

Gómez-Gutiérrez, J., R. De Silva-Dávila, and B. Lavaniegos-Espejo. 1996. Growth production of the euphausiid *Nyctiphanes simplex* on the coastal shelf off Bahía Magdalena, Baja California Sur, México. Marine Ecology Progress Series **138**: 309–314.

Gómez-Gutiérrez, J., L. Feinberg, and T. Shaw. 2007. Interannual and geographical variability of the brood size of the euphausiids *Euphausia pacifica* and *Thysanoessa spinifera* along the Oregon coast (1999-2004). Deep Sea Research Part I: Oceanographic Research Papers **54**: 2145–2169.

Gómez-Gutiérrez, J., C. Rodríguez-Jaramillo, J. Del Ángel-Rodríguez, C. Robinson, C. Zavala-Hernández, S. Martínez-Gómez, and N. Tremblay. 2010. Biology of the subtropical sac-spawning euphausiid *Nyctiphanes simplex* in the northwestern seas of Mexico: Interbrood period, gonad development, and lipid content. Deep Sea Research Part II: Topical Studies in Oceanography **57**: 616–630.

Gómez-Gutiérrez, J., S. Martínez-Gómez, and C. J. Robinson. 2012. Seasonal growth, molt, and egg production rates of *Nyctiphanes simplex* (Crustacea: Euphausiacea) juveniles and adults in the Gulf of California. Marine Ecology Progress Series **455**: 173–194.

Grant, S. M., S. L. Hill, and P. T. Fretwell. 2013. Spatial distribution of management measures, Antarctic krill catch and Southern Ocean bioregions: implications for conservation planning. Convention on the Conservation of Antarctic Marine Living Resources Science **20**: 1–19.

Guderley, H. 2004. Metabolic responses to low temperature in fish muscle. Biological Reviews **79**: 409–427.

Guderley, H., and J. St-Pierre. 2002. Going with the flow or life in the fast lane: contrasting mitochondrial responses to thermal change. The Journal of Experimental Biology **205**: 2237–2249.

Guzy, R., and P. Schumacker. 2006. Oxygen sensing by mitochondria at complex III: the paradox of increased reactive oxygen species during hypoxia. Experimental Physiology **91**: 807–819.

Habig, W. H., and W. B. Jakoby. 1981. Assays for differentiation of glutathione S-transferases. Methods in enzymology **77**: 398–405.

Hagan, M. T., H. B. Demuth, and M. H. Beale. 1996. Neural Network Design. PWS Publishing.

Halliwell, B., and J. Gutteridge. 2007. Free radicals in biology and medicine, 4th ed. Oxford University Press.

Hamanaka, R. B., and N. S. Chandel. 2009. Mitochondrial reactive oxygen species regulate hypoxic signaling. Current Opinion in Cell Biology **21**: 894–899.

Hamner, W., and P. Hamner. 2000. Behavior of Antarctic krill (*Euphausia superba*): schooling, foraging, and antipredatory behavior. Canadian Journal of Fisheries and Aquatic Sciences **57**: 192–202.

Haraldsson, M., and V. Siegel. 2014. Seasonal distribution and life history of *Thysanoessa macrura* (Euphausiacea, Crustacea) in high latitude waters of the Lazarev Sea, Antarctica. Marine Ecology Progress Series **495**: 105–118.

Harrell, Jr, F. E. 2014. Package 'Hmisc'.

Hastie, T., and R. Tibshirani. 1990. Generalized Additive Models. Chapman & Hall.

Helly, J. J., and L. A. Levin. 2004. Global distribution of naturally occurring marine hypoxia on continental margins. Deep Sea Research Part I: Oceanographic Research Papers **51**: 1159–1168.

Herlinveaux, R. H. 1962. Oceanography of Saanich Inlet in Vancouver Island, British Columbia. Journal of the Fisheries Research Board of Canada **19**: 1–37.

Hermes-Lima, M. 2004. Oxygen in biology and biochemistry: role of free radicals, pp. 319–368. *In* K. Storey [ed.], Functional metabolism: regulation and adaptation. John-Wiley & Sons, Inc.

Hill, A. D., A. C. Taylor, and R. Strang. 1991. Physiological and metabolic responses of the shore crab *Carcinus maenas* (L.) during environmental anoxia and subsequent recovery. Journal of Experimental Marine Biology and Ecology **150**: 31–50.

Hill, S. L., T. Phillips, and A. Atkinson. 2013. Potential Climate Change Effects on the Habitat of Antarctic Krill in the Weddell Quadrant of the Southern Ocean. PLoS ONE **8**: e72246.

Hochachka, P., and P. Lutz. 2001. Mechanism, origin, and evolution of anoxia tolerance in animals. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology **130**: 435–459.

Hoffman, D. L., J. D. Salter, and P. S. Brookes. 2007. Response of mitochondrial reactive oxygen species generation to steady-state oxygen tension: implications for hypoxic cell signaling. American journal of physiology. Heart and circulatory physiology **292**: H101–8.

Hofmann, G. E. 1999. Ecologically relevant variation in induction and function of heat shock proteins in marine organisms. Integrative and Comparative Biology **39**: 889–900.

Hogg, O. T., D. K. A. Barnes, and H. J. Griffiths. 2011. Highly diverse, poorly studied and uniquely threatened by climate change: an assessment of marine biodiversity on South Georgia's continental shelf. PLoS ONE **6**: e19795.

Hünerlage, K., and F. Buchholz. 2013. Krill of the northern Benguela Current and the Angola-Benguela frontal zone compared: physiological performance and short-term starvation in *Euphausia hanseni*. Journal of Plankton Research **35**: 337–351.

Ikeda, T. 1974. Nutritional ecology of marine zooplankton. Memoirs of the Faculty of Fisheries Hokkaido University **22**: 1–97.

Ikeda, T. 1977. The effect of laboratory conditions on the extrapolation of experimental measurements to the ecology of marine zooplankton II. Effect of oxygen saturation on the respiration rate . Bulletin of Plankton Society of Japan **24**: 19–28.

Ikeda, T. 1985. Metabolic rates of epipelagic marine zooplankton as a function of body mass and temperature. Marine Biology **85**: 1–11.

Ikeda, T. 2012. Respiration and ammonia excretion of euphausiid crustaceans: synthesis toward a global-bathymetric model. Marine Biology **160**: 251–262.

Ikeda, T., and A. D. McKinnon. 2012. Metabolism and chemical composition of zooplankton and hyperbenthos from the Great Barrier Reef waters, North Queensland, Australia. Plankton and Benthos Research **7**: 8–19.

Ikeda, T., J. Torres, S. Hernández-León, and S. Geiger. 2000. Metabolism, pp. 455–532. *In* R. Harris, P. Wiebe, J. Lenz, H. Skjoldal, and M. Huntley [eds.], International Council for the Exploration of the Sea (ICES) Zooplankton Methodology Manual. Academic Press.

Ito, T., and C. Deutsch. 2013. Variability of the oxygen minimum zone in the tropical North Pacific during the late twentieth century. Global Biogeochemical cycles **27**: 1119–1128.

Ivleva, I. 1980. The dependence of crustacean respiration rate on body-mass and habitat temperature. Internationale revue der gesamten hydrobiologie **65**: 1–47.

Jarman, S., N. Elliott, S. Nicol, and A. Mcminn. 2000. Molecular phylogenetics of circumglobal *Euphausia* species (Euphausiacea: Crustacea). Canadian Journal of Fisheries and Aquatic Sciences **57**: 51–58.

Joehanes, R., and J. C. Nelson. 2008. QGene 4.0, an extensible Java QTL-analysis platform. Bioinformatics **24**: 2788–2789.

Johnston, I., J. Calvo, H. GuderleyY, and d. 1998. Latitudinal variation in the abundance and oxidative capacities of muscle mitochondria in perciform fishes. Journal of Experimental Biology **201**: 1–12.

Joyner-Matos, J., L. Chapman, C. Downs, T. Hofer, C. Leeuwenburgh, and D. Julian. 2007. Stress response of a freshwater clam along an abiotic gradient: too much oxygen may limit distribution. Functional Ecology **21**: 344–355.

Judkins, D. C. 1980. Vertical distribution of zooplankton in relation to the oxygen minimum off Peru. Deep Sea Research Part A. Oceanographic Research Papers **27**: 475–487.

Kamykowski, D., and S. Zentara. 1990. Hypoxia in the world ocean as recorded in the historical data set. Deep Sea Research Part A. Oceanographic Research Papers **37**: 1861–1874.

Kang, P. J., J. Ostermann, J. Shilling, W. Neupert, E. A. Craig, and N. Pfanner. 1990. Requirement for Hsp70 in the mitochondrial matrix for translocation and folding of precursor proteins. Nature **348**: 137–143.

Karstensen, J., L. Stramma, and M. Visbeck. 2008. Oxygen minimum zones in the eastern tropical Atlantic and Pacific oceans. Progress in Oceanography **77**: 331–350.

Kawaguchi, S., T. Yoshida, L. Finley, P. Cramp, and S. Nicol. 2007. The krill maturity cycle: a conceptual model of the seasonal cycle in Antarctic krill. Polar Biology **30**: 689–698.

Kawaguchi, S., H. Kurihara, R. King, L. Hale, T. Berli, J. P. Robinson, A. Ishida, M. Wakita, P. Virtue, S. Nicol, and A. Ishimatsu. 2010. Will krill fare well under Southern Ocean acidification? Biology Letters doi:10.1098/rsbl.2010.0777.

Kawaguchi, S., A. Ishida, R. King, B. Raymond, N. Waller, A. Constable, S. Nicol, M. Wakita, and A. Ishimatsu. 2013. Risk maps for Antarctic krill under projected Southern Ocean acidification. Nature Climate Change **3**: 1–5.

Kennett, J. P. 1982. Marine geology. Prentice-Hall.

Koslow, J. A., R. Goericke, A. Lara-Lopez, and W. Watson. 2011. Impact of declining intermediate-water oxygen on deepwater fishes in the California Current. Marine Ecology Progress Series **436**: 207–218.

Kültz, D. 2005. Molecular and evolutionary basis of the cellular stress response. Annual Review of Physiology **67**: 225–257.

Kunze, E., J. Dower, I. Beveridge, R. Dewey, and K. Bartlett. 2006. Observations of biologically generated turbulence in a coastal inlet. Science **313**: 1768–1770.

Lavaniegos, B., and I. Ambriz-Arreola. 2012. Interannual variability in krill off Baja California in the period 1997-2005. Progress in Oceanography **97**: 164–173.

Lavín, M. F., P. C. Fiedler, J. A. Amador, L. T. Ballance, J. Färber-Lorda, and A. M. Mestas-Nuñez. 2006. A review of eastern tropical Pacific oceanography: Summary. Progress in Oceanography **69**: 391–398.

Lee, S. H., H. M. Kwon, Y. J. Kim, K. M. Lee, M. Kim, and B. W. Yoon. 2004. Effects of Hsp70.1 gene knockout on the mitochondrial apoptotic pathway after focal cerebral ischemia. Stroke **35**: 2195–2199.

Letessier, T., M. Cox, and A. Brierley. 2011. Drivers of variability in Euphausiid species abundance throughout the Pacific Ocean. Journal of Plankton Research **33**: 1342–1357.

Lindquist, S. 1986. The heat-shock response. Annual review of biochemistry **55**: 1151–1191.

López-Cortés, D. J., J. J. Bustillos-Guzmán, and I. Gárate-Lizárraga. 2006. Unusual mortality of krill (Crustacea: Euphausiacea) in Bahía de La Paz, Gulf of California 1. Pacific Science **60**: 235–242.

Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry **193**: 265–275.

Lushchak, V. I. 2011. Environmentally induced oxidative stress in aquatic animals. Aquatic toxicology **101**: 13–30.

Luyten, J. R., J. Pedlosky, and H. Stommel. 1983. The ventilated thermocline. Journal of Physical Oceanography **13**: 292–309.

Mackas, D. L., R. Kieser, M. Saunders, D. R. Yelland, R. M. Brown, and D. F. Moore. 1997. Aggregation of euphausiids and Pacific hake (*Merluccius productus*) along the outer continental shelf off Vancouver Island. Journal of the Fisheries Board of Canada **54**: 2080–2096.

Mangel, M., and S. Nicol. 2000. Krill and the unity of biology. Canadian Journal of Fisheries and Aquatic Sciences **57**: 1–5.

Marshall, O. J. 2004. PerlPrimer: cross-platform, graphical primer design for standard, bisulphite and real-time PCR. Bioinformatics **20**: 2471–2472.

Matear, R., A. Hirst, and B. McNeil. 2000. Changes in dissolved oxygen in the Southern Ocean with climate change. Geochemistry Geophysics Geosystems **1**: 1050.

McLaren, I. A. 1963. Effects of temperature on growth of zooplankton, and the adaptive value of vertical migration. Journal of the Fisheries Research Board of Canada **20**: 685–727.

McWhinnie, M. A. 1964. Temperature responses and tissue respiration in Antarctic Crustacea with particular reference to the krill *Euphausia superba*. Antarctic research series **1**: 63–72.

Meredith, M., E. Murphy, E. Hawker, J. King, and M. Wallace. 2008. On the interannual variability of ocean temperatures around South Georgia, Southern Ocean: Forcing by El Niño/Southern Oscillation and the southern annular mode. Deep Sea Research Part II: Topical Studies in Oceanography **55**: 2007–2022.

Meyer, B. 2011. The overwintering of Antarctic krill, *Euphausia superba*, from an ecophysiological perspective. Polar Biology **35**: 15–37.

Meyer, B., R. Saborowski, A. Atkinson, F. Buchholz, and U. Bathmann. 2002. Seasonal differences in citrate synthase and digestive enzyme activity in larval and postlarval antarctic krill, *Euphausia superba*. Marine Biology **141**: 855–862.

Meyer, B., L. Auerswald, V. Siegel, C. Spahic, C. Pape, B. Fach, M. Teschke, A. Lopata, and V. Fuentes. 2010. Seasonal variation in body composition, metabolic activity, feeding, and growth of adult krill *Euphausia superba* in the Lazarev Sea. Marine Ecology Progress Series **398**: 1–18.

Moline, M. A., H. Claustre, T. K. Frazer, O. Schofield, and M. Vernet. 2004. Alteration of the food web along the Antarctic Peninsula in response to a regional warming trend. Global Change Biology **10**: 1973–1980.

Morales, C. E., S. E. Hormazábal, and J. Blanco. 1999. Interannual variability in the mesoscale distribution of the depth of the upper boundary of the oxygen minimum layer off northern Chile (18-24°S): Implications for the pelagic system and biogeochemical cycling. Journal of Marine Research **57**: 909–932.

Moran, L., M. E. Mirault, A. P. Arrigo, M. Goldschmidt-Clermont, and A. Tissieres. 1978. Heat shock of *Drosophila melanogaster* induces the synthesis of new messenger RNAs and proteins. Philosophical Transactions of the Royal Society B: Biological Sciences **283**: 391–406.

Morimoto, R. I. 1993. Cells in stress: transcriptional activation of heat shock genes. Science **259**: 1409–1410.

Morley, S., H. Griffiths, D. Barnes, and L. Peck. 2010. South Georgia: a key location for linking physiological capacity to distributional changes in response to climate change. Antarctic Science **22**: 774–781.

Morley, S. A., G. J. Lurman, J. N. Skepper, H.-O. Pörtner, and L. S. Peck. 2009. Thermal plasticity of mitochondria: a latitudinal comparison between Southern Ocean molluscs. Comparative Biochemistry and Physiology-Part A: Molecular & Integrative Physiology **152**: 423–430.

Mukhopadhyay, I., A. Nazir, D. K. Saxena, and D. K. Chowdhuri. 2003. Heat shock response: hsp70 in environmental monitoring. Journal of Biochemical and Molecular Toxicology **17**: 249–254.

Muller, P. Y., H. Janovjak, A. R. Miserez, and Z. Dobbie. 2002. Short technical report processing of gene expression data generated by quantitative Real-Time RT-PCR. Biotechniques **32**: 1372–1379.

Müller, R. 2003. Wege der Temperaturanpassung bei marinen Crustaceen aus verschiedenen Klimazonen: Mechanismen der qualitativen und quantitativen Enzymregulation am Beispiel der Citratsynthase. Ph.D. thesis, Universität Hamburg.

Murphy, E., J. L. Watkins, P. N. Trathan, K. Reid, M. Meredith, S. E. Thorpe, N. M. Johnston, A. Clarke, G. A. Tarling, M. A. Collins, J. Forcada, R. S. Shreeve, A. Atkinson, R. Korb, M. Whitehouse, P. Ward, P. G. Rodhouse, P. Enderlein, A. G. Hirst, A. R. Martin, S. L. Hill, I. J. Staniland, D. W. Pond, D. R. Briggs, N. J. Cunningham, and A. H. Fleming. 2007. Spatial and temporal operation of the Scotia Sea ecosystem: a review of large-scale links in a krill centred food web. Philosophical Transactions of the Royal Society B: Biological Sciences **362**: 113–148.

Murphy, M. P. 2009. How mitochondria produce reactive oxygen species. Biochemical Journal **417**: 1–13.

Nicol, S., A. J. Constable, and T. Pauly. 2000. Estimates of circumpolar abundance of Antarctic krill based on recent acoustic density measurements. Commission for the Conservation of Antarctic Marine Living Resources Science **7**: 87–99.

Niu, P., L. Liu, Z. Gong, H. Tan, F. Wang, J. Yuan, Y. Feng, Q. Wei, R. M. Tanguay, and T. Wu. 2006. Overexpressed heat shock protein 70 protects cells against DNA damage caused by ultraviolet C in a dose-dependent manner. Cell stress & chaperones **11**: 162–169.

Nixon, S., and A. Thomas. 2001. On the size of the Peru upwelling ecosystem. Deep Sea Research Part I: Oceanographic Research Papers **48**: 2521–2528.

Norkko, A., S. F. Thrush, V. J. Cummings, M. M. Gibbs, N. L. Andrew, J. Norkko, and A.-M. Schwarz. 2007. Trophic structure of coastal Antarctic food webs associated with changes in sea ice and food supply. Ecology **88**: 2810–2820.

Nowacek, D. P., A. S. Friedlaender, P. N. Halpin, E. L. Hazen, D. W. Johnston, A. J. Read, B. Espinasse, M. Zhou, and Y. Zhu. 2011. Super-aggregations of krill and humpback whales in Wilhelmina Bay, Antarctic Peninsula. PLoS ONE **6**: e19173.

Ohman, M. D. 1984. Omnivory by *Euphausia pacifica*: the role of copepod prey. Marine Ecology Progress Series **19**: 125–131.

Olson, D., G. Hitchcock, R. Fine, and B. Warren. 1993. Maintenance of the low-oxygen layer in the central Arabian Sea. Deep Sea Research Part II: Topical Studies in Oceanography **40**: 673–685.

Pape, C., M. Teschke, and B. Meyer. 2008. Melatonin and its possible role in mediating seasonal metabolic changes of Antarctic krill, *Euphausia superba*. Comparative Biochemistry and Physiology-Part A: Molecular & Integrative Physiology **149**: 426–434.

Paranjape, M. A. 1967. Molting and respiration of euphausiids. Journal of the Fisheries Board of Canada **24**: 1229–1240.

Patarnello, T., L. Bargelloni, V. Varotto, and B. Battaglia. 1996. Krill evolution and the Antarctic ocean currents: evidence of vicariant speciation as inferred by molecular data. Marine Biology **126**: 603–608.

Patarnello, T., C. Papetti, and L. Zane. 2010. Genetics of Northern Krill (*Meganyctiphanes norvegica Sars*), pp. 41–57. *In* G. A. Tarling [ed.], Advances in Marine Biology. Academic Press.

Paulmier, A., and D. Ruiz-Pino. 2009. Oxygen minimum zones (OMZs) in the modern ocean. Progress in Oceanography **80**: 113–128.

Paulmier, A., D. Ruiz-Pino, V. Garçon, and L. Farías. 2006. Maintaining of the eastern south Pacific oxygen minimum zone (OMZ) off Chile. Geophysical Research Letters **33**: L20601.

Pennington, J., K. Mahoney, V. Kuwahara, D. Kolber, R. Calienes, and F. Chavez. 2006. Primary production in the eastern tropical Pacific: A review. Progress in Oceanography **69**: 285–317.

Peterson, J. O., C. A. Morgan, W. T. Peterson, and E. Di Lorenzo. 2013. Seasonal and interannual variation in the extent of hypoxia in the northern California Current from 1998–2012. Limnology and Oceanography **58**: 2279–2292.

Peterson, W. T., and D. L. Mackas. 2001. Shifts in zooplankton abundance and species composition off central Oregon and southwestern British Columbia. PICES (The North Pacific Marine Science Organization) Press **9**: 28–31.

Philipp, E., T. Brey, H.-O. Pörtner, and D. Abele. 2005a. Chronological and physiological ageing in a polar and a temperate mud clam. Mechanisms of ageing and development **126**: 598–609.

Philipp, E., H.-O. Pörtner, and D. Abele. 2005b. Mitochondrial ageing of a polar and a temperate mud clam. Mechanisms of ageing and development **126**: 610–619.

Philipp, E. E. R., and D. Abele. 2010. Masters of longevity: lessons from long-lived bivalves – A mini-review. Gerontology **56**: 55–65.

Piano, A., C. Asirelli, F. Caselli, and E. Fabbri. 2002. Hsp70 expression in thermally stressed *Ostrea edulis*, a commercially important oyster in Europe. Cell stress & chaperones **7**: 250–257.

Pinchuk, A. I., K. O. Coyle, and R. R. Hopcroft. 2008. Climate-related variability in abundance and reproduction of euphausiids in the northern Gulf of Alaska in 1998–2003. Progress in Oceanography **77**: 203–216.

Pöhlmann, K., S. Koenigstein, K. Alter, D. Abele, and C. Held. 2011. Heat-shock response and antioxidant defense during air exposure in Patagonian shallow-water limpets from different climatic habitats. Cell stress & chaperones **16**: 621–632.

Pörtner, H. O. 2010. Oxygen- and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. Journal of Experimental Biology **213**: 881–893.

Prince, E. D., and C. P. Goodyear. 2006. Hypoxia-based habitat compression of tropical pelagic fishes. Fisheries Oceanography **15**: 451–464.

Pritchard, H. D., S. R. M. Ligtenberg, H. A. Fricker, D. G. Vaughan, M. R. van den Broeke, and L. Padman. 2012. Antarctic ice-sheet loss driven by basal melting of ice shelves. Nature **484**: 502–505.

Prokhorov, A. V. 2001. Hotelling-T^2-distribution. *In* M. Hazewinkel [ed.], Encyclopedia of Mathematics. Springer.

Pyza, E., P. Mak, P. Kramarz, and R. Laskowski. 1997. Heat shock proteins (HSP70) as biomarkers in ecotoxicological studies. Ecotoxicology and Environmental Safety **38**: 244–251.

Quetin, L., and R. Ross. 1989. Effects of oxygen, temperature and age on the metabolic rate of the embryos and early larval stages of the Antarctic krill *Euphausia superba* Dana. Journal of Experimental Marine Biology and Ecology **125**: 43–62.

R Core Team. 2012. R: A language and environment for statistical computing. Vienna, Austria.

Rabalais, N. N., R. E. Turner, and W. J. Wiseman. 2002. Gulf of Mexico hypoxia, A.K.A. "The dead zone". Annual Review of Ecology and Systematics **33**: 235–263.

Ramaglia, V., and L. T. Buck. 2004. Time-dependent expression of heat shock proteins 70 and 90 in tissues of the anoxic western painted turtle. The Journal of Experimental Biology **207**: 3775–3784.

Regoli, F., M. Benedetti, A. Krell, and D. Abele. 2011. Oxidative challenges in polar seas, pp. 20–40. *In* D. Abele, T. Zenteno-Savín, and J. P. Vázquez-Medina [eds.], Oxidative Stress in Aquatic Ecosystems. Wiley-Blackwell.

Reid, J. L. 1965. Intermediate waters of the Pacific Ocean, vol. 2. Johns Hopkins Oceanographic Study.

Richardson, A. J. 2008. In hot water: zooplankton and climate change. International Council for the Exploration of the Sea (ICES) Journal of Marine Science **65**: 279–295.

Rignot, E., S. Jacobs, J. Mouginot, and B. Scheuchl. 2013. Ice-shelf melting around Antarctica. Science **341**: 266–270.

Rinehart, J. P., A. Li, G. D. Yocum, R. M. Robich, S. A. L. Hayward, and D. L. Denlinger. 2007. Up-regulation of heat shock proteins is essential for cold survival during insect diapause. Proceedings of the National Academy of Sciences of the United States of America **104**: 11130–11137.

Riquelme-Bugueño, R., S. Núñez, E. Jorquera, L. Valenzuela, R. Escribano, and S. Hormazábal. 2012. The influence of upwelling variation on the spatially-structured euphausiid community off central-southern Chile in 2007–2008. Progress in Oceanography **92**: 146–165.

Riquelme-Bugueño, R., R. Escribano, and J. Gómez-Gutiérrez. 2013. Somatic and molt production in *Euphausia mucronata* off central-southern Chile: the influence of coastal upwelling variability. Marine Ecology Progress Series **467**: 39–57.

Rivera-Ingraham, G. A., U. Bickmeyer, and D. Abele. 2013a. The physiological response of the marine platyhelminth *Macrostomum lignano* to different environmental oxygen concentrations. Journal of Experimental Biology **216**: 2741–2751.

Rivera-Ingraham, G. A., I. Rocchetta, S. Meyer, and D. Abele. 2013b. Oxygen radical formation in anoxic transgression and anoxia-reoxygenation: foe or phantom? Experiments with a hypoxia tolerant bivalve. Marine Environmental Research **92**: 110–119.

Rosa, R., and B. Seibel. 2008. Synergistic effects of climate-related variables suggest future physiological impairment in a top oceanic predator. Proceedings of the National Academy of Sciences **105**: 20776.

Ross, R., K. Daly, and T. English. 1982. Reproductive Cycle Fecundity of *Euphausia pacifica* in Puget Sound, Washington. Limnology and Oceanography **27**: 304–314.

Saba, G. K., O. Schofield, J. J. Torres, E. H. Ombres, and D. K. Steinberg. 2012. Increased feeding and nutrient excretion of adult Antarctic krill, *Euphausia superba*, exposed to enhanced carbon dioxide (CO_2). PLoS ONE **7**: e52224.

Saborowski, R., and F. Buchholz. 2002. Metabolic properties of Northern krill, *Meganyctiphanes norvegica*, from different climatic zones. II. Enzyme characteristics and activities. Marine Biology **140**: 557–565.

Saborowski, R., S. Bröhl, G. Tarling, and F. Buchholz. 2002. Metabolic properties of Northern krill, *Meganyctiphanes norvegica*, from different climatic zones. I. Respiration and excretion. Marine Biology **140**: 547–556.

Sato, M., J. Dower, E. Kunze, and R. Dewey. 2013. Second-order seasonal variability in diel vertical migration timing of euphausiids in a coastal inlet. Marine Ecology Progress Series **480**: 39–56.

Schmidt-Nielsen, K. 1984. Scaling - Why is Animal Size so Important? Cambridge University Press.

Schofield, O., H. Ducklow, D. Martinson, M. Meredith, M. Moline, and W. Fraser. 2010. How do polar marine ecosystems respond to rapid climate change? Science **328**: 1520.

Seear, P., G. A. Tarling, M. Teschke, B. Meyer, M. A. S. Thorne, M. S. Clark, E. Gaten, and E. Rosato. 2009. Effects of simulated light regimes on gene expression in Antarctic krill (*Euphausia superba* Dana). Journal of Experimental Marine Biology and Ecology **381**: 57–64.

Seibel, B., and J. Drazen. 2007. The rate of metabolism in marine animals: environmental constraints, ecological demands and energetic opportunities. Philosophical Transactions of the Royal Society B: Biological Sciences **362**: 2061.

Seibel, B. A. 2011. Critical oxygen levels and metabolic suppression in oceanic oxygen minimum zones. Journal of Experimental Biology **214**: 326–336.

Shaw, C., W. Peterson, and L. Feinberg. 2010. Growth of *Euphausia pacifica* in the upwelling zone off the Oregon coast. Deep Sea Research Part II: Topical Studies in Oceanography **57**: 584–593.

Sidell, B. D., W. R. Driedzic, D. B. Stowe, and I. A. Johnston. 1987. Biochemical correlations of power development and metabolic fuel preferenda in fish hearts. Physiological Zoology **60**: 221–232.

Siegel, V. 2000. Krill (Euphausiacea) life history and aspects of population dynamics. Journal of the Fisheries Board of Canada **57**: 130–150.

Sinclair, B. J., A. G. Gibbs, and S. P. Roberts. 2007. Gene transcription during exposure to, and recovery from, cold and desiccation stress in *Drosophila melanogaster*. Insect Molecular Biology **16**: 435–443.

Small, L., and J. Hebard. 1967. Respiration of a vertically migrating marine crustacean *Euphasia pacifica* Hansen. Limnology and Oceanography **12**: 272–280.

Smith, S. E., and P. B. Adams. 1988. Daytime surface swarms of *Thysanoessa spinifera* (Euphausiacea) in the Gulf of the Farallones, California. Bulletin of Marine Science **42**: 76–84.

Sørensen, J. G., T. N. Kristensen, and V. Loeschcke. 2003. The evolutionary and ecological role of heat shock proteins. Ecology Letters **6**: 1025–1037.

Spicer, J. I. 2013. What can an ecophysiological approach tell us about the physiological responses of marine invertebrates to hypoxia? Journal of Experimental Biology **217**: 46–56.

Spicer, J. I., M. A. Thomasson, and J. O. Stromberg. 1999. Possessing a poor anaerobic capacity does not prevent the diet vertical migration of Nordic krill *Meganyctiphanes norvegica* into hypoxic waters. Marine Ecology Progress Series **185**: 181–187.

Strahl, J., T. Brey, E. E. R. Philipp, G. Thorarinsdottir, N. Fischer, W. Wessels, and D. Abele. 2011. Physiological responses to self-induced burrowing and metabolic rate depression in the ocean quahog *Arctica islandica*. Journal of Experimental Biology **214**: 4223–4233.

Stramma, L., G. C. Johnson, J. Sprintall, and V. Mohrholz. 2008. Expanding oxygen-minimum zones in the tropical oceans. Science **320**: 655–658.

Stramma, L., E. D. Prince, S. Schmidtko, J. Luo, J. P. Hoolihan, M. Visbeck, D. W. Wallace, P. Brandt, and A. Körtzinger. 2011. Expansion of oxygen minimum zones may reduce available habitat for tropical pelagic fishes. Nature Climate Change **2**: 33–37.

Strömberg, J.-O., and J. I. Spicer. 2000. Cold comfort for krill? Respiratory consequences of diel vertical migration by *Meganyctiphanes norvegica* into deep hypoxic waters. Ophelia **53**: 213–217.

Suzuki, K. 2000. Measurement of Mn-SOD and Cu, Zn-SOD, pp. 91–95. *In* N. Taniguchi and J. Gutteridge [eds.], Experimental protocols for reactive oxygen and nitrogen species. Oxford University Press.

Sydeman, W. J., J. A. Santora, S. A. Thompson, B. Marinovic, and E. D. Lorenzo. 2013. Increasing variance in North Pacific climate relates to unprecedented ecosystem variability off California. Global Change Biology **19**: 1662–1675.

Taatjes, J. J., and C. J. Cass. 2014. Comparison of lipid, protein, and calorie content of *Thysanoessa spinifera* and *Euphausia pacifica* from Trinidad, California and Newport, Oregon. poster communication.

Taki, K. 2008. Vertical distribution and diel migration of euphausiids from Oyashio Current to Kuroshio area off northeastern Japan. Plankton and Benthos Research **3**: 27–35.

Tanasichuk, R. 1999. Interannual variation in the availability and utilization of euphausiids as prey for Pacific hake (*Merluccius productus*) along the south-west coast of Vancouver Island. Fisheries Oceanography **8**: 150–156.

Tarling, G. A., and J. Cuzin-Roudy. 2003. Synchronization in the molting and spawning activity of northern krill (*Meganyctiphanes norvegica*) and its effect on recruitment. Limnology and Oceanography **48**: 2020–2033.

Tarling, G. A., R. S. Shreeve, A. G. Hirst, A. Atkinson, D. W. Pond, E. Murphy, and J. L. Watkins. 2006. Natural growth rates in Antarctic krill (*Euphausia superba*): I. Improving methodology and predicting intermolt period. Limnology and Oceanography **51**: 959–972.

Tavaria, M., T. Gabriele, I. Kola, and R. L. Anderson. 1996. A hitchhiker's guide to the human Hsp70 family. Cell stress & chaperones **1**: 23–28.

Taylor, A. C., and J. I. Spicer. 1987. Metabolic responses of the prawns *Palaemon elegans* and *P. serratus* (Crustacea: Decapoda) to acute hypoxia and anoxia. Marine Biology **95**: 521–530.

Taylor, M. H., J. Tam, V. Blaskovic, P. Espinoza, R. Michael Ballón, C. Wosnitza-Mendo, J. Argüelles, E. Díaz, S. Purca, and N. Ochoa. 2008. Trophic modeling of the Northern Humboldt Current Ecosystem, Part II: Elucidating ecosystem dynamics from 1995 to 2004 with a focus on the impact of ENSO. Progress in Oceanography **79**: 366–378.

Teal, J., and F. Carey. 1967. Respiration of a euphausiid from the oxygen minimum layer. Limnology and Oceanography **12**: 548–550.

Tellmann, G. 2006. The E-Method: a highly accurate technique for gene-expression analysis. Nature Methods **3**: i–ii.

Teschke, M., S. Kawaguchi, and B. Meyer. 2007. Simulated light regimes affect feeding and metabolism of Antarctic krill, *Euphausia superba*. Limnology and Oceanography **52**: 1046–1054.

Teschke, M., S. Wendt, S. Kawaguchi, A. Kramer, and B. Meyer. 2011. A circadian clock in Antarctic krill: an endogenous timing system governs metabolic output rhythms in the euphausid species *Euphausia superba*. PLoS ONE **6**: e26090.

Thiel, M., E. C. Macaya, E. Acuna, W. E. Arntz, H. Bastias, K. Brokordt, P. A. Camus, J. C. Castilla, L. R. Castro, and M. Cortes. 2007. The Humboldt Current System of northern and central Chile: oceanographic processes, ecological interactions and socioeconomic feedback. Oceanography and Marine Biology **45**: 195–344.

Timmermann, A., J. Oberhuber, A. Bacher, M. Esch, M. Latif, and E. Roeckner. 1999. Increased El Niño frequency in a climate model forced by future greenhouse warming. Nature **398**: 694–697.

Tomanek, L. 2010. Variation in the heat shock response and its implication for predicting the effect of global climate change on species' biogeographical distribution ranges and metabolic costs. Journal of Experimental Biology **213**: 971–979.

Torres, J. J., A. V. Aarset, J. Donnelly, T. L. Hopkins, T. M. Lancraft, and D. G. Ainley. 1994. Metabolism of Antarctic micronektonic Crustacea as a function of depth of occurrence and season. Marine Ecology Progress Series **113**: 1–15.

Toullec, J.-Y., E. Corre, B. Bernay, M. A. S. Thorne, K. Cascella, C. Ollivaux, J. Henry, and M. S. Clark. 2013. Transcriptome and peptidome characterisation of the main neuropeptides and peptidic hormones of a euphausiid: the ice krill, *Euphausia crystallorophias*. PLoS ONE **8**: e71609.

Tremblay, N., and D. Abele. in revision. Response of three krill species to hypoxia and warming: An experimental approach to oxygen minimum zones expansion in coastal ecosystems. Marine Ecology .

Tremblay, N., J. Gómez-Gutiérrez, T. Zenteno-Savín, C. J. Robinson, and L. Sánchez-Velasco. 2010. Role of oxidative stress in seasonal and daily vertical migration of three krill species in the Gulf of California. Limnology and Oceanography **55**: 2570–2584.

Tremblay, N., T. Zenteno-Savín, J. Gómez-Gutiérrez, A. N. Maeda-Martínez, and A. N. 2011. Migrating to the oxygen minimum layer: euphausiids, pp. 89–98. *In* D. Abele, T. Zenteno-Savín, and J. P. Vázquez-Medina [eds.], Oxidative Stress in Aquatic Ecosystems. John Wiley & Sons, Ltd.

Tremblay, N., T. Werner, K. Huenerlage, F. Buchholz, D. Abele, B. Meyer, and T. Brey. 2014. Euphausiid respiration model revamped, link to model results. PANGAEA; DOI registration in progress.

Tyburczy, J., W. Peterson, J. Abell, and E. Bjorkstedt. 2013. Mass stranding and mortality of euphausiids and crustaceans in Oregon and Northern California on 15-18 June 2013, vol. 6. Climatic and Ecological Conditions in the California Current LME for April to June 2013.

Uchiyama, M., and M. Mihara. 1978. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Analytical Biochemistry **86**: 271–278.

Vandesompele, J., K. De Preter, F. Pattyn, B. Poppe, N. Van Roy, A. De Paepe, and F. Speleman. 2002. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome biology **3**: RESEARCH0034.

Weihe, E., M. Kriews, and D. Abele. 2010. Differences in heavy metal concentrations and in the response of the antioxidant system to hypoxia and air exposure in the Antarctic limpet *Nacella concinna*. Marine Environmental Research **69**: 127–135.

Welker, A. F., D. C. Moreira, É. G. Campos, and M. Hermes-Lima. 2013. Role of redox metabolism for adaptation of aquatic animals to drastic changes in oxygen availability. Comparative Biochemistry and Physiology-Part A: Molecular & Integrative Physiology **165**: 384–404.

Wells, R., G. Grigg, L. Beard, and G. Summers. 1989. Hypoxic responses in a fish from a stable environment: blood oxygen transport in the Antarctic fish *Pagothenia borchgrevinki*. Journal of Experimental Biology **141**: 97.

Werner, T. 2012. Trophic positioning, diel vertical migration behaviourand physiological traits in euphausiid species of the Namibian Upwelling system. Ph.D. thesis, Universität Hamburg.

Werner, T., and F. Buchholz. 2013. Diel vertical migration behaviour in Euphausiids of the northern Benguela current: seasonal adaptations to food availability and strong gradients of temperature and oxygen. Journal of Plankton Research **35**: 792–812.

Werner, T., K. Hünerlage, H. Verheye, and F. Buchholz. 2012. Thermal constraints on the respiration and excretion rates of krill, *Euphausia hanseni* and *Nematoscelis megalops*, in the northern Benguela upwelling system off Namibia. African Journal of Marine Science **34**: 391–399.

Whitehouse, M., M. Meredith, P. Rothery, A. Atkinson, P. Ward, and R. E. Korb. 2008. Rapid warming of the ocean around South Georgia, Southern Ocean, during the 20th century: Forcings, characteristics and implications for lower trophic levels. Deep Sea Research Part I: Oceanographic Research Papers **55**: 1218–1228.

Wishner, K. F., D. M. Outram, B. A. Seibel, K. L. Daly, and R. L. Williams. 2013. Zooplankton in the eastern tropical north Pacific: boundary effects of oxygen minimum zone expansion. Deep Sea Research Part I: Oceanographic Research Papers **79**: 122–140.

Wittenberg, A. T., A. Rosati, T. L. Delworth, G. A. Vecchi, and F. Zeng. 2014. ENSO modulation: is it decadally predictable? Journal of Climate **27**: 2667–2681.

Wong, H. R., I. Y. Menendez, M. A. Ryan, A. G. Denenberg, and J. R. Wispé. 1998. Increased expression of heat shock protein-70 protects A549 cells against hyperoxia. The American journal of physiology **275**: L836–41.

Woods, H., A. Moran, C. Arango, L. Mullen, and C. Shields. 2009. Oxygen hypothesis of polar gigantism not supported by performance of Antarctic pycnogonids in hypoxia. Proceedings of the Royal Society B: Biological Sciences **276**: 1069.

Wyrtki, K. 1962. The oxygen minima in relation to ocean circulation. Deep Sea Research and Oceanographic Abstracts **9**: 11–23.

Zane, L., and T. Patarnello. 2000. Krill: a possible model for investigating the effects of ocean currents on the genetic structure of a pelagic invertebrate. Journal of the Fisheries Board of Canada **57**: 16–23.

Zaret, T. M., and J. S. Suffern. 1976. Vertical migration in zooplankton as a predator avoidance mechanism. Limnology and Oceanography **21**: 804–813.

Zenteno-Savín, T., R. Saldierna, and M. Ahuejote-Sandoval. 2006. Superoxide radical production in response to environmental hypoxia in cultured shrimp. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology **142**: 301–308.

Zhou, M., and R. D. Dorland. 2004. Aggregation and vertical migration behavior of *Euphausia superba*. Deep Sea Research Part II: Topical Studies in Oceanography **51**: 2119–2137.

Contributions to national and international conferences

- 2014 - XI International Meeting of the Mexican Society of Planktology A.C. Aguascalientes, Mexico. May 26-30th. How climate shapes stress response of krill species. **Tremblay N** & Abele D. oral presentation.
- 2013 - European Marine Biology Symposium (EMBS). Galway, Irland. August 19-23th. Response of krill species to hypoxia and warming: a biogeographic comparison. **Tremblay N** & Abele D. oral presentation.
- 2012 - 3rd Young Marine Research network meeting. Lübeck, Germany. September 12-14th. Metabolic strategies of euphausiid species exposed to oxygen minimum zones. **Tremblay N** & Abele D. oral presentation.
- 2011 - Workshop: Antarctic krill in a changing ocean. Texel, Netherlands. April 11-15th. Role of oxidative stress in seasonal and daily vertical migration of three krill species in the Gulf of California; What's next? Tolerance mechanisms and responses of krill species to oxygen minimum zones of the Eastern Pacific compare to *Euphausia superba*: a species that has never experienced hypoxia.**Tremblay N** & Abele D. poster.

Acknowledgements

I am deeply grateful to PD Dr. Doris Abele for providing me the opportunity to work at the Alfred Wegener Institute (AWI) for my PhD, giving me the freedom to work in this subject, and to make it happens! I thank Prof. Dr. Wilhelm Hagen for his evaluation of the thesis, as well as Dr. Werner Ekau and Dr. Hauke Flores for their implication in the PhD defence committee. The Prof. Dr. Thomas Brey, Dr. Christoph Held and Dr. Hauke Flores were part of my AWI thesis committee and very important for my progress.

I would like to thank the "Fonds de recherche sur la Nature et les Technologies du Québec" (Canada) for my doctoral scholarship and the AWI, Helmholtz Centre for Polar and Marine Research (1. PACES 2.2: Integrating evolutionary ecology into coastal and shelf processes) for the funding of the project. I would also like to thank the Euromarine Mobility Fellowship 2012 for the research stay at the "Station Biologique de Roscoff" for the learning of RNA extraction, primers design and reverse transcription qPCR methods under the supervision of Kévin Cascella and Dr. Jean-Yves Toullec. I thank Kévin and Jean-Yves for their time and open collaboration. I would like to thank the AWI graduate school POLMAR for their support to PhD students and the opportunities offered to improve our formation.

I would like to thank all people that supported me during the field and laboratory work in all the different sampling areas. You all treated me like one of yours, it really felt like leaving family when the sampling adventure was over. So first, I would like to thank the crew of the R/V Kay-Kay, R/V BIPXII, R/V Elahka, and R/V James Clark Ross as well as the graduate students, technicians and researchers at the Pelagic and Mesozooplankton Laboratory from the Centro de Investigación Oceanográfica del Pacífico Sur-Oriental de la Universidad de Concepción (Concepción, Chile), the Centro de Ecología Costera de la Universidad de Guadalajara (San Patricio de Melaque, Mexico), the Centro de Investigaciones Biológicas del Noroeste, S. C. (La Paz, Mexico), the Hatfield Marine Science Center of Oregon State University (Newport, USA), and the British Antarctic Survey (Cambridge, UK) for recording environmental information, and support in collecting zooplankton samples. Special thanks to Rubén Escribano, Pamela Hidalgo, Ramiro Riquelme-Bugueño, Jocelyn Silva Aburto, Carmen Franco-Gordo, Eva Kozak, Israel Ambriz-Arreola, César Augusto Salinas Zavala, Tracy Shaw, William T. Peterson, Jay

Peterson, Sophie Fielding, and Geraint A. Tarling. I hope to work again with all of you in future collaborations.

I thank Karim Zanaty, Lara Holst, Imke Lüdeke, and especially Stefanie Meyer for their excellence and technical help in the laboratory of AWI. I also thank Kai-Uwe Ludwichowski from the ecological chemistry group of AWI for his advices for the the high-performance liquid chromatography analysis.

I thank my sometimes big, sometimes small working group at AWI for supporting me, and tell the important helping sentence every time I needed it: “Nelly, it’s normal!”. I am already missing you Valeria, Harald, Georgina! Thanks to Iara and Cyril for their presence and support in the last moments.

I thank my two “krill” colleagues, Thorsten and Kim, for the nice “krill” coffees, “krill” beers, “krill” Skyping, “krill” worldwide meeting, “krill” advices, and “krill” corrections of the manuscript. Hope we can keep this “krill” friendship and “krill” collaboration! That's the plan, no?

I thank Paula, Valentina, Eva, Christiane, Carmen, and Cyril for letting me squat in your fleet for some days, weeks or sometimes months. I really appreciated your trust and friendship! Thanks to AWI friends for the nice evenings and talks we had, most of the time around a beer, and to the ones involved with me in the Dokteam: Christiane, Verena, Mirja, Tobi, Robert, Lera, Giulia, Doro, Ella, Carmen.. and many others I might forget! Merci aussi à Kévin, Camille et Rémi pour les bonne soirées à Roscoff. Gracias a la banda hispanohablante de Bremerhaven que me abrieron los brazos como si fuera comadre latina: Georgina, Roi, Iara, Gonzalo, Luciana, Roman, Diana, Magda! I hope to keep contact with everyone and continue these friendship.

Mil besos y gracias a mi Iván. A pesar de la distancia siempre te sentí a mi lado, escuchándome, apoyando, amandome. Lo que logré aquí te lo debo en parte por tu constante soporte. Te amo, y ya, vuelvo en casa bonito! Lo hicimos, lo hicimos, lo hicimos!

Pour finir je veux remercier ma famille de leur appuis, écoute et comprehension quant à mes lieux de residences qui ne sont géneralement pas dans un périmètre de distance acceptable pour les visites fréquentes. J'espère de tout coeur que ma carrière me mènera un jour plus près de vous. Je vous aime!

Nelly Tremblay
Am Handelshafen 12
27570 Bremerhaven
Nelly.Tremblay@awi.de

Bremerhaven, den 30.04.2014

Erklärung gemäß § 6 (5) Promo (vom 14. März 2007)
Hiermit erkläre ich, dass ich die vorliegende Doktorarbeit mit dem Titel:

Tolerance mechanisms and responses of krill species of different latitudes to oxygen minimum zones

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

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

.....
(Nelly Tremblay)