Thesis for the Degree of Doctor of Philosophy

Climate Change and the Norway Lobster

Effects of Multiple Stressors on Early Development

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2014

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Akademisk avhandling för filosofie doktorsexamen i marin zoologi, som med tillstånd från Naturvetenskapliga fakulteten kommer att offentligt försvaras fredagen den 28 november 2014 kl. 10.00, Hörsalen, Sven Lovén centrum för marina vetenskaper, Kristineberg, Fiskebäckskil.

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Cover photograph by Helen Nilsson Sköld.

Published by the Department of Biological and Environmental Sciences,
University of Gothenburg, Sweden.

ISBN 978-91-628-9241-8

Printed by Ineko Creative Production Instore

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Abstract

Climate change together with anthropogenic eutrophication have led to, and will lead to, shifts in a number of abiotic factors in the oceans, such as temperature, carbon dioxide [CO_2], pH, oxygen saturation and salinity. These stressors will act simultaneously on marine organisms and may have synergistic, additive or even antagonistic effects on physiological performance and tolerance. As such, multiple stressor experiments are crucial to gain a better understanding of future vulnerability of species, populations and ecosystems.

Early life stages of invertebrates are generally considered most vulnerable to environmental stress, but only a few studies have concerned brooding species such as the Norway lobster (*Nephrops norvegicus*), which is a benthic species, of great ecological and commercial importance. The benthic stages (adults, juveniles and embryos) spend most of their time in soft sediment burrows where they may be afflicted by low pH, hypoxia and in turn an increased Mn^{2+} concentration while the subsequent pelagial stages (Zoea I-III) are exposed to elevated seawater temperature and fluctuations in salinity. This poses the question: Is the Norway lobster already at it tolerance limit or can it tolerate additional climate change related stress?

This thesis comprises four studies primarily on embryonic development but also on larval, juveniles and egg-bearing female Norway lobsters. In **Paper I**, a potential combined effect of long-term (4 months) exposure to ocean acidification (OA) and elevated temperature on embryonic physiology was investigated. Although the Norway lobster embryos rarely encounter the highest temperature tested (18°C) naturally, they were found to be tolerant to the treatment with no combined effects on development rate, metabolic rate or the level of oxidative stress. In **Paper II** an easy-to-use quantitative tool for the development staging of the Norway lobster embryos was described. Qualitative variables was fitted to the quantitative scale of amount yolk and tested against elevated temperature and OA. There was an insignificant trend of the morphological characters appearing at a lower amount yolk in the OA and 18°C combination. In **Paper III**, climate change impacts of salinity and OA tolerance in zoea larvae were studied. Tolerance to hyposalinity treatment decreased quickly with age as newly hatched zoea I larvae were more tolerant than older. However, when allowed to acclimate, tolerance and thus survival to low salinity increased. The surviving larvae of the lowest longer-term salinity treatment (17 PSU) were lighter than those exposed to higher salinities >21 PSU. Exposure to OA affected survival in some broods of zoea larvae negatively but not others, indicating genetic variation in OA tolerance. When larvae were starved, the mortality was also greater in OA indicating differences in energy usage. In **Paper IV** a higher level of OA was tested for 2 months, together with 1 week of exposure to hypoxia or manganese, on different life stages. Hypoxia drastically reduced oxygen consumption rate in all life-stages tested. Hypoxia in combination with OA also reduced metabolic rate further in embryos. Heart rate was however higher in embryos exposed to hypoxia, independent of OA but exhibited a more regular rhythm when exposed to the combination of hypoxia and OA. Females exposed to OA had a slightly increased oxygen consumption rate, but this effect was only significant in the combination with Mn^{2+}. Conversely, the combinations of OA and Mn^{2+} reduced metabolic rate of embryos. Despite the decreased metabolic rate, we found no significant effect on embryonic development rate in the combinational treatments. However, development rate was significantly lower in the control than in hypoxia and Mn^{2+}. This contradiction need to be further investigated.

In conclusion, all life stages tested seemed relatively resilient to OA alone but life-stage dependent effects were seen when treatments were combined, such as the opposite response to OA and Mn^{2+} in embryo and female metabolic rate. Previous research has shown brooding females to sense and adjust ventilation of their eggs in unfavorable conditions. If the responses seen in **Paper IV** was a result of an elevated fanning was not in the scope of this thesis but should be investigated. The synergistic effects observed in this thesis would have been overlooked in a single stressor experimental set-up, which emphasise the great need for additional multiple-stressor studies. Finally, the highest increase in pCO_2 (600 µatm) tested (**Paper IV**) still represents a moderate scenario for the end of this century, since different models predict an increase of between 500-1000 µatm pCO_2 (IPCC, 2013). Thus, the effects observed could be an underestimation of the future impact of OA. In many places the Norway lobster currently lives close to the tolerance limit of the early life stages. As such, the geographic area of suitable abiotic habitat for the Norway lobster may be severely affected in a near future.

KEYWORDS: *Nephrops norvegicus*, climate change, ocean acidification, hypoxia, salinity, temperature, manganese (Mn^{2+}), oxidative stress, metabolic rate, cardiac performance, survival, embryo, zoea.

Klimatförändringar och havskräftans tidiga utveckling

Havskräftan är en nyckelart, såväl kommersiellt som ekologiskt. Den lever hela sitt vuxna liv på mjuka havsbottnar där den syresätter sedimentet med sitt grävande och skapar därmed livsrum för många andra arter. Honan bär sin rom (ägg) i 6-10 månader och stannar under större delen av den tiden inuti sin håla där miljön kan vara tuff med perioder av syrebrist, lågt pH och hög koncentration av mangan. När larverna sedan kläckts simmar de upp i vattenmassan och kommer där i kontakt med varierande salthalt och höga temperaturer. Efter några veckor återvänder larverna ner till havsbotten och slår sig ner som postlarver/juveniler. Den här avhandlingen handlar om hur framför allt den tidiga utvecklingen, embryon och larver, påverkas av dessa yttre faktorer men också en del om hur äggbärande honor och juveniler påverkas och hur de kan förväntas klara framtida miljöförändringar orsakade av oss människor.

Förändringar i miljön sker ständigt men aldrig tidigare har den marina fysiska miljön förändrats så snabbt som den gör idag. Sedan den industriella revolutionen har allt mer koldioxid släppts ut i atmosfären, framförallt genom förbränning av fossila bränslen. Detta leder bl a till ett allt varmare klimat, uppvärmda och försurade hav. Övergödning är idag den största orsaken till perioder av syrefattiga havsbottnar men globala klimatförändringar med största sannolikhet förstärker effekten i framtiden.

I avhandlingens **Del I** studerades embryonal tolerans för hög temperatur och havsförsurning genom 4 månaders exponering av äggbärande honor för olika kombinationer av temperatur och pH. Varje månad mättes äggens syrekonsumtion, hjärtfrekvens och proteinskador (oxidativ stress). Embryonal utvecklingshastighet räknades också ut genom att mäta minskningen i förhållandet mellan äggula och ägg över tid. Trots att havskräftans tidiga utveckling sällan i naturen exponeras för den högsta undersökta temperaturen (18°C) visade det sig att de var tåliga. Negativa effekter kan dock visa sig i ett senare utvecklingsskede varför ytterligare fysiologiska mätningar och experiment som följer utvecklingen genom fler stadier är önskvärda.

Avhandlingens **Del II** fokuserade på att beskriva de morfologiska karaktärer som uppkommer under embryots utveckling och när dessa uppkommer i förhållande till hur mycket gula ägget innehåller. Syftet var att förbättra befintliga beskrivningar och stadieindelningar för att skapa ett lättanvänt verktyg för åldersbestämning av ägg/embryon. I **Del II** testades också om sekvensen av de beskrivna karaktärerna kan förskjutas till följd av att ha utvecklats i hög temperatur (18°C) och havsförsurning (-0.4 pH enheter). En sådan trend kunde urskönjas men skillnaden var inte statistiskt signifikant.

I avhandlingens **Del III** flyttades fokus till havskräftans nästa livsstadium, den frisimmande zoealarven. Här studerades tolerans för låg salthalt och havsförsurning under larvutvecklingen. Nykläckta larver var mer toleranta för akut låg salthalt än äldre larver men toleransen ökade när larverna tilläts acklimatisera sig till behandlingen. Högst överlevnad efter en tid hade larverna som exponerats för 21 PSU jämfört med i 17 och 32 PSU. Larver som exponerades för den lägsta salthalten vägde mindre än larver exponerade för de högre salthalterna. En trend av ökad ämnesomsättning med sjunkande salthalt sågs också men kunde inte styrkas statistiskt. En minskad kroppsvikt tyder dock på att det kostar mer energi för en havskräftlarv att leva i låg salthalt och detta kan potentiellt medföra negativa konsekvenser på sikt. I **Del III** exponerades larver också för försurat vatten. Här kunde man se att några kullar klarade behandlingen sämre än övriga, vilket tyder på genetiska skillnader i tolerans. Larvernas tolerans för havsförsurning minskade också om de fick svälta vilket tyder på en högre energiåtgång i försurat vatten. Larver som utsattes för försurat havsvatten och svält sågs också dö tidigare än larver i kontrollvatten. Precis som i den låga salthalten berodde antagligen detta på en högre energiåtgång i försurat vatten.

Del IV ägnades återigen åt embryonal fysiologi men inkluderade även juveniler och honor. Här exponerades havskräftor för havsförsurning och syrebrist eller mangan i ca 2 månader. Syrebrist hade den största enskilda effekten på alla inkluderade livsstadier med sänkt ämnesomsättning som följd. Alla livsstadier tolererade försurat vatten utan någon större effekt men när havsförsurning kombinerades med syrebrist eller mangan sågs effekter som var specifika för respektive livsstadium. Honorna ökade sin ämnesomsättning i kombinationen havsförsurning och mangan medan embryona hade motsatt respons med en sänkt ämnesomsättning. Embryona upplevde också en ytterligare sänkt syrekonsumtion när syrebrist kombinerades med havsförsurning vilket inte sågs i honor eller juveniler. Äggbärande honor kan genom ändring av sitt beteende öka syresättningen av sina ägg men till en högre kostnad och detta kan vara en förklaring till det motsatta förhållandet som sågs i kombinationen havsförsurning och mangan. Honans beteende låg dock inte inom ramen för denna avhandling, men bör utredas i framtiden.

Sammanfattningsvis var alla inkluderade livsstadier relativt motståndskraftiga mot enbart havsförsurning men tydliga kombinationseffekter på både honor och embryon är alarmerande och kräver fortsatta studier. Avhandlingen visar också på vikten av att kombinera olika stressfaktorer för att studera ekologiskt relevanta effekter. Den största ökningen av pCO_2 (600 µatm) som testades i **Del IV** är fortfarande ett måttligt scenario då olika modeller förutspår en ökning på mellan 500-1000 µatm pCO_2 innan slutet av århundradet. Således kan resultaten i denna studie mycket väl vara en underskattning av de framtida effekterna av havsförsurning.

Included papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals. The papers are appended at the end of the thesis.

I. **Styf HK**, Nilsson Sköld H and Eriksson SP. Embryonic response to long-term exposure of the marine crustacean *Nephrops norvegicus* to ocean acidification and elevated temperature. Ecology and evolution, 3:5055-5065, 2013.

II. **Styf HK**, Nilsson Sköld H and Eriksson SP. Qualitative variables in the *Nephrops norvegicus* (L.) embryo fitted to the quantitative scale of amount yolk and tested against elevated temperature and ocean acidification. *Manuscript.*

III. Wood HL, Eriksson S, Nordborg M and **Styf HK**. The future of *Nephrops norvegicus*: the effect of climate change on early development. *Submitted Manuscript.*

IV. **Styf HK**, Eriksson SP, Wood HL, Krång A-S. Effects of combined exposure to elevated pCO_2 and hypoxia or manganese on physiological performance in different life stages of the Norway lobster. *Manuscript.*

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1 Introduction

1.1 Stress and homeostasis

In our modern society, stress is a widely used term to describe the response to external stressors on humans, on our physical and psychological health. The external challenges are often related to a heavy workload combined with little time, which is typical for a PhD. In the aquatic environment, stressors instead relate to major changes in the physical properties surrounding the organism, e.g. salinity, temperature, oxygen saturation and pH, which are perceived through various receptors and signalling pathways. These changes challenge the ability of organisms to maintain a stable internal environment (homeostasis) and cause a chain of biological reactions, which ultimately lead to a fight or flight response (Cannon, 1929; Eriksson et al. 2013). Flight is however not always an option, e.g. not for encapsulated embryos attached to their parent or planktonic larvae that drift with waves and currents. The ability of these early life stages to maintain homeostasis and to survive periods of moderate to extreme stress is utterly important for all higher levels of organisation, e.g. populations and ecosystems. Maintaining homeostasis is energetically costly and could have a negative effect on population fitness.

At the end of the day, the survival of a species is dependent on its ability to continually develop, grow and reproduce. The outcome will depend on its plasticity and or ability to adapt quickly enough to the expected environmental changes. However, climate-change related shifts in for example temperature and pH are now happening at a historically higher rate than what have occurred in the past millions of years (IPCC, 2013).

1.2 Climate change and future predictions

Climate change together with anthropogenic eutrophication have led to, and will lead to, shifts in a number of abiotic factors in the oceans, such as temperature, pH, carbon dioxide [CO_2], oxygen saturation and salinity. In this thesis, ***climate change*** relates to CO_2 linked environmental changes. Emission of CO_2 is mostly caused by fossil fuel burning, which has increased atmospheric concentration of CO_2 with more than 100 ppm since mid 18th century. This level is higher than it has been in at least 800 000 years. Atmospheric [CO_2] is predicted to increase even further, with another 500-1000 ppm by the end of this century, and up to 2000 ppm by year 2300 (IPCC, 2013).

1.2.1 Climate change and elevated ocean temperature

Global temperature has increased the past 30 years to a level not previously recorded during the latest 160 years (IPCC, 2013). Since the industrial revolution our oceans have absorbed a third of the emitted CO_2 and 80% of the heat added to the climate system. This has reduced the greenhouse effect of increased CO_2 in the atmosphere (Sabine et al. 2004). However, one side effect of heat absorption is increased oceanic temperatures, which has been recorded all the way down to 3000 meters depth. Before the end of this century, the average surface temperature is expected to increase up to 4.8°C, depending on prediction model used and future CO_2 emission (IPCC, 2013).

1.2.2 Climate change and ocean acidification

The increased oceanic concentration of CO_2 has led to a serious shift of seawater carbonate chemistry, with a decrease in oceanic pH (i.e. $-\log[H^+]$). This process is named ocean acidification (OA) (see equilibrium reactions below).

$CO_2\,(aq) + H_2O$	⇆	H_2CO_3	(carbonic acid)
H_2CO_3	⇆	$H + HCO_3^-$	(bicarbonate ion)
$H^+ + HCO_3^-$	⇆	$2H^+ + CO_3^{-2}$	(carbonate ion)

Due to a 3-fold increase in [H^+], global oceanic pH has already decreased by 0.1 pH units, since the industrial revolution, and it is predicted to further decrease by another 0.4 pH units by 2100 (Caldeira, 2003, 2005). The phenomenon of OA has been described, as "global warming's evil twin" and "the other CO_2 problem" (Turley, 2005; Henderson, 2006; Doney et al. 2009).

1.2.3 Climate change and ocean oxygen deficiency

Anthropogenic eutrophication implicates an excessive supply of nutrients, for example from fertilizers used in agriculture and from sewage effluents as a result of inefficient sewage treatments, transported to the oceans with run-offs.

Eutrophied systems are susceptible to become oxygen limited, i.e. hypoxic (less than 2 ml dissolved oxygen/L water) or even anoxic (0 ml dissolved oxygen/L water) due to the oxidative decomposition processes carried out by bacteria (Diaz and Rosenberg 1995). The risk of oxygen depletion is enhanced if the water column is highly stratified, impeding dissolved oxygen to diffuse down towards the bottom water bodies. Research strongly points to a causal relationship between increasing benthic hypoxia and an extensive anthropogenic eutrophication (Diaz, 2001). In only a few decades, hypoxia in bottom waters has increased in frequency on spatial scale and in severity. In fact, dead costal zones have already spread to a total area of 245,000 square kilometres worldwide (Diaz and Rosenberg, 1995; Diaz and Rosenberg, 2008). Although the main cause for hypoxia today is eutrophication, in the future climate change itself is predicted to cause hypoxic events, as the elevated temperature will cause a thermal expansion and an increased stratification of the water column. Climate change may also aggravate hypoxia by the increasing rainfalls, with runoff carrying more fertilisers to the oceans and thus increasing eutrophication. Hence, oxygen tension and the amount of oxygen that will reach the bottom water will be reduced even further. Hypoxia is also likely to elevate the effect of OA in coastal zones (Melzner et al. 2013).

1.2.4 Climate change and increased manganese exposure

Changes in the mentioned physical properties of seawater can also have effects on the availability of different compounds, some of which can be highly toxic to living organisms. One such compound, which is in the scope of this thesis, is the redox-sensitive metal manganese. Soft sediments typically contain high concentrations of oxidised manganese (MnO_2) bound to sediment particles. When manganese is reduced, it is released as free ions [Mn^{2+}] into the water column where it can reach toxic concentrations of 10-20 mg/L (reviewed in Baden and Eriksson, 2006). Bioavailability of Mn^{2+} becomes higher in as oxygen saturation, pH and salinity decrease and as temperature increases (CICADS-report 2004). As there is an intensified occurrence of hypoxia along the Swedish West coast, longer periods and higher levels of bioavailable [Mn^{2+}] will be expected in the benthic coastal zone (Schiedek et al. 2007; Diaz and Rosenberg, 2008).

1.2.5 Climate change and salinity fluctuations

Elevated atmospheric and ocean temperatures also increase melting of snow and ice, which dilute the salinity of ocean water and increase stratification of the water mass. Increased precipitation also contributes to a reduced salinity (i.e. hyposalinity). In some regions an increased evaporation will instead cause a higher salinity (i.e. hypersalinity) (IPCC, 2013). Thus the effect of climate change on salinity is less predictable but is likely to cause increased salinity fluctuations.

1.3 The model organism – the Norway lobster

The benthic habitats sustain 98% of all marine species. Therefore, more knowledge is warranted on how benthic organisms will cope with climate change (Widdicombe and Spicer, 2008). The Norway lobster (*Nephrops norvegicus*) represents an ideal 'model organism', as it is a species of key ecological importance, in that it oxygenates soft sediment in the process of bioturbation. It is also already exposed to a harsh environment, such as seasonal hypoxia and elevated [Mn^{2+}], which pose the question, is it currently at its tolerance limit or can it tolerate additional climate change related stress?

The Norway lobster is a decapod crustacean, which can be found on the continental shelf and slope throughout the North-East Atlantic Ocean and in the Mediterranean Sea. It is the most extensively caught crustacean in Europe with an annual catch of 60 000 tonnes (FAO, 2012). In 2009, 290 tonnes were caught by creel on the West coast of Sweden (Sköld et al. 2011).

1.3.1 The Norway lobster life cycle

The Norway lobster life cycle is divided into several morphologically distinct stages (**Figure 1**). The embryos develop externally on the female abdomen for 6-10 months depending on temperature, latitude and habitat (reviewed in Powell and Eriksson, 2013).

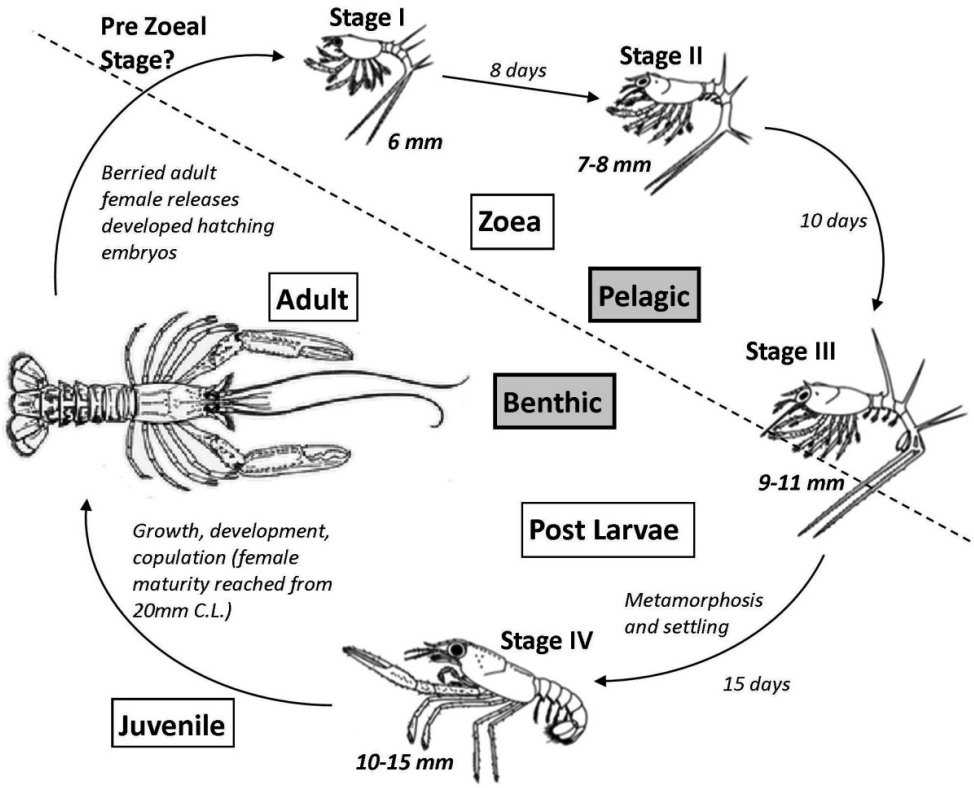

Figure 1. Life cycle of Norway lobster *Nephrops norvegicus* (not including embryonic stages). Modified after Santucci (1926) by Powell and Eriksson (2013).

During the embryonic phase it is thus confined to the ambient environment of the burrow, as the female tends to stay in the sediment during this time period. Conditions in the burrow are likely to fluctuate with regards to oxygen and pH levels but probably hold a stable high salinity and low temperature indicated at sites deeper than 30 m in **Table 1**. Although this has not yet been studied in Norway lobster burrows *in situ,* low-pH water has been documented for example in burrows of polychaete worms and thalassinidean shrimps (Torres et al. 1977; Zhu et al. 2006). This is due to the strong pH gradients at the sediment-water interface in organic-rich soft sediments. After hatching, the first stage of Norway lobster larvae (zoea I) escapes to the pelagic zone, where it develops through three larval morphs (zoea I – zoea II – zoea III). In the open water, the oxygen level is high but pH, temperature and salinity may fluctuate (**Table 1**). The Kattegat/Skagerrak region is influenced by the outflow of brackish water through the Danish straits, resulting in a strong halocline at approximately 10–15 m depth (Moksnes et al. 2014). The abundance of zoea larvae peaks in July and the majority is found at the halocline or just below it, at 10-30 m depth (Moksnes et al. 2014). Survival to the first post-larval stage in laboratory-reared mass-cultures is typically low, 3-22% (Smith, 1987) and it has been estimated that field survival is ≈30% (Nichols et al. 1987). In the end of zoea III, the larva descends back to the seafloor and finally settles as a post-larva.

In conclusion, through the whole Norway lobster life cycle the animal is naturally exposed to a range of abiotic fluctuations, that is, changes in oxygen saturation, salinity, temperature, pH and pCO_2 (**Table 1**).

1.4 Abiotic conditions in Norway lobster habitats

All animals in this thesis came from a Norway lobster population in the Gullmarsfjord, which is a sill-fjord situated on the West coast of Sweden. The water chemistry data in **Table 1** were extracted from the Swedish Meteorological and Hydrological Institute (SMHI) and represents known Norway lobster fishing grounds along the Swedish west coast (ICES, 2012). The data represent a summary of >20 years of these measurements. In the below text, data are reported as mean ± standard deviation of the stations means, minimum and maximum values.

Annual temperature at the nine SMHI stations was 9.7 ± 0.3°C at 10 meters depth and 7.6 ± 0.8°C 1 meter above sediment surface. Maximum temperature 1 meter above the sediment surface was 13.8 ± 2.9°C and maximum temperature at 10 meters was 21.2 ± 0.8°C (**Table 1**). The absolute maximum temperature measured was 22.4 (10 meter, station T4) and 18.0°C (1 meter above bottom, station T4).

Longer time series of pH measurements in the vicinity of the Gullmarsfjord are difficult to obtain as SMHI rarely measures these parameters. The average pH at the deep range was however 8.14 ± 0.11 but occasionally dropped down to pH 7.80 (September).

At 10 meters depth and 1 meter above bottom, oxygen saturation was 6.91 ± 0.29 ml/L and 5.19 ± 0.88 ml/L respectively. The minimum amount oxygen at the deep range was 2.45 ± 1.33 ml/L with the lowest measured value of 0.6 ml/L at station T5 (**Table 1**). SMHI reports their oxygen data in ml/L. To convert ml/L to mg/L (**Paper IV**) the numbers are divided by 0.7 as 7 ml/L = 10 mg/L.

At 10 meters depth, salinity was 26 ± 4 PSU and at 1 meter above the sediment surface it has been 34 ± 1 PSU. Minimum salinity at 10 meters depth was 16 ± 3 PSU with the lowest measurement at station T4 of 13.0 PSU.

Embryos develop in an ambient condition, which are here represented by the SMHI data collected at 1 meter above sediment surface. Some of the parameters are likely more severe in the actual burrows. The collected data show that embryos experience low oxygen saturation or low pH and occasionally experience a combination of the two (see station C2, C3, C4 and T5; **Table 1**). The earliest stages are also exposed to elevated temperature in some areas (see e.g. stations T3 and T4; **Table 1**). The free-swimming zoea larvae instead experience high temperature, low salinity and occasionally a combination of the two (see stations T3, T4, C4 and T5; **Table 1**). Thus in Swedish waters it is in the most southern geographic locations (Kattegat) and in the Gullmarsfjord (C2 and C3) where water quality might become a challenge to the Norway lobster.

Table 1. Field measurements extracted from SMHIs database (www.smhi.se) along the Swedish West coast, where Norway lobsters are fished.

Station	Depth (m)	pH (NBS)	Temperature (°C)	Oxygen (ml/L)	Salinity (PSU)
C1	10	NA	9.6 (-1.0-20.9)	6.8 (3.5-12.9)	28.2 (18.7-34.6)
C2	10	8.2	10.2 (-0.8-21.7)	6.4 (3.0-9.6)	27.2 (18.0-34.6)
T1	10	8.4	9.5 (-1.1-19.4)	6.8 (5.1-9.3)	30.5 (20.0-34.2)
C3	10	8.2 (7.9-8.5)	9.5 (-1.1-21.0)	6.7 (4.0-11.1)	27.7 (17.8-34.4)
T2	10	8.3 (8.0-8.9)	9.3 (-1.2-20.8)	6.8 (5.1-10.6)	29.6 (18.1-34.6)
T3	10	NA	9.7 (-0.9-21.5)	7.1 (3.3-10.6)	22.8 (14.5-33.6)
T4	10	NA	9.7 (-0.4-22.4)	7.2 (3.6-10.6)	22.2 (13.0-33.5)
C4	10	8.2 (8.1-8.4)	9.8 (-0.3-21.0)	7.2 (5.1-11.0)	22.0 (13.8-32.8)
T5	10	8.2 (8.0-8.8)	10.2 (-0.8-21.7)	7.1 (5.3-10.2)	21.8 (13.1-33.4)
C1	24 (231-248)	NA	6.5 (4.7-8.3)	5.2 (3.5-6.8)	34.8 (33.8-35.1)
C2	68 (61-74)	NA	6.5 (5.1-10.3)	3.5 (1.3-6.4)	34.3 (33.4-34.9)
T1	92 (79-120)	8.3	7.7 (3.0-14.5)	6.0 (4.5-7.4)	34.7 (32.3-35.3)
C3	65 (51-134)	8.1 (7.8-8.4)	7.2 (3.2-13.8)	4.6 (1.4-7.2)	34.1 (25.0-35.1)
T2	90 (78-105)	8.2 (8.0-8.4)	7.2 (2.8-13.8)	6.0 (3.7-7.5)	34.7 (33.3-35.3)
T3	27 (21-31)	NA	8.4 (1.6-16.4)	5.7 (3.0-9.2)	32.6 (22.5-35.6)
T4	23 (21-25)	NA	8.6 (1.8-18.0)	5.7 (3.0-9.0)	32.0 (17.6-35.5)
C4	30 (27-33)	8.1 (7.8-8.2)	8.2 (3.2-14.8)	5.4 (2.1-8.1)	32.9 (23.2-35.6)
T5	57	8.0	7.7	4.6	33.7

Mean, min and max temperature, salinity, pH and dissolved oxygen from the complete database (>20 years). Stations are either creeled (C) or trawled (T) for Norway lobsters. Data from the bottom water were taken from 1 m above the bottom surface. NA = Not Analysed. For station C1 the bottom data were collected deeper than creel depth.

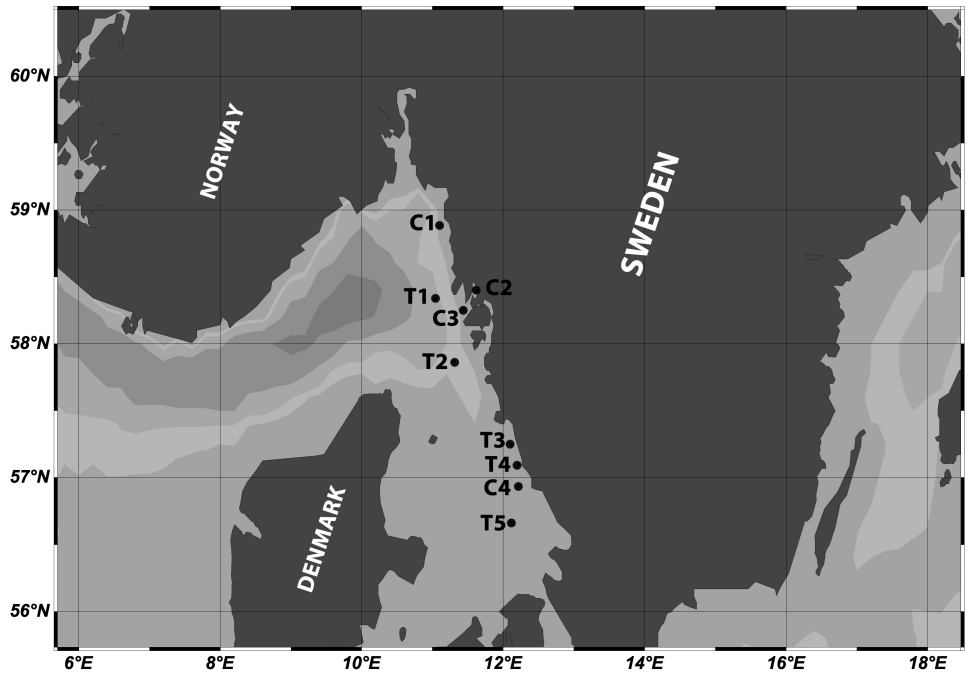

Figure 2. Map over the Swedish West coast with the SMHI stations marked. C = Creeled stations, T = trawled stations. By Mikael Dahl.

1.5 Early development in a changing environment

Previous research suggests that early development is particularly sensitive to environmental changes and that it therefore poses a bottleneck in the overall success of the species (Byrne, 2011). Combined data suggests that elevated temperature can endanger all early life stages, with both lethal and sublethal effects (e.g. Byrne, 2011; Agnalt et al. 2013; Arnberg et al. 2013). As a consequence of warmer water, oxygen solubility decreases and metabolic rate increases. This may also cause a higher uptake of certain pollutants and predispose developing organisms to their impact and vice versa (Westerhagen and Dethlefsen, 1997; Schiedek et al. 2007; Negri and Hoogenboom, 2011).

Knowledge about the effects of elevated temperature on the earliest stages of the Norway lobster is limited and mostly concern development rate of zoea larvae (e.g. Smith 1987; Dickey-Collas et al. 2000). Studies on other brooding species in the research field of climate change are also scarce (Noisette et al. 2014). Potentially, brooders offer their offspring some protection against environmental stress (Fernandez et al. 2000; Przeslawski, 2004). Female Norway lobsters are for example known to increase pleopod fanning of the egg clutch during hypoxic events (Eriksson et al. 2006).

1.5.1 Effects of OA exposure

A substantial part of previous research on biological effects of OA has focused on consequences for biomineralization, that is, the building of calcium carbonate ($CaCO_3$) shells (e.g. Orr et al. 2005). In this thesis, focus is instead on for example metabolic and developmental effects of OA. According to previous data, species tolerant to OA are generally also good ion-regulators. Although, adult crustaceans have been found more tolerant to OA than other invertebrates little is still known about tolerance of early crustacean developmental stages, which might not have the same capacity for ion regulation. If these are sensitive to the environmental changes, the species can still be at great risk (reviewed in Whiteley, 2011; Melzner et al. 2009).

Negative effects of OA on both adults and early development has been seen previously in many marine species, on for example fertilization, hatching, growth, settling success, metabolic rate, growth, immune defence and proteins biosynthesis (Kurihara et al. 2004; Havenhand et al. 2008; Wood et al. 2008; Parker et al. 2009; Hernroth et al. 2012; Dupont et al. 2013; Bradassi et al. 2013; Mukherjee et al. 2013). Species able to control for example biomineralization might also be afflicted by energetically costly trade-offs due to a shift in energy allocation (Wood et al. 2008; Findlay et al. 2010).

1.5.2 Effects of hypoxia exposure

Marine organisms will be severely challenged by eutrophication and climate change caused hypoxia. The adult crustacean is able to decrease metabolic rate and utilize stored phosphoarginine during periods of low oxygen (Hervant et al. 1999; Abe et al. 2007). Adults suffering hypoxia are also known to control the flow of body fluid towards the animal's nervous tissue in order to manage the oxygenation of these vital parts (Reiber, 1995). Short-term metabolic depression can be an advantage to the organism, as energy is saved in an unfavourable condition. However, both short term and prolonged metabolic depression has been found to negatively affect organisms with consequences such as reduced growth, development rate and a lower fecundity (Landry et al. 2007; Brown-Peterson et al. 2008). In the Norway lobster embryo, oxygen saturation below 20% induced a decreased heart rate (bradycardia) (Eriksson et al. 2006). It is however not known for how long the embryos can survive this depression and if there are associated costs or benefits that appear later on. Zoea larvae exposed to 50% (=3.1 ml/l) oxygen saturation, develop the adult type of the oxygen-transporting blood pigment haemocyanin, which has a higher affinity than the larval type (Spicer and Eriksson 2003).

1.5.3 Effects of manganese exposure

Although Mn^{2+} is an essential metal, elevated concentration of [Mn^{2+}] is known to affect immune defence in Norway lobster as it decreases the total number of haemocytes and causes a greater fraction of apoptosis (Hernroth et al. 2004; Oweson et al. 2006). Exposure to Mn^{2+} can also interfere with mitochondrial calcium uptake and expression of for example skeletogenic genes (Pinsino et al. 2014). In the sea urchin, *Paracentrotus lividus*, exposure to ~60 mg/L prevented skeleton growth and gave rise to embryos lacking skeletons (Pinsino et al. 2011). Manganese can also disturb ion-balance in muscle membranes (Baden and Eriksson, 2006).

1.5.4 Effects of hyposalinity exposure

In the current era of climatic change, tolerance to different salinity regimes may become crucial as salinity fluctuations are forecast to increase in coastal areas due to more extreme climatic events (**Table 1**; IPCC, 2013). Osmoregulation through the ATP driven N^{+}/K^{+}-ATPase enzyme is energetically costly (Santos et al. 2007). First stage zoea larvae (zoea I) of the Norway lobster have been found to contain less lipids and proteins after exposure to 20-25 PSU for two days (Torres et al. 2011). Furthermore, larvae of a gastropod, which hatched in low salinity water, exhibited for example decreased metabolic rate and reduced survival (Montory et al. 2014). In low salinity, the toxicity of metals typically increases as availability of metals increase and as metals to a higher degree will bind to for example gills. Salinity also affects survival and growth (Bianchini et al. 2008).

1.5.5 Combined exposures to the different stressors

Climate-change stressors will not occur in isolation and combined stressors can have synergistic, additive or even antagonistic effects on biology, as such multiple stressor experiments will be crucial to gain a better understanding of the vulnerability or tolerance of Norway lobster early development to future environmental changes (e.g. Darling and Cote, 2008; Holmstrup et al. 2010; Whiteley, 2011). A search on Web of Science, October 21, 2014, also reinforce the great lack of these kinds of studies (**Figure 3**). For example only 14 studies have previously dealt with physiology and the combination of OA and hypoxia and no previous studies included both OA and Mn^{2+}.

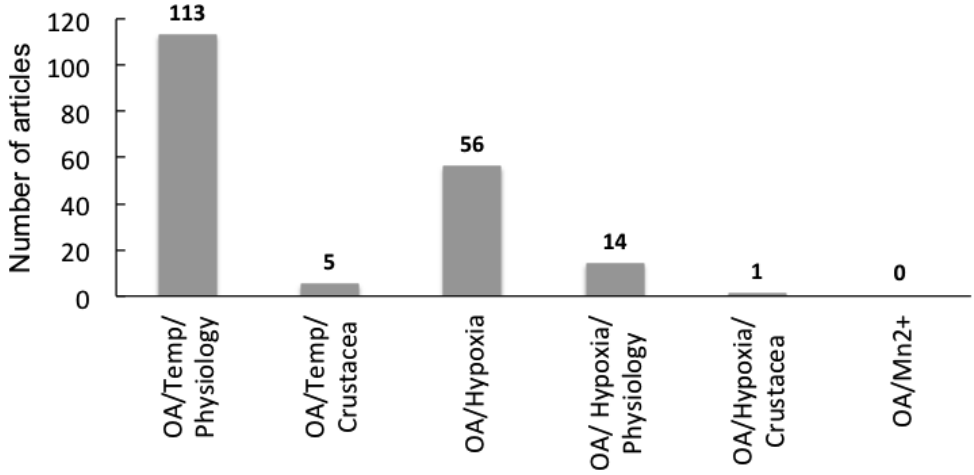

Figure 3. On the y-axis: the number of search hits on Web of Science (www.webofknowledge.com). On the x-axis different combinations of search words on topic.

2 Aim of Thesis

The overall purpose of this thesis was to provide long-term and multi-stressor experimental work on effects of near future environmental stress on an ecologically and economically important species. Effects of the following ecologically relevant stressors were investigated: elevated temperature, ocean acidification, hypoxia, hyposalinity and increased bioavailable manganese on development and physiological performance of a marine benthic invertebrate, the Norway lobster was investigated. Effects on embryos, larvae, juveniles and females are included in this thesis.

Paper I.

The aim of this paper was to investigate effects of long-term (4 months) exposure to OA and elevated temperature on embryonic development of the Norway lobster. Two levels of pH (current vs. -0.4 pH units) and six levels of temperature were used. In specific, development rate, proxies for metabolic rate (i.e. oxygen consumption and heart rate) and the level of oxidative stress were investigated. A quantitative scale of development was also constructed on the basis of amount yolk (%).

Paper II.

The aim of this paper was to develop an easy-to-use tool for age classification of Norway lobster embryos as the existing developmental schemes was found insufficient. This was done by fitting morphological and cardiac characters to the quantitative scale of development that was used in Paper I. Several of the observed characters had not been described previously in the Norway lobster: such as the on-set of heartbeats, the appearance of heartbeat regularity and the appearance of leucophores/xanthophores. In addition, the developmental sequence was tested in relation to pH (current vs. -0.4 units) and temperature (5°C vs. 18°C). Furthermore, the effect of pH and temperature on cardiac rhythm was investigated.

Paper III.

The aim of this paper was to assess vulnerability of zoea larvae to decreased salinity and pH. However, in this paper the stressors were not combined. The focus was on survival, body weight and oxygen consumption rate (OCR) of developing zoea larvae (stage I-II).

Paper IV.

The aim of this paper was to investigate effects of exposing females, juveniles and embryos to OA and hypoxia or Mn^{2+}. The duration was approximately 2 months. In specific, development rate, proxies for metabolic rate (i.e. oxygen consumption and heart rate) and effects on weight and cardiac rhythm were investigated in embryos. OCR was also determined in juveniles and females. In this paper a more severe level of OA was applied than in **Paper I-III** as it was concluded that Norway lobsters (adults and embryos) most likely experience low pH naturally in their habitat. This was also confirmed by extracting measurements from the SMHI database collected along the Swedish west coast (**Table 1**).

3 Methodologies and Parameters

For experimental treatments, life stages included and parameters analysed in each paper of this thesis please see **Table 2**.

Table 2 Summary of experimental work in the thesis

Paper	Abiotic factors and treatment levels	Exposure time	Parameters and life-stages studied
I	Temperature; 5, 10, 12, 14,16 and 18 °C pH; ambient vs. -0.4 pH units (2100)	Long-term (4 months)	Development rate (embryos) Heart rate (embryos) OCR (embryos) Oxidative stress (embryos)
II	Temperature; 5 and 18 ° pH; ambient vs. -0.4 pH units (2100)	Long-term (4 months)	Staging morphological and cardiac characters (embryos) Developmental timing Cardiac arrhythmia (embryos)
III	Salinity; 17, 21 and 34 psu pH; ambient vs. -0.4 pH units (2100)	Short term 12-18 days	Development OCR (zoea II) Weight (zoea II) Tolerance and survival (zoea I-II) Calcium content (embryos, zoea I)
IV	pCO_2; ambient vs. + 600 µatm (2100) Oxygen saturation; full vs. 23% Manganese; ambient vs. 8 mg/L $^{+}$	Medium-long term (7+1.5 weeks)	Development rate (embryos) Weight and water content (eggs) Heart rate (embryos) Cardiac arrhythmia (embryos) OCR (females, juveniles and embryos)

3.1 Collection of specimens

For all included studies (**Papers I-IV**) eggs and larvae were taken from creel caught female Norway lobster females, which arrived at the Sven Lovén Centre for Marine Sciences, Kristineberg within 2 hours after capture. At arrival the females were allowed to recover for a minimum of one week before starting the experiments.

3.2 Experimental set-up

The studies in this thesis are all derived from experimental set-ups in laboratory conditions with water supply from the fjord at a depth of 32 meters. Air and water temperature were controlled and water filtered (100 µm). Natural salinity of incoming seawater was logged.

In **Paper I** and **II**, the experimental period progressed for 4 months, as one of the major aims was to acquire long-term data for the embryonic development. Each combinational treatment of OA (ambient vs. -0.4 pH units) and temperature (5, 10, 12, 14, 16 and 18°C), n = 6. The females were held in separate 15 L aquaria's with inflow of water from a header tank system. Each brood was subsampled for eggs every month throughout the experiments. The developmental scheme was constructed using all replicates in the control pH from all temperatures. The developmental sequence was then tested by comparing two temperatures in combination with OA (5 and 18°C).

A medium to long-term experiment was also conducted in **Paper IV** where juvenile and egg-carrying Norway lobsters were exposed to either OA or ambient pCO_2 for ~7.5 weeks, combined with an additional stressor during the final week; either hypoxia or Mn^{2+}. The lobsters ($n_{females}$ = 10-12, $n_{juveniles}$ = 6-8) were held in 400 L tanks and were separated by a compartment system. Two tanks replicated each treatment. **Paper III** sums of experimental data from several different larvae experiments in OA and salinity treatments where zoea development was followed for 12-18 days. For more details on the different experimental set ups please see **Paper III** (**Table 2**).

3.3 Experimental treatments

Low pH or high pCO_2 treatments were achieved by bubbling CO_2 gas via a solenoid system controlled by a pH computer (AB Aqua Medic, Bissendorf, Germany). As the Aqua Medic computers tend to drift they had to be adjusted by measuring pH (**Paper I-III**) or CO_2 (**Paper IV**) of the experimental water on a daily basis with a WTW pH 3310 with a SenTix 41 electrode, Weilheim, Germany and a Vaisala GM70, Helsinki, Finland respectively. In all papers the simulated OA treatments corresponded to the global predicted decrease by the end of this century (IPCC, 2013). In **Paper I-III,** pH was at all times kept -0.4 pH units below the pH of ambient fjord seawater. In **Paper IV**, pCO_2 was increased by 600 µatm from the naturally occurring pCO_2 of station T5 = Anholt E (**Table 1**) where mean pH for September between 2003-2013 was 7.8 = 950 µatm pCO_2, which can be compared to the current global average of approximately 380 µatm pCO_2 (IPCC, 2013). The level of 600 µatm pCO_2 corresponds to one of the more moderate model predictions for the end of this century (predictions between 500-1000 µatm; IPCC, 2013). Water chemistry parameters that were not obtained directly were in **Paper I** calculated by using the CO_{2SYS} software (Pierrot et al. 2006) with dissociation constants from Mehrbach et al. (1973) refitted by Dickson and Millero (1987). In **Paper IV**, these data were calculated with the software CO_2calc (USGS, USA).

The salinity treatments in **Paper III** were made according to Wood et al. (2013) by diluting fully saline seawater with aerated fresh water.

The hypoxic treatment was achieved by bubbling nitrogen (N_2) gas into header tanks. The water was thereafter fed to the animal tanks (**Paper IV**). The amount N_2 fed to the header tanks was controlled through a dissolved oxygen (DO) control system equipped with four DO optodes (Qubit Systems Inc, Kingston, Canada), which continuously measured the oxygen saturation. Basically this system bubble the water with N_2 through a solenoid whenever the oxygen saturation exceeds a given set point.

3.4 Parameters in focus

An organism will direct energy towards different vital functions, throughout its life cycle, such as growth, reproduction and homeostasis. In times of stress, certain traits or mechanisms will require additional energy, energy that will require a higher intake of food or allocation from other less vital functions (Stearns, 1992). In this thesis the following parameters were studied: development rate, development of morphological characters, OCR, heart rate and cardiac rhythm, calcium content, oxidative stress, weight change and survival, as they all and together reflect upon an organisms sensitivity to environmental change.

3.4.1 Cardiorespiratory development and performance

The cardiovascular system is of great importance to homeostasis. As development progresses, oxygen demand increases and at some point, diffusion will not be sufficient. Further development will require a functional convective system to keep the tissues oxygenated (Spicer, 1994). The exact **onset of heartbeats** in the Norway lobster is not previously known but should be coupled with development of thoracic segmentation (Spicer, 2001). Patterns in cardiac performance and rate of **oxygen consumption** also reflect upon metabolic rate and may reveal critical environmental conditions (Frederich and Pörtner, 2000; Brown et al. 2004).

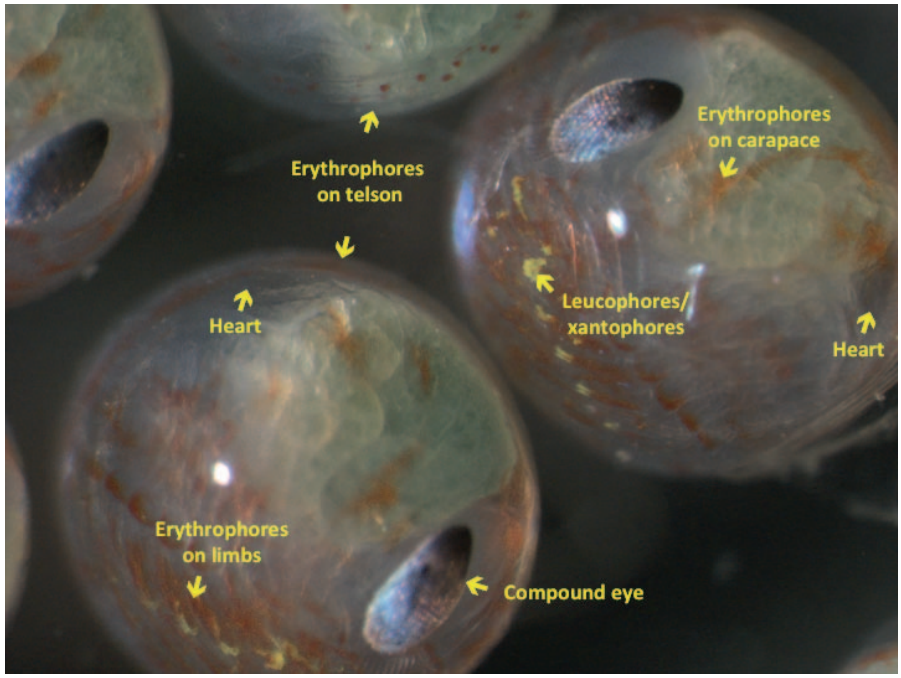

Figure 4. Eggs from Norway lobster with developing embryos. Chromatophores and other characters are pointed out.

During early crustacean development, **heart rate** equals cardiac output (Harper and Reiber, 2004) making heart rate an easy and relevant parameter to quantify. This is also easily done with Norway lobster eggs, as they are transparent (**Figure 4**). When studying cardiac performance in Norway lobster embryos, two kinds of cardiac irregularities can be observed, that is **intermittence (IM)** and **double beats (DB)**. The IM equals cardiac arrest, such as when the heart temporally stops but resume beating. The DB occurs when the heart performs double beats instead of single beats. These two may be important health traits. In Norway lobster embryos, IM has earlier been shown to increase in high oxygen saturation (>95%) while DB were more common during hypoxia (Eriksson et al. 2006).

Cardiac arrest has also been described in adult Norway lobster (Hagerman and Uglow, 1985). IM and DB are also found in other adult crustaceans (McMahon and Wilkens, 1983; Kuramoto and Ebara, 1991).

Cardiac parameters (i.e. heart rate and cardiac rhythm) were studied in eggs placed under an inverted microscope in water filled flat surface flasks. Temperature during these measurements did not deviate more than 1°C from experimental temperature. OCR (µg O2/egg/h) of embryos and larvae were investigated by measuring oxygen saturation (%) in closed vials containing eggs or larvae, pre and post incubation in a temperature-set water bath or a thermo-constant room. Measurements were performed with an Oxy 4, fibre-optic system (PreSens, Regensburg, Germany) (**Papers I**, **III** and **IV**). Oxygen saturation in the vials with zoea larvae exposed to different salinities was measured continuously (**Paper III).**

Standard metabolic rate (mg O2/kg/h) of females and juveniles (**Paper IV**) were measured using static intermittent-flow respirometry on individuals (Clark et al. 2013). In short, the lobsters were placed into custom-made 1.1-liter respirometers (inner diameter 7 cm, inner length 26 cm) equipped with a circulation pump with the stream of water passing across an in-line robust 3 mm oxygen optode (Firesting, Pyroscience, Aachen, Germany). For the adults, the measurements consisted of 10-minute measurements followed by 10 minutes of flushing to replenish the respiration water. For juveniles the measurement took 30 minutes with 10 minutes of flushing in-between. In total 3-6 subsequent runs were performed. Each measurement was corrected for background respiration.

3.4.2 Weight

Through development, egg **dry weight** decreases as the embryo consume its yolk and egg **wet weight** increases as water is absorbed, especially close to hatching (Smith, 1987). An increased or decreased weight could be indicative on the energetic demand of the organism (Schiffer et al., 2013). Increased wet weight could also indicate an adaptive swelling of the egg to facilitate gas exchange (Dorey et al. 2013).

Wet and dry weights of eggs and larvae were scaled on a Mettler XP205 (1 µg accuracy) (**Paper III** and **IV**). Animals were blotted dry prior to wet weighing. For larvae, single individuals were weighed and freeze dried in a Scanvac Coolsafe^{TM}. Eggs were weighed in small clusters taken from the same replicate female and the number of eggs in each sample was counted to estimate average dry and wet weigh per egg. For females and juveniles only wet weight was obtained (Mettler Toledo, 0.1 g accuracy) (**Paper III** and **IV**)

3.4.3 Development

Effects on **development rate** can have far-reaching effects on species biogeography and recruitment patterns (e.g. Pörtner and Farrell 2008). For example, earlier hatching could subject the larvae to the risk of a mismatch in food source timing (Gotceitas et al. 1996; Byrne, 2011; Pochelon et al. 2011). Environmental stress are known to

To measure development rate eggs were photographed (Leica stereomicroscope, MZ16 A, Leica Microsystems, Wetzlar, Germany) and analysed with the ImageJ software (Wayne Rasband, National Institute of Health, USA) by calculating pixels covering yolk: pixels covering entire egg. Development rate was estimated by dividing total yolk consumption with the number of experimental days (**Paper I** and **IV**). In **Paper IV** development rate was also assessed as the rate of increased eye index (average of length and width of eye) according to Perkins (1972) and Helluy and Beltz (1991).

Developing embryos can be accurately stage classified with the help of easily recognizable morphological features such as chromatophores (**Figure 4**; **Paper II**). This has been done previously in Dunthorn (1967) but as that scale was considered too gross it was developed further in this thesis. In **Paper I** and **II**, the amount yolk was used as a continuous measure of embryonic age instead of eye index, which was used by Perkins (1972) and Helluy and Beltz (1991) on the closely related *Homarus americanus.* This was because the eggs at start of experiment had no visible eyes and in many not even an embryo.

3.4.4 Calcium content

Crustaceans build calcium carbonate ($CaCO_3$, calcite) exoskeletons and calcification has been seen to accelerate after zoea III (Spicer and Eriksson, 2003). So far, **calcium content** in embryos and in the first larval stage has not been reported.

Calcium content was measured by flame atomic absorption spectrophotometry (GBC 932 Scientific Equipment PTY Ltd, Victoria Australia). In brief, five eggs and newly hatched larvae (zoea I) were sampled and immediately frozen in -80°C. The larvae were <5 hours post hatching when sampled. Samples were freeze-dried before placed in an ultrasound water bath for five hours (Eriksson and Baden, 1998) where after calcium content was measured. For more details, see **Paper III**.

3.4.5 Oxidative stress

Metabolism produces potent reactive oxygen species (ROS), which are both important signalling factors for homeostasis, as well as the cause of cellular damage, such as **oxidative stress** (Finkel and Holbrook, 2000; Hamdoun and Epel, 2007).

Oxidized proteins are known to accumulate in tissues with age and exposure to certain pollutants and therefore are commonly used as biomarkers for oxidative stress (Harman, 1956; Dalle-Donne et al. 2003; Carney-Almroth et al. 2005). Tolerance towards oxidative stress depends on the organism's ability to mitigate damaging effects of ROS. This can be done through antioxidant enzymes (e.g. superoxide dismutase, catalase and glutathione peroxidase), antioxidant compounds (e.g. carotenoids) and/or through the composition of tissues (Finkel and Holbrook, 2000; Monaghan et al. 2009). Oxidative stress can be looked upon as a negative trade-off to energy consuming activities. Whether organisms will be able to mitigate an increased oxidative stress in a changing environment is of great interest to study (Monaghan et al. 2009).

In **Paper I**, oxidative stress was assessed with a protein carbonyl method (Levine et al. 1990; Reznick and Packer 1994; Yan et al. 1996). Protein Assay Reagent Kit was purchased from Pierce, Thermo Scientific. All other chemicals were from Sigma-Aldrich (St Louis, MO).

3.4.6 Survival rate

The ability to maintain homeostasis at periods of environmental stress is energetically costly and could have a negative effect on population fitness. Homeostasis may be upheld up to a critical point at which physiological functions break down, and the animal dies. Survival rate is an ultimate end-point and the outcome will depend on the species plasticity and or ability to adapt quickly enough to the expected environmental changes.

Survival of Zoea I-II in different salinity and OA treatments were assessed in **Paper III**.

3.4.7 Data analysis

Statistical analyses were performed in either R, SPSS Statistics or PRIMER 6 with the PERMANOVA+ add-on. PERMANOVA provides analyses without the limitations of normally distributed datasets. All analyses held a 95% significance threshold. For example ANOVA, ANCOVA and MANOVA was used. For details, please see **Paper I-IV**.

4 Results and Discussion

4.1 Morphology during embryonic development

Through egg development, the embryos' main source of energy is the yolk, which shrinks from 100 % to 0 % of the egg space. Meanwhile, **chromatophores** appear successively on different parts of the embryo (**Figure 3; Paper II**). In this section the appearance of these characters on the scale of amount yolk is presented. The amount yolk was chosen instead of eye index (Perkins, 1972; Helluy and Beltz, 1991) as it can be quantified already from day 1 of development. In **Paper II**, the dark eye pigment was observed at 87±13% yolk. In **Paper IV**, amount yolk and eye index was found to be interchangeable as they were linearly related.

Each character's first appearance was represented by the average between observed maximum and minimum amount yolk. The embryo was visible at 95 ± 5 % yolk (mean ± max/min) and its eye pigment was first observed at 87 ± 13% yolk. The red chromatophores (erythrophores) appeared in close sequence first on the limbs (70 ± 4% yolk), then on the carapace (69 ± 6 % yolk) and last on the terminal segment (64 ± 5% yolk). From about 49 ± 1% yolk, when the leucophores/xanthophores appeared and to the time of hatching, all morphological features that could be visually examined seemed to be in place (**Table 2**; **Figure 3a**; **Paper II**). The aim of **Paper II** was to scheme visually different types of chromatophores in order to create a tool for age classification rather than to fully identify the type of chromatophores seen, such as leucophores or xanthophores.

4.2 Cardiorespiratory physiology

In times of stress, an organism can either increase metabolic rate in order to maintain its performance level or it can reduce metabolic rate in order to save energy.

4.2.1 Cardiorespiratory development in embryos

The embryonic heart started to beat at 79 ± 8% yolk and it becomes regular at 72 ± 3% yolk (**Paper II**). In the closely related species American lobster (*Homarus americanus*), onset of heartbeats was shown to take place when the egg is filled with approximately 90% yolk (Helluy and Beltz, 1991). This might be explained by the fact that eggs from American lobsters have a lower surface to volume ratio of 3,3 (recalculated from Sibert et al 2004) compared to eggs from the Norway lobster of 4,0 (recalculated from Powell and Eriksson 2013), which makes the American lobster require an active circulation system at an earlier developmental stage. At a temperature of 12°C, **heart rate** was found to increase from ~55 beats per minute (BPM) at 70% yolk to ~150 BPM at 20% yolk and **OCR** was seen to increase from 1.9 nmol O_2/egg/h in eggs with 70 % yolk to 3.3 nmol O_2/egg/h in eggs with 20% yolk. This is in contrast to the crayfish, *Procamabrus clarki*, where heart rate decreased through embryo development but in agreement with for example the crab, *Cancer pagurus* (Reiber, 1997; Naylor et al. 1999) and many other crustaceans. Furthermore, IM was the most common cardiac arrhythmia through embryonic development of the Norway lobster (**Paper II** and **IV**; **Figure 5**).

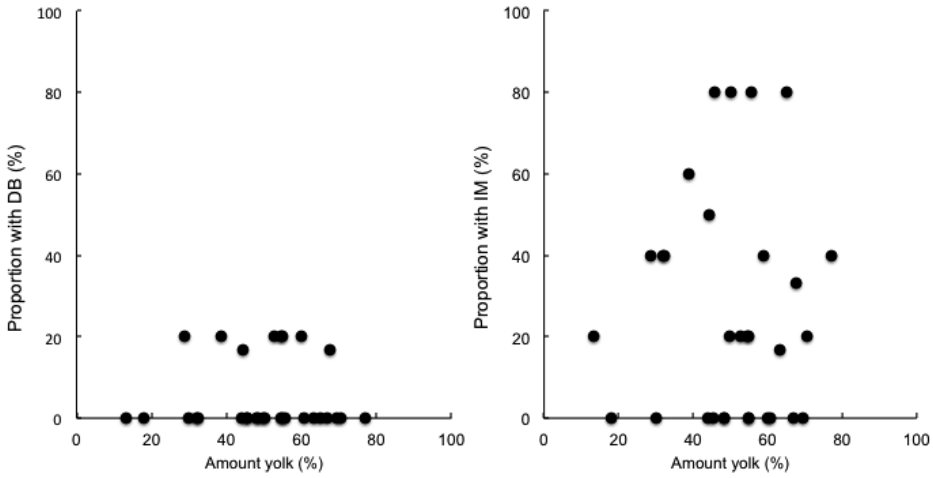

Figure 5. Proportion of DB and IM during embryonic development in 12°C and fully oxygen saturated water.

4.2.2 Combined effects of OA and elevated temperature

Elevated temperature decreases solubility of oxygen in seawater and increases metabolic rate up to a thermal limit. Simultaneous exposure to OA has previously been seen to lower this limit in many organisms (e.g. Metzger et al. 2007). In response to elevated temperature, mean heart rate in Norway lobster embryos increased linearly from 78 BPM at 5°C to 155 BPM at 18°C in eggs with 50% yolk (**Paper I**). Exposure to OA (-0.4 pH units) did not change this pattern. Thus heart rate in the Norway lobster embryos were not limited in the conditions tested. However, a decreased proportion of DB was seen as temperature was elevated (**Paper III**). In contrast to mean heart rate, OCR reached a maximum at 14°C (**Paper I**). The same pattern has been observed in adult spider crab (*Maja squinado*) and in kelp crab larvae (*Taliepus dentatus*), that is, an increased heart rate with temperature above the level where ventilation no longer increases (Frederich and Pörtner, 2000; Storch et al. 2009). This indicates that the cardiovascular system was insufficient at providing oxygen. However, in the embryos it may also reflect upon the number of metabolically active cells able to take up oxygen (Guppy and Withers, 1999).

Exposure to OA at the level of -0.4 pH units (=1279 ±44 µatm CO_2 at 12°C) had no main or interactive effects with temperature on OCR, (**Paper I**). This is in contrast to previous studies on embryos, for example, *Littorina obtusata* and *Petrolisthes cinctipes*, which both showed depressed OCR as well as heart rate during exposure to OA (Ellis et al. 2009; Carter et al. 2012; Ceballos-Osuna et al. 2013).

4.2.3 Combined effects of OA and hypoxia or Mn^{2+}

Oxygen consumption in the juveniles and embryos studied in **Paper IV** were unaffected by OA as a single stressor. This was in accordance with the results seen in **Paper I**, although a milder but longer CO_2 exposure was applied in **Paper I**. However, in the females, a slight increase in OCR following exposure to OA (1550 µatm CO_2) was seen (**Paper IV**).

Metabolic suppression is considered an adaptive strategy for the survival of short-term stress (Fabry et al. 2008). A greatly reduced OCR was found at hypoxia for all life stages tested in **Paper IV**. During exposure to declining oxygen saturations, adult decapod crustaceans are known to regulate their oxygen uptake and thereby maintain oxygen consumption. This is achieved by for example an increased ventilation rate and an increased perfusion of hemolymph through the gills but also through an increased biosynthesis and optimisation of the respiratory pigment (haemocyanin) (DeFur et al. 1990; Baden et al., 1990; Hagerman and Uglow, 1985; Taylor, 1981; Spicer and Baden, 2001). Acclimated adult Norway lobster maintain oxygen uptake down to 5kPa (~25% O_2-saturation) after which it declines (Spicer, 1995). In **Paper IV** the hypoxic treatment averaged ca. 23% saturation, which corresponds to approximately 4 kPa, and it was maintained for over a week.

Interestingly, combined exposure to OA and hypoxia further reduced OCR in embryos. This could possibly be attributed to a reduced gas exchange across the eggshell due to the initial metabolic depression induced by hypoxia, leading to an accumulation of CO_2 inside the egg, which lowered pH and further steepened the depression (Pörtner et al. 2005; Reipschläger and Pörtner, 1996; Guppy and Withers, 1999). The combined effect was not reflected on heart rate. However, cardiac performance became more rhythmic in the combination of OA and hypoxia than in hypoxia alone (**Paper IV**). Physiological stress has previously been seen to cause a more regular cardiac rhythm in crustaceans (McMahon, 1999).

Embryonic heart rate was higher in hypoxia than in the other groups although OCR was lower. The increased proportion of DB also suggested that heart rate could not be increased further, and that cardiac output had to be elevated through the means of DB instead (**Paper IV**). When average mean and average maximum heart rate was compared a smaller difference in hypoxia compared to in the other groups was evident. This indicates that the eggs in hypoxia were closer to their tolerance limit, with less capacity to further increase heart rate. As a caveat, the maximum heart rate observed may not be the maximum possible heart rate. However, this heart rate corresponds well to the rate seen embryos in **Paper I** at 18°C and with the same amount yolk. Pre-hatch *N. norvegicus* embryos have earlier been seen to induce bradycardia at low oxygen saturation (Eriksson et al. 2006). Though the authors could show a linear decrease at oxygen saturation of <30%, the data only showed a clear bradycardia at oxygen saturation <20%. This is then in agreement with the findings of the present study on less developed embryos exposed to 23% oxygen saturation. However, differing response to environmental stress depending on development stage has been seen previously; red swamp crayfish (*Procambarus clarki*) exposed to hypoxia exhibited decreased heart rate of 16 days old embryos and third instar larvae whereas no effect on heart rate was seen during the embryonic and larval development in-between (Reiber, 1997; Spicer, 2001). Earlier developmental stages of Norway lobster embryos have also been found to be more tolerant to hypoxia, as they will survive chronic progressive exposure down to 5% O_2-saturation, compared to later stage embryos that had decreased survival rates below 7% O_2-saturation as well as premature hatching below 16% O_2-saturation (Eriksson et al. 2006). Insensitivity to hypoxia has also been seen in for example early development of the grass shrimp (*Palaemonetes pugio*) (Reiber et al. 2000).

Manganese was also seen to depress metabolism in females. Previous research has not found any inhibiting effects of manganese on oxygen uptake of the crustacean respiratory pigment, haemocyanin (Baden and Neil, 1998). Although manganese has the potential to block haemocyanin production, this has little influence on oxygen transport at normoxic levels (Baden et al. 2003). In several of the lobsters exposed to manganese, a black layer of precipitated manganese dioxide was observed on the exoskeleton (**Figure 6)** including the gills, similar to what has been observed in Norway lobsters from hypoxic areas in the field (SE Kattegat) (Baden et al. 1990). Effects of the precipitated layer of manganese on respiration is not yet investigated but may impede normal function of the gills and cause internal hypoxia, as has been found for crustaceans when exposed to other essential metals like Cu and Zn (Spicer and Weber, 1991).

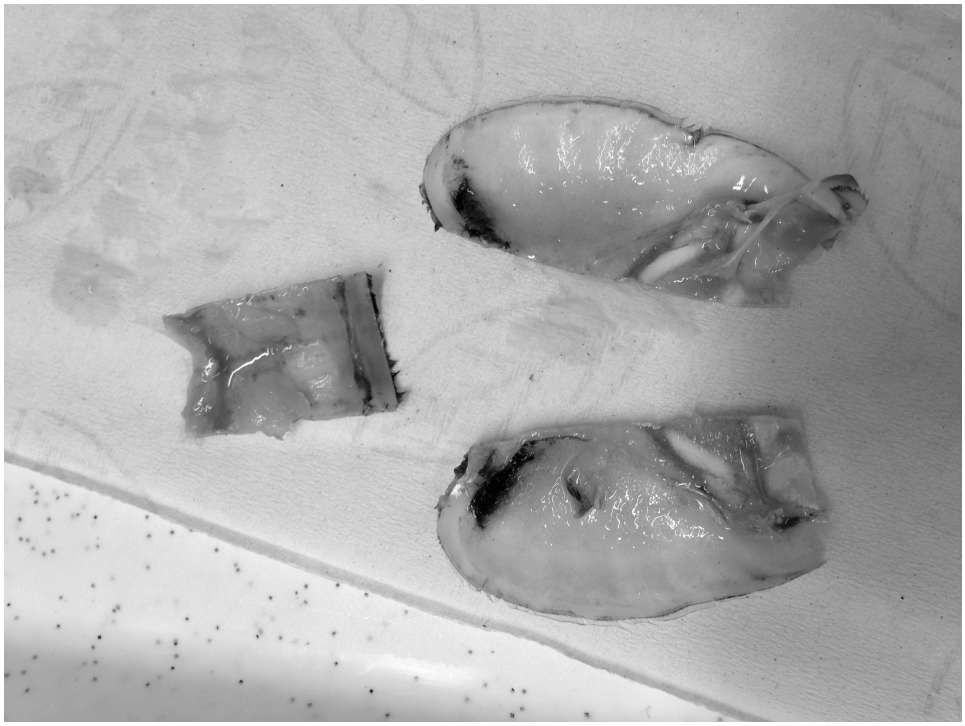

Figure 6. Inside of a carapace from an adult Norway lobster exposed to 8 mg Mn/L during 1.5 week. The black layers are precipitated manganese dioxide.

Exposure of embryos to Mn^{2+} had no significant effects, neither on heart rate, cardiac rhythm nor OCR. The level of OCR was although higher in the Mn^{2+} embryos. Norway lobster embryos have previously been found to take up manganese through their eggshells during exposure to manganese-enriched water of similar concentration and duration (i.e. 10 mg Mn L^{-1} for x days) as was used in **Paper IV** (Eriksson, 2000). Due to the known neurotoxic effects of Mn^{2+}, it is plausible that negative effects of exposure during embryonic development will be seen first later in development, e.g. on hatching success, metamorphosis, swimming and feeding (Baden and Eriksson 2006).

Combined exposure of females to OA and Mn^{2+} increased metabolic rate significantly. This was probably linked to a higher cost of ion regulation, which could have implications on physiological performance and ultimately survival (Wood et al. 2008; Li and Gao, 2012; Stumpp et al. 2012). An organism under stress can reallocate energy from other physiological functions such as protein synthesis and growth (e.g. Guppy and Withers, 1999; Pörtner et al, 2005; Gorr et al. 2006) but also increase feeding in order to compensate for the higher energetic demand (Thomsen et al. 2013). Whether the increased metabolic rate in females had negative consequences for other physiological endpoints are still to be tested. Data on for example weight and lipid content of the hepatopancrease will be analysed. In contrast, the embryos exhibited a lower oxygen consumption in the combination of Mn^{2+} and OA, suggesting severe stress (**Paper IV**). A lower uptake of Mn^{2+} in low pH (CICADS-report, 2004) has previously been seen in invertebrates. This however, does not explain the effect seen on embryos in **Paper IV**. It remains to be investigated whether it was caused by a higher uptake or a higher toxicity in combination with OA.

As the treatment effects in **Paper IV** was seen to increase with embryonic age it would be very interesting to redo the experimental set up with embryos closer to hatching. I would then expect to see a significant higher oxygen consumption rate in Mn^{2+} compared to in the control due to the higher metabolic demand and higher permeability of the eggshell with development.

4.2.4 Effects of OA and hyposalinity on zoea larvae

The level of OCR of developing zoea larvae (in 12°C), in zoea I - zoea II - zoea III increased on average from about 40, 60 and 140 nmol O_2/larva/h respectively (**Figure 7**). These data are not included in any of the papers.

No significant difference due to exposure to OA could be seen in OCR, not after 12 or 18 days of exposure.

A high variance in OCR was however seen between larvae from different broods. This high variance in OCR was also seen in both embryos and larvae of the porcelain crab as well as in embryos of another crab, *Cancer pagurus* (Naylor et al. 1999; Carter et al. 2012). This is likely true also for embryos of Norway lobster but in this thesis OCR was not measured for individual eggs/embryos due to methodological constraints.

Exposure to hyposaline water increased OCR, although not significantly (**Paper III**). An increased OCR in response to osmotic stress has been seen in other crustaceans, as the energetic demand for osmoregulation increases. This might lead to decreased energy stores and changed energy substrate utilization (Torres et al. 2011).

Larvae of the porcelain crab were not affected by either OA or salinity stress as single factors but when sequentially (OA then salinity) added an increased OCR was measured (Miller et al. 2014). Future studies on Norway lobster larvae should aim at combining low salinity with elevated temperature and OA, as it is a highly relevant condition for the pelagic life stages (**Table 1**).

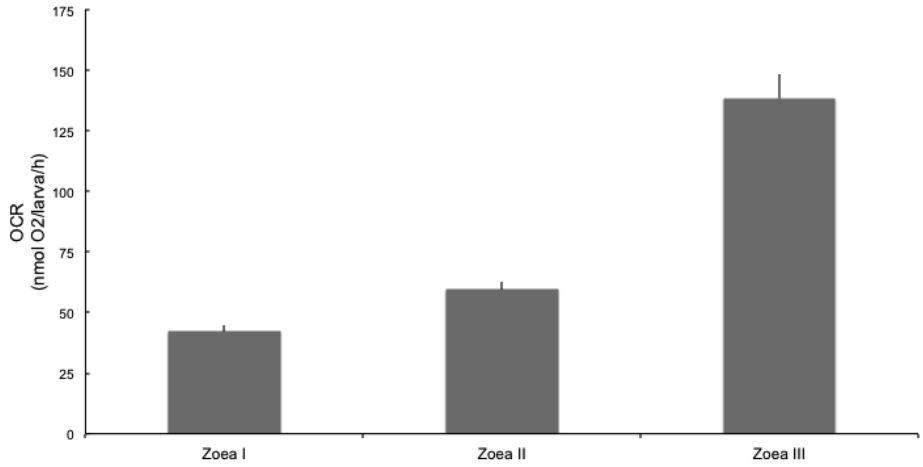

Figure 7. Oxygen consumption rate (OCR) in the three different stages of developing Norway lobster zoea larvae. N = 18 (ZI), 12 (ZII) and 2 (ZIII).. Data from both control pH and low pH combined.

4.3 Weight and water content of eggs and larvae

In **Paper III**, zoea larvae (from zoea I to zoea II) exposed to different salinities for 12 days had a significantly different dry weight. Larvae exposed to 34 or 21 PSU weighed 2.1 mg while larvae exposed to 17 PSU only weighed 1.7 mg. This is in agreement with Torres et al., 2011 who saw a decrease in lipid and protein content in Norway lobster zoea I larvae exposed acutely to 20-25 PSU for 2 days. Wet weight of larvae exposed to OA was not affected, although there was a general trend towards greater weight in the acidified condition. This is in contrast to *H. araneus* larvae, which exhibited a lower weight in 2400 µatm pCO_2 at day 8 (Schiffer et al. 2013).

In **Paper IV** dry weight was not affected by treatment. Wet weight was however significantly lower in the hypoxia eggs also exposed to OA. Eggs exposed to the combination of OA and Mn and OA and hypoxia also had significantly lower water content than the others. The lower water content was likely an effect of the reduced metabolic rate also seen in these groups, as a lower production of metabolic water would be expected (Petersen and Anger, 1997).

4.4 Development rate of embryos and larvae

In this thesis, **development rate** during the egg phase was quantified using two methods, 1) consumed amount yolk day^{-1} (**Papers I** and **IV**) and 2) increase in eyeindex/day (**Paper IV**). By measuring yolk consumption; the embryos energy requirement was in focus and by studying eye growth; the energy converted into embryonic growth was in focus. There was a significant relationship between the amount yolk and eye index, making them both suitable as continuous age equivalents (**Paper IV**). The second method was however more time efficient, as it demanded less analytic time per image. As the eye pigment is not seen until 87±13% yolk and as it can be difficult to measure early on I propose to calculate yolk percentage in eggs pre-appearance of erythrophores on limbs (≈ 70% yolk) after which the eye-pigment is easily seen.

Temperature is the major controlling factor in invertebrates of development rate (e.g. Byrne, 2011). Exposure to a temperature outside the thermal limit of a species can cause developmental abnormalities, reduced fitness and also negatively affect higher levels of organisation, such as population and ecosystem (Wang and Overgaard, 2010; Pörtner and Farrell, 2008; Byrne, 2011). In the Norway lobster, development rate of embryos was found to increase from 0.14% yolk/day at 5 °C to 0.77% yolk/day in 18°C (**Paper I**).

By including a recalculated value on development rate from Farmer (1974), 0.83% yolk/day in 21 °C could be included in the graph (**Figure 8**). By comparing Q_{10} values between the range of 10-18°C (Q_{10} = 2.9) and 18-21°C (Q_{10} = 1.3) an upper limit to an increased development rate close to 21°C is suggested but this however need further investigations by experiments at higher temperatures (**Figure 8**). Farmer (1974) suggested that the increase in development rate should stabilizes above 15°C. **Paper I** supports no such limit as development rate increased linearly up to the highest temperature tested, 18°C. At 5°C, development proceeded, although at a slow rate. This contradicts earlier literature, where it has been suggested that development may arrest for up to 7 months at winter temperatures (Farmer 1974).

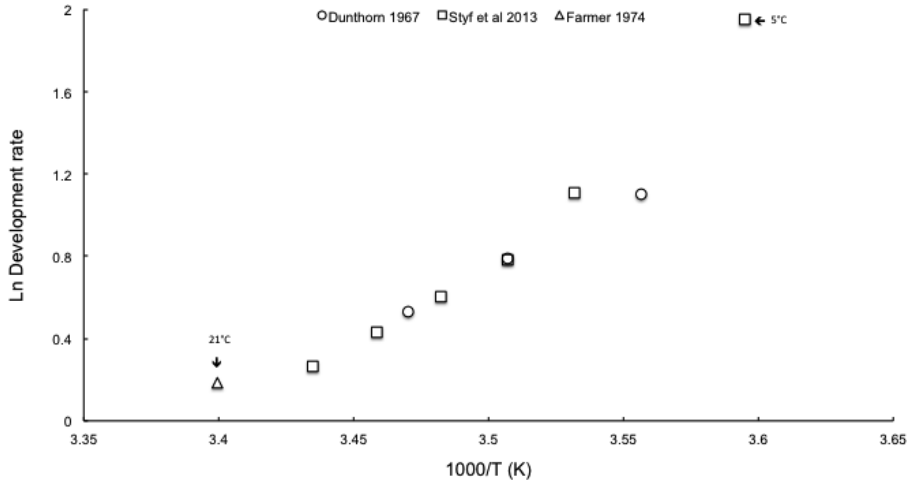

Figure 8. Arrhenius plot of the average development rate (Ln % yolk consumption/clutch/day) vs. incubation temperature (1000/Kelvin) for 5, 8, 10, 12, 14, 15, 16, 18 and 21°C.

Development rate of Norway lobster embryos was not affected by exposure to either the -0.4 pH units (or 1280 µatm in 12°C) exposure for 4 months in **Paper I** or the 1570 µatm CO_2 exposure in **Paper IV**. Also, there were no interactive effects of exposure to OA in combination with temperature on development rate. The insensitivity of development rate to OA is in line with previous studies on early development in a range of crustaceans, for example, *Acartia tsuensis*, *Homarus gammarus*, ,*Petrolisthes cinctipes* and *Pandalus borealis* (Kurihara and Ishimatsu 2008; Arnold et al. 2009; Ceballos-Osuna et al. 2013; Arnberg et al. 2013). However, low pH has been found to reduce development rate in several other crustacean species, for example, *Semibalanus balanoides*, *Hyas araneus*, and *Homarus americanus* (Findlay et al. 2009; Walther et al. 2010; Keppel et al. 2012). Exposure to OA also reduced embryonic development rate in the barnacle, *Semibalanus balanoides* and decreased growth of rock oyster (*Saccostrea glomerata*) embryos and larvae with elevated negative effects seen in combination with an increased temperature (Findlay et al. 2009; Parker et al. 2009). Exposure to OA in combination with low salinity increased development rate in the amphipod *Echinogammarus marinus (*Egilsdottir et al. 2009*)*.

Exposure to hyposaline water (i.e. 17 PSU) caused zoea I larvae to start moulting into zoea II one day earlier (i.e. on day 7) compared to zoea larvae exposed to 21 or 34 PSU. Moulting was however completed in all treatment groups at day 11 (**Paper III**). Furthermore, three larvae in zoea stage III were found in the control pH treatment at termination of the OA exposure experiment (**Paper III**). These finding were not significant but suggests an increased zoea development rate in hyposaline and OA treatment. Additional studies are needed to further evaluate this.

In **Paper IV** where both yolk consumption rate and eye growth rate was used as measures for development rate the results where contradictive. For yolk consumption rate a significantly higher utilization of yolk was seen in both hypoxia and in manganese compared to in the control while no significant difference could be seen for eye growth. This suggests a higher energy demand, which could have implications later in the life cycle, such as low energy reserves in the hatched zoea larvae. This effect however was not reflected in patterns of metabolic rate, which would be expected. Therefor this need to be further explored before making assertive conclusions.

4.5 Calcium content of embryos and larvae

As exoskeletons of crustaceans are mostly in the form of calcite, crustaceans are believed to be less sensitive to future lowered ocean pH (reviewed in Whiteley, 2011). In **Paper III,** the **calcium content** [Ca^{2+}] of the egg increased through embryonic development from 0.063 to 1.37 µg Ca^{2+}/egg between 95% yolk and close to hatching. Calcium content was 22-fold higher (i.e. 25.8 µg Ca^{2+}/larva) in newly hatched zoea I larvae when compared with in eggs (**Table 3**; **Paper III**). Crustaceans typically take up Ca^{2+} by absorbing ambient water and newly hatched zoea I larvae contain 12% more water than the final egg stage (Greenaway 1985; Smith 1987). However, the absorbed water volume consists of no more than 1.18% Ca^{2+} at an experimental salinity of 31 PSU (Castro and Huber 2005). Consequently, a great increase in [Ca^{2+}] was seen between late stage eggs and newly hatched larvae (within < 5 hours) that cannot only be explained by the increased wet weight of the larvae. Furthermore, in **Paper III**, exposure to low pH caused a slightly higher [Ca^{2+}] through egg development but did not affect calcium concentration in the newly hatched larvae. If to speculate, the result seen at metamorphosis is not likely due to calcification of the cuticle, as this is first seen at zoea III (Spicer and Eriksson, 2003). Although, an active uptake up Ca^{2+}, accumulated for later use in calcification is plausible. After hatch the larva becomes carnivorous and is thus in need of a well functioning alimentary canal. In adult lobsters ingested food is grinded by a calcified structure in the stomach, the gastric mill (Patwardhan, 1935). Whether this exists in the larvae is not known but the formation of this structure could be a reason for the rapid uptake of Ca^{2+} directly after hatch.

The increasing effect of pH on [Ca^{2+}] in eggs was not likely related to changes in structural calcification as the effect then also would have been seen in the newly hatched larvae (**Paper III**). Although, an increase in [H^{+}] which reacts with $CaCO_3$ in the shell or other calcified structures would cause a higher internal [Ca^{2+}] (Booth et al. 1984).

The data collected, together with the limited published data on mechanistic physiology and chemistry of embryonic development are not enough to draw conclusions as to the meaning of these results seen at metamorphosis (from embryo to zoea I) and under OA. However, these are interesting findings that should be followed up with additional experiments.

4.6 Ageing and oxidative stress in embryos and larvae

Oxidative stress is coupled with energetically costly activities (Alonso-Alvarez et al. 2004). In this thesis an initial low level of oxidative stress, in eggs with close to a 100% yolk was found (**Paper I**). This is in accordance with earlier finding where eggs seemed to be actively cleared from metabolic rest products, so that new generations started physiologically young (Hernebring et al. 2006; Fredriksson et al. 2012). Closer to hatching, oxidative stress was higher, probably due to the increase in metabolic rate causing a mismatch between oxygen demand and delivery (Monaghan et al. 2009). After hatching, oxidative stress declined to 1.03 nmol protein carbonyls/mg proteins. It is possible that the larva at hatching can rid it self of cellular damage as this has been seen in for example mice during early embryonic development (Hernebring et al. 2006). Through larvae development oxidative stress was not seen to increase, although OCR did (**Figure 7** and **9**).

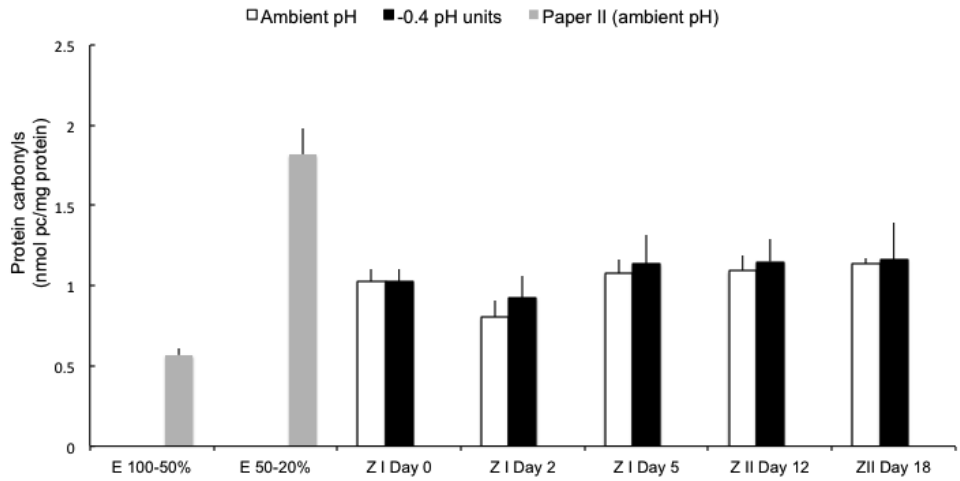

Figure 9. Level of oxidative stress (protein carbonyls/mg proteins) for the first and the second half of embryo development (first = E100-50%, second = E50-20%) and the first 18 days of zoea development (zoea I = ZI and zoea II = ZII) at 12°C. White columns = control pH and black columns = -0.4 pH units. Protein carbonyl measurements performed by Dr. Helen Nilsson Sköld. Data on larvae is not included in any of the attached papers. N_{broods} = x (E100-50%), x (50-20%), 7 (ZIday0), 6 (ZI day2), 6 and 4 (ZIday5), 6 and 4 (ZIIday12) , 4 and 3 (ZIIday18).

An increased embryonic development rate might infer a lower tolerance to oxidative stress (Metcalfe and Monaghan, 2001). In Norway lobster embryos, development rate increased as temperature did (up to 18°C) but the level of oxidative stress did not. However, effects of elevated temperature on oxidative stress have been observed in many other invertebrates (Lesser, 2006). Furthermore, oxidative stress was not seen to increase in either eggs or larvae exposed to -0.4 pH units. In fact, oxidative stress was higher in the control pH eggs (**Paper I**).

Adult Norway lobsters, exposed to the same low pH treatment as the eggs in **Paper I** exhibited a higher level of oxidative stress in their hepatopancreas than the control animals (Hernroth et al. 2012). Although antioxidant levels were never analyzed for the embryos in this thesis, their insensitivity compared to the adults and through larval development, suggests a high antioxidant capacity. Whether developing Norway lobsters use a separate antioxidant system than the adults is not currently known. However, developing sea urchins utilize a more potent embryo-specific antioxidant, mercaptohistidine (Shapiro, 1991; Hamdoun and Epel, 2007).

The role of chromatophores (i.e. carotenoids) in ROS defense is also of interest. In birds, carotenoids are important in protection against cellular damage (Surai, 2007). This is also likely in the Norway lobster (Sagi et al. 1995; Liñán-Cabello et al. 2002).

It has been suggested that negative effects of low pH seen in for example bivalves, could be coupled with a persistent oxidative stress signal (Tomanek et al. 2011). However, this explanation was not supported by Matoo et al (2013). The link between oxidative stress and pH is thus not straightforward. As oxidative stress was measured using different methods in Norway lobster embryos (**Paper I**, protein carbonyls) and adults (Hernroth et al. 2012, advanced glycation end products), the potential effect of method choice also needs further examination.

4.7 Survival of embryos and larvae

Although not quantified, mortality was not apparent in the experimental eggs. Thus, by all accounts, there has not been any lethal effect of treatments on the embryos. However, there is a slight possibility that females have removed dead eggs as other brooding crustaceans have been observed to do (Factor, 1995). So far, however, no female Norway lobster has been observed with this behaviour.

Survival of fed larvae exposed to OA was brood specific with a large effect in 2 broods but no effect in 3 other broods. Survival of starved zoea I larvae, was significantly lower at day 6 in the group exposed to OA (**Paper III**). Newly hatched larvae need to reach shallower water in order to start exogenous feeding and survive the intervening period by catabolising endogenous proteins. Starved larvae increase their protease activity, which suggests an ability to utilize protein reserves during periods of starvation (Pochelon et al. 2011). At day 6 in the OA treatment, either this capability dropped or the protein reserve was close to depleted due to a higher utilization rate in low pH. Either way, this was indicative of a higher energetic demand in OA. The first stage zoea larvae should however reach food-rich water within 24 h from hatching (Pochelon et al 2011). Exposure to OA is therefore not likely to pose a threat to this aspect of the larvae success. However, OA could affect the predation skill of the larvae as elevated pCO_2 has been found to disturb olfaction and behaviour in several species of fish and crustaceans (Munday et al. 2009; Dixson et al. 2010; Briffa et al. 2012). Survival of larval porcelain crabs was not affected by exposure to seawater with pH 7.6, although survival of juveniles of the same species was reduced by 30% (Ceballos-Osuna et al. 2013). Exposure of larvae to OA has also been seen to decrease survival in for example scallops (e.g. *Argopecten irradians*) and fish (Baumann et al. 2012; Gobler et al. 2014)

Larvae, which were allowed to acclimate to a decreasing salinity over a couple of days, were fairly tolerant to exposure to salinities down to 17 PSU. Survival of larvae exposed to 34, 21 or 17 PSU was all significantly different. Larvae exposed to 21 PSU had the highest survival (84%) and larvae exposed to 17PSU had the lowest survival (41%) (**Paper III**). This indicates that the optimum salinity for early larvae is below fully saline seawater, possibly somewhere between 20-30 PSU. The exact optimal level needs to be further explored but it corresponds to the lower salinity of surface water along the Swedish West coast (**Table 1**). Post-hatching, the larva ascends up through the water column where it acutely is exposed to a different water body than that at the sea floor.

This exposure is instant and in **Paper III** young zoea larvae were more sensitive than the older to acute exposure to hyposaline water. Thus, the field situation might not allow for any acclimation to occur.

Larvae that hatched in the highest temperature (18°C) in **Paper I** were all viable, although they were only followed < 1 day. It would be interesting to follow larvae exposed to the stressors in this thesis during embryonic life through zoea development.

4.8 Maternal protection during early development

The Norway lobster female invests massive amounts of energy into breeding as does all brooding species (Menge 1974; Clarke 1987; Pandian 1994; Fernandez et al 2000). The on-set of female maturity occurs at approximately 20 mm carapace length (for review see Powell and Eriksson 2013). Each brood takes two years to develop from immature oocytes to hatched larvae. The female also supply each egg with high-energy yolk, which sustains the embryos for the entire incubation time (6-10 months), this is highly energetically demanding (Rosa and Nunes, 2002). There seems to be a relationship between the amount yolk that an egg is filled with and performance of the later adult (Heming and Buddington, 1988). During incubation, the female also invest energy by physically ventilating the brood with her pleopods. It is known that the frequency in pleopod beating increases as the embryos develop and also during periods of hypoxia (Eriksson et al. 2006). This is also seen in other brooders such as goobies and crabs (Naylor et al. 1999; Pollock et al. 2007; Brante et al. 2003). Females have also been seen to transfer for example hormones, immune factors, mRNAs and other beneficial biomolecules to her offspring (Burggren and Blank 2009). Brood specific variability in tolerance to environmental stress, which was seen in **Paper III** could also be related to genotype/ maternal tolerance. This has previously been demonstrated in for example the urchin *Heliocidaris erythrogramma* (Schlegel *et al.,* 2012).

5 Main Conclusions and Future Perspectives

This thesis has investigated the potential effect by climate change-induced stressors on the Norway lobster early development. It comprises studies on embryonic and larval tolerance in response to multiple stressors: ocean acidification (OA), temperature, hypoxia, Mn^{2+} and salinity. It also includes data for juveniles and egg carrying females.

Norway lobster embryos of the Swedish west coast population were found to be tolerant to 4 months of exposure to elevated temperature (up to 18°C), although naturally exposed to an annual average temperature of 8°C and seldom to the highest temperature tested here. Between 2010-2014 Norway lobsters in the Gullmarsfjord were exposed to 18°C or just above for only 19 days in total, with the majority of these days in August or September. It was thus concluded that initially, the Norway lobster would benefit from global warming. However, global warming will continue to increase seawater temperature, which will extend the number of warm days as well as elevate the maximum temperature the lobsters will encounter.

Embryos were also tolerant to OA and the combination of OA and elevated temperature. No negative effects were seen on development rate, development timing, cardiac performance, metabolic rate or the level of oxidative stress. However, negative carry-over effects of condition to subsequent stages are plausible, this warrant further experimental work.

Metabolic rate in embryos exposed to hypoxia was supressed as expected. Thereto, combined exposure to OA and hypoxia suppressed oxygen consumption rate (OCR) even further. This was however not statistically significant but as the difference between the treatments increased with embryonic age it is likely that it would have been with a slightly different experimental set up, including more developed embryos. Metabolic rate in the embryos also dropped in the combinational treatment of OA and Mn^{2+}. A prolonged metabolic suppression would reduce development rate in an early organism but this was not seen in this thesis. Possibly the experimental period was too short for the method applied but since development rate actually seemed to increase in both hypoxia and Mn^{2+} these results cannot be interpreted from the data in this thesis alone. The physiological consequences of the metabolic depressions observed are thus still to be unravelled as well as the increased development rate.

Larvae, which were hatched in ambient pH and then transferred to -0.4 pH units showed a strong brood specific difference in OA tolerance, possibly genetic. Larvae that were kept unfed also displayed mortality at an earlier time point in OA than in ambient pH, thus the OA larvae probably experienced a higher energetic demand probably connected to ion regulation. Furthermore, newly hatched zoea I larvae exposed to acute hyposalinity were found to be more tolerant than older larvae. When allowed to acclimate, tolerance and thus survival increased. A higher survival was then seen in 21 compared to in 17 or 32 PSU. In lowest salinity the larvae had a significantly lower dry weight, which suggests higher energy expenditure. The pelagic zoea stages are naturally exposed to the combination of elevated temperature and hyposalinity and effects of further shifts in these abiotic stressors together with OA need to be thoroughly investigated. I therefor recommend these stressors to be combined in a future experimental set up for this species.

The juveniles in this thesis were exposed to OA and hypoxia as well as the combination of the two. Hypoxia led to a metabolic depression but no effect of OA was observed. In the egg carrying females OCR was also suppressed in hypoxia but increased in the combination of OA and Mn^{2+}. Due to practical constraints the combination of hypoxia and manganese could not be tested in this thesis although in the natural world these will act simultaneously on all the benthic Norway lobster life stages. Hence, future studies of this sort, also including OA, would be highly interesting.

In conclusion, all stages seemed relatively resilient to OA alone but life-stage dependent effects were seen when treatments were combined, such as the opposite response to OA and Mn^{2+} in embryo and female OCR. Previous research has seen brooding females to sense and adjust ventilation of their eggs in unfavorable conditions. If the increased OCR seen in females exposed to OA and Mn^{2+} is a result of an elevated fanning was not in the scope of this thesis but should be investigated.

The synergistic effects seen in this thesis would have been overlooked in a single stressor experimental set-up, which emphasise the great need for additional multiple-stressor studies. Finally, the highest increase in pCO_2 (600 µatm) tested in this thesis still represents a moderate scenario for the end of this century, since different models predict an increase of between 500-1000 µatm pCO_2 (IPCC, 2013). Thus, the effects observed could be an underestimation of the future impact of OA. In many places the Norway lobster currently lives close to the tolerance limit of the early life stages. As such, the geographic area of suitable abiotic habitat for Norway lobster may be severely affected in a near future.

6 References

Abe H, Hirai S and Okada S (2007) Metabolic responses and arginine kinase expression under hypoxic stress of the kuruma prawn *Marsupenaeus japonicus*. Comp Biochem Phsyiol A, 146:40-46.

Agnalt A- L, Grefsrud ES, Farestveit E, Larsen M and Keulder F (2013) Deformities in larvae and juvenile European lobster (*Homarus gammarus*) exposed to lower pH at two different temperatures. Biogeosciences, 10:7883–7895.

Alonso-Alvarez C, Bertrand S, Devevey G, Prost J, Faivre B and Sorci G (2004) Increased susceptibility to oxidative stress as a proximate cost of reproduction. Ecol Lett, 7:363-368.

Arnberg M, Calosi P, Spicer JI, Tandberg AHS, Nilsen M, Westerlund S and Bechmann (2013) Elevated temperature elicits greater effects than decreased pH on the development, feeding and metabolism of northern shrimp (*Pandalus borealis*) larvae. Mar Biol, 160:2037-2048.

Arnold KE, Findlay HS, Spicer JI, Daniels CL and Boothroyd D (2009) Effect of CO_2-related acidification on aspects of the larval development of the European lobster. Biogeosciences, 6:1747-1754.

Baden SP, Pihl L, Rosenberg R (1990) Effects of oxygen depletion on the ecology blood physiology and fishery of the Norway lobster, *Nephrops norvegicus*. Mar Ecol-Prog Ser, 67:141–155.

Baden SP and Neil DM (1998) Accumulation of manganese in the haemolymph, nerve and muscle tissue of *Nephrops norvegicus* (L.) and its effect on neuromuscular performance. Comp Biochem Physiol A, 119:351–359.

Baden SP, Håkansson CLJ and Spicer JI (2003) Between-individual variation in haemocyanin concentrations in the Norway lobster *Nephrops norvegicus* following exposure to hypoxia and manganese. Mar Biol, 143:267–273.

Baden SP and Eriksson SP (2006) Role, routes and effects of manganese in crustaceans. Oceanogr Mar Biol, 44:61-83.

Baumann H, Talmage SC and Gobler CJ (2012) Reduced early life growth and survival in a fish in direct response to increased carbon dioxide. Nature Clim Change, 2:38-41.

Bianchini A, Lauer MM, Nery LEM, Colares EP, Monserrat JM and dos Santos Filho EA (2008) Biochemical and physiological adaptations in the estuarine crab *Neohelice granulata* during salinity acclimation. Comp Biochem Physiol A 151:423-436.

Booth CE, McDonald DG, Walsh PJ (1984) Acid-base balance in the sea mussel *Mytilus edulis*. Effects of hypoxia on air-exposure on hemolymph and acid-base status. Mar Biol Lett, 5:347-358.

Bradassi F, Cumani F, Bressan G and Dupont S (2013). Early reproductive stages in the crustose coralline alga *Phymatolithon lenormandii* are strongly affected by mild ocean acidification. Mar Biol, 160: 2261-2269.

Brante A, Fernandez A, Eckerle L, Mark F, Pörtner H-O and Arntz W (2003) Reproductive investment in the crab *Cancer setosus* along a latitudinal cline egg production, embryo losses and embryo ventilation. Mar Ecol-Prog Ser, 251:221–232.

Briffa M, de la Haye K and Munday PL (2012) High CO_2 and marine animal behaviour: Potential mechanisms and ecological consequences. Mar Pollut Bull, 64:1519-1528.

Brown JH (2004) Towards a metabolic theory of ecology. Ecology, 85:1771-1789.

Brown-Peterson NJ, Manning S, Patel V, Denslow ND and Brouwer M (2008) Effects of cyclic hypoxia on gene expression and reproduction in a grass shrimp, *Palaemonetes pugio*. Biol Bull, 214: 6-16.

Burggren W and Blank T (2009) Physiological study of larval fishes: challenges and opportunities. Sci Mar, 73S1.

Byrne M (2011) Impact of ocean warming and ocean acidification on marine invertebrate life history stages: vulnerabilities and potential persistence in a changing ocean. Oceanogr Mar Biol, 49:1–42.

Caldeira K and Wickett ME (2003) Anthropogenic carbon and ocean pH. Nature, 425:365–365.

Caldeira K and Wickett ME (2005) Ocean model predictions of chemistry changes from carbon dioxide emissions to the atmosphere and ocean. J Geophys Res -Oceans 110:11.

Cannon WB (1926) Physiological regulation of normal states: some tentative postulates concerning biological homeostatics. In: Pettit, A. (Ed.), A Charles Richet: ses amis, ses collègues, ses élèves. Éditions Médicales, Paris, p. 91.

Carney-Almroth B, Sturve J, Berglund Å and Förlin L (2005) Oxidative damage in eelpout (*Zoarces viviparus*), measured as protein carbonyls and TBARS, as biomarkers. Aquat Toxicol, 73:171-180.

Carter HA, Ceballos-Osuna L, Miller NA and Stillman JH (2012) Impact of ocean acidification on metabolism and energetics during early life stages of the intertidal porcelain crab *Petrolisthes cinctipes*. J Exp Biol, 216:1412-1422.

Castro P and Huber ME (2005). Marine biology, Fifth edition. The McGraw-Hill Companies, Inc. 1221 Avenue of the Americas, New York, U.S.A.

Ceballos-Osuna L, Carter HA, Miller NA and Stillman JH (2013) Effects of ocean acidification on early life-history stages of the intertidal porcelain crab *Petrolisthes cinctipes*. J Exp Biol 216:1405-1411.

CICADS-report (2004) Concise International Chemical Assessment Document 63. Manganese and its compounds: Environmental aspects. Cicads 63.

Clarke A (1983). Life in cold water: the physiological ecology of polar marine ectotherms. Oceanogr. Mar. Biol, 21:341–453.

Clarke A (1987) Temperature, latitude and reproductive output. Mar Ecol-Prog Ser, 38:89–99.

Clark TD, Sandblom E and Jutfelt F (2013). Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. J Exp Biol, 216:2771-2782.

Cossins AR and Bowler K (1987) in Temperature Biology of Animals (Chapman & Hall, London), pp. 210–220.

Dalle-Donne I, Ranieri R, Giustarini D, Milzani A and Colombo R (2003) Protein carbonyl groups as biomarkers of oxidative stress. Clin Chim Acta, 329:23-38.

Darling, ES and Côté, IM (2008) Quantifying the evidence for ecological synergies. Ecol Lett, 11:1278-86.

DeFur PL, Mangum CP and Reese JE (1990) Respiratory responses of the blue crab *Callinectes sapidus* to long-term hypoxia. Biol Bull, 178:46-54.

Diaz RJ and Rosenberg R (1995) Marine benthic hypoxia: A review of its ecological effects and the behavioural responses of benthic macrofauna. Oceanog Mar Biol 33, 245-303.

Diaz RJ (2001) Overview of hypoxia around the world. J Environ Qual, 30:275-281.

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

Dickey-Collas M, McQuaid N, Armstrong MJ, Allen M and Briggs RP (2000) Temperature-dependent stage durations of Irish Sea Nephrops larvae. J Plankton Res, 22: 749-760.

Dickson AG and Millero FJ (1987) A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. Deep-Sea Res, 34:1733–1743.

Dixson DL, Munday PL and Jones GP (2010) Ocean acidification disrupts the innate ability of fish to detect predator olfactory cues. Ecol Lett, 13:68–75.

Doney SC, Fabry VJ, Feely RA and Kleypas JA (2009) Ocean acidification: the other CO_2 problem. Annu Rev Mar Sci, 1:169–192.

Dorey N, Melzner F, Martin S, Oberhänsli F, Teyssié JL, Bustamante P, et al. (2013). Ocean acidification and temperature rise: effects on calcification during early development of the cuttlefish *Sepia officinalis*. Mar Biol, 160:2007–2022.

Dunthorn AA (1967) Some Observations on the Behaviour and Development of the Norway lobster. ICES C.M.K:5 (mimeo).

Dupont S, Dorey N, Stumpp M, Melzner F and Thorndyke M (2013) Long-term and trans-life-cycle effects of exposure to ocean acidification in the green sea urchin *Strongylocentrotus droebachiensis*. Mar Biol, 160:1835-1843.

Egilsdottir H, Spicer, JI and Rundle SD (2009) The effect of CO_2 acidified seawater and reduced salinity on aspects of the embryonic development of the amphipod *Echinogammarus marinus* (Leach). Mar Pollut Bull, 58:1187-1191.

Ellis RP, Bersey J, Rundle SD, Hall-Spencer JM and Spicer JI (2009) Subtle but significant effects of CO_2 acidified seawater on embryos of the intertidal snail, *Littorina obtusata*. Aquat Biol, 5:41-48.

Eriksson SP and Baden SP (1998) Manganese in the haemolymph and tissues of the Norway lobster, *Nephrops norvegicus* (L.), along the Swedish west coast, 1993–95. Hydrobiologia, 375–376.

Eriksson SP (2000) Variations of manganese in the eggs of the Norway lobster, *Nephrops norvegicus*. Aquat Toxicol, 48:291–295.

Eriksson SP, Nabbing M and Sjöman E (2006) Is brood care in *Nephrops norvegicus* during hypoxia adaptive or a waste of energy? Funct Ecol, 20:1097–1104.

Eriksson SP, Hernroth B and Baden SP (2013) Stress biology and immunology in *Nephrops norvegicus*. Adv Mar Biol, 64:149-200.

Fabry VJ, Seibel BA, Feely RA and Orr JC (2008) Impacts of ocean acidification on marine fauna and ecosystem processes. ICES J Mar Sci 65:414–432.

Factor JR (1995) Biology of the lobster *Homarus americanus*. London: Academic Press. pp. 528.

FAO (2012) Global capture production 1950–2010. (http://www.fao.org/fishery/statistics/global-capture-production/query/en2012).

Farmer ASD (1974) Reproduction in *Nephrops norvegicus* (Decapoda: Nephropidae) J Zool Lond 174:161-183.

Fernández M, Bock C and Pörtner H-O (2000) The cost of being a caring mother: the ignored factor in the reproduction of marine invertebrates. Ecol Lett, 3:487-494.

Findlay HS, Kendall MA, Spicer JI and Widdicombe S (2009) Future high CO_2 in the intertidal may compromise adult barnacle *Semibalanus balanoides* survival and embryonic development rate. Mar Ecol-Prog Ser, 389:193–202.

Findlay HS, Kendall MA, Spicer JI and Widdicombe S (2010) Relative influence of ocean acidification and temperature on intertidal post-larvae at the northern edge of their geographic distribution. Estuar Coast Shelf Sci, 86:675–682.

Finkel T and Holbrook NJ (2000) Oxidants, oxidative stress and the biology of ageing. Nature, 408:239–247.

Frederich M and Pörtner H-O (2000) Oxygen limitation of thermal tolerance defined by cardiac and ventilatory performance in spider crab, *Maja squinado*. Am J Physiol. Reg I 279:1531–1538.

Fredriksson Å, Johansson Krogh E, Hernebring M, Pettersson E, Javadi A, Almstedt A and Nyström T (2012). Effects of aging and reproduction on protein quality control in soma and gametes of Drosophila melanogaster. Aging Cell, 11:634-643.

Gobler CJ, DePasquale EL, Griffith AW, Baumann H (2014) Hypoxia and acidification have additive and synergistic negative effects on the growth, survival, and metamorphosis of early life stage bivalves. PloS One, 9: e83648. doi:10.1371/journal.pone.0083648.

Gorr TA, Gassmann M and Wappner P (2006) Sensing and responding to hypoxia via HIF in model invertebrates. J Insect Physiol, 52:349-364.

Gotceitas V, Puvanendran V, Leader LL and Brown JA (1996) An experimental investigation of the 'match/mismatch' hypothesis using larval Atlantic cod. Mar Ecol-Prog Ser, 130: 29-37.

Greenaway P (1985) Calcium balance and molting in the Crustacea. Biol Rev Camb Philos Soc, 60:425–454.

Guppy M and Withers P (1999) Metabolic depression in animals: physiological perspectives and biochemical generalizations. Biol Rev Camb Philos Soc 74: 1-40.

Hagerman L and Uglow RF (1985) Effects of hypoxia on the respiratory and circulatory regulation of *Nephrops norvegicus*. Mar Biol, 87:273–278.

Hamdoun A and Epel D (2007) Embryo stability and vulnerability in an always changing world. PNAS, 104:1745–1750.

Harman D (1956) Aging: a theory based on free radical and radiation chemistry. J Gerontol, 11: 298–300.

Harper SL and Reiber CL (2004) Physiological development of the embryonic and larval crayfish heart. Biol Bull, 206:78-86.

Havenhand JN, Buttler F-R, Thorndyke MC and Williamson JE (2008) Near-future levels of ocean acidification reduce fertilization success in a sea urchin. Curr Biol, 18: R651-R652.

Helluy S and Beltz BS (1991) Embryonic development of the American lobster *Homarus americanus*: Quantitative staging and characterizations of an embryonic molt cycle. Biol Bull, 180:355–371.

Heming TA and Buddington RK (1988) Yolk absorption in embryonic and larval fishes. In: WS Hoar and DJ Randall (eds.), Fish Physiology, Vol XI, pp. 407-446. Academic Press, London.

Henderson C (2006) Ocean acidification: the other CO_2 problem. New Scientist http://environment.newscientist. com/article/mg19125631.200

Hernroth B, Baden SP, Holm K, André T, Söderhäll I (2004). Manganese induced immune suppression of the lobster, *Nephrops norvegicus*. Aquat Toxicol. 70:223–231.

Hernroth B, Sköld HN, Wiklander K, Jutfelt F and Baden S (2012) Simulated change causes immune suppression and protein damage in the crustacean *Nephrops norvegicus*. Fish Shellfish Immun, 33:1095-101.

Hernebring M, Brolen G, Aguilaniu H, Semb, H and Nystrom T (2006) Elimination of damaged proteins during differentiation of embryonic stem cells. PNAS, 16:7700–7705.

Hervant F, Garin D, Mathieu J and Freminet A (1999) Lactat metabolism and glucose turnover in the subterranean crustacean *Niphargus virei* during post-hypoxic recovery. J Exp Biol, 202:579-592.

Holmstrup M, Bindesbøl AM, Oostingh GJ, Duschl A, Scheil V, Köhler HR, Loureiro, *et al*. (2010) Interactions between effects of environmental chemicals and natural stressors: a review. Sci Total Environ, 408: 3746-62.

ICES (2012) Report of the Working Group on the Assessment of Demersal Stocks in the North Sea and Skagerrak (WGNSSK). ACOM:13, 1384 pp.

IPCC (2013) Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Stocker TFD, Qin GK, Plattner M, Tignor SK, Allen J, Boschung A, Nauels Y, Xia V, Bex PM, Midgley PM (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp 1535.

Keppel EA, Scrosati RA and Courtenay SC (2012) Ocean acidification decreases growth and development in American lobster (*Homarus americanus*) larvae. J Northwest Atl Fish Sci, 44:61–66.

Kuramoto, T and Ebara, A (1991) Combined effects of octopamine and filling pressure on the isolated heart of the lobster, *Panulirus japonicus*. J Comp Physiol 161B, 339–347.

Kurihara H, Shimode S and Shirayama Y (2004) Effects of raised CO_2 concentration on the egg production rate and early development of two marine copepods (*Acartia steueri* and *Acartia erythraea*). Mar Pollut Bull, 49:721–727.

Kurihara H and Ishimatsu A (2008) Effects of high CO_2 seawater on the copepod (*Acartia tsuensis*) through all life stages and subsequent generations. Mar Pollut Bull, 56:1086–1090.

Landry CA, Steele SL, Manning S and Cheek AO (2007) Long term hypoxia suppresses reproductive capacity in the estuarine fish, *Fundulus grandis*. Comp Biochem and Physiol A, 148A:317–323.

Lesser MP (2006) Oxidative stress in marine environments: biochemistry and physiological ecology. Annu Rev Physiol, 68:253e78.

Levine RL, Garland D, Oliver CN *et al.* (1990) Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol, 186:464–478.

Liñán-Cabello MA, Paniagua-Michel J and Hopkins PM (2002) Bioactive roles of carotenoids and retinoids in crustaceans. Aquac Nut 8:299-309.

Li W and Gao K (2012) A marine secondary producer respires and feeds more in a high CO_2 ocean. Mar Pollut Bull, 64:699-703.

Matoo OB, Ivanina AV, Ullstad C, Beniash E and Sokolova IM (2013) Interactive effects of elevated temperature and CO_2 levels on metabolism and oxidative stress in two common marine bivalves (*Crassostrea virginica* and *Mercenaria mercenaria*).Comp Biochem Physiol A, 164:545-553.

McMahon, BR and Wilkens, JL (1983) Ventilation, perfusion, and oxygen uptake. The Biology of Crustacea, Vol. 5: International Anatomy and Physiological Regulation (ed. D.E. Bliss), pp. 289–372. Academic Press, New York.

Mehrbach C, Cullberson CH, Hawley JE and Pytkowicz RM (1973) Measurements of the apparent dissociation constants of carbonic acids in seawater at atmospheric pressure. Limnol Oceanogr, 18:897–907.

Melzner F, Gutowska MA, Langenbuch M, Dupont S, Lucassen M, Thorndyke MC, Bleich M and Pörtner H-O (2009) Physiological basis for high CO_2 tolerance in marine ectothermic animals: pre-adaptation through lifestyle and ontogeny? Biogeosciences, 6:2313-2331.

Melzner F, Thomsen J, Koeve W, Oschlies A, Gutowska MA, Bange HW, Hansen HP and Körtzinger A (2013) Future ocean acidification will be amplified by hypoxia in coastal habitats. Mar Biol, 160:1875-1888.

Menge BA (1974) Effect of wave action and competition on brooding and reproductive effort in the seastar. *Leptasterias hexactis*. Ecology, 55:84-93.

Metcalfe NB and Monaghan P (2001) Compensation for a bad start: grow now, pay later? Trends Ecol Evol, 16: 254–260.

Miller SH, Zarate S, Smith EH, Gaylord B, Hosfelt JD and Hill TM (2014) Effect of Elevated pCO_2 on metabolic responses of porcelain crab (*Petrolisthes cinctipes*) larvae exposed to subsequent salinity stress. PLoS One, 9: e109167. doi:10.1371/journal.pone.0109167.

Moksnes P-O, Jonsson P, Nilsson Jacobi M and Vikström K (2014). Larval connectivity and ecological coherence of marine protected areas (MPAs) in the Kattegat-Skagerrak region. Swedish Institute or the Marine Environment Report no 2014:2. Pp 226.

Monaghan P, Metcalfe NB and Torres R (2009) Oxidative stress as a mediator of life history trade-offs: mechanisms, measurement and interpretation. Ecol Lett, 12:75–92.

Montory JA, Pechenik JA, Diederich CM and Chaparro OR (2014) Effects of Low Salinity on Adult Behavior and Larval Performance in the Intertidal Gastropod *Crepipatella peruviana* (Calyptraeidae). PLoS ONE, 9: e103820. doi:10.1371/journal.pone.

Mukherjee J, Wong KKW, Chandramouli KH, Qian P-Y, Leung PTY, Wu RSS and Thiyagaranjan V (2013). Proteomic response of marine invertebrate larvae to ocean acidification and hypoxia during metamorphosis and calcification. J Exp Biol, 216:4580-4589.

Munday PL, Dixson DL, Donelson JM, Jones GP, Pratchett MS, Devitsina GV and Døving KB (2009) Ocean acidification impairs olfactory discrimination and homing ability of a marine fish. PNAS 106: 1848–1852.

Naylor JK, Taylor EW and Bennett DB (1999) Oxygen uptake of developing eggs of *Cancer pagurus* (Crustacea: Decapoda: Cancridae) and consequent behavior of the ovigerous female. J Mar Biol Ass, 79:305-315.

Nichols H, Bennett DB, Symonds DJ and Grainger R (1987) Estimation of the stock size of adult *Nephrops norvegicus* (L.) from larvae surveys in the western Irish Sea in 1982, J Nat Hist, 21:1433-1450.

Orr JC, Fabry VJ, Aumont O, Bopp L *et al.* (2005) Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. Nature, 437:681–686.

Oweson CAM, Baden SP and Hernroth B (2006) Manganese induced apoptosis in haematopoietic cells of *Nephrops norvegicus* (L.). Aquat Toxicol, 77:322-328.

Pandian TJ (1994) Arthropods—Crustacea. In: Adiyodi KG, Adiyodi RG (eds) Reproductive biology of invertebrates. Oxford and IBH Publishing, Kerala, p 1–165.

Parker LM, Ross PM and O'Connor WA (2009) The effect of ocean acidification and temperature on the fertilization and embryonic development of the Sydney rock oyster *Saccostrea glomerata* (Gould 1850). Glob Change Biol, 15:2123-2136.

Patwardhan SS (1935) On the structure and mechanism of the gastric mill in decapoda.

Perkins HC (1972) Developement rates at various temperatures of embryos of the northern lobster (*Homarus americanus* Milne-Edwards). Fish Bull, 70: 95-90.

Petersen S and Anger K (1997) Chemical and physiological changes during the embryonic development of the spider crab *Hyas araneus* L. (Depapoda: Majidae). Comp Biochem Physiol B, 117:299-306.

Phillips BF and Sastry AN (1980) Larval ecology. In: The Biology and Management of Lobsters, Vol. 11. J.S. Cobb and B.F. Phillips, eds. New York, Academic Press, pp. 143-178.

Pinsino A, Roccheri MC, Costa C and Matranga V (2011) Manganese Interferes with Calcium, Perturbs ERK Signaling, and Produces Embryos with No Skeleton. Toxicol Sci, 123:217-230.

Pinsino A, Roccheri MC and Matranga V (2014). Manganese overload affects p38 MAPK phosphorylation and metalloproteinase activity during sea urchin embryonic development. Mar Environ Res, 93:64-69.

Pochelon PN, Queiroga H, Rotllant G, dos Santos A and Calado R (2011) Effect of unfavorable trophic scenarios on amylase and protease activity of Nephrops norvegicus (L.) larvae during their first vertical migration: a laboratory approach. Mar Biol, 158:2079-2085.

Pochelon PN, Lopes da Silva T, Reis A, dos Santos A, Queiroga H and Calado R (2011) Inter-individual and within brood variability in the fatty acid profiles of Norway lobster, *Nephrops norvegicus* (L.) embryos. Mar Biol, 158:2825-2833

Pollock MS, Clarke LMJ and Dubé MG (2007) The effects of hypoxia on fishes: from ecological relevance to physiological effects. Environ Rev, 15:1-14.

Powell A and Eriksson SP (2013) Reproduction: Life cycle, larvae and larviculture. Adv Mar Biol, 64:201-247.

Przeslawski R (2004) A review of the effects of environmental stress on embryonic development within intertidal gastropod egg masses. Molluscan Res, 24: 43–63.

Pörtner HO, Langenbuch M and Michaelidis B (2005) Synergistic effects of temperature extremes, hypoxia and increases in CO_2 on marine animals: from Earth history to global change. J Geophys Res, 110:1–15.

Pörtner HO and Farrell AP (2008) Physiology and climate change. Science, 322:690-692.

Reiber CL (1995) Physiological adaptations of crayfish to the hypoxic environment. Amer Zool, 35:1-11.

Reiber CL (1997) Ontogeny of cardiac and ventilatory function in the crayfish *Procambarus clarkii*. Amer Zool, 37:82-91.

Reipschläger A and Pörtner H-O (1996) Metabolic depression during environmental stress: The role of extracellular versus intracellular pH in *Sipunculus nudus*. J Exp Biol, 199:1801-1807.

Reznick AZ and Packer L (1994) Oxidative damage to proteins: spectrophotometric method for carbonyl assay. Methods Enzymol, 233:263–357.

Ries JB, Cohen AL and McCorkle DC (2009) Marine calcifiers exhibit mixed responses to CO_2-induced ocean acidification. Geology, 37:1131-1134.

Rosa R and Nunes ML (2002) Biochemical changes during the reproductive cycle of the deep-sea decapod Nephrops norvegicus on the south coast of Portugal. Mar Biol, 141:1001–1009.

Sabine CL, Feely RA, Gruber N, Key RM, Lee K, Bullister JL, Wanninkhof R, Wong CS, Wallace DWR and Tilbrook B (2004) The oceanic sink for anthropogenic CO_2. Science, 305:367-371.

Sagi A, Rise M, Isam K and Arad S (1995) Carotenoids and their derivatives in organs of maturing female crayfish *Cherax quadricarinatus*. Comp Biochem Physiol, 112:309-313.

Santos LCF, Belli NM, Augusto A, Masui DC, Leone FA, McNamara JC and Furriel RPM (2007) Gill (Na^+, K^+)-ATPase in diadromous, freshwater palaemonid shrimps: species-specific kinetic characteristics and alpha-subunit expression. Comp Biochem Physiol A,148:178-188.

Schiedek D, Sundelin B, Readman JW and Macdonald RW (2007) Interactions between climate change and contaminants. Mar Pollut Bull, 54:1845-1856.

Schiffer M, Harms L, Pörtner H-O, Lucassen M, Mark FC and Storch D (2013) Tolerance of *Hyas araneus* zoea I larvae to elevated seawater pCO_2 despite elevated metabolic costs. Mar Biol, 160:1943-1953.

Shapiro BM (1991) The control of oxidant stress at fertilization. Science, 252:533–536.

Sibert V, Ouellet P and Brêthes JC (2004) Changes in yolk total proteins and lipid components and embryonic growth rates during lobster (*Homarus americanus*) egg development under a simulated seasonal temperature cycle. Mar Biol, 144:1075–1086.

Sköld M, Svedäng H, Valentinsson D, Jonsson P, Börjesson P, Lövgren J, Nilsson HC, Svenson A and Hjelm J (2011) Fiskbestånd och bottenmiljö vid svenska västkusten 2004-2009 –effekter av trålgränsutflyttning och andra fiskeregleringar. Page 8.

Smith RSM (1987) The biology of larval and juvenile. *Nephrops norvegicus* (L.) in the Firth of Clyde. PhD Thesis, Glasgow University, Scotland.

Spicer JI and Weber RE (1991) Respiratory impairment in crustaceans and molluscs due to exposure to heavy-metals. Comp Biochem Physiol C, 100:339–342.

Spicer JI (1994) Ontogeny of cardiac function in the brine shrimp *Artemia fransicana* Kellogg 1906 (Branchiopoda: Anostraca). J Exp Zool, 270:508-516.

Spicer JI and Baden SP (2001) Environmental hypoxia and haemocyanin between-individual variability in Norway lobsters *Nephrops norvegicus* (L.). Mar Biol, 139:727-734.

Spicer JI, Eriksson SP (2003) Does the development of respiratory regulation always accompany the transition from pelagic larvae to benthic fossorial post-larvae in the Norway lobster *N. norvegicus* (L.)? J Exp Mar Biol Ecol, 295:219–243.

Spicer JI (1995) Ontogeny of respiratory function in crustaceans exhibiting either direct or indirect development. J Exp Zool, 272:413-418.

Spicer JI (2001) Development of cardiac function in crustaceans: Patterns and Processes. Amer Zool, 41:1068-1077.

Stearns S (1992) The evolution of life histories. Oxford University Press, New York.

Storch D, Santelices P, Barria J, Cabeza K, Pörtner H-O and Fernández M (2009) Thermal tolerance of crustacean larvae (zoea I) in two different populations of the kelp crab *Taliepus dentatus* (Milne-Edwards). J Exp Biol, 212:1371-1376.

Stumpp M, Hu MY, Melzner F, Gutowska MA, Dorey N, Himmerkus N, Holtmann WC, Dupont ST, Thorndyke MC and Bleich M (2012) Acidified seawater impacts sea urchin larvae pH regulatory systems relevant for calcification. PNAS, 109:18192-18197.

Taylor EW (1981) Some effects of temperature on respiration in decapodan crustaceans. J Therm Biol, 6:239–248

Thomsen J, Casties I, Pansch C, Körtzinger A and Melzner F (2013) Food availability outweighs ocean acidification effects in juvenile *Mytilus edulis*: laboratory and field experiments. Glob Change Biol, 19:1017–1027.

Tomanek L, Zuzow MJ, Ivanina AV, Beniash E and Sokolova IM (2011) Proteomic response to elevated pCO_2 level in eastern oysters, *Crassostrea virginica*: evidence for oxidative stress. J Exp Biol, 214:1836 1844.

Torres JJ, Gluck DL and Childress JJ (1977) Activity and physiological significance of the pleopods in the respiration of *Callianassa californiensis* (Dana) (Crustacea-Thalassinidea). Biol Bull, 152:134–146.

Turley C. (2005) The other CO_2 problem. openDemocracy. http://www.acamedia.info/sciences/sciliterature/globalw/reference/carol turley.html

Walther K, Sartoris FJ and Pörtner HO (2011) Impacts of temperature and acidification on larval calcium incorporation of the spider crab *Hyas araneus* from different latitudes (54° vs. 79°N). Mar Biol, 158:2043-2053.

Walther H, Anger K and Pörtner H (2010) Effects of ocean acidification and warming on the larval development of the spider crab *Hyas araneus* from different latitudes (54° vs. 79° N). Mar Ecol-Prog Ser, 417:159-170.

Wang T and Overgaard J (2007) The heartbreak of adapting to global warming. Science, 315:49-50.

Westerhagen H and Dethlefsen V (1997) The use of malformations in pelagic fish embryos for pollution assessment. Hydrobiologia, 352:241-250.

Whiteley NM (2011) Physiological and ecological responses of crustaceans to ocean acidification. Mar Ecol-Prog Ser, 430:257-271.

Widdicombe S and Spicer JI (2008) Predicting the impact of ocean acidification on benthic biodiversity: what can animal physiology tell us? *Journal of Experimental Marine Biology and Ecology*, **366**, 187–197.

Wood HL, Spicer JI, Widdicombe S (2008) Ocean acidification may increase calcification rates, but at a cost. Proc R Soc B Biol Sci, 275:1767.

Wu RSS, Zhou BS, Randall DJ, Woo NYS and Lam PKS (2003) Aquatic hypoxia is an endocrine disruptor and impairs fish reproduction. Environ Sci Technol, 37:1137-1141.

Yan B, Dai Q, Liu X, Huang S and Wang Z (1996) Flooding-induced membrane damage, lipid oxidation and activated oxygen generation in corn leaves. Plant and Soil, 179, 261–268.

Zhu Q, Aller RC and Fa Y (2006) Two-dimensional pH distributions and dynamics in bioturbated marine sediments. Geochim Cosmochim A, 70:4933-494

7. Acknowledgements

Det har varit några väldigt speciella år, två flyttar, två barn, en del krämpor och en väldans massa kräftor och alldeles snart en avhandling. Det känns overkligt att nästan vara i mål. Vad ska man då säga. Jo, tack! Tack till alla er som funnits där efter den mycket krokiga vägen. Som stöttat, hjälpt, inspirerat och motiverat. Som har stått ut med humörsvängningar, självupptagenhet, hysteriska sammanbrott och euforiska rus, om vartannat. Ett stort tack till mina närmaste medarbetare och strålande medförfattare. Ni är ena riktiga superkvinnor. Tack till dig **Sanna** för att du gav mig chansen att doktorera och för alla år som vi nu har känt varandra. Jag hoppas att vi snart kommer iväg på den där konferensen tillsammans. Tack **Hannah W**, **Helen S** och **Anna-Sara**. Det har varit en förmån att få arbeta tillsammans med er och jag är väldigt tacksam för ert engagemang. Tack också till dig **Malin C** för dina råd, ditt redigerande och stöd, speciellt de sista veckorna. Det har varit väldigt skönt att få höra din röst när det känts extra kämpigt.

I would also like to thank you, Professor **John Spicer** for doing me the honour of being my oponent. Thank you!

Tack till **GRIP** (Göteborg University Research Platform on Integrative Physiology) för finansiering och för vårt stora gemensamma experiment. Speciellt tack till **Britt W**, **Andreas K** och **Albin G** på zoologen. Tack också till **Fredrik J**. Det har varit roligt att få lära känna dig, trots lunginflammation!

Tack till alla på Sven Lovén! **Marie**, **Kalle**, **Lars**, **Pia**, **Hasse**, **Marita**, **Niklas, Mässen, Klages** med flera.

Tack till **Kerstin W**, **Gerry Q** och **Bob C** för statistikhjälp. Tack till **Tony** för alla kräftor.

Tack till det underbara doktorandgänget på Kristineberg som jag haft förmånen att lära känna. Ni har alla redan flugit vidare men ni vet vilka ni är, **Maria A**, **Nari**, **Matilda**, **Sonja**, **Ida**, **Erika.** Stor kram! Ni ska veta att det var mycket värt att få ha er nära = Facebook, de sista veckorna.

Tack till dig **Olga**, rumskamrat och labchef. Stor kram till **Rick**. Tack också till dig **Geraldine** för små pratstunder på kontoret.

Linda S min fina vän. I Löv You! Jag är så glad för att jag fann dig. Tack för alla sköna små stunder på ditt kontor och för mindre sköna stunder i din allt för korta soffa. Tack för att du alltid får mig att må bra.

Tack också till **Ida** och **Andreas**, goa vänner som gör livet roligt! Är så tacksam för att ha funnit er. Hoppas att vi snart kommer iväg på den där resan tillsammans.

Peter och Mia, tänk att vi skulle behöva flytta 200 mil för att verkligen lära känna varandra. Jag är väldigt glad att vi gjorde det. Stora kramar till er och tack för ert stöd och tålamod. Stora kramar också till alla er som jag saknar i Lysekil. **Marie** och **Kalle**, **Erika** och **Andreas**, **Malin** och **Håkan**, **Jonatan** och **Lisa**, **Maria** och **Martin**.

Stina L, vi gjorde det!! Lycka till i NZ! **Iris** och **Elisabet**, puss och kram. När ska man få festa med era rosa flamingos mån tro? Tack också till **Linda D**, **Amalen**, **Malin H** och resten av Göteborgsgänget för att det alltid är roligt att träffa er även om det inte blir så ofta.

Tack **Lina R** för att du är en trogen vän som alltid finns där och som det alltid kommer att vara lätt att prata med, om allt! Så roligt vi har haft och så många minnen jag har där du finns med! Ta med dig **Jonas** och kom upp till oss i vinter vetja så får vi dricka varm choklad i en snödriva och skåda norrsken tillsammans.

Tack **Josefine** för att du är världens bästa lillasyster. Puss! Tack **Isak** och **Lena** för att ni är världens bästa storasyskon. **Mamma** och **pappa**. Jag hoppas att ni förstår hur viktiga ni är för mig. Tack för allt stöd. Utan er hade inget varit möjligt. Tack också till **Erik** och **Cecilia**. Jag älskar er alla väldigt mycket!

Slutligen vill jag rikta ett särskilt stort tack till mina gullungar, **Alva** och **Vincent**, som har stått ut med både mammas och pappas känsloliv de senaste åren. Puss, puss, puss på er. Tack **Mikael** för att du alltid tror på mig, pushar och håller min puls uppe, på gott och ont! Vi har aldrig tidigare varit en familj utan minst en avhandling inblandad så hur ska livet nu bli kan man undra? Alldeles underbart hoppas jag! Puss & Kram.