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By

DANIEL PETER SMALL

A thesis submitted to Plymouth University
in partial fulfilment for the degree of

DOCTOR OF PHILOSOPHY

School of Marine Science and Engineering
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In collaboration with;
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The National Lobster Hatchery

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i. Abstract

The successful completion of the early developmental stages in organisms with complex life cycles is crucial to the persistence of a species both at the local and global scale. Thus changes in the abiotic environment experienced during larval and early benthic development can have profound effects on the development and ultimately dynamics of populations of marine invertebrates.

The effects of elevated temperature and pCO_2 in line with future predictions of anthropogenic climate change, ocean warming and ocean acidification (OA), on the survivorship and growth during early development of marine invertebrates is beginning to be understood, yet the underlying physiological ontogeny driving such changes, and the more subtle effects on physiological performance of climate change drivers, has yet to be distinguished. Therefore the aim of the present study is to investigate the effects of elevated temperature and pCO_2 on the developmental eco-physiology of an economically and ecologically important species, the European lobster, *Homarus gammarus*, to characterise the underlying physiological responses of early development behind responses of survival and growth.

The main findings relate to how changing optimal temperature conditions during larval development results in changes in metabolic performance and therefore aerobic scope, ultimately driving survival and growth. Larval stages which exhibit narrower aerobic scope were also sensitive to elevated pCO_2 evident as reduced survival, changes to energetic demands and organic content, and reduced calcification. Furthermore, this is the first attempt to characterise the physiological response of early benthic juveniles to climate change drivers. Early benthic juveniles are quite different in underlying physiology to later juveniles and adults, cumulating in this stage being energy limited. Such limitations are expressed as a reduction in aerobic scope in relation to elevated temperature and pCO_2, and associated sensitiveness to elevated pCO_2 resulting in increased moult related mortalities and the breakdown of haemolymph buffering capacity under combinations of elevated temperature and pCO_2. Throughout early development, elevated temperature and pCO_2, through underlying physiological responses, may have dramatic effects on the geographic range and successful development of *H. gammarus*.

ii. Contents

iii. Abbreviations

OA – Ocean acidification

CCS – Carbon capture and storage

pCO_2 – Partial pressure of carbon dioxide

TCO_2 – Total concentration or carbon dioxide

$[HCO_3^-]$ – Bicarbonate concentration

$[CO_3^{2-}]$ – Carbonate concentration

pO_2 – Partial pressure of oxygen

DO_2 – Dissolved oxygen

Ω_{cal} – Calcite saturation

Ω_{ara} – Aragonite saturation

T_O – Optimum temperature

T_P – Pejus temperature threshold

T_C – Critical temperature threshold

T_D – Denaturation temperature threshold

WBM – Wet body mass

DM – Dry mass

C – Carbon

H – Hydrogen

N – Nitrogen

A_T – Total Alkalinity

MDS – Moult Death Syndrome

SD – Shell Dissolution

S.E. – Standard Error

TL – Total body length

CL – Carapace length

AL – Abdomen length

RL – Rostrum length

Chel – Chelae length

$[Ca^{2+}]$ – Calcium concentration

$[Mg^{2+}]$ – Magnesium concentration

$[Sr^{2+}]$ – Strontium concentration

$[Cu^{2+}]$ – Copper concentration

$[Mn^{2+}]$ – Manganese concentration

$[Na^+]$ – Sodium concentration

$[K^+]$ – Potassium concentration

$[P^+]$ – Phosphorus concentration

Vol. - Volume

iv. Figure Legends

Figure 1.1 Estimated mortality of the grey marsh crab, *Sesarma cinereum* (Bosc 1802) larvae under combinations of temperature and salinity: (a) Zoea III, (b) Zoea IV, and (c) Megalopa. Isopleths represent fitted response curves based on % mortality under 12 combinations of temperature and salinity. Figures from Costlow *et al*. (1960).

Figure 1.2 Partial pressure of haemolymph oxygen (pO_2 - mmHg) in the spiny spider crab, *Maja squinado* (Herbst 1788) during progressive cooling and warming (Means ± S.D.): Tp I and Tp II indicate lower and upper *pejus* temperature thresholds respectively while Tc I and Tc II indicate lower and upper critical temperature thresholds respectively. Figure and definitions from Frederich and Pörtner (2000).

Figure 1.3 The aerobic thermal window during life history: (a) Theorised model of changes in aerobic thermal window throughout life history, from Pörtner and Farrell (2008). (b) The same model adapted to include differences in aerobic thermal window between larvae and megalopa stages, from Walther *et al*. (2010).

Figure 1.4 Theoretical model of changes in an organism's aerobic thermal scope due to elevated pCO_2: Solid line indicates aerobic thermal scope under normocapnia, dashed line indicates aerobic thermal scope under hypercapnia. T_C indicates critical temperature thresholds, T_P indicates *pejus* temperature thresholds, and T_O indicates optimum temperature. Dashed green arrows indicate changes in T_C, T_P, and T_O due to hypercapnia. Figure and definitions from Pörtner and Farrell (2008).

Figure 1.5 Time line of developmental events during the life history of *Homarus gammarus*: Comparison of developmental stages and ecological phases, sourced from Cobb and Wahle (1994).

Figure 1.6 Juvenile *H. gammarus*: Photo courtesy of David Liittschwager, National Geographic.

Figure 2.1 Diagram representing the recirculating experimental design, focussed on temperature control: Blue lines and arrows indicate the flow of control (17 °C) treatment water, red lines and arrows indicate the flow of elevated temperature (21 °C) treatment water. A = Header tanks with filtration. B = Chiller units with heating elements to control water temperature. C = Water inflow into individual rearing cones. Note that there is a 'swapping' of treatment water to aid temperature control within tanks. D = Sump tanks with filtration. E = Waste water overflow. F = Daily 8 h flow of new, clean sea water to ensure water quality.

Figure 2.2 Diagram representing an individual larval rearing cone: A = Water inflow (Set to designated temperature level). B = Air inflow (Set to designated pCO_2 level). C = Water height in cone. D = Water overflow through mesh to allow broken food removal but retain larvae and larger food particles. E = Cone lid to prevent excess evaporation and spray. F = Water level in surrounding tank.

Figure 2.3 Survival of larval *Homarus gammarus* to each developmental stage under elevated temperature and pCO_2 (Mean ± S.E., d.f. = 3, N = 35): Survival calculated as % of live individuals in the previous developmental stage. (a) Stage II, (b) Stage III and (c)

Stage IV. Grey bars indicate 17 °C. White bars indicate 21 °C. Clear bars indicate 420 µatm pCO_2. Striped bars indicate 1,100 µatm pCO_2. Numbers indicate significant differences between treatments within stages.

Figure 2.4 Growth status of Stage IV *H. gammarus* larvae reared under elevated temperature and pCO_2 (Means ± S.E., d.f = 1, N = 57): (a) Wet body mass (mg) and (b) carapace length (mm). Grey bars indicate 17 °C and white bars indicate 21 °C. Numbers indicate significant differences between temperature treatments.

Figure 2.5 Oxygen consumption of larval *H. gammarus* reared under elevated temperature and pCO_2 (Means ± S.E., d.f = 1, N = 31): Oxygen consumption (µmol O_2 min^{-1} g^{-1} S.T.P) of (a) Stage I, (b) Stage II, (c) Stage III, and (d) Stage IV larvae. Grey bars indicate 17 °C and white bars indicate 21 °C. Numbers indicate significant differences between temperature treatments.

Figure 2.6 Organic content and oxygen consumption of Stage IV larvae *H. gammarus* reared under elevated pCO_2 (Mean ± S.E., d.f = 1, N = 24): (a) Dry body mass (DM, % of wet body mass), (b) nitrogen levels (% of Dry Mass), (c) carbon to nitrogen ratio (C:N), and (d) oxygen consumption (µmol O_2 min^{-1} g^{-1} S.T.P). Grey bars indicate 420 µatm pCO_2 and white bars indicate 1,100 µatm pCO_2. Numbers indicate significant differences between pCO_2 treatments.

Figure 3.1 Representative *Homarus gammarus* larvae indicating morphometric measurements: (a) Stage I, (b) Stage II, (c) Stage III, and (d) Stage IV. Morphometric

traits measured were carapace length (CL – yellow), abdomen length (AL - blue), rostrum length (RL – red) and chelae length (Chel – green). Scale = 1 mm.

Figure 3.2 Key morphometric aspects of Stage IV larvae *H. gammarus* reared under elevated temperature and pCO_2 (Means ± S.E., d.f= 3, N = 57): (a) Total body length (mm), (b) carapace length (mm), and (c) abdomen length (mm). White bars indicate 17 °C, grey bars indicate 21 °C. Clear bars indicate 420 µatm pCO_2 conditions; striped bars indicate 1,100 µatm pCO_2 conditions. Capital letters indicate significant differences between treatments.

Figure 3.3 Carapace mineral content of Stage IV larvae *H. gammarus* reared under elevated temperature and pCO_2 (Means ± S.E., d.f = 3, N = 19): (a) Carapace [Ca^{2+}] (µmol g^{-1}) and (b) carapace [Mg^{2+}] (µmol g^{-1}). White bars indicate 17 °C, grey bars indicate 21 °C. Clear bars indicate 420 µatm pCO_2; striped bars indicate 1,100 µatm pCO_2. Capital letters indicate significant differences between treatments.

Figure 4.1 Survival of early benthic juvenile *Homarus gammarus* after five week exposure to elevated temperature and pCO_2 (Means ± S.E.): (a) 10 °C and (b) 13 °C. Diamonds with full lines indicate 450 µatm pCO_2, squares with dashed lines indicate 1,100 µatm pCO_2 and triangles with dotted lines indicate 9,000 µatm pCO_2.

Figure 4.2 Growth status of juvenile *H. gammarus* after 5 weeks exposure to elevated temperature and pCO_2 (Means ± S.E., d.f = 2, N = 93): Growth in terms of % Wet Body Mass (WMB) gain. White bars indicate 10 °C, grey bars indicate 13 °C. Clear bars

indicate 450 µatm pCO_2, thick striped bars indicate 1,100 µatm pCO_2, and thin striped bars indicate 9,000 µatm pCO_2. Numbers indicate significant differences.

Figure 4.3 The carapace calcium content of early benthic juvenile *Homarus gammarus* after five week exposure to elevated temperature and pCO_2 (Means ± S.E., d.f = 1, N = 48): (a) The effect of elevated temperature and pCO_2 on carapace calcium concentration ($[Ca^{2+}]$ - µmol mg^{-1}). White bars indicate 10 °C, and grey bars indicate 13. Clear bars indicate 450 µatm pCO_2, striped bars indicate 1,100 µatm pCO_2. (b) The effect of moulting and elevated pCO_2 on carapace $[Ca^{2+}]$ in individuals exposed to 13 °C. White bars indicate 450 µatm pCO_2, and grey bars indicate 1,100 µatm pCO_2. Clear bars indicate non-moulted individuals, striped bars indicate moulted individuals. Numbers indicate significant differences.

Figure 4.4 Rates of oxygen consumption and feeding of early benthic juvenile *H. gammarus* after five weeks exposure to elevated temperature and pCO_2 (Means ± S.E., d.f = 2, N = 69): (a) Oxygen consumption (µmol O_2 min^{-1} g^{-1} S.T.P.), (b) feeding rate (µg min^{-1} g^{-1}). White bars indicate 10 °C and grey bars indicate 13 °C. Clear bars indicate 450 µatm pCO_2, thick striped bars indicate 1,100 µatm pCO_2, and thin striped bars indicate 9,000 µatm pCO_2. Numbers indicate significant differences.

Figure 4.5 Examples of early benthic juvenile *H. gammarus* exposed to elevated pCO_2 at 10 °C for five weeks: (a) 450 µatm pCO2 and (b) 9,000 µatm pCO_2. SD indicates shell dissolution. Scale = 5 mm.

Figure 5.1 Haemolymph acid–base status of 1 year old juvenile *Homarus gammarus* after two week exposure to elevated temperature and pCO_2 (Means ± S.E., d.f = 2, N = 49): A) Haemolymph pH (NBS scale), B) haemolymph carbon dioxide partial pressure (pCO_2 - kPa), and C) haemolymph bicarbonate concentration ($[HCO_3^-]$ - mmol L^{-1}). White bars indicate 13 °C, grey bars indicate 17 °C. Clear bars indicate 450 µatm pCO_2, thick striped bars indicate 1,100 µatm pCO_2, and thin striped bars indicate 8,000 µatm pCO_2. Numbers indicate significant differences between treatments.

Figure 5.2 Oxygen consumption and feeding rates of 1 year old juvenile *H. gammarus* after two week exposure to elevated temperature and pCO_2 (Means ± S.E., d.f = 2, N = 50): (a) Oxygen consumption (µmol O_2 min^{-1} g^{-1} S.T.P.) and (b) food consumption (mg g^{-1} h^{-1}). White bars indicate 13 °C, grey bars indicate 17 °C. Clear bars indicate 450 µatm pCO_2, thick striped bars indicate 1,100 µatm pCO_2, and thin striped bars indicate 8,000 µatm pCO_2. Numbers indicate significant differences between treatments.

Figure 5.3 Epipodite activity of 1 year old juvenile *H. gammarus* after two week exposure to elevated temperature and pCO_2 (Means ± S.E., d.f = 2, N = 49): Epipodite Na^+/K^+-ATPase activity (nmol mg^{-1} h^{-1}). White bars indicate 13 °C, grey bars indicate 17 °C. Clear bars indicate 450 µatm pCO_2, thick striped bars indicate 1,100 µatm pCO_2, and thin striped bars indicate 8,000 µatm pCO_2. Numbers indicate significant differences between treatments.

Figure 5.4 Davenport diagram of 1 year old juvenile *H. gammarus* after two week exposure to elevated pCO_2 at 13 °C (Means ± S.E., N = 24): Diamond indicates 450 µatm pCO_2, square indicates 1,100 µatm pCO_2, and triangle indicates 8,000 µatm pCO_2.

Isopleths indicate haemolymph pCO_2 (kPa). Dashed line indicates haemolymph non bicarbonate buffering capacity.

Figure 6.1 Time line of developmental events during the life history of *Homarus gammarus*: (a) Comparison of developmental stages and ecological phases, sourced from Cobb and Wahle (1994). (b) Adaptation to include aspects of physiological development. Red text and lines indicate ontogeny of aerobic scope. Blue text and lines indicate the effects of elevated temperature and pCO_2. Dashed arrows indicate the effects of temperature (Red) and pCO_2 (Blue) on the survivorship of *H. gammarus*. Up pointing arrows indicate positive comparative survival, and down pointing arrows indicate negative comparative survival.

v. Table Legends

Table 2.1 Water chemistry parameters over the course of the experimental period (Means ± S.E., d.f = 3, N = 89): Temperature (°C), salinity, pH (NBS Scale), total alkalinity (A_T - µEq kg^{-1}), carbon dioxide partial pressure (pCO_2 - µatm), total carbon dioxide (TCO_2 - µmol kg^{-1}) bicarbonate concentration ($[HCO_3^-]$ - µmol kg^{-1}), carbonate concentration ($[CO_3^{2-}]$ - µmol kg^{-1}), calcite saturation (Ω_{cal}) and aragonite saturation (Ω_{ara}). Superscript capital letters indicate significant differences between treatments. [1]Parameters calculated using CO_2SYS program (Lewis and Wallace 1998) with constants provided by Mehrbach *et al.* (1973) refitted by Dickson and Millero (1987) and KSO_4 constants from Dickson (1990).

Table 2.2 Life history traits of *Homarus gammarus* throughout larval development under elevated temperature and pCO_2 (Means ± S.E.): Survival (% of initial number), stage duration (Duration - d), and growth parameters wet body mass (WBM – mg, d.f = 1, N = 57) and carapace length (CL – mm, d.f = 1, N = 57). Superscript capital letters represent significant differences between treatments.

Table 2.3 The organic content of Stage IV *H. gammarus* reared under elevated temperature and pCO_2 (Means ± S.E., d.f = 3, N = 24): Ash content (Ash - % DM), total carbon, hydrogen, and nitrogen (TCHN - % DM), carbon (C - % DM), hydrogen (H - % DM). Superscript capital letters indicate significant differences between treatments.

Table 3.1 Morphometric aspects of larval H. gammarus reared under elevated temperature and pCO_2 (Means ± S.E., d.f= 3, N = 57): Total length (TL - mm), carapace length (CL - mm), abdomen length (AL - mm), chelae length (Chel - mm), and rostrum

length (RL - mm). Superscript capital letters indicate significant differences between treatments.

Table 3.2 Carapace mineralisation of larval *H. gammarus* reared under elevated temperature and pCO_2 (Mean ± S.E. ., d.f = 3, N = 18): Carapace concentrations of calcium ($[Ca^{2+}]$ - µmol g^{-1}), magnesium ($[Mg^{2+}]$ - µmol g^{-1}), Strontium ($[Sr^{2+}]$ µmol g^{-1}), manganese ($[Mn^{2+}]$ - µmol g^{-1}), sodium ($[Na^{+}]$ - µmol g^{-1}), potassium ($[K^{+}]$ - µmol g^{-1}), and phosphorus ($[P^{+}]$ - µmol g^{-1}). Superscript capital letters indicate significant differences between treatments.

Table 4.1 Water chemistry parameters throughout the exposure period (Means ± S.E., d.f = 5, N = 76): Temperature (°C), salinity, pH (NBS Scale), total alkalinity (A_T - µEq kg^{-1}), total carbon dioxide content (TCO_2 - µmol kg^{-1}), carbon dioxide partial pressure (pCO_2 - µatm), bicarbonate concentration ($[HCO_3^{-}]$ - µmol kg^{-1}), carbonate concentration ($[CO_3^{2-}]$ - µmol kg^{-1}), calcite saturation (Ω_{cal}) and aragonite saturation (Ω_{ara}). Superscript capital letters indicate significant differences between treatments. [1]Parameters calculated using CO_2SYS program (Lewis and Wallace 1998) with constants provided by Mehrbach *et al.* (1973) refitted by Dickson and Millero (1987).

Table 4.2 Organic content of early benthic juvenile *Homarus gammarus* after five week exposure to elevated temperature and pCO_2 (Means ± S.E., d.f = 1, N = 48): Dry mass (DM - mg), carbon (C - %), nitrogen (N - %), carbon:nitrogen ratio (C:N), hydrogen (H - %), total CHN (TCHN -%), and Ash (%). Superscript capital letters indicate significant differences between treatments.

Table 4.3 Carapace mineral content of early benthic juvenile *H. gammarus* after five week exposure to elevated temperature and pCO_2 (Means ± S.E., d.f = 1, N = 48): Concentration of carapace calcium ($[Ca^{2+}]$ - µmol g^{-1}), magnesium ($[Mg^{2+}]$ - µmol g^{-1}), strontium ($[Sr^{2+}]$ - µmol g^{-1}), manganese ($[Mn^{2+}]$ - µmol g^{-1}), sodium ($[Na^{+}]$ - µmol g^{-1}), potassium ($[K^{+}]$ - µmol g^{-1}), and phosphorus ($[P^{+}]$ - µmol g^{-1}). All mineral contents calculated as *per* g of carapace dry mass. Superscript capital letters indicate significant differences between treatments.

Table 5.1 Water chemistry parameters throughout the exposure period (Means ± S.E., d.f = 5, N = 76): Temperature (°C), salinity, pH (NBS Scale), total alkalinity (A_T - µEq kg^{-1}), total carbon dioxide content (TCO_2 - µmol kg^{-1}), carbon dioxide partial pressure (pCO_2 - µatm), bicarbonate concentration ($[HCO_3^-]$ - µmol kg^{-1}), carbonate concentration ($[CO_3^{2-}]$ - µmol kg^{-1}), calcite saturation (Ω_{cal}) and aragonite saturation (Ω_{ara}). Superscript capital letters indicate significant differences between treatments. [1]Parameters calculated using CO_2SYS program (Lewis and Wallace 1998) with constants provided by Mehrbach *et al.* (1973) refitted by Dickson and Millero (1987).

Table 5.2 Haemolymph biochemistry of juvenile *Homarus gammarus* after two week exposure to elevated temperature and pCO_2 (Means ± S.E., d.f = 2, N = 48): Haemolymph calcium ($[Ca^{2+}]$ - µmol L^{-1}), magnesium ($[Mg^{2+}]$ - µmol L^{-1}), copper ($[Cu^{2+}]$ - µmol L^{-1}), sodium ($[Na^{+}]$ - µmol L^{-1}), potassium ($[K^{+}]$ - µmol L^{-1}), protein ([protein] – mg mL^{-1}), and *L*-lactate ([*L*-lactate] – mmol L^{-1}) concentrations. Superscript capital letters indicate significant differences between treatments.

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vii. Author declaration

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award without prior agreement of the Graduate Committee.

Work submitted for this research degree at the Plymouth University has not formed part of any other degree either at Plymouth University or at another establishment.

This study was financed with the aid of a studentship form the Plymouth University and carried out in collaboration with Plymouth Marine Laboratory and the National Lobster Hatchery, with additional funding awarded to the National Lobster Hatchery from the National Marine Aquarium.

Relevant scientific seminars and conferences, including those organised by the Marine Biology and Ecology Research Centre, were regularly attended at which work was often presented.

External institutions were visited for conferences and workshops.

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Date

Chapter 1

General introduction

The persistence of a species at local and global scales requires all ontogenetic stages of development to be successfully completed (Byrne 2012) which itself is dependent on the ability of each development stage to adapt to its environment (Charmantier 1998, Burggren 2006, Pelster and Schwerte 2006). The larval and juvenile development of many marine invertebrates often involves transitions between very different environments and dramatic changes in behaviour, ecology, morphology, size, weight, chemical composition, and physiology (Thorson 1950, Strathmann 1993, Anger 1998, 2001, Hadfield *et al.* 2001). The ontogeny of physiological functions in marine invertebrates and the subsequent sensitivities to environmental drivers due to changes in physiological development can often translate into developmental bottlenecks during complex life cycles (Nasrolahi *et al.* 2013). Consequently, the ability of each developmental stage to adapt to its environment and the abiotic fluctuations experienced, within the limitations defined by the ontogenetic development of physiological functions, represents a challenge for marine invertebrates with complex life histories (Charmantier 1998).

1.1. The ontogeny of physiological functions during larval development

The larval phase of marine invertebrates is thought to be particularly sensitive to environmental drivers (Thorson 1950, Byrne 2012), and the ontogeny of physiological functions throughout larval development can provide insights into why such sensitivities to environmental drivers occur (Spicer and Gaston 1999).

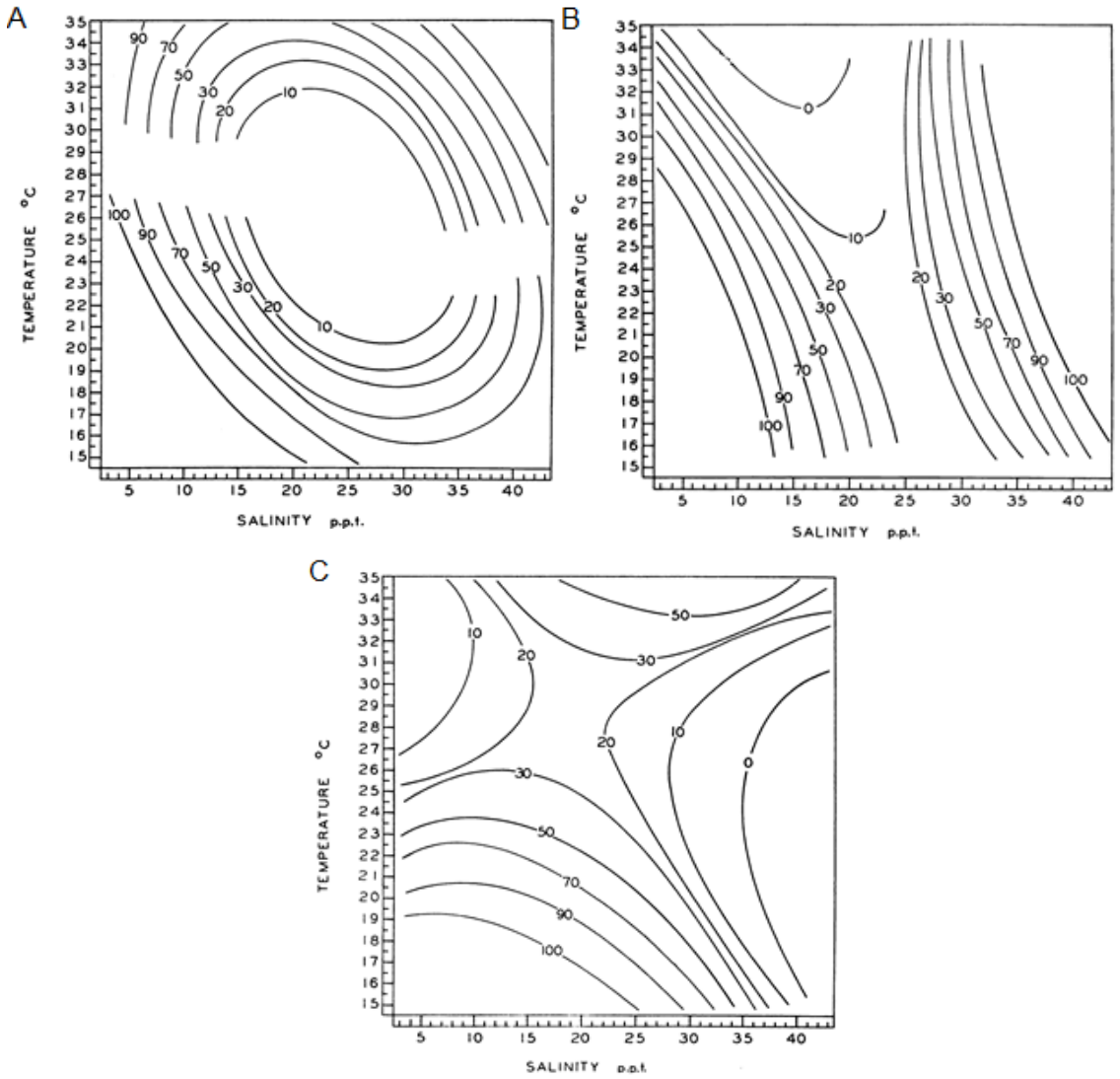

Figure 1.1 Estimated mortality of the grey marsh crab, *Sesarma cinereum* (Bosc 1802) larvae under combinations of temperature and salinity: (a) Zoea III, (b) Zoea IV, and (c) Megalopa. Isopleths represent fitted response curves based on % mortality under 12 combinations of temperature and salinity. Figures from Costlow *et al*. (1960).

The larval development and associated ontogeny of physiological functions of marine crustaceans, for example, has received a lot of attention. Key examples can be found in relation to the responses of larval development to abiotic factors such as temperature and salinity. For example, Costlow *et al.* (1960) investigated the larval development of the grey marsh crab, *Sesarma cinereum* (Bosc 1802) under a range of salinity * temperature combinations (Fig. 1.1). Using survivorship at each larval stage, the authors

documented shifts in the optimal temperature and salinity conditions during *S. cinereum* larval development, with particularly dramatic shifts occurring between Zoea III, IV, and megalopa stages (Costlow *et al.* 1960). Similar ontogenetic shifts in optimal temperature and salinity combinations have been observed in a range of decapod crustaceans (Costlow *et al*. 1962, 1966, Anger 1991, Agard 1999), and may be the result of the development of new physiological milestones and associated energetic demands (Agard 1999). Such physiological developments occur during the transition between the final zoeal stage and the megalopa stage in a range of decapod crustaceans, including grapsid crabs and the European and American lobsters, *Homarus gammarus* (Linnaeus 1758) and *H. americanus* (Milne-Edwards 1837) respectively, where this transition is coupled with the development of, and changes to, osmoregulatory functions and processes (Charmantier *et al.* 1988, 1998, 2001, 2002). The same transition in the Norway lobster, *Nephrops norvegicus* (Linnaeus 1758), and blue crab, *Callinectes sapidus* (Rathburn 1896), is coupled with the development of, and changes to, oxyregulatory capabilities (Tankersley and Wieber 2000, Spicer and Eriksson 2003). Even in groups which do not undergo such a pronounced metamorphic event during development, such as amphipods and anostracans, there are often profound shifts in osmoregulation (Morritt and Spicer 1995, 1996, 1999) and respiratory regulation (Spicer 1994, 1995a,b, Spicer and Morritt 1996).

The ontogeny of such physiological functions throughout crustacean development could be interpreted as an adaptive factor to maximise survival in the environments which larvae are exposed to (Charmantier 1998). For example, the larval development of the freshwater prawn, *Macrobrachium petersi* (Hilgendorf 1879) is defined by strong hyper- /hypo- osmoregulatory abilities of Stage I larvae, followed by reduced ability to regulate in fresh waters in later larval stages, and the re-appearance of strong hyper-

/hypo- osmoregulatory abilities in megalopa (Read 1984). These changes in osmoregulatory types and abilities are strongly representative of the environments occupied by individual larval stages, as larvae are released by freshwater adults, develop in increasingly saline estuaries and coastal regions, and settle once more as freshwater post larvae (Read 1984). Such links between larval dispersal strategies, regulatory capabilities, and stage specific sensitivities to abiotic factors is a common pattern in decapod crustaceans (Costlow *et al.* 1960, 1962, 1966, Dawirs 1985, Anger 1991, Agard 1999, Charmantier *et al.* 2002, Cieluch *et al.* 2004) affirming the link between physiological development and larval environment.

It is therefore clear that the physiological development of larval crustaceans drives the sensitivity of life history traits such as survival and growth throughout larval development, and can explain changes in larval sensitive to abiotic factors (Charmantier *et al.* 1988, Charmantier and Charmantier-Daures 1991, Charmantier 1998). As the development of such physiological milestones occur during the transitory megalopa stage of marine invertebrates (Agard 1999), it is important to consider this stage in an ecological context and how physiological development and sensitivity of larvae can have profound effects on population dynamics. The physiological condition of settling megalopa has a large effect on subsequent size, condition, growth and survival of juveniles (Jarrett and Pechenik 1997, Pechenik *et al.* 2002, Jarrett 2003, Gimenez *et al.* 2004) while the environmental drivers experienced during larval development can impact the sensitivity to abiotic factors of later juvenile stages (Qiu and Qian 1999, Beckerman *et al.* 2002, Nasrolahi *et al.* 2012). The modification of physiological functions and associated energetic demands accompanying transitions to megalopa stages, therefore, can be linked to the subsequent high mortalities, > 90 %, of early juvenile populations (Gosselin and Qian 1997, Charmantier 1998). This provides a clear

link between the physiological development and condition of larval stages, the abiotic factors experienced during larval development, and the performance and success of juvenile populations, highlighting the importance of larval physiological development (Jarrett and Pechenik 1997, Tankersley and Wieber 2000, Pechenik *et al.* 2002, Jarrett 2003, Gimenez *et al.* 2004).

1.2. The ontogeny of physiological functions during juvenile development

The post-larval juvenile stage of benthic marine invertebrates in its own right represents a challenging transition between larval and adult life stages. The mortality rates of early juveniles are extremely high, > 90 % (Gosselin and Qian 1997), and early juveniles of many marine invertebrates are ecologically distinct from late juveniles and adults in terms of behaviour, morphology and appearance, habitat, and predation risks (Wahle and Steneck 1991, Smith and Herrnkind 1992, Gosselin 1997).

Along with this ecological distinction, there also appears to be a physiological distinction between early (post-larvae) juvenile and late juveniles. This distinction is evident when reviewing the sparse literature on the physiological development of juvenile marine crustaceans. Osmoregulation, in terms of structure of organs and function of regulatory capacity, operates in a manner similar to that of adults from the transition to megalopa larvae in decapods (Incze and Wahle 1991, Charmantier 1998, Charmantier *et al.* 1998, 2001, 2002, Cieluch *et al.* 2004). However, the ability of juveniles to fully utilise osmoregulatory capabilities to the level of adults progressively improves during juvenile development often representing that of adults in late juvenile stages (Charmantier *et al.* 1998, 2001, 2002, Cieluch *et al.* 2004). The structure and function of the respiratory pigment, haemocyanin, also follows a similar pattern, as adult type haemocyanin molecules appeared in late juvenile development of the

Dungeness crab, *Cancer magister* (Dana 1852) (Terwilliger and Dumler 2001, Terwilliger and Ryan 2001), along with increasing haemocyanin oxygen affinity (Terwilliger and Brown 1993, Terwilliger and Ryan 2001). Coupled with haemocyanin development of *C. magister* is the development of ionoregulatory capabilities, in this case the regulation of haemolymph [Ca^{2+}] and [Mg^{2+}] (Brown and Terwilliger 1992, Terwilliger and Brown 1993) which has also been demonstrated in *H. gammarus* (Newton and Potts 1993). In *N. norvegicus*, the oxyregulatory capabilities represent adult type upon metamorphosis to the megalopa stage; however the gill structures do not fully develop until later in juvenile development (Spicer and Eriksson 2003). Such progressive development of regulatory structures and functions throughout juvenile development is reflected in the increasing tolerance of juveniles to environmental drivers, as for example early juveniles have narrower salinity and temperature tolerances than later juveniles, supporting the idea of a physiological distinction between early and late benthic marine invertebrates.

How this complex physiological development throughout the early life history of marine invertebrates responds to environmental drivers, and perhaps most pressingly climate change drivers in particular, however, is still to be determined, although we are beginning to understand some of the developmental responses.

1.3. Climate change and marine invertebrate development

The early life history of calcifying marine invertebrates is thought to be particularly sensitive to climate change drivers such as ocean warming and ocean acidification (OA) (Kurihara 2008, Pörtner and Farrell 2008, Melzner *et al.* 2009, Byrne 2011).

Increased atmospheric CO_2 due to anthropogenic emissions is a major driver of climate change, including global warming (Levitus *et al.* 2001). During the past 100 years, atmospheric CO_2 levels have increased by 100 ppm (Raven *et al.* 2005), and the temperature of the Earth's surface has increased by 0.6 °C (IPCC 1995), corresponding to an increase in mean ocean temperature of 0.31 °C (Levitus *et al.* 2000). Future elevated global temperatures due to anthropogenic CO_2 emissions will translate into further oceanic warming, and sea surface temperatures are expected to increase by 3 °C – 5 °C by the year 2100 (Sokolov *et al.* 2009).

Temperature is the major abiotic driver of larval development in marine invertebrates (e.g. Anger 1998, 2001). In marine crustaceans, development time decreases with increasing temperature (Dawirs 1985, Mackenzie 1988, Lindley 1998, Weiss *et al.* 2009a), however maximum growth and survival is limited to narrow optimal mid-range temperatures (Mackenzie 1988, Weiss *et al.* 2009a, b, 2010). Such ranges of temperature tolerance are dependent on mitochondrial function and oxygen limitation (Pörtner 2002) as has been shown in the spiny spider crab, *Maja squinado* (Herbst 1788) where the maintenance of high oxygen levels and oxygen supply to match oxygen demand was only achieved in a narrow optimum temperature window (Fig. 1.2) (Frederich and Pörtner 2000). Outside of this optimum window, haemolymph pO_2 fell due to an uncompensated demand for oxygen until critical levels are reached where metabolism is anaerobic (Frederich and Pörtner 2000). Such limitations in thermal windows have been also demonstrated for larval stages of marine crustaceans (Storch *et al.* 2009a, b). Due to the temperature dependent limitations of aerobic capacity of marine organisms, changes in environmental temperatures have consequences for species' geographical distributions (Pörtner 2002, Pörtner and Knust 2007). Indeed, increasing seawater temperature over the past 50 years has been accompanied by the

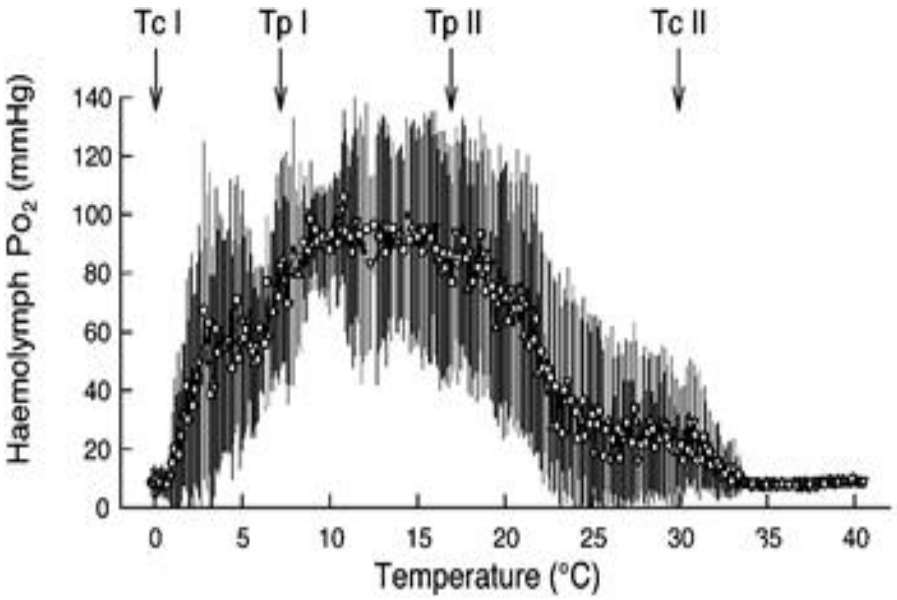

Figure 1.2 Partial pressure of haemolymph oxygen (pO_2 - mmHg) in the spiny spider crab, *Maja squinado* (Herbst 1788) during progressive cooling and warming (Means ± S.D.): Tp I and Tp II indicate lower and upper *pejus* temperature thresholds respectively while Tc I and Tc II indicate lower and upper critical temperature thresholds respectively. Figure and definitions from Frederich and Pörtner (2000).

pole ward shift in rocky shore species distributions throughout northern Europe (Hawkins *et al.* 2009). The distributions, abundance, and biomass of decapod larvae, for example, are expected to change under increasing temperatures related to ocean warming (Lindley 1998), a pattern which can be linked to the changing distribution of rocky shore species. This can be seen by the differentiation of larval aerobic thermal windows shown by southern and central populations of the Chilean kelp crab, *Taliepus dentatus* (Milne-Edwards 1834) (Storch *et al.* 2009b).

Coupled with the predictions of ocean warming due to increased atmospheric CO_2 is OA. The oceans have been estimated to absorb 48 % of anthropogenic emissions over the last 100 years (Sabine *et al.* 2004, Raven *et al.* 2005) which has resulted in a decrease in oceanic pH by 0.1 units, equivalent to a 30 % increase in hydrogen ions

(Sabine *et al.* 2004, Raven *et al.* 2005). Future predictions of anthropogenic CO_2 emissions will result in an increase of oceanic pCO_2 and a decrease in oceanic pH of 0.4 units by the year 2100 (Caldeira and Wickett 2003, 2005). In association with decreasing oceanic pH are alterations to the oceans' carbonate chemistry (Feely *et al.* 2004) which will result in the under-saturation of aragonite and calcite states of the worlds' oceans. Asides from sea water acidification due to anthropogenic emissions, sea water acidification also occurs due to the release of CO_2 from natural volcanic vent systems (Hall-Spencer *et al.* 2008) and the upwelling of acidified water at continental shelves, the extent of which is predicted to be increased by OA (Feely *et al.* 2008). Carbon capture and storage (CCS) represents a means of climate mitigation, *via* capturing atmospheric CO_2 and depositing it in liquid form in sub-sea storage facilities, such as exhausted gas and oil fields (Marchetti 1977, Caldeira 2003, Orr *et al.* 2003). CO_2 leakages during the transport or storage stage of nsuch operations possess threats of localised severe hypercapnia to benthic marine organisms and ecosystems (Blackford *et al.* 2009, Kano *et al.* 2010).

The early larval development of a range of marine invertebrates is negatively impacted by elevated pCO_2 associated with OA. Bivalve molluscs and echinoderms experience protracted developmental times, high mortality rates, and morphological abnormalities associated with mineralised shell formation suggesting calcification implications (Kurihara and Shirayama 2004, Kurihara *et al.* 2007, 2008a, Martin *et al.* 2011, Nakamura *et al.* 2011, Stumpp *et al.* 2011a, b, Gonzalez-Bernat *et al.* 2012). Crustaceans are proposed to be amongst the most tolerant groups to OA (Kroeker *et al.* 2010, 2013) although negative effects on their larval development as a result of exposure to elevated pCO_2 have been demonstrated. Larval survival decreased in the king crab, *Paralithodes camtschaticus* (Tilesius 1815) due to elevated pCO_2, despite no

apparent changes in mineralisation and an increase in body size (Long *et al.* 2013). Survival also decreased in great spider crab, *Hyas araneus* (Linnaeus 1758) larvae, along with decreased growth and lipid content (determined by C:N ratios) and increased development time (Walther *et al.* 2010) with possible implications for key life history traits. Furthermore, the megalopa stage of crustacean development appeared particularly sensitive due to reductions in calcification in *H. gammarus* (Arnold *et al.* 2009) and *H. araneus* (Walther *et al.* 2011). In the case of *H. araneus*, the megalopa stage appeared especially sensitive to combinations of elevated temperature and pCO_2 (Walther *et al.* 2010, 2011). Indeed, while there is a range of responses of the developmental stages of marine invertebrates to elevated temperature and pCO_2, ocean warming and OA will occur simultaneously in the near future due to anthropogenic climate change and therefore need to be considered together (Przeslawski *et al.* 2005, Pörtner 2008, Pörtner and Farrell 2008, Widdicombe and Spicer 2008). The interactive effects of elevated temperature and pCO_2 on marine organisms are beginning to be described (Reynaud *et al.* 2003, Przeslawski *et al.* 2005, Rosa and Seibel 2008, Dissanayake and Ishimatsu 2011, Melatunan *et al.* 2011, 2013, Zittier *et al.* 2012) however our knowledge on the larval development of marine invertebrates under elevated temperature and pCO_2 is more limited. The larval development of *H. araneus* showed complex sensitivities to elevated temperature and pCO_2 (Walther *et al.* 2010, 2011) while the hard clam, *Mercenaria mercenaria* (Linnaeus 1758) and the bay scallop, *Argopecten irradians* (Lamarck 1819) experienced additive effects of reduced survival, development, growth, and lipid synthesis under elevated temperature and pCO_2 (Talmage and Gobler 2011). The larval development of the northern shrimp, *Pandalus borealis* (Krøyer 1838), however, was more sensitive to elevated temperature than pCO_2 (Arnberg *et al.* 2013).

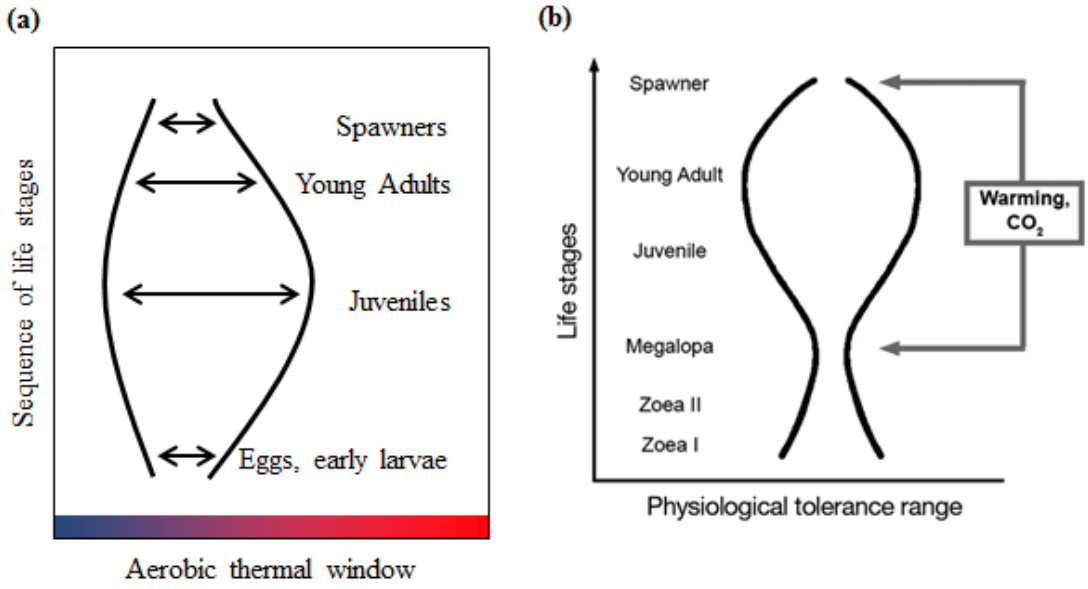

Figure 1.3 The aerobic thermal window during life history: (a) Theorised model of changes in aerobic thermal window throughout life history, from Pörtner and Farrell (2008). (b) The same model adapted to include differences in aerobic thermal window between larvae and megalopa stages, from Walther *et al*. (2010).

One theory as to why early life history stages are more sensitive to climate change than latter stages is due to changes in a species aerobic thermal window throughout development (Fig. 1.3). Early life-history stages are proposed to have the narrowest aerobic thermal window, as opposed to juveniles and growing adults which are thought to possess the widest (Pörtner and Farrell 2008). Exposure to hypercapnia increases and organism's sensitivity to heat, resulting in a narrowing of the organism's aerobic thermal window (Fig. 1.4) (Pörtner and Farrell 2008) as has been shown in the adult stage of the edible crab, *Cancer pagurus* (Linnaeus 1758) (Metzger *et al.* 2007). Therefore, constrictions to the aerobic thermal windows and scope for aerobic performance of organisms due to elevated pCO_2 would have a larger effect on eggs and larvae than later juvenile stages (Pörtner and Farrell 2008). Walther *et al.* (2010) expand on this theory (Fig. 1.3b) by stating that amongst the larval stages, megalopa have the

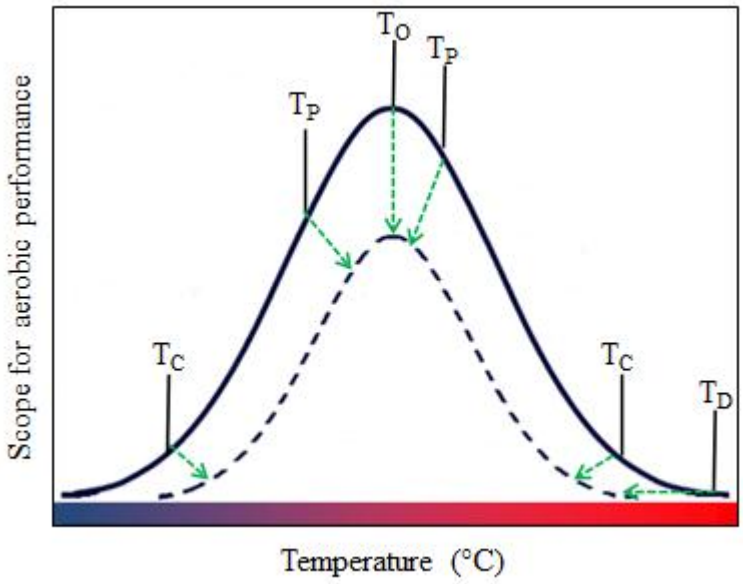

Figure 1.4 Theoretical model of changes in an organism's aerobic thermal scope due to elevated pCO_2: Solid line indicates aerobic thermal scope under normocapnia, dashed line indicates aerobic thermal scope under hypercapnia. T_C indicates critical temperature thresholds, T_P indicates *pejus* temperature thresholds, and T_O indicates optimum temperature. Dashed green arrows indicate changes in T_C, T_P, and T_O due to hypercapnia. Figure and definitions from Pörtner and Farrell (2008).

narrowest aerobic thermal window and it is this stage which is the major early life history bottleneck in relation to climate change.

Such abrupt and distinct changes during larval development, and associated sensitivities to abiotic factors, may explain why this life history stage has received a lot of attention. Furthermore, there are indications also that the effects of elevated pCO_2 on larvae carry over to juveniles (Dupont *et al.* 2012, Hettinger *et al.* 2012).

Juvenile stages also undergo dramatic changes in physiology and can be classed as two distinct developmental stages in their own right; early benthic juveniles and late benthic juveniles (Sect. 1.2). As with larval development, temperature appears to be the most

important abiotic factor determining the growth and survival throughout juvenile development of marine invertebrates.

With regards to OA, however, juvenile development has been largely overlooked. Juvenile stages of marine invertebrate life histories are proposed to possess the widest aerobic thermal window in comparison to other stages and so are perceived to be the most tolerant stage to global change drivers (Fig. 1.3) (Pörtner and Farrell 2008). However, as previously discussed the distinction between early and late juvenile stages may warrant them being assessed as two separate stages, as is apparent with regards to megalopa stages during larval development (Walther *et al.* 2010). While this has not occurred in the context of OA, Green *et al.* (2004) showed survival due to shell dissolution in *M. mercenaria* buried in aragonite under-saturated sediments was significantly lower in early benthic juveniles than later juveniles, indicating the potential for differences in sensitivity between early and late juvenile stages to changes in abiotic factors. As such a distinction has yet to be made for juvenile stages in relation to elevated pCO_2 associated with OA; this discussion must consider the juvenile stage as a whole and propose how considering two distinct phases in juvenile development may help clarify our understanding of the effects of OA on juvenile populations. Scope for growth and physiological energetics of the grooved carpet shell clam, *Ruditapes decussatus* (Linnaeus 1758) were reduced due to elevated pCO_2 (Fernandez-Reiriz *et al.* 2011). However, certain bivalve populations appear more tolerant to elevated pCO_2, with no effects on mortality, growth and mineralisation in estuarine populations of *R. decussatus* (Range *et al*. 2011) and an increase in scope for growth in the blue mussel, *Mytilus edulis* (Linnaeus 1758) from high alkaline coastal waters (Fernandez-Reiriz *et al.* 2012). Similarly there were differences between closely-related species, as while growth and development decreased in the barnacles, *Semibalanus balanoides* (Linnaeus

1767) and *Elminius modestus* (Darwin 1854) under elevated pCO_2 (Findlay *et al.* 2010a, b), there were no effects of elevated pCO_2 on juvenile barnacles, *Amphibalanus amphitrite* (Darwin 1854) (McDonald *et al.* 2009). Furthermore, when ocean warming and OA are considered in concert, the few studies on juvenile stages indicate similar variability in responses. Juvenile molluscs were sensitive to both elevated temperature and elevated pCO_2 but there were no interactions between the two drivers (Lischka *et al.* 2011, Talmage and Gobler 2011) while juvenile barnacles were sensitive to elevated pCO_2 not temperature (Findlay *et al.* 2010a, b) or elevated pCO_2 only at elevated temperature (Findlay *et al.* 2010a). The ochra starfish, *Pisaster ochraceus* (Brandt 1835), showed positive growth responses under elevated temperature and pCO_2 with only a marginal decrease in mineralisation due to exposure to elevated pCO_2 (Gooding *et al.* 2009) while juveniles of the common cuttlefish, *Sepia officinalis* (Linnaeus 1758), experienced increased mineralisation (Dorey *et al.* 2012). While there is a lot of variability between studies with relation to the effects of elevated temperature and pCO_2 in lines with ocean warming and OA predictions on juvenile development, none of the above studies distinguish between early and late juveniles, which may account for some of the variability seen as early stages of juvenile development are more sensitive to abiotic factors than later stages (Gosselin and Qian 1997). For example, by assessing the juvenile phase as a whole, any impacts on the sensitive early benthic phase may be disguised by the responses of the more tolerant late benthic phase.

In conclusion the sensitivity of larval and juvenile development of marine invertebrates to elevated temperature and pCO_2 due to ocean warming, ocean acidification, and the combinations of the two, is beginning to be understood. However, we still do not know enough of the ontogeny of physiological functions and how this can drive sensitivities in life history traits to ocean change, with many of the studies available restricted to

single species or single physiological and life-history traits. While the effects of elevated temperature and pCO_2 on the early development of marine invertebrates are comparatively well known, little is known of the interactive effects of these drivers, combined as they will be in the near future.

1.4 Thesis aims and objectives

Consequently, the aim of this thesis is to:

Investigate the eco-physiological sensitivities and potential life history bottlenecks of larval and early juvenile development to elevated temperature and pCO_2 in line with future predictions of anthropogenic climate change in a key marine invertebrate, the European lobster *H. gammarus*.

The objectives of the present thesis are five-fold:

Objective 1. To investigate the sensitivities of lobster larval development in terms of key life history traits (survival and growth) together with physiological responses (oxygen consumption and organic content) when exposed to elevated temperature and pCO_2, and to discuss how physiological sensitivities throughout larval development may be linked to changes in life history traits (Chapter 2).

This will be achieved by rearing larvae under normocapnic conditions, representing current conditions (420 µatm pCO_2, pH 8.1), and hypercapnic conditions in relation to predictions of Ocean Acidification in the year 2100 (1,100 µatm pCO_2, pH 7.7) made by Caldeira and Wickett (2003 and 2005). The two temperature treatments chosen are a control of 17 °C, representing current seasonal average when the experiment was carried out, and 21 °C representing + 4 °C associated with ocean warming (Sokolov *et*

al. 2009). Individuals will be sampled at each inter-moult stage and survival enumerated, growth determined by increases in wet mass, dry mass and organic content, *via* elemental analysis, and oxygen consumption determined using closed respirometry techniques. Such proxies will allow the evaluation of life history sensitivities, along with changes in underlying physiology which can link life history traits to environmental drivers.

Objective 2. To investigate the morphometric development and carapace mineralisation of larval *H. gammarus* under conditions of elevated temperature and pCO_2 (Chapter 3).

Using individuals sampled in Chapter 2, Morphometric development will be assessed in terms of total body length, carapace length, abdomen length, chelae length, and rostrum length, to determine the plasticity of morphological traits during exposure to elevated temperature and pCO_2. Mineralisation will be characterised at each inter-moult period of larval development in terms of carapace $[Ca^{2+}]$, $[Mg^{2+}]$, $[Sr^{2+}]$, $[Mn^{2+}]$, $[Na^{+}]$, $[K^{+}]$, and $[P^{+}]$ to assess the ability of *H. gammarus* larvae to calcify under elevated temperature and pCO_2, and the integrity of the mineralised structures produced under such conditions. Changes in morphological traits and mineralisation investment can often be related to functional energetic trade-offs in order to provide energy for the maintenance of internal functions and to ensure growth and survival under unfavourable conditions. Therefore, coupled with the findings of Chapter 2, any changes in morphometric traits or mineralisation throughout larval development can provide insights to the energetic costs of larval development under elevated temperature and pCO_2 and the functional trade-offs which occur in order to achieve maximum survival and growth under unfavourable conditions.

Objective 3. To assess the sensitivity of survival, growth, and underlying physiological and functional biology of early benthic juvenile *H. gammarus* under elevated temperature and pCO_2 conditions (Chapter 4).

Early benthic juveniles (Carapace length = 7.5 ± 0.1 mm, as defined by Wahle and Steneck (1991)) will be exposed for five weeks to normocapnia (450 µatm pCO_2, pH 8.1) representing current environmental conditions, 'mild' hypercapnia (1,100 µatm pCO_2, pH 7.7) representing future predictions of OA in the year 2100 (Caldeira and Wickett 2003, 2005) and 'extreme' hypercapnia (8,000 µatm pCO_2, pH 6.9) representing predictions of CCS leakage scenarios (Blackford *et al.* 2009, Kano *et al.* 2010) at 9 °C representing current winter temperatures when the experiment was performed, and 13 °C representing + 4 °C associated with predictions of future ocean warming (Sokolov *et al.* 2009). The life history traits of survival and growth, including moulting frequency, wet body mass, dry body mass and organic content, will be assessed after 5 weeks along with oxygen consumption, feeding rates, and carapace mineralisation to determine the underlying functional and physiological responses of early juveniles to elevated temperature and pCO_2. Such measurements will allow discussion on how these physiological and functional aspects can drive life history responses, and will provide information on the sensitivity of this stage as a further potential life history bottle neck in relation to anthropogenic climate change.

Objective 4. To assess the ability of early juvenile *H. gammarus* to regulate internal acid-base balance under elevated temperature and pCO_2, and the energetic consequences of such regulation (Chapter 5).

One year old individuals will be exposed for two weeks to the same pCO_2 conditions as recorded in Chapter 4, but at 13 °C representing current summer temperatures at the

time of the experiment, and 17 °C representing + 4 °C associated with predictions of future ocean warming (Sokolov *et al.* 2009). Acid-base balance will be determined after 2 weeks, while gill Na^+/K^+-ATPase will also be determined as a proxy for energy demand related to bicarbonate buffering. Oxygen consumption and feeding rates will be determined to assess the functional and physiological responses related to energy acquisition and demand, while haemolymph biochemistry including *L*-lactate levels, protein levels, $[Ca^{2+}]$, $[Mg^{2+}]$, $[Cu^{2+}]$, $[Na^+]$, and $[K^+]$ will be determined as proxies for anaerobic end products, non-bicarbonate buffering capacity and haemocyanin content, and ionic regulatory processes associated with acid-base regulation respectively. Such in-depth analysis of physiological and functional biology of early juveniles will allow not only for the assessment of their ability of haemolymph acid-base and ionic regulation, but also the energetic demands and state of ontogenetic development of physiological regulatory functions during early juvenile development.

Objective 5. To bring together Objectives 1-4 in order to discuss the developmental eco-physiology of *H. gammarus*, and the sensitivity of development and ontogeny of physiological functions to elevated temperature and pCO_2 in relation to future predictions of ocean warming, OA, and in the case of juveniles, CCS leakage scenarios (Chapter 6).

The discussion aims to link the eco-physiological development and life history responses to *H. gammarus* larvae and early juveniles under future predictions of ocean change to key ecological processes such as recruitment and life history stage transitions to discuss the potential effects of ocean change on future populations of this commercially important marine invertebrate.

The approach undertaken throughout the study is to use mesocosms specifically designed for the rearing of larval and juvenile lobsters under conditions of elevated temperature and pCO_2 in line with predictions of ocean warming (Sokolov *et al*. 2009), OA (Caldeira and Wickett 2003, 2005), and in the case of juveniles, CCS leakage scenarios (Blackford *et al.* 2009, Kano *et al.* 2010).

1.5 Study species

The European lobster, *Homarus gammarus* (Linnaeus 1758), is an economically important decapod crustacean, with 394 t landed in Europe during 2010 alone (FAO 2013). The geographical range of *H. gammarus* extends from north of the Arctic circle, along the European and Mediterranean coasts as far south as Morocco, from shallow waters to 200 m depth on the edge of the continental shelf (Cobb and Wahle 1994, Cobb and Castro 2007). Owing to its economic importance, the life history of *H. gammarus* has been well studied and a vast quantity of information is available on the nutrition and optimum abiotic factors for the survival and hence production of this species for commercial gain, *via* hatchery based stock replenishment strategies (Beard *et al.* 1992, Addison and Bannister 1994, Wickins *et al.* 1995, Daniels *et al.* 2010, 2013, Scolding *et al.* 2012).

Homarus gammarus has a complex life cycle (Fig 1.5) encompassing pelagic larval stages, relatively sedentary benthic juvenile and adults stages, and the dramatic behavioural, anatomical and physiological transition between such stages as seen in the majority of decapod crustaceans (Gruffydd *et al.* 1975, Cobb and Wahle 1994, Cobb and Castro 2007). Post hatching, *H. gammarus* undergoes four larval moults, three of which are fully pelagic with the fourth representing a bottom seeking megalopa stage (Charmantier *et al.* 1991), which in total last 20 days at 16 °C (Schmalenbach and

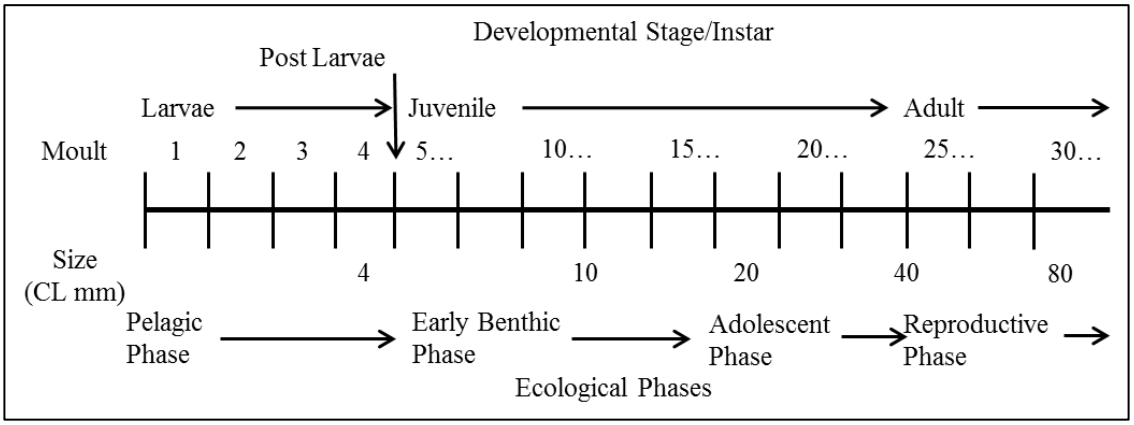

Figure 1.5 Time line of developmental events during the life history of *H. gammarus*: Comparison of developmental stages and ecological phases, sourced from Cobb and Wahle (1994).

Franke 2010) and is dependent on the prevalent environmental conditions, mainly temperature and nutritional status (Cobb and Wahle 1994, Cobb and Castro 2007).

Stage I larvae, the newly released zoea, which last in the water column for 4-5 days at 16 °C (Schmalenbach and Franke 2010), exhibit strong positive phototactic behaviour (Schmalenbach and Buchholz 2009). This behaviour results in a migration of larvae to the sea surface and in doing so represents a transition between benthic (embryonic) and pelagic (larval) environments. Stages II and III last for 6 and 9 days respectively at 16 °C (Schmalenback and Franke 2010) and show diminishing positive phototactic behaviour with increasing swimming ability (Schmalenbach and Buchholz 2009). In the closely related American lobster, *Homarus americanus*, digestive enzyme activity (protease, amylase, and lipase) doubles between Stages I and II, which along with the morphological development of hepatopancreas show a large development of digestive ability (Biesiot and Capuzzo 1990) which is accompanied by energetic demands of Stages II and III compared to Stage I (Capuzzo and Lancaster 1979). The fourth and final larval stage, Stage IV, represents a bottom seeking megalopa and a further transition between pelagic (larval) and benthic (juvenile) environments. As previously

discussed in Section 1.1, in marine crustaceans the moult between the penultimate larval stage and megalopa stage is accompanied by the development of a range of physiological and morphological milestones, which in the case *H. gammarus* and *H. americanus* are known to include the development of osmoregulatory structures and functions (see Charmantier *et al*. 2001 for review) and increased dependence on protein catabolism for energy (Capuzzo and Lancaster 1979). Upon settlement to the benthos, a further moult is obtained as lobsters complete the transition from larvae to juveniles.

Within juvenile development of clawed lobsters, there is a further ontogenetic shift in behaviour and habitat representing an ecological distinction between early and late benthic juveniles (Wahle and Steneck 1991, Cobb and Wahle 1994). As discussed previously (Sect. 1.2), such ecological distinctions may be representative of physiological distinctions between early and late juvenile crustaceans due to the development of physiological functions. There are two examples of such physiological development within the clawed lobsters, the development of ionoregulatory abilities during juvenile stages of *H. gammarus* (Newton and Potts 1993) and the development of gill structures during the juvenile stages of *Nephrops norvegicus* gill structures develop (Spicer and Eriksson 2003) which suggest that such a physiological differentiation between early and late juvenile stages is relevant for clawed lobsters as for other crustaceans.

Our vast knowledge of lobster larvae and juveniles provides a base for assessing the development of this species under future predictions of ocean warming and ocean acidification, while the economic importance of the species provides an additional importance to assess the sensitivity of future populations. Further to this, the ontogeny of physiological functions throughout the larval and juvenile development of lobsters has been assessed in terms of osmoregulation (Charmantier *et al.* 1988, 2001) and oxy-

regulation in the closely-related *N. norvegicus* (Spicer and Eriksson 2003). We also know that the mineralisation of Stage IV *H. gammarus* is sensitive to elevated pCO_2 (Arnold *et al.* 2009). While less is known of juvenile physiology, we may get insight on adult physiology in terms of acid-base regulation (Taylor and Whiteley 1989, Whiteley *et al.* 1990, Whiteley and Taylor 1990) and while this knowledge is based on aerial exposure, the processes involved are directly applicable to elevated pCO_2 due to OA and therefore, along with the knowledge on ontogeny of physiological functions of lobsters, provide a foundation for further research into the sensitivities of *H. gammarus* larval and early juvenile development under elevated temperature and pCO_2.

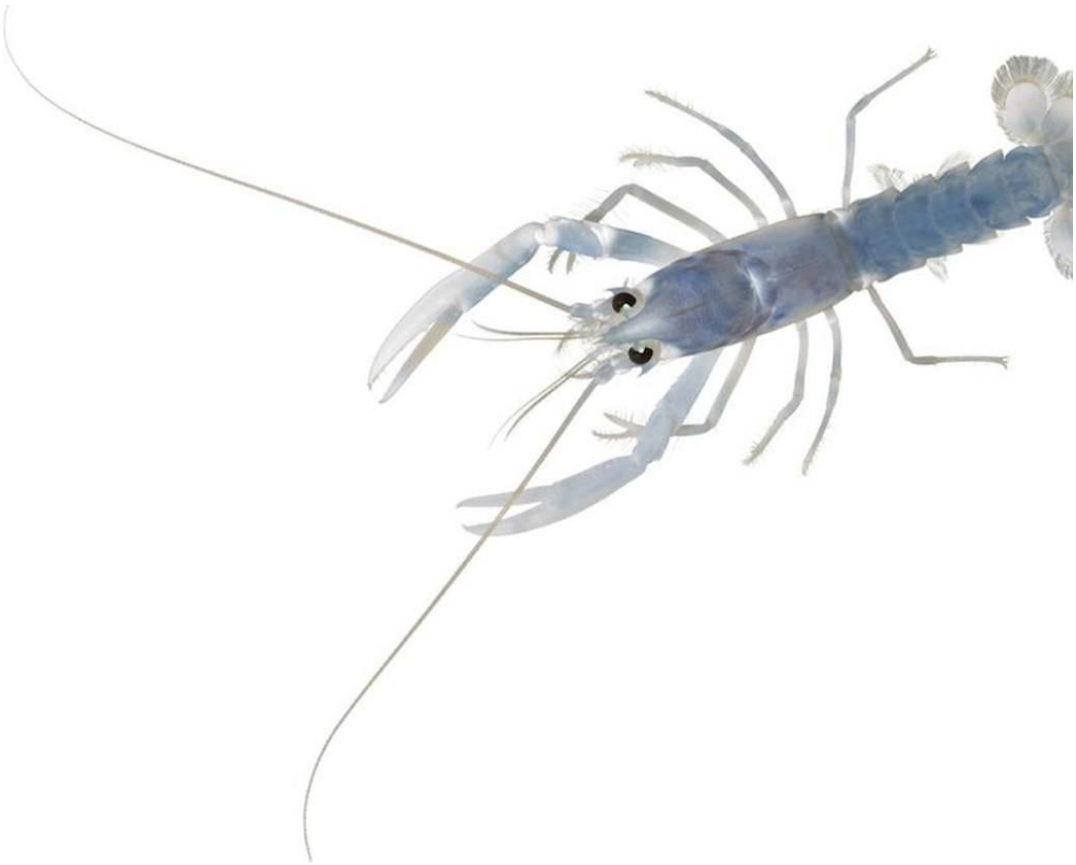

Figure 1.6 Juvenile *H. gammarus*: Photo courtesy of David Liittschwager, National Geographic.

Chapter 2

Physiological responses to elevated temperature and pCO_2 during larval development

2.1 Introduction

The early developmental stages of marine organisms have been identified as potential life-history bottlenecks when we consider the impact of climate change on biological systems (Kurihara 2008, Pörtner and Farrell 2008, Byrne 2011, 2012). Life history traits, such as survivorship and growth, are linked to the environment *via* physiological processes (Calow and Forbes 1998, Ricklefs and Wikelski 2002, Young *et al.* 2006, Sunday *et al.* 2011). Therefore understanding physiological responses behind such life history traits is a critical process in linking organism and population changes to environmental changes (Pörtner 2010) and formulating successful stock management and species conservation strategies (Young *et al.* 2006). This is particularly relevant for major decapod crustacean fishery species, which rely on larval dispersal and development as a key factor of their life history (Anger 2001, Gaylord *et al.* 2005), yet the physiological processes underlying and mediating larvae responses to environmental changes, such as elevated temperature, elevated pCO_2, and reduced pO_2, are understudied compared with more conventional growth and survivorship proxies.

Among these environmental parameters, temperature is a major driver of crustacean larval development (Anger 1998, 2001, Lindley 1998, Hartnoll 2001). Increasing atmospheric pCO_2 due to anthropogenic CO_2 emissions results in global warming, which will translate into ocean warming as surface waters are expected to increase by 3 - 5 °C by the year 2100 (Sokolov *et al.* 2009). While crustacean developmental time increases with increasing temperature, this can be accompanied by costs in terms of

survival and growth (Anger 2001, Hartnoll 2001) as there can be an energetic trade-offs experienced at higher temperature (Atkinson 1995, Weiss *et al.* 2010). Indeed, despite increased developmental time with temperature, maximum larval growth and survival are obtained at mid-range optimum temperatures (Sastry and McCarthy 1973, Mackenzie 1988). While the responses of growth and survival to temperature are well known, we know little of the physiological drivers of the temperature responses of larvae (Hartnoll 2001, Storch *et al.* 2009a, b). Where the energetics of larval development have been studied, elevated temperature increases respiration rates until above optimum conditions where rates cease to rise and begin to decrease (Schatzlein and Costlow 1978, Vernberg *et al.* 1981, Anger 1987). At this point larvae exhibit oxygen- and capacity-limited thermal tolerance similar to that of adults (Storch *et al.* 2009a, b) defining optimal thermal windows.

Along with elevated temperature, increasing atmospheric CO_2 levels are predicted to cause a rise in oceanic pCO_2 (Sabine *et al.* 2004) and an associated decrease in pH and alteration of the carbonate chemistry of sea water (Caldeira and Wickett 2003, 2005, Feely *et al.* 2004); a process referred to as OA. OA is a relatively new, but extremely prolific, field in climate change research. However, within this context, most studies of larval development in crustaceans to date still focus primarily on survivorship and growth/rates (Whiteley 2011). Larval studies of this nature show increased mortality in some species (Kurihara *et al.* 2004, Long *et al.* 2013), decreases in body size (Kurihara *et al.* 2008b) or no effect on survival or growth in others (Arnold *et al.* 2009, Egilsdottir *et al.* 2009). There are fewer studies which have investigated the underlying physiology of larval development within the context of OA, often revealing the presence of subtle but important effects of elevated pCO_2. Walther *et al.* (2010) found that beyond pCO_2 induced increases in development time and reduced growth and survival, performance

was affected due to changes in C:N ratios. In the king crab, *Paralithodes camtschaticus* (Tilesius 1815) body size increased, yet organic content did not change, driving negative survival (Long *et al*. 2013). Likewise Arnold *et al*. (2009) demonstrated that despite no significant pCO_2 effects on survivorship or growth, Stage IV European lobster *Homarus gammarus* (Linnaeus 1758) larvae exposed to elevated pCO_2 possessed exoskeletons characterised by reduced $[Ca^{2+}]$.

While much has been already achieved in understanding the effect of warming and OA on the physiology of marine invertebrates, the fact that these stressors occur together in nature makes it imperative to study what happens when they are manipulated in concert (Pörtner and Farrell 2008, Widdicombe and Spicer 2008). We know how multiple stressors can interact upon larval development through in depth studies on the relationships between temperature and salinity on survivorship resulting in ontogenetic shifts in optimum conditions between developmental stages (Costlow *et al*. 1960, 1962, 1966, Anger 1991, Agard 1999, Cieluch *et al.* 2004). Such ontogenetic shifts can be linked with to the larval ecology and export strategies associated with particular crustacean species as they define the environmental conditions which the larvae experience during development (Read 1984). A good example is that of Charmanteir *et al.* (2002) who demonstrate that the grapsid crab, *Chasmagnathus granulata* (Dana 1851), populates brackish lagoons and estuarine enviernments as adults and juveniles, while larval export to coastal marine zones in order to distribute to other estuarine environments leads larvae to be exposed to more saline environments. Such changes in salinity reigines throughout larval development due to this export strategy is mirrored in the ontogeny of osmoregulatory capabilities in *C. granulata* larvae and subsequent optimum salinity conditions (Charmantier *et al.* 2002). These ontogenetic shifts in optimum conditions are, however, mostly based upon *per cent* survival at each

developmental stage, and little is known of the underlying physiology behind such shifts. Less is known with regards to the interactions between temperature and OA, yet we are beginning to understand that there are important physiological implications when the two are combined (Findlay *et al*. 2010a, b, Walther *et al*. 2010, 2011, Arnberg *et al*. 2013).

Here, I investigate the sensitivity of crustacean larval development to elevated temperature and pCO_2 conditions in terms of key life history traits (survivorship and growth), together with physiological responses (metabolic rate) and condition (organic content), and discuss how physiological sensitivity may link to, and interact with, changes in life history traits. *H. gammarus,* is a particularly good model organism for such investigations due to its complex life cycle, in which early stages include a transition from pelagic larvae to benthic juveniles (Cobb and Wahle 1994) representing coupling between pelagic and benthic habitats. Furthermore, the larval stages of *H. gammarus* are relatively large in comparison to many other invertebrate larvae, allowing detailed measurements of proxies which may otherwise be difficult. In addition, larval responses to OA (Arnold *et al.* 2009) and temperature have been already investigated individually (Gruffydd *et al.* 1975, Mackenzie 1988), allowing us to develop testable predictions on the effect of the interaction between these two drivers driving these developmental responses. The present study will advance our understanding of the effects of climate change on this species by linking physiology to growth, survivorship, and condition, which will give us a holistic view on the performance of individuals and viability of future populations of this economically important species, which may be attributed to other species with similar life histories. This is done by studying the larval development of *H. gammarus* under future climate change predictions due to increased atmospheric pCO_2, including an OA related reduction of seawater pH and an increase in

ocean surface temperatures. A fully quadratic experiment was designed consisting of temperature levels chosen to represent current monthly averages during larval occurrence in surface waters when the experiment was performed (17 °C) and + 4 °C associated with predicted surface water warming (21 °C, Sokolov *et al.* (2009)) and pCO_2 levels to represent current conditions (420 µatm, pH 8.1) and decrease of 0.4 pH units due to OA predictions (1,100 µatm, pH 7.7, Caldeira and Wickett (2003, 2005)).

2.2 Materials and methods

2.2.1 Animal husbandry

Newly-hatched *H. gammarus* larvae (N = 4,320) were obtained within 6 h of hatching from ovigerous females at the National Lobster Hatchery (Padstow, UK). Females were caught off the South Cornwall coast during July 2011 and kept in an aquarium (vol. = 1,200 L) with sea water sourced from the Camel estuary (Padstow, UK -50°32'19.67"N, 4°56'5.85"W). Stock sea water was mechanically and biologically filtered with weekly water changes (Salinity = 35, T = 19 °C, DO_2 = 8 mg L^{-1}) and light:dark regimes throughout the rearing and experimental periods remained constant at 12:12h. Females were fed twice a week on whole mussels (*Mytilus edulis* Linnaeus 1758) and larvae were fed twice daily *ad-libitum* throughout the exposure period on a mixture of frozen copepod and krill.

To prevent contamination of the larval system, any possible bacterial agents from the maternal tank were removed by immediately transferring larvae, *via* a sieve (0.5 x 0.5 mm square mesh) into a large rearing cone (vol. = 80 L) filled with aerated sea water containing *N*-chloro tosylamide (conc. = 2 mg L^{-1}). Larvae were ‘bathed’ in this tank for 1 h before being transferred, *via* the sieve, into the experimental system.

2.2.2 Experiment design

The experimental system (Fig. 2.1) consisted of six aquaria (vol. = 20 L) each containing 6 rearing cones (vol. = 2 L, Fig. 2.2). The design of the rearing cones is displayed in Fig. 2.2. Each cone had a flow through of sea water (flow rate = 10 mL min^{-1}) which was re-circulated through the system, *via* mechanical and biological filtering mechanisms. Of the six aquaria, three were designated haphazardly to the control temperature condition, and three to the elevated temperature condition, with the water inflow to the cones being controlled by a water chiller with heating elements

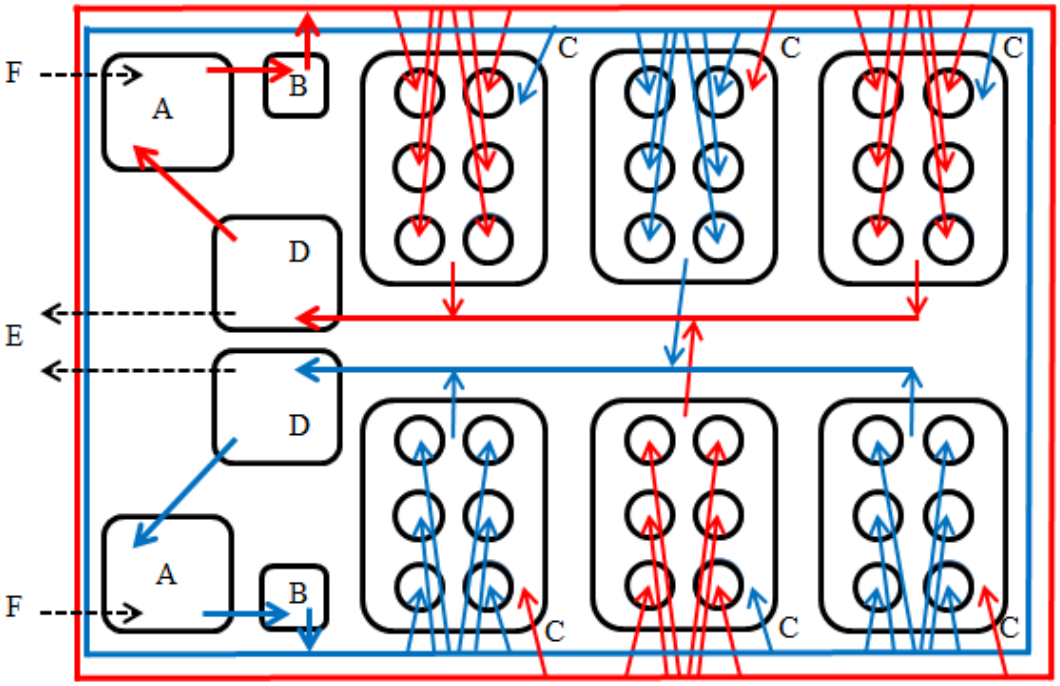

Figure 2.1 Diagram representing the recirculating experimental design, focussed on temperature control: Blue lines and arrows indicate the flow of control (17 °C) treatment water, red lines and arrows indicate the flow of elevated temperature (21 °C) treatment water. A = Header tanks with filtration. B = Chiller units with heating elements to control water temperature. C = Water inflow into individual rearing cones. Note that there is a 'swapping' of treatment water to aid temperature control within tanks. D = Sump tanks with filtration. E = Waste water overflow. F = Daily 8 h flow of new, clean sea water to ensure water quality.

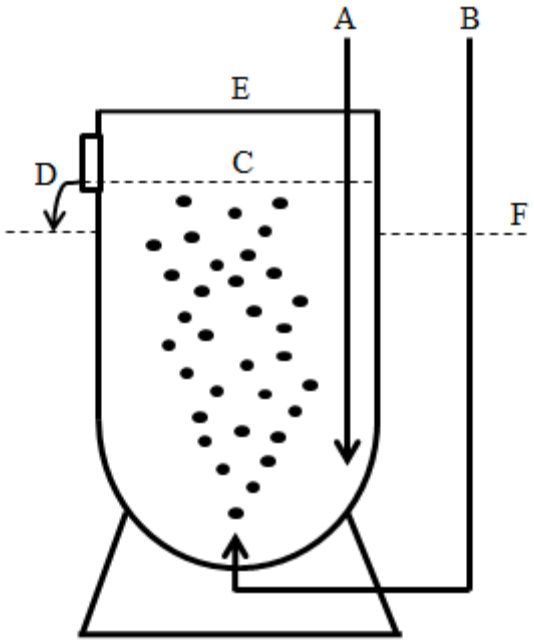

Figure 2.2 Diagram representing an individual larval rearing cone: A = Water inflow (Set to designated temperature level). B = Air inflow (Set to designated pCO_2 level). C = Water height in cone. D = Water overflow through mesh to allow broken food removal but retain larvae and larger food particles. E = Cone lid to prevent excess evaporation and spray. F = Water level in surrounding tank.

(SeaChill TR10, Teco s.r.l., Ravenna, Italy) and water outflow into the aquaria acting as a water bath to allow constant stable temperatures (Fig. 2.1). Each cone also received constant aeration, and had a mesh (0.5 mm^2 Square Mesh) on the outflow to allow small broken down food waste to be removed while larger food particles and larvae were kept in. To ensure high water quality, the system received a flow through of fresh sea water (flow rate = 10 mL min^{-1}) for 8 h each day.

Equilibration of sea water with the desired level of pCO_2 was achieved by bubbling an appropriate gas mixture into the water contained in each cone. Control pCO_2 (420 µatm) was achieved by bubbling untreated air into the sea water contained in three of the six

cones in each aquaria, while elevated pCO_2 levels were achieved by bubbling CO_2-enriched air (ambient air mixed with pure CO_2 to achieve a pCO_2 of 1,100 µatm following methods of Findlay *et al.* (2008)) into the sea water within the remaining six cones in each aquaria. Levels of pCO_2 in the air supplied to acidified cones was measured continuously throughout the exposure period using a CO_2 gas analyser (Li-820, Li-Cor Biosciences, Lincoln, Nebraska, USA) with the flow of the CO_2 input being adjusted as necessary.

Water chemistry was monitored daily with measurements of pH (NBS scale) taken using a pH electrode (HI-1210B/5 Hanna Instruments Ltd., Leighton Buzzard, UK) connected to a hand-held pH meter (HI-98160, Hanna Instruments Ltd. Leighton Buzzard, UK), temperature using a thermocouple (HH802U Omega Engineering Inc. Stamford, USA), and salinity using a refractometer (S/Mill Hand Refractometer, Atago, Tokyo, Japan). Water samples were taken every 5 d, and were filtered, fixed with $HgCl_2$ (Conc. = 0.02 %) and stored in borosilicate flasks (vol. = 250 mL). Total alkalinity (A_T) was measured by gran titration method using an alkalinity titrator (As-Alk2, Apollo SciTech Inc., Bogart, USA). Carbonate system parameters of carbon dioxide partial pressure (pCO_2 - µatm), total carbon dioxide (TCO_2 - µmol kg^{-1}) bicarbonate concentration ($[HCO_3^-]$ - µmol kg^{-1}), carbonate concentration ($[CO_3^{2-}]$ - µmol kg^{-1}), calcite saturation (Ω_{cal}), and aragonite saturation (Ω_{ara}) were calculated from A_T, pH, temperature, and salinity using CO_2SYS program (Lewis and Wallace 2006) with constants provided by Mehrbach *et al.* (1973) refitted by Dickson and Millero (1987) and KSO_4 constants from Dickson (1990). Water chemistry parameters for all treatments during the exposure period are displayed in Table 2.1.

2.2.3 Determination of survivorship

The number of live individuals in each cone at each inter-moult period was counted and cumulative survival expressed as a percentage of the number of individuals introduced into the cone at day 0, also taking into account of individuals removed for analysis and not replaced. Survival was also expressed as the percentage of individuals counted in each cone during the previous stage, to highlight stage specific changes in survivorship.

2.2.4 Determination of rates of oxygen consumption

The rates of oxygen consumption (as a proxy for metabolic rate) for one individual from each cone (N = 9 *per* treatment) at each inter-moult period was measured closely following the semi-closed respirometry method of Spicer and Eriksson (2003). Individuals were placed in blacked out respiration chambers (vol. = 40 mL) containing filtered sea water at the same temperature and pCO_2 as throughout the exposure period. Individuals were left in open chambers for 30 min prior to the commencement of oxygen measurements, which lasted for 2 h for Stages I, II, and III and 1.5 h for Stage IV. Preliminary trials indicated that the time left in open chambers prior to the first measurement did not affect oxygen consumption, and random blanks showed negligible background changes in oxygen levels. Oxygen concentration was measured at the beginning and end of the respiration period using an oxygen electrode (1302, Strathkelvin Instruments, Glasgow, UK) housed in a temperature controlled chamber (TC50, Strathkelvin Instruments, Glasgow, UK) coupled to an oxygen meter (781, Strathkelvin Instruments, Glasgow, UK). Rates of oxygen consumption were calculated as the difference in concentrations, expressed as μmol O_2 g(wet body mass)$^{-1}$ min^{-1} S.T.P. using oxygen solubility coefficients obtained from Green and Carritt (1967) and wet body mass as calculated below.

2.2.5 Determination of growth

Two individuals of each inter-moult period, including those used for the determination of oxygen consumption, were taken from each cone (N = 18 *per* treatment), dried of exosomatic water by carefully dabbing the individuals with tissue paper, and weighed using a precision balance (3719MP, Sartorius, Germany, d = 0.1 mg) for wet body mass (WBM) in mg. After weighing, all individuals were laid straight and flat on their right hand side to be photographed with a macro enabled digital camera (Powershot A710 IS, Canon, Reigate, UK). Photographs were analysed with ImageJ software (Rasband WS, U.S. National Institutes of Health, Bethesda, USA) and carapace length (CL) measurements were obtained by measuring the distance from the rear of the eye socket to the rear of the carapace. Immediately after photographing, all individuals were rinsed in ultra-pure water, dabbed dry, and frozen at – 20 °C for future analysis of organic content and mineralisation (Chapter 3).

2.2.6 Determination of organic content

The organic content (Carbon, Hydrogen, and Nitrogen, CHN levels) of larval *H. gammarus* at each inter-moult stage was analysed in one individual from each cone (N = 9 *per* treatment). Individuals were freeze dried and weighed with a high precision balance (AT201 Mettler-Toledo Ltd, Leicester, UK, d = 0.01 mg). If dry mass was less than 2.5 mg, the whole animal was placed into a tin cup and crushed. If dry mass was greater than 2.5 mg, it was ground into a uniform powder using a mortar and pestle, and 2 mg sub-samples placed into a tin cup and analysed using an elemental micro analyser (EA1110 CHNS, Carlo Erba, Italy, modified by Elemental Analysis, Okehampton, UK) calibrated using cyclohexanone 2,4-dinitrophenylhydrazone and standardised against L-Cystine.

2.2.7 Statistical analysis

Data were first tested for normal and homogenous distributions using a Kolmogorov – Smirnov Test for normality followed by a Levene's test for equal variances. For data where assumptions were not met, residuals were analysed against treatment to determine how much residual variation was not due to the assigned treatment, where all assumptions were met. A two-way ANOVA was performed to analyse the effects of temperature, pCO_2, and their interactions on all proxies at each inter-moult stage. For oxygen consumption, a two-way ANCOVA was performed with WBM as a covariant. Tank effects were analysed as a random factor and if not significant, were discarded from analysis. CHN results were also analysed for correlations using a Spearman's rank order correlation.

Table 2.1 Water chemistry parameters over the course of the experimental period (Means ± S.E., d.f = 3, N = 89): Temperature (°C), salinity, pH (NBS Scale), total alkalinity (A_T - µEq kg^{-1}), carbon dioxide partial pressure (pCO_2 - µatm), total carbon dioxide (TCO_2 - µmol kg^{-1}) bicarbonate concentration ($[HCO_3^-]$ - µmol kg^{-1}), carbonate concentration ($[CO_3^{2-}]$ - µmol kg^{-1}), calcite saturation (Ω_{cal}) and aragonite saturation (Ω_{ara}). Superscript capital letters indicate significant differences between treatments. [1]Parameters calculated using CO_2SYS program (Lewis and Wallace 1998) with constants provided by Mehrbach *et al.* (1973) refitted by Dickson and Millero (1987) and KSO_4 constants from Dickson (1990).

	17 °C		21 °C	
	420 µatm	1,100 µatm	420 µatm	1,100 µatm
Temperature	17.2 ± 0.03^A	17.2 ± 0.03^A	21.3 ± 0.03^B	21.3 ± 0.03^B
Salinity	33.0 ± 0.01^A	33.0 ± 0.01^A	33.1 ± 0.02^B	33.1 ± 0.02^B
pH	8.08 ± 0.01^A	7.73 ± 0.01^B	8.11 ± 0.01^A	7.76 ± 0.01^B
A_T	2.17 ± 0.04	2.18 ± 0.04	2.22 ± 0.03	2.23 ± 0.03
$^1pCO_2$	454 ± 11^A	1154 ± 17^B	426 ± 11^A	1181 ± 36^B
$^1TCO_2$	1981 ± 42^A	2121 ± 34^B	1987 ± 20^A	2154 ± 27^B
$^1[HCO_3^-]$	1829 ± 40^A	2015 ± 32^B	1806 ± 17^A	2039 ± 26^B
$^1[CO_3^{2-}]$	135 ± 1.6^A	65 ± 1.5^B	167 ± 4.9^C	76 ± 1.3^B
$^1\Omega_{cal}$	$3.26 \pm .04^A$	$1.57 .04^B$	$4.06 \pm .12^C$	$1.87 \pm .03^B$
$^1\Omega_{ara}$	$2.10 \pm .03^A$	$1.01 \pm .02^B$	$2.6 \pm .08^C$	$1.25 \pm .02^D$

2.3 Results

2.3.1 Survivorship

The survival of *H. gammarus* throughout larval development under elevated temperature and pCO_2, in terms of % survival from previous stage, is displayed in Figure 2.3 while cumulative survival displayed in Table 2.2. There were no significant interactions between elevated temperature and pCO_2 on the survival of *H. gammarus* at any stage of larval development, however there were significant effects of elevated temperature and pCO_2 individually at various developmental stages. In detail, the survival of larvae to Stage II was significantly reduced by elevated temperature ($F_{1, 35}$ =

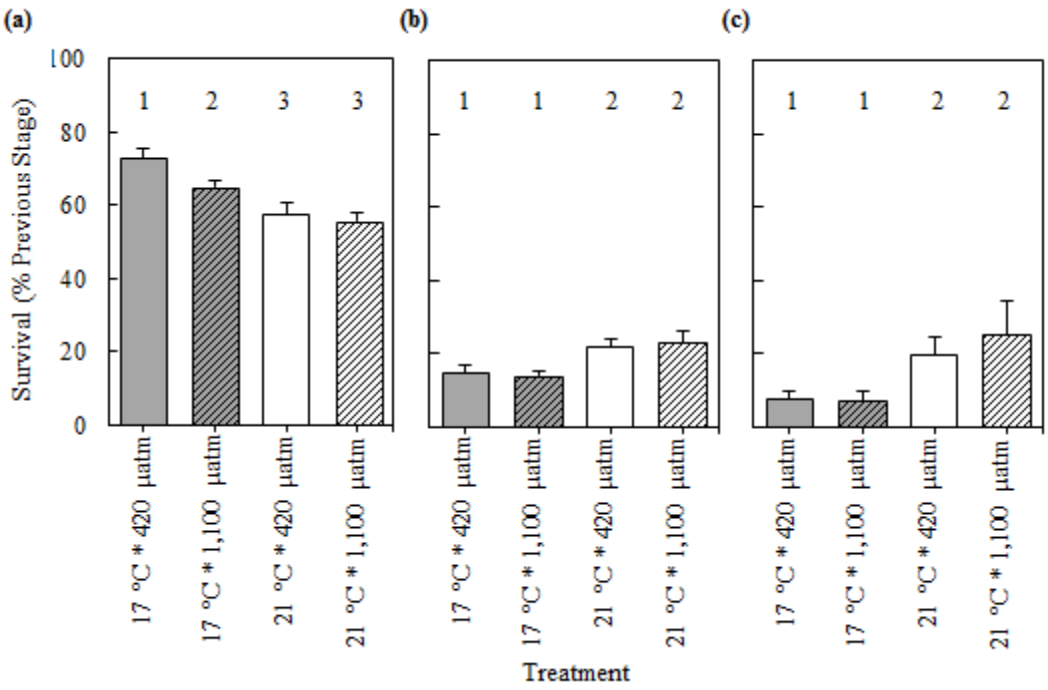

Figure 2.3 Survival of larval *Homarus gammarus* to each developmental stage under elevated temperature and pCO_2 (Mean ± S.E., d.f. = 3, N = 35): Survival calculated as % of live individuals in the previous developmental stage. (a) Stage II, (b) Stage III and (c) Stage IV. Grey bars indicate 17 °C. White bars indicate 21 °C. Clear bars indicate 420 µatm pCO_2. Striped bars indicate 1,100 µatm pCO_2. Numbers indicate significant differences between treatments within stages.

25.911, $p < 0.001$, Fig. 2.3, Table 2.2) and pCO_2 ($F_{1, 35} = 4.960$, $p = 0.033$, Fig. 2.3, Table 2.2), however the decrease in survival due to elevated pCO_2 was only detectable at T = 17 °C. There were no further effects of elevated pCO_2 on survival throughout development, although elevated temperature was accompanied by a greater survival from Stage II to Stage III ($F_{1, 35} = 7.443$, $p = 0.026$, Fig. 2.3) and from Stage III to Stage IV ($F_{1, 35} = 6.107$, $p = 0.039$, Fig. 2.3), which together resulted in an overall significantly greater Stage IV survival ($F_{1, 35} = 36.029$, $p < 0.001$, Table 2.2).

2.3.2 Growth

Growth, in terms of wet body mass (WBM) and carapace length (CL), of larval *H. gammarus* throughout development under elevated temperature and pCO_2 is summarised in Table 2.2 while the WBM and CL of Stage IV individuals are displayed in Figure 2.4. There were no significant interactions between elevated temperature and

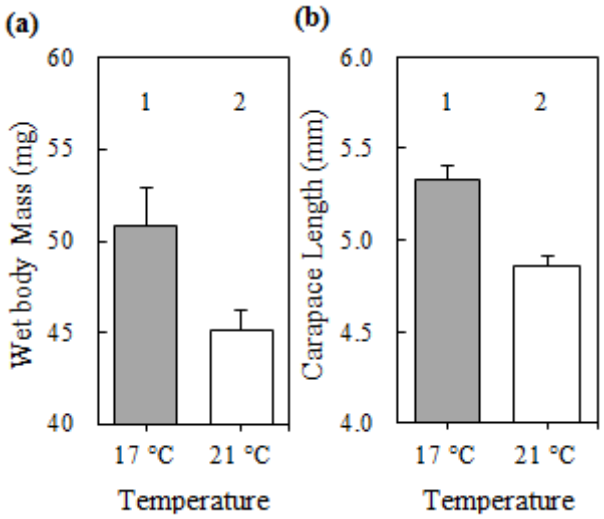

Figure 2.4 Growth status of Stage IV *H. gammarus* larvae reared under elevated temperature and pCO_2 (Means ± S.E., d.f = 1, N = 57): (a) Wet body mass (mg) and (b) carapace length (mm). Grey bars indicate 17 °C and white bars indicate 21 °C. Numbers indicate significant differences between temperature treatments.

pCO_2 on WBM or CL at any stage of larval development, nor were there any effects of elevated temperature or pCO_2 on WBM or CL at Stages I, II, or III (Table 2.2). At Stage IV, WBM was significantly lower in individuals exposed to elevated temperature ($F_{1, 57}$ = 6.480, p = 0.014) as was CL ($F_{1, 57}$ = 2.391, p < 0.001). Overall, total larval wet mass growth between Stage I and Stage IV was 350 % at 21 °C when compared with 400 % at 17 °C, with body length growth of 170 % compared to 185 %. Development time, expressed as duration of each larval stage, was shorter at T = 21 °C than at T = 17 °C, resulting in a total larval duration of 16 days and 24 days respectively with no effect of elevated pCO_2.

2.3.3 Rates of oxygen consumption

Rates of oxygen consumption during *H. gammarus* larval development under elevated temperature are displayed in Figure 2.5, while oxygen consumption of Stage IV individuals under elevated pCO_2 are displayed in Figure 2.6d. There were no significant interactions between elevated temperature and pCO_2 on oxygen consumption at any stage of larval development, however there were significant effects of elevated temperature and pCO_2 individually at varous developmental stages. In detail, rates of oxygen consumption of Stage I individuals significantly decreased due to elevated temperature ($F_{1, 31}$ = 9.930, p = 0.001, Fig. 2.5), while at Stage II, oxygen consumption significantly increased with elevated temperature ($F_{1, 31}$ = 25.793, p < 0.001, Fig 2.5) along with at Stage III ($F_{1, 31}$ = 40.470, p < 0.001, Fig 2.5). At stage IV there was a significant increase in rates of oxygen consumption due to elevated pCO_2 ($F_{1, 30}$ = 4.550, p = 0.042). Elevated pCO_2 had no significant effect on the oxygen consumption of Stages I, II, and III, while elevated temperature had no significant effect on the oxygen consumption of Stage IV. There was a significant tank effect at Stage I, yet when tank 1

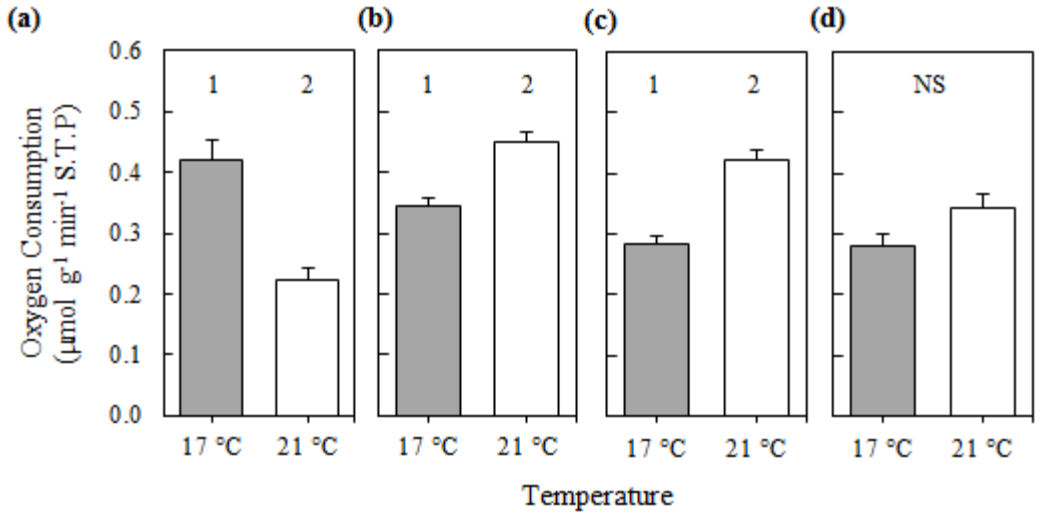

Figure 2.5 Oxygen consumption of larval *H. gammarus* reared under elevated temperature and pCO_2 (Means ± S.E., d.f = 1, N = 31): Oxygen consumption (µmol O_2 min^{-1} g^{-1} S.T.P) of (a) Stage I, (b) Stage II, (c) Stage III, and (d) Stage IV larvae. Grey bars indicate 17 °C and white bars indicate 21 °C. Numbers indicate significant differences between temperature treatments.

was removed from the analysis, the tank effect was no longer significant, while all other results remain the same. Wet body mass was not significant as a covariant throughout, while Stage IV rates of oxygen consumption significantly positively correlated with increased dry mass.

2.3.4 Organic content

Dry mass (DM), Nitrogen content (N), and Carbon:Nitrogen ratio (C:N) of Stage IV *H. gammarus* reared under elevated temperature and pCO_2 are displayed in Figure 2.6, while remaining organic content parameters are displayed in Table 2.3. There were no significant interactions between elevated temperature and pCO_2 on any parameter of organic content at any stage of larval development. There were also no significant effects of elevated temperature and pCO_2 individually at Stages I, II, and III, nor of

temperature at Stage IV. At Stage IV there was a significant increase in dry mass, in terms of % of wet mass, ($F_{1, 24}$ = 5.249, p = 0.032) due to elevated pCO_2. In terms of the organic content of dry mass, the nitrogen portion decreased significantly with elevated pCO_2 ($F_{1, 24}$ = 5.430, p = 0.030), and while carbon and hydrogen did not change significantly, there was a strong positive correlation between the three elements (Carbon v Nitrogen N = 25, ρ = .803, p < 0.001, Hydrogen v Nitrogen N = 25, ρ = .610, p = 0.001, Carbon v Hydrogen N = 25, ρ = .858, p < 0.001) suggesting that they also decrease along side decreasing Nitrogen but to a lesser extent.

Due to the slight reduction in Carbon and the large reduction in Nitrogen, there was a significant increase in the C:N ratio ($F_{1, 24}$ = 4.958, p = 0.037). There is a total significant decrease in the % organic (CHN) portion of the total animal under elevated pCO_2, and so a significant increase in the ash content ($F_{1, 24}$ = 5.564, p = 0.028).

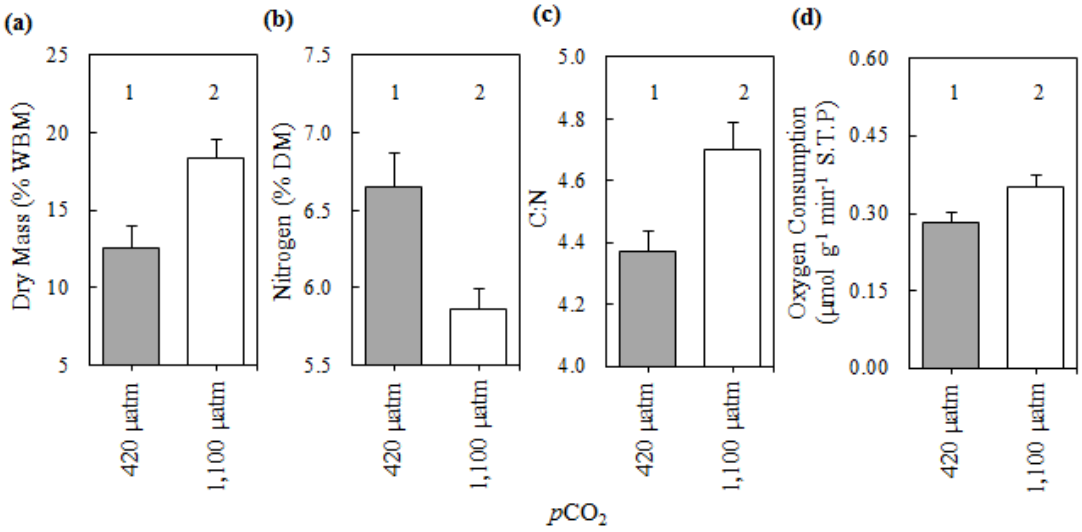

Figure 2.6 Organic content and oxygen consumption of Stage IV larvae *H. gammarus* reared under elevated pCO_2 (Mean ± S.E., d.f = 1, N = 24): (a) Dry body mass (DM, % of wet body mass), (b) nitrogen levels (% of Dry Mass), (c) carbon to nitrogen ratio (C:N), and (d) oxygen consumption (µmol O_2 min^{-1} g^{-1} S.T.P). Grey bars indicate 420 µatm pCO_2 and white bars indicate 1,100 µatm pCO_2. Numbers indicate significant differences between pCO_2 treatments.

Table 2.2 Life history traits of *Homarus gammarus* throughout larval development under elevated temperature and pCO_2 (Means ± S.E.): Survival (% of initial number), stage duration (Duration - d), and growth parameters wet body mass (WBM – mg, d.f = 1, N = 57) and carapace length (CL – mm, d.f = 1, N = 57). Superscript capital letters represent significant differences between treatments.

	13 °C		21 °C	
	420 µatm	1,100 µatm	420 µatm	1,100 µatm
Survival				
Stage II	72.9^{A}	64.2^{B}	57.5^{C}	55.8^{C}
Stage III	11	9.1	12.9	12.4
Stage IV	0.6^{A}	0.6^{A}	2.1^{B}	2.2^{B}
Duration				
Stage I	4	4	3	3
Stage II	6.7 ± .1	6.6 ± .2	5	5
Stage III	13.8 ± 1.7^{A}	14.2 ± 1.7^{A}	8.2 ± 0.3^{B}	7.4 ± 0.2^{B}
Total	24.6	24.9	16.2	15.4
WBM				
Stage I	12.6 ± 0.4	12.6 ± 0.3	12.8 ± 0.3	12.7 ± 0.3
Stage II	21.4 ± 0.4	21.9 ± 0.4	22.3 ± 0.4	22.7 ± 0.5
Stage III	31.6 ± 1.0	31.8 ± 1.0	31.9 ± 1.0	32.2 ± 1.2
Stage IV	51.3 ± 1.5^{A}	50.4 ± 1.6^{A}	44.5 ± 1.4^{B}	45.6 ± 1.6^{B}
% Increase	407.1	400	347.7	359.1

Table 2.2 Continued

CL				
Stage I	2.9 ± 0.1	2.8 ± 0.1	2.8 ± 0.1	2.9 ± 0.1
Stage II	3.7 ± 0.1	3.8 ± 0.1	3.8 ± 0.1	4.0 ± 0.1
Stage III	4.6 ± 0.1	4.7 ± 0.1	4.7 ± 0.1	4.8 ± 0.1
Stage IV	5.4 ± 0.1^{A}	5.2 ± 0.1^{A}	4.8 ± 0.1^{B}	4.9 ± 0.1^{B}
% Increase	186.2	185.7	171.4	170.0

Table 2.3 The organic content of Stage IV *H. gammarus* reared under elevated temperature and pCO_2 (Means ± S.E., d.f = 3, N = 24): Ash content (Ash - % DM), total carbon, hydrogen, and nitrogen (TCHN - % DM), carbon (C - % DM), hydrogen (H - % DM). Superscript capital letters indicate significant differences between treatments.

	17 °C		21 °C	
	420 µatm	1,100 µatm	420 µatm	1,100 µatm
Ash	59.58 ± 1.20^{A}	62.43 ± 1.27^{B}	60.28 ± 1.38^{A}	62.58 ± 0.42^{B}
TCHN	40.42 ± 1.20	37.57 ± 1.27	39.72 ± 1.38	37.42 ± 0.28
C	29.38 ± 0.87	27.38 ± 0.90	28.74 ± 0.92	27.45 ± 0.25
H	4.39 ± 0.12	4.13 ± 0.20	4.33± 0.16	4.18 ± 0.05

2.4 Discussion

2.4.1 Overview

Homarus gammarus larval development is sensitive to elevated temperature and pCO_2 levels predicted to occur by the end of this century, yet these drivers do not exert a synergistic or additive effect. The developmental sensitivity to elevated temperature and pCO_2 appears highly stage specific, with the metabolic effects of elevated temperature varying between positive at Stages II and III and negative at Stages I and IV, and the organic content effects of elevated pCO_2 confined to Stage IV and survival effects to Stage II. Changes in the physiological developmental trajectory of larval *H. gammarus* drive changes in life history traits, as the sensitivity of Stage I larvae to elevated temperature precedes a reduction in survival to Stage II, while increases in metabolic performance with elevated temperature in Stages II and III precedes improvements in survival to Stages III and IV. Finally, the developmental patterns reported here highlight the complexity of developmental responses of *H. gammarus* to climate change drivers, indicating that future global change scenarios may have important consequences for species of marine organisms which possess similarly complex life cycles to that characteristic of *H. gammarus.* Below I discuss my findings on the changes in developmental trajectory of larvae of *H. gammarus* exposed to future climate change scenarios separately for temperature and pCO_2, while finally considering the possible ecological and economic implications of the potential effects of ocean warming and ocean acidification on larval development.

2.4.2 Thermal sensitivity of lobster larvae

Elevated temperature has major effects on the survivorship of *H. gammarus*, as survival to Stage IV of larval decapods tends to increase with increased temperature until a critical point where, thereafter, there is a decrease in survival with increasing temperature (Sastry and McCarthy 1973, Johns 1981b, Mackenzie 1988). However, despite this overall increase in survival to Stage IV due to elevated temperature, there are complex stage dependant responses in terms of survival and metabolic rates which need to be considered for an accurate image of the effects of elevated temperature on larval development. Individual larval stages of *H. gammarus* show differing sensitivities to elevated temperature with regards to metabolism and survival, which can be translated as ontogenetic shifts in optimum temperature between stages. The metabolic performance of Stage I individuals decreases due to elevated temperature, coupled with a decrease in survival to Stage II. However at Stages II and III there is an increase in metabolic performance with elevated temperature, resulting in a subsequently higher survival to Stages III and IV. Ontogenetic shifts in optimum temperatures between larval stages have been reported for a number of species of marine Crustacea (e.g. Costlow *et al*. 1960, 1962, 1966). The ontogenetic shifts observed for *H. gammarus* larvae in the present chapter may be explained by changes in the energetic physiology of different stages. Crustaceans living above their optimal temperature range often experience levelling off or even decreases in rates of oxygen consumption (Dehnel 1960, Sastry and McCarthy 1973, Vernberg *et al.* 1981, Anger 1987). This is shown here by Stage I larvae reared at 21 °C, as oxygen consumption is lower than at 17 °C, which indicates a decrease in their capacity to perform aerobically (Storch *et al.* 2009a, b) which has also been demonstrated in the Atlantic rock crab, *Cancer irroratus* (Say 1817) larvae (Sastry and McCarthy 1973). As larval respiration rates are not exponentially

dependant on temperature they represent an aspect of both active and maintenance metabolism due to the constant activity of larvae (Storch *et al.* 2009a, b), and so are a good indication that at 21 °C, Stage I larvae are reaching *pejus* temperatures rather than critical temperatures (Pörtner and Farrell 2008). The increase in rates of oxygen consumption due to elevated temperature in Stages II and III can represent an increase in aerobic scope, resulting in higher survival to Stages III and IV**,** thus indicating an ontogenetic shift in aerobic scope and optimum temperature during *H. gammarus* larval development (Sastry and McCarthy 1973).

Shifts in the optimal temperature and salinity conditions for maximal survival during larval development of many marine crustaceans are linked with the changing thermal and saline environments the stages are exposed to through larval export strategies (Costlow *et al.* 1960, 1962, 1966, Dawirs 1985, Anger 1991, Charmantier 1998, Agard 1999, Charmantier *et al.* 2002, Cieluch *et al.* 2004). In the wild, hatching of *H. gammarus* larvae begins in spring and early summer as sea water temperatures reach above 8 °C (Richards and Wickins 1979, Charmantier and Mounetguillaume 1992, Cobb and Wahle 1994). Stage I larvae then rapidly concentrate at the surface (Cobb and Wahle 1994), and so would potentially be exposed to temperatures between 8 °C upon hatching and up to 17 °C in surface waters during late summer (Western Channel Observatory temperature data and *pers. obs.*). Such environmental temperature shifts during larval export may be indicative of the shifting optimum temperatures between stages described in the present study. Stages I and IV in the present study exhibit reduced metabolic performance, and so aerobic scope and optimum temperature, are transitory stages between colder and warmer environments. While such changes in thermal physiology appear in concert with expected changes in temperature during such transitions, the narrower aerobic scope and lower optimum temperature makes them

more sensitive to thermal stress during these stages, therefore, will produce potential bottlenecks in larval development and recruitment.

Rates of larval development, in terms of stage duration, generally increased with increasing temperature (Johns 1981a, Mackenzie 1988, Anger 2001, Weiss *et al.* 2009b), our findings on *H. gammarus* being consistent with this pattern. In detail, total development to Stage IV occurs in 16 d at 21 °C compared to 24 d at 17 °C. Growth of larval *H. gammarus*, however, decreased with elevated temperature as Stage IV individuals were significantly smaller, both in terms of carapace length and wet body mass, with a *circa* 20 % reduction in total growth between control and elevated temperature treatments. In the closely-related American lobster, *H. americanus* (Milne-Edwards 1837), larvae reared under elevated temperature were smaller with maximum growth rates occurring at mid-range temperatures (Mackenzie 1988), similar to what is reported for other decapod crustaceans (Johns 1981a, Weiss *et al.* 2010). Temperature-resultant reductions in size have been attributed to the temperature-related decreases in development rates and moult frequencies (Templeman 1936). However as there were no significant effects of temperature on body size in Stages I, II, and III larvae, the decrease in body mass due to elevated temperature appears confined to Stage IV. This decrease can therefore be attributed to the sensitivity of this stage to elevated temperature. Once more, changes in metabolic performance appear to inform changes in life history responses. Wet body mass and carapace length are limited to and defined by the post-moult hardening phase in marine Crustacea (Anger 2001). Stage-specific temperature-related changes in growth can be attributed to energetic demands of that developmental stage. In this case, megalopa *H. gammarus* larvae did not exhibit a significant metabolic response to elevated temperature, likely due to a decrease in metabolic performance with elevated temperature and therefore aerobic scope (Storch *et*

al. 2009a, b). This indicates another example of a shift in optimal temperature conditions along *H .gammarus* developmental trajectory: here specifically between Stages III and IV. Shifts in optimal temperature conditions may be attributed to the redirection of energy to the development of physiological milestones (Agard 1999), such as the development of oxyregulatory and osmoregulatory capabilities and structures which occurs at the transition from Stage III to Stage IV larval Norway lobster, *Nephrops norvegicus* (Linnaeus 1758) and *H. americanus* (Charmantier *et al.* 1988, 2001, Spicer and Eriksson 2003) or respiratory regulation which accompanies the same transition in *N. norvegicus* (Spicer and Eriksson 2003). Such transitions would therefore divert energy from processes such as growth, and when combined with decreased aerobic performance at elevated temperature will result in smaller megalopa due to high energetic demands not being met by energy supply as part of the energetic trade off experienced by ectotherms at high temperature (Atkinson 1995).

2.4.3 Effect of elevated pCO_2 on larval metabolism and condition

Temperature appears to have the dominant effect on development, however the larval stages exhibiting reduced metabolic performance under elevated temperature mentioned above are also sensitive to elevated pCO_2, as these stages are living close to or beyond *pejus* temperatures (*sensu* Pörtner and Farrell 2008, Storch *et al*. 2009a, b) which become narrower due to elevated pCO_2 (Metzger *et al.* 2007, Pörtner and Farrell 2008). Elevated pCO_2 affected survival at Stage II and condition, in terms of dry mass and CHN ratios, of Stage IV animals which will be discussed here. The 10 % lower survival to Stage II under elevated pCO_2 at control temperature (17 °C) suggests that Stage I larvae are sensitive to elevated pCO_2 at lower temperature. This sensitivity may not be apparent at high temperature due to the high temperature-dependent mortality during

this transition between Stages I and II. Stage IV individuals are sensitive to changes in pCO_2 in terms of metabolism and condition, as suggested by the apparent proximity to *pejus* temperatures that Stage IV larvae at 21 °C experience due to the lack of temperature-related increase in oxygen consumption (Anger 1987, Storch *et al.* 2009a, b) and temperature dependant reductions in body size and mass. Elevated oxygen consumption due to elevated pCO_2 suggests increased energetic demands, which occur with a net increase in dry mass and, as % C remains stable, the increase in total C content. This appears to be a positive investment into growth under elevated pCO_2, however the ratios of organic material, and therefore the condition of larvae, are affected by elevated pCO_2 with a significant decrease in % N causing an increase in the C:N ratio. This suggests a higher level of protein turnover which can be associated with living in a stressful environment (Anger 2001). Despite an increase in net growth, total organic content as a percentage of dry mass decreases with an increase in ash content under elevated pCO_2 conditions. These results contradict those of Arnold *et al.* (2009) who reported a decrease in carapace dry mass and Ca^{2+} content of Stage IV *H. gammarus*, and Walther *et al.* (2010) who report a trend for decreasing dry mass and C:N ratios at the megalopa stage of the great spider crab, *Hyas araneus* (L.). The decrease in carapace dry mass and Ca^{2+} content reported by Arnold *et al*. (2009) compared with the increase in total animal dry mass reported here may be due to a change in resource partitioning of *H. gammarus* when exposed to elevated pCO_2, resulting in reduced carapace mineralisation due to investment into other structures and functions which may regulate the physiological implications of elevated pCO_2. When comparing the positive investment to growth expressed by *H. gammarus* with the negative responses of dry mass and C:N ratios of *H. araneus* (Walther *et al.* 2010) I can highlight the complexity of larval responses to global change, as Walther *et al.* (2010)

reported pCO_2 effects only for one of the two populations, and significant results differing between temperature treatments. Alterations to larval C:N ratios may have profound ecological consequences as the condition of settling megalopa larvae impacts the survival, growth, and performance of juvenile stages (Jarrett and Pechenik 1997, Pechenik *et al.* 2002, Jarrett 2003, Gimenez *et al.* 2004, Nasrolahi *et al.* 2012, Pansch *et al.* 2012a). Furthermore, altered C:N ratios may also have implications for trophic interactions, as changes in protein content compared to carbon will alter the nutritional value of larvae for predators.

2.4.4 Conclusions

Here I demonstrate the complicated responses of larval development and physiology to elevated temperature and pCO_2. Pörtner and Farrell (2008) propose that fish eggs and early larval stages have the narrowest thermal window and will therefore be more susceptible to global change. Walther *et al.* (2010) expand on this by stating that the megalopa of *Hyas araneus* had the narrowest thermal window and so is where the major early life stage bottleneck occurs. As in Walther *et al.* (2010), the megalopa (Stage IV) of *H. gammarus* in the present study also represents a bottleneck in its thermal window. However, Stage I larvae similarly appear to have a narrow aerobic thermal window, and thus represent another potential developmental bottleneck under elevated temperature and pCO_2. In the life cycle of *H. gammarus*, Stage I represents a major transition from benthic to pelagic and Stage IV a major transition the opposite way, from pelagic to benthic. In the case of Stage IV, in particular, the transition is accompanied by morphological, behavioural, and physiological metamorphosis (Cobb and Wahle 1994) large changes in physiology such as the development of adult-type abilities to oxy-regulate and osmoregulate (Charmantier *et al.* 1988, 2001, Spicer and Eriksson 2003).

The ecological implications of such elevated temperature and pCO_2 effects throughout larval development, particularly the transition phases (Stage I and IV) may indicate that environmental drivers may impair particularly the function and life history of these more sensitive life stages, disrupting fundamental ecological processes facilitating the benthic-pelagic coupling of marine ecosystem.

These results should also be considered within an economic context, *H. gammarus* being an important species in numerous regional fisheries. The temperature and pCO_2-related mortalities at Stage I and Stage II will result in a narrowing of the geographic range of this population, possibly constraining early larval dispersal. The temperature-related decrease in megalopa body size could also be crucial, as demographic processes of lobster stocks and populations are body size dependant (Wahle 1992, Wahle and Steneck 1992, Cobb and Wahle 1994, Wahle and Fogarty 2006) , and along with timing of settlement of megalopa, can have a large effect on the subsequent size, condition, and survival of juveniles (Phillips *et al.* 2006) with larger settlement size and earlier transition to the benthos leading to a greater survival advantage (Wilbur 1980). This is coupled with the indications that Stage IV larvae are under extreme energetic stress due to a narrowing of their aerobic window under global change scenarios which drives such changes in body mass and highlights the potential for carry over effects of climate change between life stages. Larval growth, survival, and development, are central to population dynamics, ecological function, and essential for a sustainable management (Wahle and Fogarty 2006), and I have demonstrated here that the observed changes in life history traits due to elevated temperature and pCO_2 can be explained by the ontogeny of physiological functions.

Chapter 3

The effects of elevated temperature and pCO_2 on aspects of larval morphometric development and carapace mineralisation

3.1 Introduction

The variability of morphometric development of decapod crustacean larvae, in terms of the size increments of various body compartments and appendages between stages, may be an ecological strategy to deal with changing environmental conditions (Anger 2001), while the mineralisation of crustacean larvae can be seen as critical to larval and post larval function as larval condition can impact post larval success (Svensson *et al.* 2004, Phillips *et al.* 2006, Wahle and Fogarty 2006). However, the effects of ocean warming and OA on the morphometric development and mineralisation of marine crustacean larvae are poorly studied.

Temperature is the most important abiotic factor affecting larval growth (e.g. Anger 1998, 2001). Morphometric aspects of crustacean larvae, such as body size, carapace length, and abdomen length, decrease with increasing temperature (Shirley *et al.* 1987, Weiss *et al.* 2010) while extreme temperatures impact morphogenesis in the brown shrimps, *Crangon crangon* (Linnaeus 1758) and *C. allmanni* (Kinahan 1860) (Criales and Anger 1986). Elevated pCO_2, on the other hand, has been shown to have no impact on the morphometric development, in terms of carapace length, on European lobster, *Homarus gammarus* (Linnaeus 1758) larvae (Arnold *et al.* 2009), while body length increases in the king crab, *Paralithodes camtschaticus* (Tilesius 1815) (Long *et al.* 2013). These are the only studies to date on crustacean larval morphometrics in relation to elevated pCO_2. The morphometric development of bivalve and echinoderm larvae,

however, have been shown to be sensitive to elevated pCO_2, with effects manifested as morphometric deformities and shell abnormalities (Kurihara and Shirayama 2004, Kurihara *et al*. 2007, 2008a, Dupont *et al*. 2008, Clark *et al*. 2009, Gazeau *et al*. 2010, O'Donnell *et al*. 2010). Further investigation into the morphological development of crustacean larvae is needed to determine the sensitivity of this aspect of development to elevated pCO_2.

With regards to mineralisation, elevated temperature stimulates calcification in barnacles (Clavier *et al.* 2009, Pansch *et al.* 2012b), crayfish (Lahti 1988), ostracods (Roca and Wansard 1997, Mezquita *et al.* 1999), and decapod larvae (Walther *et al.* 2011). However, the stimulation of calcification in marine invertebrates is defined by threshold temperature levels, beyond which calcification is compromised (Roca and Wansard 1997, Howe and Marshall 2002) possibly due to temperature dependant enzymatic transport processes of calcification minerals (Walther *et al.* 2011). Mineralisation during larval development of marine Crustacea is sensitive to elevated pCO_2 (Arnold *et al.* 2009, Walther *et al.* 2011), exposure to which resulted in a decrease in carapace Ca^{2+} mineralization at the megalopa stage of development in both *H. gammarus* (Arnold *et al.* 2009) and the great spider crab, *Hyas araneus* (Linnaeus 1758) (Walther *et al.* 2011). However in *P. camtschaticus* there was no effect of elevated pCO_2 on larval mineralisation (Long *et al.* 2013). Ca^{2+}:Mg^{2+} ratios increased in megalopa *H. gammarus* (Arnold *et al.* 2009) which may be detrimental to larval survival as some of the World's surface oceans are predicted to become under saturated with aragonite by the year 2100 due to OA related changes in oceanic carbonate chemistry (Feely *et al.* 2004, Orr *et al.* 2005) with periodic up-welling of under-saturated water already impacting coastal areas (Feely *et al.* 2004). We do not know how decreases in calcification will impact later juvenile stages; however larval

condition, body size, and recruitment success are key aspects of population growth (Wahle and Steneck 1991, Svensson *et al*. 2004).

The above mentioned studies do show that the larval development of marine crustaceans, in terms of morphometric aspects, is sensitive to elevated temperature while mineralisation is sensitive to temperature and sensitive to elevated pCO_2. This is despite crustaceans being less sensitive to OA than many other marine taxa (Kroeker *et al.* 2010, 2013). Alongside the individual effects of elevated temperature and pCO_2, there is also evidence for interactive effects of elevated temperature and pCO_2 on, for example, calcium incorporation into larval crustacean exoskeletons (Walther *et al*. 2011). However, despite the above mentioned sensitivity of crustacean larvae, in terms of morphological development and carapace mineralisation to elevated temperature and pCO_2, our knowledge is limited.

Chapter 2 investigated the growth, survival, condition, and metabolism of larvae *H. gammarus*, indicating changes in thermal sensitivity and aerobic scope between stages driving stage specific sensitivities to elevated temperature and pCO_2. The present chapter aimed to expand upon this and characterise the sensitivity of morphometric aspects of development and carapace mineralisation of *H. gammarus* larvae to elevated temperature and pCO_2. In addition to the body mass changes observed in Chapter 2, this will provide a detailed view of the growth responses of larval *H. gammarus* to elevated temperature and pCO_2. The morphometric development of larvae was defined as total body length, carapace length, abdomen length, chelae length, and rostrum length to determine the effect of elevated temperature and pCO_2 on various aspects of body size. Carapace mineralisation, in terms of Ca^{2+}, Mg^{2+}, Sr^{2+}, Mn^{2+}, Na^{+}, K^{+}, and P^{+} incorporation, was also assessed to determine calcification investment and carapace integrity throughout larval development. This will provide further information on the

potential physiological costs associated with the ontogeny of physiological function and sensitivities during larval development under elevated temperature and pCO_2 as demonstrated in Chapter 2.

Homarus gammarus larvae were reared from hatching under normocapnic (420 µatm pCO_2, pH 8.1) and hypercapnic (1,100 µatm pCO_2, pH 7.7) conditions associated with predictions of OA for the year 2100 (Caldeira and Wickett 2003, 2005) at current seasonal temperature (17 °C) and + 4 °C (21 °C) associated with ocean warming predictions for the year 2100 (Sokolov *et al.* 2009).

3.2 Materials and Methods

3.2.1 Animal husbandry and experimental exposure

Individuals used for the present chapter were the same as those used in the experiments described in Chapter 2. In brief, *H. gammarus* larvae (N = 4,320) were collected within 6 h of hatching from ovigerous females at the National Lobster Hatchery, Padstow, UK. Stock conditions are reported in Section 2.2.1.

The experimental system used is displayed in Figures 2.1 and 2.2 and explained in Section 2.2.2. Briefly, the system consisted of 36 larval rearing cones (N = 120 individuals *per* cone) nested within six aquaria (N = 6 cones *per* tank). Each aquarium was allocated to one of two experimental temperatures, with water inflow into the respective cones controlled at the desired temperature using chiller units with heating elements (SeaChill TR10, Teco S.r.l., Ravenna, Italy). Additional temperature controlled sea water flowed directly into the aquaria so that they acted like a water bath. Temperature treatments were chosen as 17 °C to represent current seasonal average

temperatures, and 21 °C to represent the predicted increase of + 4 °C by the year 2100 due to ocean warming (Sokolov *et al*. 2009).

The six cones in each aquarium were randomly designated to one of two pCO_2 levels (three cones designated as control pCO_2 and three cones designated as elevated pCO_2), as in Section 2.2.2. Control pCO_2 levels (420 µatm pCO_2, pH 8.1) were achieved by bubbling untreated atmospheric air into the water in the cones. Elevated pCO_2 levels (1,100 µatm, pH 7.7) were chosen to represent future predictions of Ocean Acidification (Caldeira and Wickett 2003, 2005) and were achieved by bubbling atmospheric air mixed with pure CO_2 gas into the acidified cones as described in Findlay *et al.* (2008). For details of water chemistry monitoring parameters throughout the exposure period, refer to Section 2.3.2 and Table 2.1.

3.2.2 Determination of morphometric traits

Throughout the exposure period, inter-moult larvae of each developmental stage were removed from the experimental set up (N = 18 *per* treatment), carefully dabbed dry with tissue paper and photographed using a digital camera (Powershot A710 IS, Canon, Reigate, UK) in order to determine a number of morphometric measurements (see below). Larvae were laid flat on their right hand side (Fig. 3.1) as this was the most convenient for the analysis of photographs for morphometric traits. The morphometric traits measured are highlighted in Figure 3.1 and included total body length (TL), carapace length (CL), abdomen length (AL), chelae length (Chel), and rostrum length (RL). Individuals were also inspected for abnormalities and damage. Individuals were then rinsed with fresh ultra-pure water, dabbed dry with tissue paper, and frozen (T = -20 °C) for future determination of carapace mineralisation.

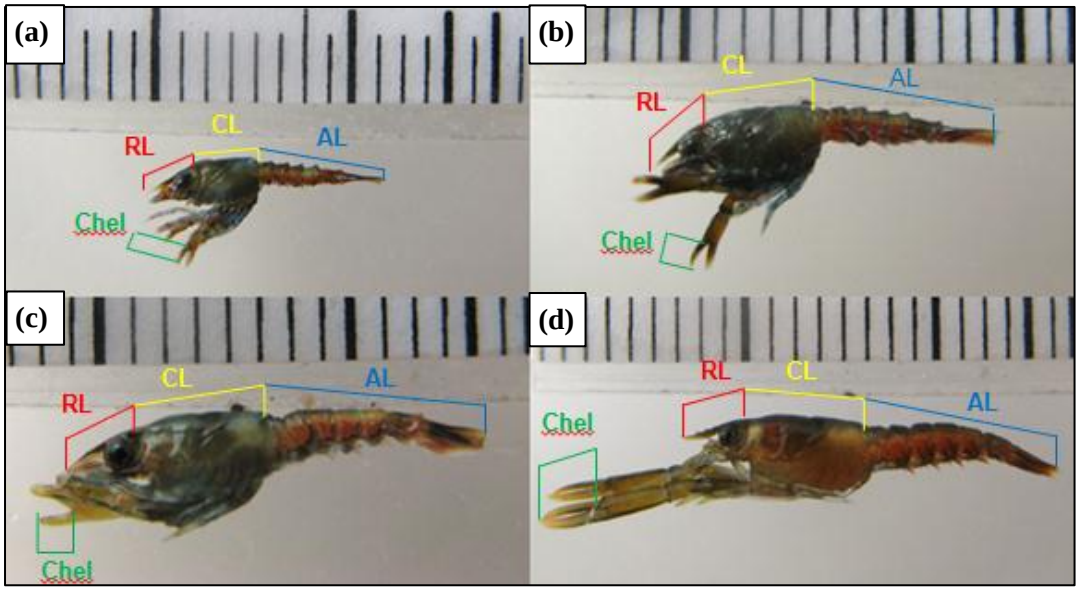

Figure 3.1 Representative *Homarus gammarus* larvae indicating morphometric measurements: (a) Stage I, (b) Stage II, (c) Stage III, and (d) Stage IV. Morphometric traits measured were carapace length (CL – yellow), abdomen length (AL - blue), rostrum length (RL – red) and chelae length (Chel – green). Scale = 1 mm.

3.2.3 Determination of carapace mineralisation

The carapace of frozen individuals was carefully removed using fine forceps and cleaned of all tissue. Carapaces were then weighed using a high precision balance (AT201 Mettler-Toledo Ltd, Leicester, UK, d = 0.01 mg), before being freeze dried (Modulyo, Thermo Electron Corp. UK) at -50 °C for 24 h. Freeze dried carapaces were then weighed once more using a high precision balance (AT201 Mettler-Toledo Ltd, Leicester, UK, d = 0.01 mg), before being digested in 2 mL nitric acid (79 % concentration, trace analysis grade) in a microwave digestion unit (MarsXpress, CEM Corp, Matthews, USA). Digests were then diluted to 10 mL with ultra-pure water and analysed for [Ca^{2+}], [Mg^{2+}], [Sr^{2+}], [Mn^{2+}], [Na^{+}], [K^{+}], and [P^{+}] using ICP-Optical Emission Spectrometer (Varian 725-ES, Agilent Technologies Inc, Santa Clara, USA.

Detection limits for Ca^{2+}, Mg^{2+}, K^{+}, P^{+} = 10 µg L^{-1}, Na^{+} = 40 µg L^{-1}, and Mn^{2+}, Sr^{2+} = 0.2 µg L^{-1}). Carapace mineral content was expressed as µmol g^{-1} (carapace dry mass).

3.2.4 Statistical analysis

All data were initially analysed for normality of distribution, using a Kolmogorov-Smirnov Test, and homogeneity, using a Levene's Test of Equality of Error. All data passed the assumptions of the normality and homogeneity tests. Data were then analysed using two-way ANOVA to test for the effects of pCO_2, temperature, and pCO_2 * temperature, with the term 'tank' as a random factor nested within pCO_2 and temperature. As 'tank' had no significant effect on any of the traits tested it was removed from the analysis.

3.3 Results

3.3.1 Larval morphometric traits

Traits of the morphometric development of *H. gammarus* larvae reared under elevated temperature and pCO_2 are presented in Table 3.1, while key morphometric aspects of Stage IV *H. gammarus* are displayed in Figure 3.2. There were neither significant interactions between elevated temperature and pCO_2 on any morphometric traits at any stage of larval development, nor any effects of elevated pCO_2 alone (Table 3.1). There was a significant negative effect of elevated temperature on total length ($F_{1, 57}$ = 15.143, p < 0.001, Fig. 3.2), carapace length (CL, $F_{1, 57}$ = 22.391, p < 0.001, Fig 3.2) and abdomen length (AL, $F_{1, 57}$ = 8.658, p = 0.005, Fig 3.2) of Stage IV individuals.

There were no visible abnormalities or damage attributable to elevated pCO_2 and temperature.

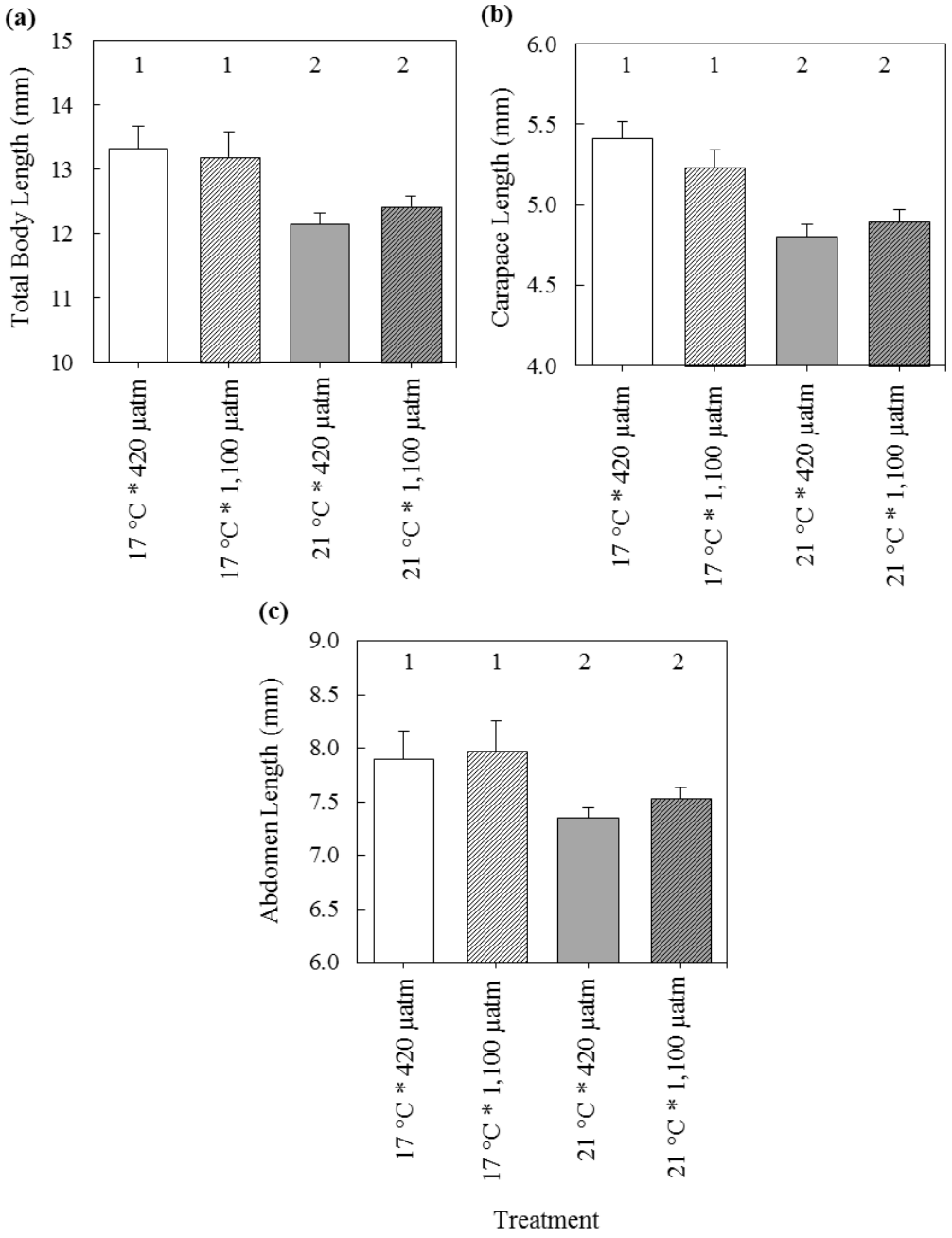

Figure 3.2 Key morphometric aspects of Stage IV larvae *H. gammarus* reared under elevated temperature and pCO_2 (Means ± S.E., d.f= 3, N = 57): (a) Total body length (mm), (b) carapace length (mm), and (c) abdomen length (mm). White bars indicate 17 °C, grey bars indicate 21 °C. Clear bars indicate 420 µatm pCO_2 conditions; striped bars indicate 1,100 µatm pCO_2 conditions. Capital letters indicate significant differences between treatments.

3.3.2 Larval carapace mineralisation

Concentrations of ions associated with the carapace of inter-moult *H. gammarus* throughout larval development under elevated temperature and pCO_2 are displayed in Table 3.2, while carapace [Ca^{2+}] and [Mg^{2+}] of Stage IV individuals are displayed in Figure 3.3. There were significant interactions between elevated temperature and pCO_2 on Stage III carapace [K^+] ($F_{1\ 35}$ = 5.342, p = 0.027, Table 3.2) and on Stage IV carapace [Mg^{2+}] ($F_{1,\ 19}$ = 9.906, p = 0.006, Fig. 3.3, Table 3.2). There were no further significant interactions between elevated temperature and pCO_2 on any other aspect of carapace mineral content at any stage of larval development. There was a significant decrease of carapace [K^+] in Stage I individuals due to elevated temperature ($F_{1,\ 35}$ = 4.663, p = 0.038), but no further effects of elevated temperature or pCO_2 individually on the carapace mineral content at any stage of larval development.

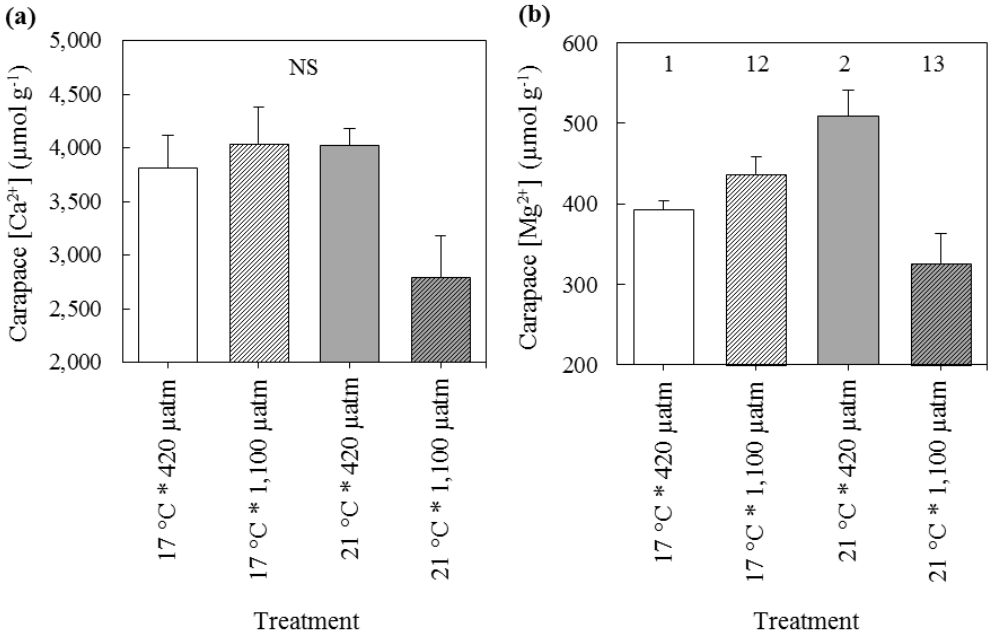

Figure 3.3 Carapace mineral content of Stage IV larvae *H. gammarus* reared under elevated temperature and pCO_2 (Means ± S.E., d.f = 3, N = 19): (a) Carapace [Ca^{2+}] (µmol g^{-1}) and (b) carapace [Mg^{2+}] (µmol g^{-1}). White bars indicate 17 °C, grey bars indicate 21 °C. Clear bars indicate 420 µatm pCO_2; striped bars indicate 1,100 µatm pCO_2. Capital letters indicate significant differences between treatments.

Table 3.1 Morphometric aspects of larval H. gammarus reared under elevated temperature and pCO_2 (Means ± S.E., d.f= 3, N = 57): Total length (TL - mm), carapace length (CL - mm), abdomen length (AL - mm), chelae length (Chel - mm), and rostrum length (RL - mm). Superscript capital letters indicate significant differences between treatments.

	17 °C		21 °C	
	420 µatm	1,100 µatm	420 µatm	1,100 µatm
TL				
Stage I	8.05 ± 0.10	7.99 ± 0.11	7.95 ± 0.14	8.25 ± 0.15
Stage II	9.84 ± 0.12	9.97 ± 0.14	10.18 ± 0.10	10.43 ± 0.09
Stage III	11.36 ± 0.16	11.61 ± 0.20	11.30 ± 0.13	11.60 ± 0.18
Stage IV	13.31 ± 0.36^{A}	13.20 ± 0.39^{A}	12.15 ± 0.16^{B}	12.42 ± 0.16^{B}
CL				
Stage I	2.86 ± 0.05	2.83 ± 0.05	2.82 ± 0.06	2.88 ± 0.06
Stage II	3.73 ± 0.45	3.82 ± 0.07	3.82 ± 0.03	4.04 ± 0.08
Stage III	4.62 ± 0.07	4.68 ± 0.09	4.73 ± 0.08	4.83 ± 0.08
Stage IV	5.41 ± 0.11^{A}	5.23 ± 0.11^{A}	4.80 ± 0.08^{B}	4.90 ± 0.07^{B}
AL				
Stage I	5.19 ± 0.08	5.16 ± 0.08	5.13 ± 0.09	5.37 ± 0.10
Stage II	6.11 ± 0.11	6.15 ± 0.10	6.37 ± 0.09	6.38 ± 0.08
Stage III	6.73 ± 0.11	6.92 ± 0.14	6.57 ± 0.07	6.77 ± 0.11
Stage IV	7.90 ± 0.26^{A}	7.97 ± 0.28^{A}	7.34 ± 0.10^{B}	7.52 ± 0.11^{B}

Table 3.1 Continued

Chel				
Stage I	0.67 ± 0.02	0.68 ± 0.02	0.71 ± 0.02	0.68 ± 0.03
Stage II	0.80 ± 0.02	0.80 ± 0.05	0.83 ± 0.02	0.83 ± 0.03
Stage III	1.27 ± 0.05	1.42 ± 0.05	1.30 ± 0.09	1.27 ± 0.06
Stage IV	2.15 ± 0.13	2.09 ± 0.11	2.00 ± 0.07	2.04 ± 0.06
RL				
Stage I	1.53 ± 0.06	1.60 ± 0.07	1.58 ± 0.05	1.60 ± 0.06
Stage II	1.79 ± 0.09	1.86 ± 0.08	1.91 ± 0.09	1.77 ± 0.05
Stage III	1.73 ± 0.09	1.81 ± 0.09	1.90 ± 0.08	1.94 ± 0.09
Stage IV	2.23 ± 0.10	2.19 ± 0.10	1.94 ± 0.09	2.06 ± 0.08

Table 3.2 Carapace mineralisation of larval *H. gammarus* reared under elevated temperature and pCO_2 (Mean ± S.E. ., d.f = 3, N = 18): Carapace concentrations of calcium ($[Ca^{2+}]$ - µmol g^{-1}), magnesium ($[Mg^{2+}]$ - µmol g^{-1}), Strontium ($[Sr^{2+}]$ µmol g^{-1}), manganese ($[Mn^{2+}]$ - µmol g^{-1}), sodium ($[Na^{+}]$ - µmol g^{-1}), potassium ($[K^{+}]$ - µmol g^{-1}), and phosphorus ($[P^{+}]$ - µmol g^{-1}). Superscript capital letters indicate significant differences between treatments.

	17 °C		21 °C	
	420 µatm	1,100 µatm	420 µatm	1,100 µatm
$[Ca^{2+}]$				
Stage I	1509 ± 77	1541 ± 90	1649 ± 149	1762 ± 170
Stage II	2618 ± 148	2509 ± 153	2266 ± 67	2625 ± 130
Stage III	3101 ± 116	3353 ± 87	3109 ± 279	3128 ± 161
Stage IV	3810 ± 311	4040 ± 343	4026 ± 158	2792 ± 393
$[Mg^{2+}]$				
Stage I	256 ± 8	257 ± 12	274 ± 17	293 ± 24
Stage II	368 ± 16	346 ± 19	341 ± 11	377 ± 13
Stage III	398 ± 17	421 ± 13	409 ± 45	421 ± 20
Stage IV	393 ± 11^{A}	437 ± 21^{AB}	509 ± 32^{AB}	326 ± 37^{AC}
$[Sr^{2+}]$				
Stage I	9.19 ± 0.49	9.25 ± 0.54	10.08 ± 0.77	10.75 ± 1.05
Stage II	16.24 ± 0.93	15.49 ± 0.91	13.48 ± 0.62	15.61 ± 0.70
Stage III	20.19 ± 0.82	21.69 ± 0.58	18.90 ± 2.46	19.83 ± 1.14
Stage IV	23.58 ± 1.87	24.68 ± 2.31	26.65 ± 1.38	20.47 ± 2.51

Table 3.2 Continued

$[Mn^{2+}]$				
Stage I	0.275 ± 0.25	0.254 ± 0.029	0.224± 0.023	0.273 ± 0.034
Stage II	0.161 ± 0.019	0.170 ± 0.013	0.103 ± 0.010	0.149 ± 0.021
Stage III	0.151 ± 0.019	0.141 ± 0.020	0.207 ± 0.035	0.140 ± 0.018
Stage IV	0.182 ± 0.062	0.107 ± 0.028	0.206 ± 0.030	0.289 ± 0.075
$[Na^{+}]$				
Stage I	1202 ± 62	1237 ± 92	1133 ± 66	1081 ± 78
Stage II	1174 ± 86	1008 ± 63	1113 ± 71	1093 ± 105
Stage III	284 ± 82	225 ± 47	436 ± 182	572 ± 133
Stage IV	161 ± 38	565 ± 180	482 ± 118	433 ± 62
$[K^{+}]$				
Stage I	289 ± 21^{A}	298 ± 20^{A}	267 ± 19^{B}	237 ± 16^{B}
Stage II	186 ± 12	169 ± 9	199 ± 8	212 ± 22
Stage III	73.5 ± 11.2^{A}	57.7 ± 6.3^{A}	65.9 ± 14.3^{A}	115.1 ± 20.4^{B}
Stage IV	44.0 ± 11.1	81.4 ± 23.3	95.8 ± 21.2	84.7 ± 14.1
$[P^{+}]$				
Stage I	310 ± 22	261 ± 10	298 ± 17	287 ± 15
Stage II	295 ± 21	276 ± 14	264 ± 22	256 ± 14
Stage III	415 ± 21	426 ± 23	359 ± 44	399 ± 41
Stage IV	295 ± 10	237 ± 18	321 ± 24	222 ± 25

3.4 Discussion

3.4.1 Overview

The present chapter investigated the effects of elevated temperature associated with ocean warming and elevated pCO_2 associated with OA on some key morphometrics and carapace mineralisation of *H. gammarus* throughout larval development. There were no significant effects of elevated temperature or pCO_2 on the morphometrics of Stage I, II, and III larvae. Stage IV, the final larval stage (megalopa), did, however, show a significant decrease in total body length, along with decreases in carapace length and abdomen length, at the elevated temperature. Rostrum length and chelae length were not affected by elevated temperature, and there was no effect of elevated pCO_2 on any aspect of Stage IV morphology.

Carapace mineralisation, in terms of the major calcification minerals Ca^{2+} and Mg^{2+} was not affected by elevated temperature or pCO_2 during larval Stages I, II, and III. At Stage IV, there was a significant interaction between elevated temperature and pCO_2 on carapace [Mg^{2+}], as [Mg^{2+}] decreased in individuals exposed to elevated pCO_2 at 21 °C. Carapace [Ca^{2+}] was not affected by temperature or pCO_2. Carapace [K^{+}] was negatively affected by temperature at Stage I, while at Stage III there was a significant interaction between temperature and pCO_2 resulting in a pCO_2-related increase but only at 21 °C. Consequently it is suggested that the morphological development of larval *H. gammarus* is sensitive to elevated temperature, while in terms of mineralisation there is an interaction between pCO_2 and temperature as larval *H. gammarus* are sensitive to elevated pCO_2 but only at elevated temperatures.

3.4.2 The effect of elevated temperature and pCO_2 on larval morphometric development

There was no significant effect of OA on any aspect of larval morphometrics throughout development, nor were there any signs of OA related abnormalities or damages, suggesting that, at least in morphometric terms, lobster larval development is relatively tolerant to OA. This is in contrast with many marine invertebrate larvae studied to date, as larval abnormalities and alterations in morphologies due to OA have been well documented in bivalve molluscs and echinoderms (Kurihara and Shirayama 2004, Kurihara *et al.* 2007, 2008a, Kurihara 2008, Bechmann *et al.* 2011). The morphological results presented here support comparable data for *H. gammarus* larvae from Arnold *et al.* (2009) where there was no effect of pCO_2 on carapace length. These are the only studies to investigate the effects of elevated pCO_2 on the morphometric development of crustacean larvae, so conclusions as to the tolerance cannot be made. Despite a tolerance to elevated pCO_2, larval morphometric development is sensitive to elevated temperature. Stage IV larvae reared at 21 °C showed significant decreases in total body length, carapace length, and abdomen length, compared to those reared at 17 °C, while the morphology of Stages I, II, and III larvae were unaffected by temperature. As previously mentioned (Sect. 2.4), Stage IV larval *H. gammarus* reared at temperatures less than 21 °C showed decreased growth in terms of Wet Body Mass. The present findings indicate that all aspects of body size decreased with elevated temperature, as has been demonstrated in other crustacean species (Shirley *et al.* 1987, Weiss *et al.* 2010). This reduction in growth may be due to the temperature- related decrease in moult duration and larval development time (Templeman 1936). However, there was no effect of temperature on body size of larval stages I, II, or III, all of which have decreased moult durations at 21 °C compared to 17 °C (Sect. 2.3), therefore the decrease in body size is restricted to Stage IV individuals. As body size of crustacean

larvae is dependent on a short post moult hardening phase (Anger 2001) any reductions in size which are stage dependant can be attributed to increased costs during the post moult period. Therefore the decreases body size seen in Stage IV individuals at 21 °C may be due to the thermal sensitivity of this stage (suggested previously in Sect. 2.5). Body size is ecologically important (Werner and Gilliam 1984) and particularly for larval stages can be important in feeding, in terms of food encounter and clearance rates (Hines 1986). Decreased body size in the present study was only found for Stage IV, the benthic-seeking, megalopa stage, and so morphological effects of temperature may have implications for future populations, as successful transitioning between larval and juvenile populations is strongly dependant on body size in many marine invertebrates (Hines 1986). Adult size is positively correlated with megalopa and first juvenile size in Brachyuran crabs (Hines 1986) while smaller megalopa are more susceptible to, for example, abiotic factors (Green *et al.* 2004) and predation (Wahle 1992). Despite the decreased body size, there was no significant effect of temperature on chelae length or rostrum length. The rostrum provides a defensive structure for the sensory organs around the head, while the chelae of marine crustaceans provides a tool for defence, attack, and environment and food manipulation, and so these structures are ecologically important. That their size remains constant despite a decrease in body size due to elevated temperature may represent investment into maintaining the integrity of certain functional aspects of morphology, potentially resulting in a change in energy investment into various processes (Findlay *et al* 2010b).

I have shown temperature-related-effects on key morphometric characteristics of Stage IV lobster larvae, and the mineralisation of these aspects may provide some insights into whether there are any functional changes to the integrity of these aspects in relation to elevated pCO_2 and temperature.

3.4.3 The effect of elevated temperature and pCO_2 on larval carapace mineralisation

In the present study, I found no effect of elevated temperature or pCO_2 on the composition of major exoskeleton constituents Ca^{2+} and Mg^{2+} throughout larval Stages I, II, and III. For Stage IV, there was no significant effect of pCO_2 or temperature on carapace [Ca^{2+}]; however there was the hint of a decrease due to exposure to elevated pCO_2 at 21 °C. There was a significant interaction between pCO_2 and temperature on Stage IV carapace [Mg^{2+}] resulting in an increase due to elevated temperature under normocapnic conditions, and a decrease due to elevated pCO_2 at 21 °C (Table 3, Fig. 3). Temperature also increased mineralisation of *H. araneus* larvae while elevated pCO_2 caused reductions in mineralisation at all temperatures (Walther *et al.* 2011). In the present study, the increase in mineralisation at 21 °C may be indicative of either no change or an increase in calcification investment despite decreases in carapace length, resulting in a more densely calcified exoskeleton. A previous study on larval *H. gammarus* mineralisation under elevated pCO_2 by Arnold *et al.* (2009) recorded a significant decrease in both carapace [Ca^{2+}] and [Mg^{2+}] at Stage IV in larvae reared at 19 °C. While there is a general consensus for decreased calcification rates of Stage IV *H. gammarus*, the findings of Arnold *et al.* (2009) contrast with those of the present study; Arnold *et al.* (2009) found decreases in [Ca^{2+}] and marginal decreases in [Mg^{2+}], while the present study found significant decreases in [Mg^{2+}] and marginal decreases in [Ca^{2+}]. However, these studies together do suggest that the mineralisation of Stage IV *H. gammarus* is sensitive to elevated pCO_2 at higher temperatures. This is in line with the predictions made in Chapter 2 that Stage IV *H. gammarus* are above their optimum temperature range at 21 °C, and possibly even 19 °C. Crustaceans larvae, therefore, appear no different to other phyla as the calcification of a range of marine larvae is compromised under elevated pCO_2 (Kurihara and Shirayama 2004, Kurihara *et al.* 2007,

Arnold *et al*. 2009, Walther *et al*. 2011) supporting the notion that the larval stages of calcifying marine invertebrates are particularly sensitive to OA (Kurihara 2008, Byrne 2011).

3.4.4 Conclusions

The results of the present study on the morphology and mineralisation of larval *H. gammarus* under elevated temperature and pCO_2 provide additional insights into the costs associated with the changes in temperature and pCO_2 sensitivities highlighted in Chapter 2. Stage IV (Megalopa) *H. gammarus* have a narrower aerobic scope compared with Stages II and III, resulting in pCO_2-related sensitivities to organic content and oxygen consumption (Chapter 2). Similarly, this crucial transitional stage between pelagic and benthic habitats, and between larval and juvenile life stages, is sensitive to elevated temperature in terms of growth in line with the decreases in growth seen in Chapter 2. It is also sensitive in terms of mineralisation to elevated temperature and pCO_2, resulting in decreased mineralisation under elevated pCO_2 at 21 °C. This supports the idea by Walther *et al*. (2010), building on that from Pörtner and Farrell (2008), that the megalopa stage of crustacean larval development represents a bottleneck due to a narrow aerobic thermal window compared to other larval stages which may be due to the changes in physiological and ecological development that occur during this transition (Charmantier et al. 1988, 2001, Spicer and Eriksson 2003).

As mentioned in Chapter 2, the economic and ecological context of these results must be considered given the importance of *H. gammarus* in regional fisheries. The temperature and pCO_2 effects on morphologies and mineralisation demonstrated in the present study provides us with further evidence of the potential demographic and recruitment effects of climate change drivers on lobster populations. The success of the

transition between pelagic larvae and benthic juveniles in lobsters is body size dependant as larger individuals have a higher survival advantage during this period while a larger settling body size also has positive carry over effects to juvenile condition and size (Wahle 1992, Wahle and Staneck 1992, Cobb and Wahle 1994, Phillips *et al.* 2006, Wahle and Fogarty 2006). Therefore the smaller body size due to elevated temperature shown in the present study may cause shifts in geographic ranges in warmer areas. This will be further impacted by the reduction in mineralisation of settling Stage IV larvae due to elevated pCO_2 at warmer temperatures.

Chapter 4

The effect of elevated temperature and pCO_2 on the survival, growth, and aspects of physiology of early benthic juveniles

4.1 Introduction

The juvenile stage of benthic marine invertebrates represents a challenging transition between larval and adult forms. Early juveniles of many marine invertebrate species are ecologically distinct from late juveniles and adults (Wahle and Steneck 1991, Gosselin 1997) and mortality in such early juvenile populations is often extremely high (Gosselin and Qian 1997). Along with an ecological distinction, there also appears to be a physiological distinction between early (post-larvae) juvenile and late juveniles. For example, many physiological aspects of crustacean biology such as osmo-regulation, ion-regulation, oxy-regulation, and haemocyanin structure and function are continuously developing throughout early juvenile development before reaching true adult function in late juveniles (Brown and Terwilliger 1992, Terwilliger and Brown 1993, Tankersley and Wieber 2000, Charmantier *et al*. 2001, Terwilliger and Dumler 2001, Terwilliger and Ryan 2001, Spicer and Eriksson 2003, Cieluch *et al*. 2004). Despite these important distinctions, juvenile development has not been considered as two distinct phases when investigating the effects of ocean warming and OA. Juveniles of marine organisms are believed to exhibit the widest aerobic scope in relation to eggs, larvae, and spawning adults, and thus to be more tolerant to environmental changes than earlier or later forms (Pörtner and Farrell 2008). This said, recent investigations of the sensitive larval development phase of marine crustaceans have shown how individual larval stages are amongst the most sensitive (Walther *et al*. 2010, 2011). Walther *et al*. (2010) proposed a modification to the model by Pörtner and Farrell (2008) to

distinguish between the changes in aerobic scope between individual larval stages as the megalopa larvae have the narrowest aerobic thermal window. In Chapters 2 and 3 this is expanded in the case of *H. gammarus* to include Stage I. A similar modification may also be relevant when considering juvenile development as two distinct phases.

The few studies focussing on juvenile stages of calcifying marine invertebrates show a range of responses to elevated pCO_2. Scope for growth and physiological energetics are reduced in juvenile bivalves (Fernandez-Reiriz *et al*. 2011). However, certain juvenile populations of what bivalves are more tolerant to OA, with no detectable effects on mortality, growth, or mineralisation (Range *et al*. 2011) and sometimes even increases in scope for growth (Fernandez-Reiriz *et al*. 2012). Similarly, juvenile barnacle species show decreased growth in relation to elevated pCO_2 as a trade of for maintaining mineralisation (Findlay *et al*. 2010b) while others show no effects (McDonald *et al*. 2009). Juvenile amphipods also show no responses to OA (Hauton *et al*. 2009) while juvenile American lobsters *Homarus americanus* (Milne-Edwards 1837) and blue crabs *Callinectes sapidus* (Rathburn 1896) exhibit increased calcification when exposed to elevated pCO_2 and associated reductions in aragonite saturation (Ries *et al.* 2009) but with no change in Mg^{2+}:Ca^{2+} ratios (Ries 2011).

Aside from OA, temperature is known to increase juvenile invertebrate growth and metabolism until threshold levels have been reached (Hartnoll 2001, Portner 2001, Talmage and Gobler 2011, Kinne 1963), yet only a few studies on juvenile development have considered the combine exposure to elevated temperature and pCO_2 and the responses are very variable. For example, juvenile molluscs are sensitive to both elevated temperature and pCO_2, but no interactions between the two factors (Lischka *et al.* 2011, Talmage and Gobler 2011) while some juvenile crustaceans are only sensitive to elevated pCO_2 not temperature (Findlay *et al.* 2010a, b) or pCO_2 at elevated

temperature (Findlay *et al.* 2010a). The ochra starfish, *Pisaster ochraceus* (Brandt 1835) shows positive growth responses under elevated pCO_2 and temperature, with a slight reduction in calcified material under elevated pCO_2 conditions (Gooding *et al*. 2009).

These few studies highlight the need for further investigation into the effects of co-occurring climate change drivers due to the large variability in juvenile responses. Furthermore, despite the range of juvenile calcifying marine organisms studied in relation to OA and warming, no studies have differentiated between early and late juveniles. This is an oversight which may account for some variability between studies. For example, although not focussed on OA, Green *et al*. (2004) showed reduced survival due to shell dissolution in aragonite under-saturated sediments in early benthic hard clam, *Mercenaria mercenaria* (Linnaeus 1758) compared to later juveniles, indicating the potential for differences in sensitivity between early and late juvenile stages under predictions of future OA and temperature conditions.

The present study investigates the sensitivity of early benthic stage juvenile *H. gammarus* (Linnaeus 1758) to elevated temperature and pCO_2 in terms of life history traits (survival and growth), carapace mineralisation, and key aspects of the underlying functional biology, i.e. oxygen consumption and food consumption, behind these traits. Early benthic juvenile *H. gammarus* were exposed for five weeks to combinations of elevated pCO_2 conditions representing OA (Caldeira and Wickett 2003, 2005) and CCS (Blackford *et al.* 2009, Kano *et al.* 2010) scenarios, at temperatures representing current winter conditions and ocean warming predictions of +3 °C (Sokolov *et al*. 2009). Rates of survival and moulting frequency were measured throughout, while growth, including organic content, and carapace mineralisation were determined after five weeks exposure. Rates of oxygen consumption, as a proxy for metabolic rate, and feeding rate, as a proxy for energy acquisition, were also measured after five weeks exposure to determine any

underlying functional effects of elevated temperature and pCO_2 on the performance of early juvenile lobsters. Early benthic juvenile (mean carapace length (± S.E.) = 7.5 ± 0.1 mm, as defined by Wahle and Steneck (1991)) *H. gammarus* were chosen due to the clear ecological distinction between early benthic lobsters and later juvenile lobsters (Wahle and Steneck 1991) and the apparent physiological distinction between these stages in metamorphic marine crustaceans studied to date. The results of this study will allow a discussion on sensitivity to climate change drivers during this specific stage of juvenile development.

4.2 Materials and methods

4.2.1 Animal collection, experimental exposure, and husbandry

Early benthic juvenile (5 month old), *H. gammarus* (N = 108) were reared at the National Lobster Hatchery, Padstow, UK from ovigerous females caught from South Cornwall during July 2011. Ovigerous females were kept in aquaria (vol. = 1,200 L) in sea water that was mechanically and biologically filtered with weekly water changes (salinity = 35, T = 19 °C, DO_2 = 8 mg L^{-1}) and sourced from the Camel estuary, Padstow, UK. Upon hatching, larvae were transferred to a batch culture system (Burton 2003) as described by Scolding et al. (2012). Briefly, larvae were reared in Kreisel-like hoppers (vol. = 90 L) containing vigorously aerated, re-circulated, mechanically and biologically filtered sea water (salinity = 35, T = 19 °C, DO_2 = 8 mg L^{-1}). Larvae were fed live brine shrimp (*Artemia salina* Linnaeus 1758), frozen copepods, and frozen krill (*Euphausia superba* Dana 1850). Upon metamorphosis to Stage IV, individuals were transferred to individual 'Orkney' pots (vol. = 100 mL, Orkney Lobster Hatchery, Orkney, UK) held in 250 L trays supplied with constantly aerated, re-circulated, mechanically and biologically filtered sea water (salinity = 35, T = 19 °C, DO_2 = 8 mg

L^{-1}) as described by Scolding et al. (2012). Juveniles were fed twice a week on small mussels (*Mytilus edulis* Linnaeus 1758).

Juveniles were transported by car to Plymouth Marine Laboratory, Plymouth, UK. During transportation, juveniles were kept in individual 'Orkney' pots (vol. = 100 mL, Salinity = 35, T = 15 °C, pH = 8.1, DO_2 = 8 mg L^{-1}) and on arrival they were haphazardly designated to one of two temperature treatments, a control temperature treatment of 10 °C representing the current seasonal temperature, and an elevated temperature treatment of 13 °C representing +3 °C associated with ocean warming (Sokolov *et al*. 2009). Lobsters were initially introduced to the system at 19 °C and water temperature was subsequently lowered by 1 °C *per* day until the designated treatment level was reached, and acclimated to their designated temperature conditions for three weeks prior to pCO_2 exposure to minimise the effect of sudden temperature changes on the proxies measured.

The experimental system consisted of six large tanks (vol. = 24 L), three of which being designated to one of the two temperatures. Each tank held 18 individual pots, each containing one lobster and individually supplied with re-circulated, mechanically filtered sea water (10 mL min^{-1}) which was gently bubbled with air. Water temperature of the entire system was set to control levels of 10 °C while water flowing into the hot designated pots was heated to 13 °C using a chiller unit with a heating element (Seachill TR10, Teco S.r.l., Ravenna, Italy). After three weeks of exposure to designated temperatures, acidification commenced with six out of the 18 pots in each tank being haphazardly designated to one of three pCO_2 treatments (six individuals *per* pCO_2 *per* tank, 18 individuals *per* temperature*pCO_2 combination). The pCO_2 levels were chosen to achieve pH levels of nominal control pH 8.1, pH 7.7 associated with -0.4 unit decrease due to OA (Caldeira and Wickett 2003, 2005), and pH 6.9 to represent Carbon

Capture and Storage (CCS) leakage scenarios (Blackford *et al*. 2009, Kano *et al*. 2010). Acidification was achieved by mixing pure CO_2 gas with atmospheric air to a suitable pCO_2 level to achieve nominal pH, which was bubbled into each pot individually. CO_2 levels in the air were measured constantly using a CO_2 gas analyser (Li-820, Li-Cor Biosciences, Lincoln, USA), and adjusted as needed. Water parameters of pH (NBS scale, 826 Mobile pH meter, Metrohm, Switzerland), temperature (using an HH802U thermo couple, Omega Engineering Inc. Stamford, USA), and salinity (using a S/Mill Hand Refractometer, Atago, Tokyo Japan) were measured daily, with water samples (vol. = 150 mL) taken every 5 d, which were poisoned with $HgCl_2^-$, and stored for future analysis of Total Alkalinity (using an As-Alk2 Titrator, Apollo SciTech Inc., Bogart, USA) and subsequent calculation of sea water pCO_2, HCO_3^-, CO_3^-, calcite saturation, and aragonite saturation values using the CO_2Sys program (Lewis and Wallace 1998) with constants provided by Mehrbach *et al*. (1973) refitted by Dickson and Millero (1987) and KSO_4 constants from Dickson (1990). Water parameters for the exposure period are reported in Table 1.

4.2.2 Determination of survival and growth

Survival and moult frequency was monitored daily, with mortalities being removed from the system immediately and the number of cases of Moult Death Syndrome (MDS) determined. MDS was identified due to mortality occurring during moulting with the carapace being half removed from the thorax, still attached at the head. At the beginning and end of the five week pCO_2 exposure period, wet body mass (WBM) was measured for each individual using a micro balance (3719MP, Sartoris, Göttingen, Germany, d = 0.001g). Growth was expressed as % change in WBM over the five week period.

Table 4.1 Water chemistry parameters throughout the exposure period (Means ± S.E., d.f = 5, N = 76): Temperature (°C), salinity, pH (NBS Scale), total alkalinity (A_T - µEq kg^{-1}), total carbon dioxide content (TCO_2 - µmol kg^{-1}), carbon dioxide partial pressure (pCO_2 - µatm), bicarbonate concentration ($[HCO_3^-]$ - µmol kg^{-1}), carbonate concentration ($[CO_3^{2-}]$ - µmol kg^{-1}), calcite saturation (Ω_{cal}) and aragonite saturation (Ω_{ara}). Superscript capital letters indicate significant differences between treatments. [1]Parameters calculated using CO_2SYS program (Lewis and Wallace 1998) with constants provided by Mehrbach *et al.* (1973) refitted by Dickson and Millero (1987).

	10 °C			13 °C		
	450 µatm	1,100 µatm	9,000 µatm	450 µatm	1,100 µatm	9,000 µatm
Temperature	9.54 ± 0.11^{A}	9.61 ± 0.11^{A}	9.64 ± 0.12^{A}	13.12 ± 0.04^{B}	13.11 ± 0.04^{B}	13.19 ± 0.06^{B}
pH	8.07 ± 0.01^{A}	7.74 ± 0.01^{B}	6.90 ± 0.02^{C}	8.05 ± 0.01^{A}	7.73 ± 0.01^{B}	6.89 ± 0.01^{C}
Salinity	33.71 ± 0.04	33.73 ± 0.04	33.72 ± 0.04	33.76 ± 0.04	33.79 ± 0.04	33.78 ± 0.04
A_T	2.32 ± 0.06	2.23 ± 0.05	2.29 ± 0.06	2.38 ± 0.06	2.32 ± 0.06	2.29 ± 0.04
$^{1}TCO_2$	2180 ± 64^{A}	2197 ± 8^{A}	2666 ± 70^{B}	2224 ± 65^{A}	2280 ± 64^{A}	2628 ± 42^{B}
$^{1}pCO_2$	497 ± 26^{A}	1086 ± 51^{B}	8773 ± 614^{C}	559 ± 35^{A}	1258 ± 134^{B}	8827 ± 225^{C}
$^{1}[HCO_3^-]$	2046 ± 61^{A}	2094 ± 79^{A}	2269 ± 54^{B}	2080 ± 63^{A}	2169 ± 61^{A}	2267 ± 38^{B}
$^{1}[CO_3^{2-}]$	111.8 ± 3.3^{A}	54.1 ± 2.2^{B}	8.4 ± 0.9^{C}	121.4 ± 2.6^{D}	60.2 ± 39^{B}	9.1 ± 0.3^{C}
$^{1}\Omega_{cal}$	2.69 ± 0.08^{A}	1.30 ± 0.05^{B}	0.20 ± 0.02^{C}	2.91 ± 0.06^{D}	1.45 ± 0.09^{B}	0.22 ± 0.01^{C}
$^{1}\Omega_{ara}$	1.70 ± 0.05^{A}	0.82 ± 0.03^{B}	0.13 ± 0.01^{C}	1.86 ± 0.04^{D}	0.92 ± 0.06^{B}	0.14 ± 0.01^{C}

4.2.3 Determination of rates of oxygen and food consumption

The rates of oxygen and food consumption of early juvenile *H. gammarus* were determined after five week exposure to elevated temperature and pCO_2. Individuals were transferred to flow-through respirometry chambers (N = 12 *per* treatment) with filtered (Ø = 2.2 µ) sea water (S = 35) set to designated treatment levels. Respiration chambers were blacked out using black plastic sheets and each chamber was equipped with a magnetic flea and placed over a magnetic stirrer plate (MS53M, Jeiotech, Seoul, Korea) to ensure even mixing of O_2. After 60 min, water flow into the chambers was stopped, and oxygen levels within the chambers allowed to decline, with the partial pressure of oxygen recorded every 15 min using an optical oxygen analyser (101, Oxysense Dallas, USA) as described in Rastrick and Whiteley (2011). Rates of oxygen consumption were expressed as µmol O_2 mg $(WBM)^{-1}$ min^{-1} S.T.P. Juveniles were not fed for 48 h prior to determination of oxygen and food consumption rates to prevent past feeding experiences impacting on the proxies measured.

Immediately after respiration trials, individuals were gently dabbed dry with tissue paper and weighed to determine WBM, before being transferred back into their exposure containers and allowed to rest for 2 h. Juvenile lobsters were then fed with pieces of pre-weighed squid, *Loligo vulgaris* (Lamark 1798) (mean wet mass (± S.E.) = 143 ± 7.4 mg) and allowed to feed for 1 h. All uneaten food after 1 h was removed, weighed, and food consumption rates calculated *per* individual as µg min^{-1} g $(WBM)^{-1}$. Individuals remained in treatment conditions for a further 48 h to allow removal of food from the gut before being removed, rinsed with ultra-pure water, dabbed dry with tissue paper, and frozen at -80 °C for subsequent determination of organic content and carapace mineralisation.

4.2.4 Determination of organic content and mineralisation

Due to the limited material available, organic content and carapace mineralisation were determined using the same individuals, with the abdomens being used for organic content and carapace for mineralisation. Organic content and mineralisation was not determined for individuals exposed to 9,000 µatm pCO_2 due to the unknown cause of shell disease and possible bacterial infection, which was later found to be shell dissolution due to high pCO_2.

The abdomen of frozen lobsters was removed, freeze dried at -50 °C for 24 h (Modulyo, Thermo Electron Corp., Cambridge, UK), and weighed for determination of dry mass (DM) using a high precision balance (AT201, Mettler-Toledo Ltd, Leicester, UK, d = 0.01 mg). Abdomens were then ground into a uniform powder using a mortar and pestle, and 2 mg subsamples were measured into tin cups using a high precision balance (AT201, Mettler-Toledo Ltd, Leicester, UK, d = 0.01 mg). Tin cups were then crushed and % Carbon (C), Hydrogen (H), and Nitrogen (N) levels of the abdomen were determined using a using an elemental micro analyser (EA1110 CHNS, Carlo Erba, Italy, modified by Elemental Analysis, Okehampton, UK) calibrated using cyclohexanone 2,4-dinitrophenylhydrazone and standardised against L-Cystine.

To determine carapace mineralisation after five weeks exposure to elevated temperature and pCO_2, whole carapace of frozen lobsters were removed, cleaned of residual tissue, freeze dried at -50 °C for 24 h (Modulyo, Thermo Electron Corp. UK), and weighed using a high precision balance (AT201 Mettler-Toledo Ltd, Leicester, UK, d = 0.01 mg). Carapaces were then dissolved using 2 mL nitric acid (79 % concentration, trace analysis grade) in a microwave digestion unit (MarsXpress, CEM Corp, Matthews, USA). Solutions were subsequently diluted to 10 mL and analysed for $[Ca^{2+}]$, $[Mg^{2+}]$,

[Sr^{2+}], [Mn^{2+}], [Na^{+}], [K^{+}], and [P^{+}] using an ICP-OES (Varian 725-ES, Agilent Technologies Inc, Santa Clara, USA. Detection limits for Ca^{2+}, Mg^{2+}, K^{+}, P^{+} = 10 µg L^{-1}, Na^{+} = 40 µg L^{-1}, and Mn^{2+}, Sr^{2+} = 0.2 µg L^{-1}).

4.2.5 Statistical analysis

All data were analysed for normality of distribution, using a Kolmogorov-Smirnov Test, and homogeneity, using a Levene's Test of Equality of Error. All data passed the assumptions of the normality and homogeneity tests. Data were analysed for treatment effects using an ANCOVA where WBM was used as a covariate. The term 'tank', nested within temperature * pCO_2, was included as a random factor in all analyses, while moulting was used as a random factor for carapace mineralisation and organic content. There was no significant effect of tank throughout, and where WBM and moult were not significant they were removed from subsequent analysis.

4.3 Results

4.3.1 Survival and growth

Survivorship of juvenile *H. gammarus* exposed to elevated pCO_2 at 10 °C and 13 °C for 5 weeks is displayed in Figure 4.1. After five weeks exposure, survival was 100 % under control pCO_2 conditions at both temperatures. At 10 °C, survival also decreased, but decreased to 83.3 and 72.2 % under 1,100 and 9,000 µatm pCO_2 respectively. At 10 °C, 22.2 % of individuals moulted, which increased to 33.3 % under control conditions at 13 °C. All control individuals which attempted to moult at both temperatures succeeded. Moult frequency increased to 61.1 % under 1,100 µatm pCO_2 at 10 °C, with 27.3 % of these resulting in MDS, and slightly increased to 27.8 % under

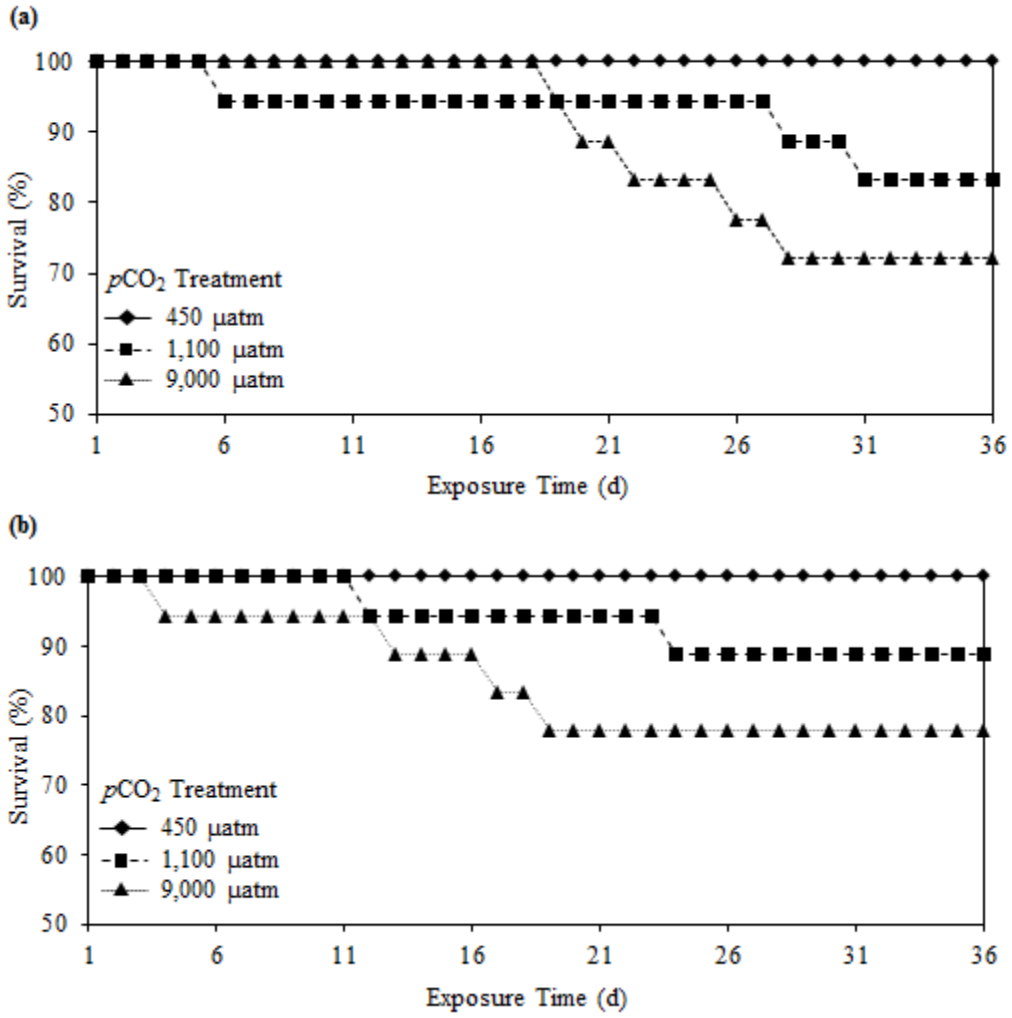

Figure 4.1 Survival of early benthic juvenile *Homarus gammarus* after five week exposure to elevated temperature and pCO_2 (Means ± S.E.): (a) 10 °C and (b) 13 °C. Diamonds with full lines indicate 450 µatm pCO_2, squares with dashed lines indicate 1,100 µatm pCO_2 and triangles with dotted lines indicate 9,000 µatm pCO_2.

9,000 µatm pCO_2, with 100 % of these resulting in MDS. At 13 °C moult frequency increased to 38.9 % under both 1,100 µatm pCO_2 and 9,000 µatm pCO_2, with 14.3 % and 57.1 % resulting in MDS respectively.

Growth, in terms of changes in WBM throughout the exposure period, is displayed in Figure 4.2. There was no significant interaction between elevated temperature and pCO_2 on growth. WBM significantly increased due to elevated temperature ($F_{1, 93} = 6.058$, p =

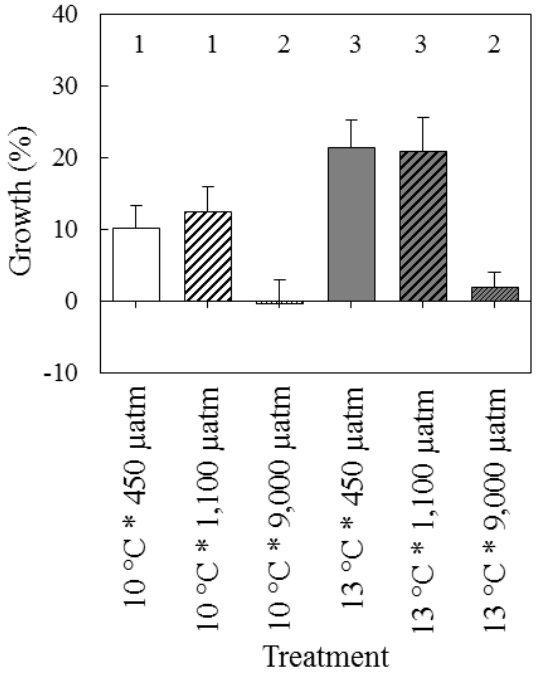

Figure 4.2 Growth status of juvenile *H. gammarus* after 5 weeks exposure to elevated temperature and pCO_2 (Means ± S.E., d.f = 2, N = 93): Growth in terms of % Wet Body Mass (WMB) gain. White bars indicate 10 °C, grey bars indicate 13 °C. Clear bars indicate 450 µatm pCO_2, thick striped bars indicate 1,100 µatm pCO_2, and thin striped bars indicate 9,000 µatm pCO_2. Numbers indicate significant differences.

0.016, Figure 4.2) and decreased due to elevated pCO_2 ($F_{2, 93}$ = 11.258, p < 0.001, Figure 4.2)

4.3.2 Organic content

Aspects of the organic content of the abdomen of juvenile *H. gammarus* after five week exposure to elevated pCO_2 at 10 °C and 13 °C is summarised in Table 4.2. There was a significant interaction between elevated temperature and pCO_2 on abdomen C:N ratio (($F_{1, 48}$ = 4.707, p = 0.015, Table 4.2, however no further significant interactions occurred on any other aspect of organic content of juvenile lobsters. There were significant effects of elevated temperature on various aspects of juvenile organic content,

Table 4.2 Organic content of early benthic juvenile *Homarus gammarus* after five week exposure to elevated temperature and pCO_2 (Means ± S.E., d.f = 1, N = 48): Dry mass (DM - mg), carbon (C - %), nitrogen (N - %), carbon:nitrogen ratio (C:N), hydrogen (H - %), total CHN (TCHN -%), and Ash (%). Superscript capital letters indicate significant differences between treatments.

	10 °C		13 °C	
	450 µatm	1,100 µatm	450 µatm	1,100 µatm
DM				
C	32.35 ± 0.29^{A}	32.89 ± 0.40^{A}	30.92 ± 0.38^{B}	31.36 ± 0.49^{B}
N	8.17 ± 0.08^{A}	8.15 ± 0.17^{A}	7.60 ± 0.10^{B}	7.99 ± 0.20^{B}
C:N	3.96	4.05	4.07	3.94
H	5.06 ± 0.03^{A}	5.08 ± 0.07^{A}	4.78 ± 0.06^{B}	4.82 ± 0.08^{B}
TCHN	45.58 ± 0.37^{A}	46.12 ± 0.62^{A}	43.31 ± 0.48^{B}	44.19 ± 0.75^{B}
Ash	54.42 ± 0.37^{A}	53.88 ± 0.62^{A}	56.69 ± 0.48^{B}	55.82 ± 0.75^{B}

but no significant effects of elevated pCO_2. In detail, total % CHN significantly decreased due to elevated temperature ($F_{1, 48} = 13.892$, $p < .001$, Table 4.3) along with a significant increase in ash content. Carbon content significantly decreased due to elevated temperature ($F_{1, 48} = 14.042$, $p < .001$) as did nitrogen content ($F_{1, 48} = 6.434$, $p = 0.015$) and hydrogen content ($F_{1, 48} = 18.347$, $p < 0.001$).

4.3.3 Carapace mineralisation

The carapace mineral content of juveniles exposed to elevated temperature and pCO_2 for five weeks is displayed in Table 4.3, while the effects of elevated temperature and pCO_2 and the effects of elevated pCO_2 and moulting on carapace [Ca^{2+}] are displayed in

Figure 4.3a and b respectively. Carapace [Ca^{2+}] content significantly decreased due to elevated pCO_2 ($F_{1, 28}$ = 5.740, p = 0.021, Fig 4.3a), and significantly increased due to moulting ($F_{2, 48}$ = 4.182, p = 0.047, Fig 4.2b).

There was a significant increase in Carapace [Mg^{2+}] due to moulting ($F_{1, 48}$ = 4.902, p = 0.032) with a significant interaction between moulting and pCO_2 ($F_{1, 48}$ = 4.879, p = 0.033). This interaction resulted in no difference between individuals that moulted and those that did not in all treatments, except high pCO_2 at 13 °C where individuals were able to incorporate more [Mg^{2+}] into their exoskeletons after moulting, while those who

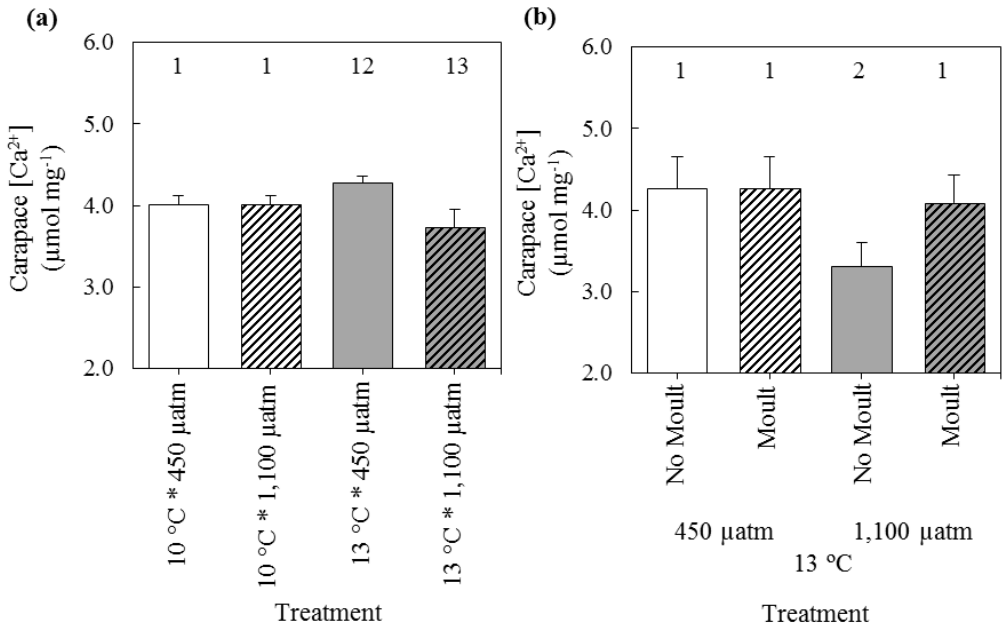

Figure 4.3 The carapace calcium content of early benthic juvenile *Homarus gammarus* after five week exposure to elevated temperature and pCO_2 (Means ± S.E., d.f = 1, N = 48): (a) The effect of elevated temperature and pCO_2 on carapace calcium concentration ([Ca^{2+}] - µmol mg^{-1}). White bars indicate 10 °C, and grey bars indicate 13. Clear bars indicate 450 µatm pCO_2, striped bars indicate 1,100 µatm pCO_2. (b) The effect of moulting and elevated pCO_2 on carapace [Ca^{2+}] in individuals exposed to 13 °C. White bars indicate 450 µatm pCO_2, and grey bars indicate 1,100 µatm pCO_2. Clear bars indicate non-moulted individuals, striped bars indicate moulted individuals. Numbers indicate significant differences.

did not moult exhibited a decrease in carapace [Mg^{2+}]. Carapace [Sr^{2+}] significantly increased due to moulting ($F_{1, 48}$ = 4.257, p = 0.045) with a significant interaction between pCO_2 and temperature ($F_{2, 48}$ = 4,429, p = 0.042). Individuals which moulted under elevated pCO_2 at 13 °C had no difference in [Sr^{2+}] to those in control treatments. There was no significant effect of elevated temperature, pCO_2, or their interaction on carapace [Mn^{2+}], [Na^{+}], [K^{+}], or [P^{+}].

Table 4.3 Carapace mineral content of early benthic juvenile *H. gammarus* after five week exposure to elevated temperature and pCO_2 (Means ± S.E., d.f = 1, N = 48): Concentration of carapace calcium ([Ca^{2+}] - µmol g^{-1}), magnesium ([Mg^{2+}] - µmol g^{-1}), strontium ([Sr^{2+}] - µmol g^{-1}), manganese ([Mn^{2+}] - µmol g^{-1}), sodium ([Na^{+}] - µmol g^{-1}), potassium ([K^{+}] - µmol g^{-1}), and phosphorus ([P^{+}] - µmol g^{-1}). All mineral contents calculated as *per* g of carapace dry mass. Superscript capital letters indicate significant differences between treatments.

	10 °C		13 °C	
	420 µatm	1,100 µatm	420 µatm	1,100 µatm
[Ca^{2+}]	4007 ± 123^{A}	4009 ± 106^{A}	4268 ± 89^{AB}	3733 ± 225^{AC}
[Mg^{2+}]	584 ± 22	599 ± 23	663 ± 16	623 ± 44
[Sr^{2+}]	31.9 ± 0.6^{A}	32.0 ± 0.9^{A}	34.1 ± 0.7^{AB}	29.4 ± 1.7^{AC}
[Mn^{2+}]	0.543 ± 0.067	0.399 ± 0.028	0.397 ± 0.021	0.336 ± 0.021
[Na^{+}]	248 ± 9	229 ± 9	251 ± 12	237 ± 12
[K^{+}]	40.7 ± 2.3	36.5 ± 1.7	34.6 ± 2.2	37.9 ± 3.5
[P^{+}]	451 ± 39	464 ± 34	434 ± 14	405 ± 31

4.3.4 Rates of oxygen and food consumption

Rates of oxygen and food consumptions of early benthic juvenile *H. gammarus* exposed to elevated temperature and pCO_2 for five weeks are displayed in Figure 4.4. Rates of oxygen consumption were marginally affected by an interaction between elevated temperature and pCO_2 ($p = 0.066$) and significantly affected by elevated pCO_2 alone ($F_{2, 69} = 4.397$, $p = 0.016$, Fig. 4.4a). There was no significant effect of elevated temperature on juvenile oxygen consumption.

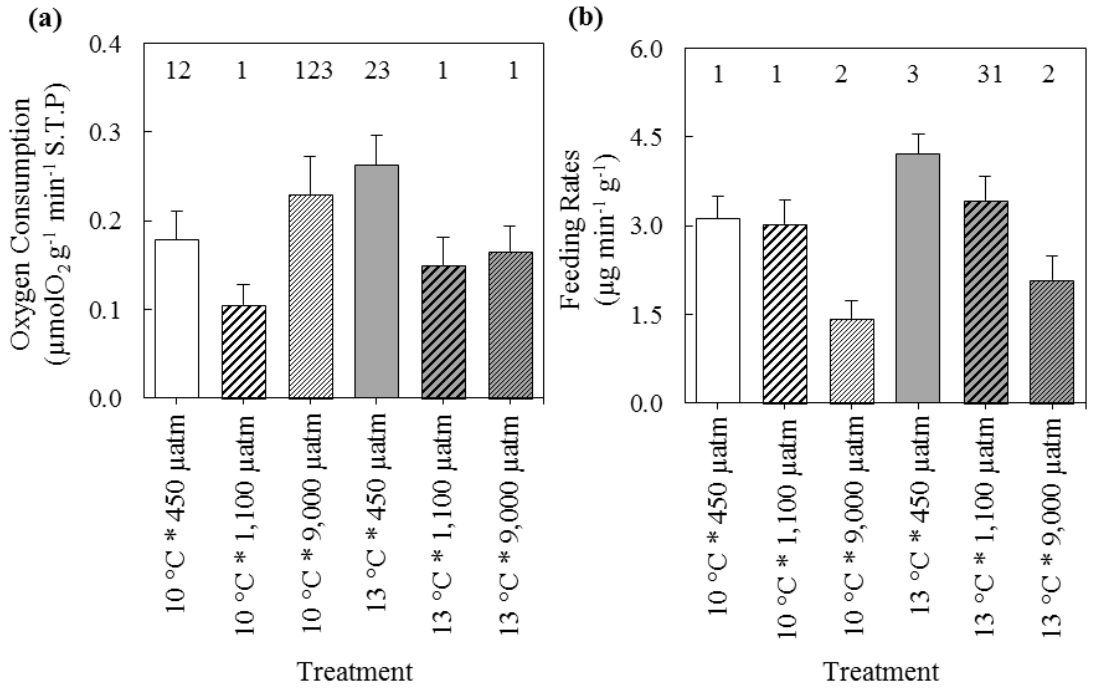

Figure 4.4 Rates of oxygen consumption and feeding of early benthic juvenile *H. gammarus* after five weeks exposure to elevated temperature and pCO_2 (Means ± S.E., d.f = 2, N = 69): (a) Oxygen consumption (µmol O_2 min^{-1} g^{-1} S.T.P.), (b) feeding rate (µg min^{-1} g^{-1}). White bars indicate 10 °C and grey bars indicate 13 °C. Clear bars indicate 450 µatm pCO_2, thick striped bars indicate 1,100 µatm pCO_2, and thin striped bars indicate 9,000 µatm pCO_2. Numbers indicate significant differences.

There was no significant interaction between elevated temperaute and pCO_2 on juvenile *H. gammarus* food consumption, however food consumption significantly decreased due to elevated pCO_2 ($F_{2, 69}$ = 14.145, p > 0.001, Fig. 4.4b) and increased due to elevated temperature ($F_{1, 69}$ = 5.408, p = 0.023, Fig. 4.4b).

4.4 Discussion

4.4.1 Overview

The present chapter investigated the sensitivity of early benthic juvenile *H. gammarus* to elevated temperature and pCO_2 in terms of life history traits (survival and growth), carapace mineralisation, organic content, metabolism, and feeding rates.

Five week exposure to elevated pCO_2 comparable with OA and CCS leakage scenarios resulted in decreased survival rates of early benthic juvenile *H. gammarus* due to moult related mortalities at both 10 °C and 13 °C, made more apparent by pCO_2-dependent increase in moulting frequency. Temperature increased growth rates and minimally influences organic content with no effect of OA, while exposure to CCS conditions resulted in no growth. Exposure to OA-related levels of pCO_2 results in shell dissolution of individuals at 13 °C, but moulting individuals were able to re-mineralise carapaces to control levels, while under CCS conditions individuals show severe shell dissolution at both temperatures. Oxygen consumption and food consumption both increased with elevated temperature and at both temperatures metabolic depression occurs with elevated pCO_2. Metabolic depression under elevated pCO_2 levels along with decreased feeding rates and potential acid-base disturbances are likely the main causes for disruptions in moulting abilities and shell dissolution. In the five week time frame of the experiment, there appears to be no direct costs, asides from moult related mortalities, of

metabolic depression and increased calcification investment in terms of growth rates under OA conditions, yet severe mortality and shell dissolution occurs under extreme hypercapnia associated with CCS.

4.4.2 The effect of elevated temperature and pCO_2 on juvenile survival

The survival of juvenile *H. gammarus* after five week exposure to elevated pCO_2 decreased at pCO_2 levels corresponding to future predictions of both OA and CCS compared to those exposed to control levels. Temperature had no effect on the survival of individuals under control pCO_2 conditions, survival rates were higher under OA and CCS conditions at 13 °C compared to 10 °C. Survival has shown to be negatively affected by elevated pCO_2 in marine crustaceans (Kurihara *et al.* 2004, 2008b, Spicer *et al*. 2007) along with a range of marine invertebrates (Shirayama and Thornton 2005, Talmage and Gobler 2011). In the present study the direct cause of all cases of mortality was due to complications during moulting. Moult Death Syndrome (MDS), a term used to encompass mortalities just before, during, or soon after ecdysis, is a common syndrome in crustaceans (Shields *et al.* 2006). The cause of MDS in juvenile lobsters is unclear and may have a number of triggers including unfavourable environmental conditions (Shields *et al.* 2006). Increased occurrence MDS has been reported due to OA and elevated temperature in the shiba shrimp, *Metapenaeus joyneri* (Miers 1880) (Dissanayake and Ishimatsu 2011). Haemolymph acidosis and related disturbances to haemolymph carbonate chemistry due to elevated temperature is known to cause abnormal calcium deposits in or beneath the exoskeleton of *H. americanus* resulting in MDS (Bowser and Rosemark 1981, Dove *et al*. 2004, Dove *et al.* 2005). Mean reductions in haemolymph pH recorded by Dove *et al.* (2005) which caused MDS and calcinosis were from pH 7.4 to pH 7.2 (Δ -0.2 pH units), which was within the range of

reductions seen in crustaceans under elevated pCO_2 (Pane and Barry 2007, Spicer *et al.* 2007, Small *et al.* 2010) or combinations of elevated pCO_2 and temperature (Zittier *et al.* 2012) indicating that acid-base disturbances may be the cause of moult related mortalities under elevated pCO_2 conditions (Kurihara *et al.* 2008b). How exactly elevated sea water pCO_2 will interact with moulting is not known but could occur in a number of ways. For example, lobsters are known to utilise internal stores of HCO_3^- for buffering of acid-base disturbances resulting from aerial exposure (Taylor and Whiteley 1989, Whiteley and Taylor 1990) and whether they use similar mechanisms for acid-base regulation in hypercapnic sea water is yet to be determined. Such HCO_3^- regulation involves the acidification of internal carapace compartments in order to release Ca^{2+} and HCO_3^- (Whiteley 1999). Such an acidification also occurs during pre-moult phases and may be compromised due to already elevated pCO_2 and associated haemolymph disturbances due to sea water acidification. Alternatively, immediately prior to ecdysis the haemolymph pH of crustaceans increases due to an influx of HCO_3^- (Truchot 1976, Dejours and Beekenkamp 1978, Mangum *et al.* 1985) and an efflux of CO_2, despite uptake of H^+ (Wheatly and Ignaszewski 1990). This process may be compromised during exposure to hypercapnia due to the already elevated levels of haemolymph HCO_3^- regulation required for pH buffering. Finally, moulting crustaceans experience metabolic acidosis due to an increase in haemolymph pCO_2 (Mangum *et al.* 1985) and an increase in metabolic and anaerobic metabolism end products during apolysis and ecdysis as respiratory surfaces isolated form the surrounding water (Whiteley 1999). This may also be compromised by acidification due to elevated seawater pCO_2 resulting in a lethal internal acidosis. Increasing the prevalence of MDS in the present study was the increase in moult frequency seen under OA and CCS conditions at both temperatures, which has previously been reported in marine shrimp (Kurihara *et al.*

2008b). Despite the prevalence of MDS, growth, in terms of WBM and organic content, was not significantly affected by OA but increased due to elevated temperature. Juveniles exposed to CCS conditions at both temperatures, however, exhibited no detectable growth during the five weeks exposure. Within optimum temperature ranges, increasing temperature is known to increase growth rates in marine crustaceans (Anger 2001, Hartnoll 2001) including lobsters (MacKenzie 1988). A range of growth responses to elevated pCO_2 have been reported for crustaceans: these include reductions (Wickins 1984a, Kurihara *et al*. 2008b), no change (Kurihara and Ishimatsu 2008, Arnold *et al.* 2009), and increases (Ries *et al.* 2009). However, further to changes in WBM, growth can also be expressed as changes in Dry Mass, organic content, and ash content (Anger 2001) which includes mineralised structures. To understand the effects of elevated temperature and pCO_2 on growth these factors also need to be taken into consideration.

4.4.3 The effect of elevated temperature and pCO_2 on organic content and shell mineralisation

Coupled with the maintenance of growth rates in terms of WBM, the organic content of juvenile lobsters was not affected by elevated pCO_2. Changes in CHN content and ratios could indicate underlying costs associated with maintaining body size and mass; however this is not the case for juvenile *H. gammarus*. There was a slight change in organic content due to elevated temperature, total CHN content did decrease by ~ 2 %, but such a small change is unlikely to be biologically relevant. While OA has no effect on organic content, juveniles exposed to OA conditions do exhibit a complex response in relation to carapace mineralisation and moulting. At 10 °C there is no effect of pCO_2 on carapace mineralisation, however at 13 °C there are significant effects of pCO_2 and

moulting. Individuals exposed to OA conditions at 13 °C have decreased levels of carapace [Ca^{2+}], [Mg^{2+}], and [Sr^{2+}] compared to all other conditions. This shell dissolution may be attributed to the previously mentioned utilisation of calcified structures as a HCO_3^- source for haemolymph pH regulation (Taylor and Whiteley 1989, Whiteley and Taylor 1990). However, individuals who successfully moult under elevated OA conditions at 13 °C are able to re-incorporate control levels of these ions into their carapace. Interestingly, post moult *C. sapidus* undergo a net uptake of CO_2 from the surrounding water along with Ca^{2+} in order to mineralise the new exoskeleton (Cameron and Wood 1985a, Wood and Cameron 1985) which may be easier under conditions of elevated sea water pCO_2 explaining the increase in calcification after moulting under OA conditions relative to controls. While OA results in decreased mineralisation in *H. gammarus* megalopa (Arnold *et al.* 2009), increases in calcification have been demonstrated in most crustaceans studied to date, including juvenile *H. americanus* and *C. sapidus*, along with adult western white shrimp, *Penaeus occidentalis* (Streets 1871) and eastern king prawn, *P. plebejus* (Hess 1865) (Wickins 1984a, Ries *et al.* 2009, Ries 2011). Adults of the giant tiger prawn, *P. monodon* (Fabricus 1798) also increase calcification under elevated pCO_2 (Wickins 1984a) however juveniles of the same species responded with reduced carapace mass and changes in Ca^{2+}:Mg^{2+} ratios (Wickins 1984b). Conversely, lobsters exposed to extreme pCO_2 levels associated with CCS scenarios in the current study experienced severe dissolution of the mineralised exoskeleton (Fig. 4.5) which results in MDS (Floreto *et al.* 2000). Whether the increase in mineral incorporation by *H. gammarus* in the present

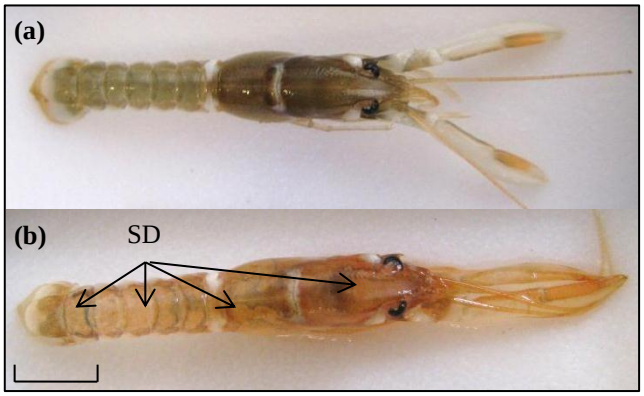

Figure 4.5 Examples of early benthic juvenile *H. gammarus* exposed to elevated pCO_2 at 10 °C for five weeks: (a) 450 µatm pCO2 and (b) 9,000 µatm pCO_2. SD indicates shell dissolution. Scale = 5 mm.

study under OA conditions results in a total increase in calcification over longer term as in *H. americanus* (Ries *et al.* 2009, Ries 2011) remains to be determined, however the energetic basis for pCO_2 responses can inform on any potential costs associated with increased calcification and maintained growth (Wood *et al.* 2008, Melatunan *et al*. 2011, 2013).

4.4.4 The effect of elevated temperature and pCO_2 on juvenile growth and energy metabolism

The metabolism and rates of food acquisition of early juvenile *H. gammarus* exposed to elevated temperature and pCO_2 may provide insights into the occurrence of shell disease and MDS mortalities, and also the costs of responses in shell mineralisation and maintenance of growth rates. Rates of oxygen and food consumption and growth of juvenile *H. gammarus* increase with increasing temperature as is found for other marine crustaceans living within optimum temperature ranges. However, when exposed to OA conditions, rates of oxygen and food consumption decrease at both temperatures. Metabolic depression is a known response of some marine invertebrates to increased

pCO_2 conditions (Reipschlager and Portner 1996, Langenbuch and Portner 2002, Small *et al.* 2010, Melatunan *et al.* 2011) and comes as a price of reduced energy availability for routine activity (Dissanayake and Ishimatsu 2011). In the present study we see reductions in food consumption rates which may be the result of less energy availability for active food acquisition. While metabolic depression and reduced feeding rates appears to have no impact on growth rates and mineralisation during the short term of this experiment, metabolic and acid-base disturbances due to unfavourable environmental conditions are known to cause MDS (Shields *et al*. 2006). Whether this will manifest itself in implications for growth and mineralisation during longer term exposure periods remains to be identified, yet the results from the present study can be used to give a clear indication as to the viability of future *H. gammarus* populations.

4.4.5 Conclusions

The present chapter shows that early benthic juvenile *H. gammarus* are sensitive to elevated pCO_2 in line with OA and CCS predictions at both 10 °C and 13 °C, primarily due to increased moult related mortalities, yet whether this stage is more sensitive than later juvenile stages has yet to be determined. As early benthic phases of marine invertebrates are defined by naturally high mortalities, > 90 % (Gosselin and Qian 1997), further increased mortality due to OA and CCS will have detrimental effects on recruitment from larval to adult populations of this commercially important species. The temperatures used in the present study, 10 °C and 13 °C, are within the optimal thermal range of this species and life history stage, as shown by the temperature-related increases in growth, metabolism, and feeding rates, which may be why there is no interactions between temperature and pCO_2 on any aspects measured. However, even within optimal temperature ranges there are negative effects of elevated pCO_2 on

metabolism and feeding rates, indicating a reduction in metabolic performance possibly due to the energetic demands of internal acid-base regulation. However, despite exposure temperature being within optimal thermal range, the limited aerobic capacity of juveniles at 13 °C in relation to elevated pCO_2 may be due to the lobsters being seasonally acclimatised to lower temperatures, making them sensitive to temperature increases.

The energetic demands of internal acid-base regulation and limited aerobic scope of early juvenile lobsters can explain the apparent shell dissolution which occurs with elevated pCO_2, especially under CCS levels, and may be the reason for increased moult related mortalities. Interestingly, under OA conditions, individuals are able to re-incorporate 'lost' Ca^{2+}, Mg^{2+}, and Sr^{2+} into the carapace through moulting, possibly allowing them to maintain shell integrity, i.e. in terms of strength and brittleness. Further investigations into the interactions of environmental pCO_2 levels and haemolymph HCO_3^- buffering mechanisms during moulting in crustaceans will allow insights into how crustaceans are seen as one of the few groups of calcifying marine invertebrates which are able to increase calcification rates under OA (Ries *et al.* 2009, Ries 2011, Kroeker *et al.* 2011, 2013, but see also Findlay *et al* 2012). While this appears beneficiary, however, we have also shown some of the functional energetic consequences of OA exposure to crustaceans, due to decreases in metabolism and associated decreases in feeding rates which suggest that early juvenile lobsters are energy limited under OA, which may be the cost associated with internal pH regulation and carapace mineralisation in a high CO_2 world.

Chapter 5

The energetic consequences of acid-base regulation under elevated temperature and pCO_2 in juveniles

5.1 Introduction

Reductions in seawater pH and associated changes to the oceans' carbonate chemistry due to elevated pCO_2 pose a challenge to marine invertebrates, especially to the maintenance of acid-base balance homeostasis and ion-regulation (Cameron 1978, 1985, Truchot 1979, Henry *et al*. 1981, Spicer *et al*. 2007, Dissanayake *et al*. 2010, Small *et al*. 2010, Dissanayake and Ishimatsu 2011). Seawater acidification could result in decreases in haemolymph pH and associated extra-cellular acid-base disturbances (Michaelidis *et al.* 2005, Pane and Barry 2007). This decrease in haemolymph pH can reduce the oxygen affinity of haemocyanin (Pörtner 1990), while an increase in intracellular H^+ can disrupt biological processes such as metabolism, protein synthesis, ion-regulation, and cell volume control (Grainger *et al.* 1979, Madshus 1988, Whiteley 1999, 2011). Therefore, haemolymph buffering capacity and the ability to regulate haemolymph pH is crucial for marine organisms to function under conditions of elevated pCO_2. Furthermore, elevated pCO_2 can impact upon an organism's aerobic scope in relation to temperature (Metzger *et al.* 2007, Pörtner 2008, Pörtner and Farrell 2008, Walther *et al.* 2009), with temperature itself also affecting the acid-base status of marine invertebrates (Wood and Cameron 1985, Qadri *et al.* 2007).

Marine crustaceans possess effective extracellular buffering mechanisms to maintain haemolymph homeostasis, predominantly in the form of the physiological regulation of HCO_3^- (Cameron 1978, 1985, Truchot 1979, Wood and Cameron 1985, Whiteley 1999), and have been perceived to be amongst the most 'tolerant' groups of marine

invertebrates to elevated pCO_2 (Melzner *et al*. 2009, Kroeker *et al*. 2010, 2013). Crustaceans' buffer haemolymph acid-base disturbances by the accumulation of HCO_3^- in haemolymph (Cameron 1978, 1985, Truchot 1979, Wood and Cameron 1985) however the mechanisms and source of HCO_3^- buffering is poorly known and varies between species. Some species, such as the European lobster, *Homarus gammarus* (Linnaeus 1758), are known to be able to mobilise HCO_3^- from internal stores to buffer acid-base disturbances, such as in response to aerial exposure (Defur *et al*. 1980, Henry *et al*. 1981, Taylor and Whiteley 1989, Whiteley and Taylor 1990). In this case, the source of internal HCO_3^- is presumably dissolution of the mineralised exoskeleton evidenced by the observed increases in haemolymph $[Ca^{2+}]$ which accompanies increases in haemolymph $[HCO_3^-]$ (Taylor and Whiteley 1989, Whiteley *et al.* 1990). In short, the acidification of the carapace fluid compartment leads to the formation of HCO_3^- and Ca^{2+} (Whiteley 1999), which can effectively buffer acidosis in the short-term but may in turn have negative long-term effects on carapace mineralisation, internal ion-regulation, and moulting abilities. Other species, such as the blue crab, *Callinectes sapidus* (Rathburn 1896) and the velvet crab, *Necora puber* (Linnaeus 1767) are able to utilise external sea water as a source of HCO_3^- for haemolymph buffering with little effect on mineralisation of the exoskeleton (Cameron 1978, 1985, Henry *et al.* 1981, Spicer *et al.* 2007, Small *et al.* 2010). This form of HCO_3^- regulation occurs across gill membranes and is driven by electroneutral ion exchange of HCO_3^- for Cl^- and H^+ for Na^+ by Na^+/K^+- and H^+-ATPases (Cameron 1978, Whiteley 2011).

Haemolymph HCO_3^- buffering, whether *via* internal shell dissolution or external sea water HCO_3^-, is a metabolically expensive process (Whiteley 2011), and there are energetic consequences and trade-offs associated with possessing such regulatory mechanisms (Calow and Forbes 1998), which may have an underlying functional effect

on this ‘tolerant’ group of organisms (Widdicombe and Spicer 2008, Whiteley 2011). For example, thermal sensitivity increases with exposure to hypercapnia in the great spider crab, *Hyas araneus* (Linnaeus 1758) (Walther *et al.* 2009) and the edible crab, *Cancer pagurus* (Linnaeus 1758) (Metzger *et al*. 2007), while scope for aerobic activity and osmo-regulation and ion-regulation is compromised in prawns (Dissanayake *et al*. 2010, Dissanayake and Ishimatsu 2011). Such investigations into the costs associated with acid-base regulation are limited to a few studies and focus mainly on pCO_2, not temperature. When the two factors are assessed together elevated temperature can be seen to have a negative effect on a species ability to regulate acid-base balance as, for example, *H. araneus* experiences more severe acidosis due to elevated pCO_2 at 4 °C than at 1 °C (Zittier *et al.* 2012) while *H. gammarus* is unable to compensate acidosis due to aerial exposure at 20 °C compared with complete compensation at 10 °C (Whiteley and Taylor 1990). These studies show the potential for interaction between global warming and seawater acidification when impacting upon organism acid-base physiology and, as both drivers will occur simultaneously, there is a pressing need to understand how organisms respond under combinations of elevated temperature and pCO_2.

Juvenile stages of marine organisms are proposed to be the most tolerant life stage to the effects of climate change due to their wide aerobic scope (Pörtner and Farrell 2008), and are thought to possess adult type regulatory pathways and abilities. For example, juvenile *H. gammarus* and the American lobster, *H. americanus* (Milne-Edwards) develop adult type structures for osmoregulation through the transition from pelagic larvae to benthic juveniles (Charmantier *et al.* 2001), which are presumed to function as adults throughout all of their juvenile development. The same applies to the oxy-regulatory abilities of Norway lobster, *Nephrops norvegicus* (Linnaeus 1758) (Spicer

and Eriksson 2003). The juvenile stage of marine crustaceans, as mentioned in Chapters 1 and 4, can be separated into two physiologically distinct stages, early benthic juveniles and late juveniles, due to the continuous development of physiological functions throughout this phase, such as osmo-regulation (Charmantier *et al.* 1998, 2001, 2002, Cieluch *et al*. 2004), oxy-regulation (Spicer and Eriksson 2003), ion-regulation and haemolymph haemocyanin development (Brown and Terwilliger 1992, Newton and Potts 1993, Terwilliger and Brown 1993, Terwilliger and Dumler 2001, Terwilliger and Ryan 2001). However, little is known of the haemolymph biochemistry of these juvenile stages, or of their acid-base regulatory abilities in general, especially in relation to the more subtle changes in pCO_2 associated with OA. If we are to understand the effects of climate change on marine organisms, we need to understand what makes animals tolerant and others susceptible (Widdicombe and Spicer 2008); a concept which can also be applied to life history stages.

The aim of this present chapter was to assess the ability of early juvenile *H. gammarus* (as defined by Wahle and Steneck (1992)) to regulate haemolymph acid-base status when challenged with elevated pCO_2 levels at both current and elevated temperatures, and the energetic consequences of such regulation. A secondary aim of the chapter was to gather data on juvenile lobster physiology and physiological sensitivities and to discuss how these compare to those of adults. Chapter 3 of the present thesis indicated that the early benthic juvenile stage of *H. gammarus* development is energetically demanding and therefore susceptible to elevated temperature and pCO_2 in winter acclimated individuals, and the present chapter will assess the implications of such energetic demands in terms of acid-base regulation and metabolic performance at elevated summer temperatures indicative of the upper range of this species' thermal window. This was achieved by exposing 12 month old juvenile lobsters, acclimatised to

13 °C (representing current summer averages) and 17 °C (+ 4 °C representing ocean warming scenarios Sokolov *et al*. (2009)), to elevated pCO_2 conditions of 1,100 μatm and 8,000 μatm, in line with predictions of pCO_2 increases due to OA and CCS scenarios (Caldeira and Wickett 2003, 2005, Raven *et al*. 2005, Blackford *et al.* 2009, Kano *et al*. 2010). After two weeks exposure, the haemolymph acid-base status of individuals was assessed in terms of haemolymph pH, pCO_2, and [HCO_3^-]. To express the energetic consequences of haemolymph acid-base regulation, oxygen consumption was measured as a proxy for metabolic energy demand, along with gill Na^+/K^+-ATPase activity, and feeding rates were quantified as a proxy for energy acquisition. Haemolymph ion levels were measured as a proxy for ion homeostasis, while *L*-lactate (anaerobic metabolic end product) and protein levels were measured as proxies for condition. *H. gammarus* is an ecologically and economically important species, so their ability to compensate for environmental changes, and the associated energetic costs, would potentially have a wide socio-economic impacts.

5.2 Materials and methods

5.2.1 Animal collection, husbandry, and exposure

Eleven month old *H. gammarus* (N = 54) were supplied by the National Lobster Hatchery (Padstow, UK). Individuals were hatched from wild caught berried hens and reared under constant conditions (S = 35, T = 19 °C, DO_2 = 8 mg L^{-1}) as described in Chapters 2 and 4. Individuals were transported to the Plymouth Marine Laboratory (Plymouth, UK), where they were haphazardly separated into two temperature treatments (N = 27 *per* treatment), a nominal 13 °C representing current seasonal temperatures, and a nominal 17 °C representing future predictions of ocean warming (Sokolov *et al.* 2009). Individuals were kept under these temperature conditions for

three weeks prior to elevated pCO_2 exposure in order to minimise the effect of sudden temperature changes on proxies gathered during the experimental period.

The experimental system consisted of six tanks, three *per* temperature; within each were nine pots, three *per* pCO_2 treatment each containing one individual (N = 9 individuals *per* temperature*pCO_2 combination). Temperature of the whole system was maintained at 13 °C (13.19 ± 0.02 °C), while water inflow into the hot tanks was heated to nominal 17 °C (17.67 ± .03 °C) using an aquarium heater (3614 Aquarium Heater, Eheim GmbH & Co. KG, Deizisau, DE) in conjunction with a chiller (L Series, Guangdong Boyu Group Co. LTD., Guangdong, China) to achieve a stable temperature. Each lobster was individually supplied with a constant flow of mechanically filtered, re-circulated sea water (10 mL min^{-1}) at the appropriate temperature, along with gently bubbling air. Upon commencement of acidification, nine pots in each tank were haphazardly allocated to one of three pCO_2 treatments. Elevated pCO_2 levels in the bubbling air into each pot were achieved by mixing pure CO_2 with atmospheric air, following methods described by Findlay *et al*. (2008), with CO_2 supply being adjusted as necessary. Air CO_2 levels were continuously measured using a CO_2 gas analyser (Li-820, Li-Cor Biosciences, Lincoln, USA) and adjusted as necessary. Elevated pCO_2 levels were set to achieve a control pCO_2 of 450 µatm, a predicted increase in pCO_2 associated with Ocean Acidification of 1,100 µatm (Caldeira and Wickett 2003, 2005), and a predicted increase associated with Carbon Capture and Storage leakage scenarios, 8,000 µatm pCO_2 (Blackford *et al*. 2009, Kano *et al.* 2010). Individuals were fed every three days with a selection of squid (*Logilo vulgaris* Linnaeus 1798), mussel (*Mytilus edulis* 1758) and krill (*Euphausia superba* Dana 1850) *ad-libitum*, and all uneaten food was removed from the chambers after 3 h.

Temperature, pH (NBS scale), and salinity were recorded daily, while water samples were collected and poisoned with $HgCl_2^-$ every 7 d for future determination of sea water total alkalinity (A_T). A_T was measured by gran titration method using an alkalinity titrator (As-Alk2 Titrator, Apollo SciTech Inc., Bogart, USA), and the values, along with values of pH, temperature, and salinity, were used to calculate sea water carbon dioxide partial pressure (pCO_2 - µatm), total carbon dioxide (TCO_2 - µmol kg^{-1}), bicarbonate concentration ($[HCO_3^-]$ - µmol kg^{-1}), carbonate concentration ($[CO_3^{2-}]$ - µmol kg^{-1}), aragonite saturation (Ω_{ara}), and calcite saturation (Ω_{cal}) using CO_2Sys programme (Lewis and Wallace 2006) with constants provided by Mehrbach *et al*. (1973) refitted by Dickson and Millero (1987) and KSO_4 constants from Dickson (1990). Seawater parameters throughout the exposure period are displayed in Table 5.1.

5.2.2 Determination of rates of oxygen and food consumption

After two week exposure to elevated temperature and pCO_2, the rates of oxygen consumption were determined for individual juvenile lobsters. Individuals were not fed for 48 h prior to determination of oxygen and feed consumption. Individuals were transferred into stop flow respiration chambers (vol. = 195 mL) blacked out with black plastic and containing glass marbles (Ø = 1.0 cm) to reduce water volume and provide shelter, where they were allowed to settle for 30 min in their designated treatment conditions. After 30 min resting, water flow to each respirometer was stopped, and rates of oxygen consumption recorded for 2 h by determining oxygen partial pressure (pO_2) every 15 min. pO_2 was determined using an optical oxygen analyser system (101, OxySense, Dallas, USA) as described in Rastrick and Whiteley (2011).

After measurements of oxygen consumption, individuals were returned to their designated treatment containers, where individuals were left to rest for 2 h before

feeding rates were determined as follows. Individuals were fed with pre –weighed fresh squid, and left to feed for 1 h. At the end of the trial, remaining squid was removed and weighed, and consumption rates calculated as g squid eaten *per* g of body mass.

5.2.3 Determination of haemolymph acid-base status

After feeding trials had finished, individuals remained within their designated treatments for a further 48 h before determination of haemolymph acid-base status. 100 µL haemolymph samples were taken by direct cardiac puncture using a gas tight syringe (Gastight 1710 100 µL syringe, Hamilton Co. Bonaduz, Switzerland) and needle (RN Needle, Hamilton Co. Bonaduz, Switzerland). Samples were obtained anaerobically while the individuals were still submerged in treatment water to avoid any effects of emersion on haemolymph acid-base parameters (Calosi *et al.* 2013). A subsample of 70 µL of haemolymph was transferred within 2 sec into a microcentrifuge tube (Eppendorf, vol. = 1.6 mL), the size and shape of which allowed for a tight fit onto the end of a micro pH electrode (Micro-InLab pH combination electrode, Mettler Toledo, Colombus, USA) connected to a calibrated pH meter (Seven Easy, Mettler Toledo, Colombus, USA) in order to anaerobically determine haemolymph pH (NBS scale). During pH measurements, the sample was placed in a water bath set to the designated temperature treatment. Any remaining haemolymph from pH measurements (*circa* 60 µL) was immediately frozen at -80 °C for future analysis.

The remaining 30 µL was injected within 2 sec of pH measurements into a CO_2 analyser (965D, Corning Diagnostics, Cambridge, USA) to determine haemolymph TCO_2.

Using haemolymph pH and TCO_2, haemolymph acid–base variables of pCO_2 and $[HCO_3^-]$ were calculated using the Henderson-Hasselbalch equation in the forms

(1) $pCO_2 = TCO_2 / \alpha\ (10^{\text{pH-PK'1}} + 1)$

(2) $[HCO_3^-] = TCO_2 - \alpha\ pCO_2$

where α is the solubility coefficient of CO_2 in the shore crab, *Carcinus maenas* (Linnaeus 1758) haemolymph (13 °C, α = 0.4050 mmol L^{-1} kPa^{-1}, while at 17 °C, α = 0.3375 mmol K^{-1} kPa^{-1}, calculated from Truchot (1976)) and pK'_1 is the first apparent dissociation constant of carbonic acid in *C. maenas* haemolymph (13 °C, pK'_1 = 6.04, while at 17 °C, pK'_1 = 6.015, calculated from Truchot (1976)).

5.2.4 Determination of epipodite Na^+/K^+-ATPase activity

Immediately following haemolymph acid-base status measurements, all epipodites were carefully removed from each individual for the determination of Na^+/K^+ ATPase activity following Brooks and Mills (2003) with the following modifications. All epipodites from each individual were sonicated (Vibracell, Sonics and Materials Inc., Danbury, USA) in 250 µL of ice-cold sonication buffer containing 100 mmol L^{-1} HEPES, 100 mmol L^{-1} NaCl and 0.1 % sodium deoxycholate, pH 7.2. Activity was determined in two different buffers: 30 µL of sonicatant was added to 500 µL of buffer (1) containing 10 mmol L^{-1} $MgCl_2$, 100 mmol L^{-1} NaCl, 15 mmol L^{-1} KCl, and 100 mmol L^{-1} HEPES, pH 7.2 and (2) the same buffer without KCl but containing 10 mmol L^{-1} ouabain, which specifically inhibits Na^+/K^+-ATPase. Samples were prepared in triplicate. The reaction was started by the adding of 27 µL ATP (100 mmol L^{-1}) and incubated in a hot block (Dry Block Thermostat, Grant) at 37°C for 20 min. After 20 min the reaction was stopped with 1 mL Bonting's reagent (560 mmol L^{-1} H_2SO_4, 8.1 mmol L^{-1} ammonium molybdate and 176 mmol L^{-1} $FeSO_4$). Colour, arising from the reaction of free phosphate with Bonting's reagent, was allowed to develop for 20 min at room temperature before absorbance was measured at 700 nm using a

spectrophotometer (Novaspec II, Pharmacia Biotech, Piscataway, USA) using 0.65 mmol L^{-1} phosphorus standard solution (Sigma-Aldrich, St Louis, USA) as the standard. The difference between ATP concentrations in the two buffers can be attributed to Na^+/K^+ ATPase activity.

Protein concentration was also determined for the gill sonicatant in a microplate format (VersaMax ELISA microplate reader, Molecular Devices, Sunnyvale, USA) using the method of Bradford (1976) with 200 mg mL^{-1} bovine serum albumin (Sigma-Aldrich, St Louis, USA) as the standard.

5.2.5 Ionic and biochemical analysis of haemolymph

Frozen haemolymph samples were centrifuged at 10,000 rpm for 5 min to remove cells and coagulates before protein, *L*-lactate, and ionic concentrations were determined.

L-lactate was determined using 8 µL subsamples of haemolymph from each individual, diluted (x 3) and mixed *L*-lactate reagent (Sigma-Aldrich, St Louis, USA), then read at λ = 550 nm using a plate reader (VersaMax ELISA microplate reader, Molecular Devices, Sunnyvale, USA).

Haemolymph protein concentrations were determined in 5 µL subsamples diluted (x 10) using the Coomassie Brilliant Blue dye binding method (Bradford 1976) using bovine serum albumin (Sigma-Aldrich, St Louis, USA) as the standard. Optical density was read at λ = 595 nm using a microplate reader (VersaMax Microplate Reader, Molecular Devices LLC, Sunnyvale USA). A further 10 µL subsample of haemolymph from each individual was diluted (x 150), then analysed for $[Ca^{2+}]$, $[Mg^{2+}]$, $[Cu^{2+}]$, $[Na^+]$, $[K^+]$, and $[P^+]$, using an ICP-OES (Varian 725-ES, Agilent Technologies Inc, Santa Clara, USA. Detection limits for Ca^{2+}, Mg^{2+}, K^+, P^+ = 10 µg L^{-1}, Na^+ = 40 µg L^{-1}, Cu^{2+} = 0.2 µg L^{-1}).

Table 5.1 Water chemistry parameters throughout the exposure period (Means ± S.E., d.f = 5, N = 76): Temperature (°C), salinity, pH (NBS Scale), total alkalinity (A_T - µEq kg^{-1}), total carbon dioxide content (TCO_2 - µmol kg^{-1}), carbon dioxide partial pressure (pCO_2 - µatm), bicarbonate concentration ($[HCO_3^-]$ - µmol kg^{-1}), carbonate concentration ($[CO_3^{2-}]$ - µmol kg^{-1}), calcite saturation (Ω_{cal}) and aragonite saturation (Ω_{ara}). Superscript capital letters indicate significant differences between treatments. [1]Parameters calculated using CO_2SYS program (Lewis and Wallace 1998) with constants provided by Mehrbach *et al.* (1973) refitted by Dickson and Millero (1987).

	13 °C			17 °C		
	450 µatm	1,100 µatm	8,000 µatm	450 µatm	1,100 µatm	8,000 µatm
Temperature	13.1 ± 0.02^{A}	13.2 ± 0.04^{A}	13.2 ± 0.06^{A}	17.6 ± 0.06^{B}	17.7 ± 0.04^{B}	17.7 ± 0.07^{B}
pH	8.12 ± 0.01^{A}	7.73 ± 0.01^{B}	6.91 ± 0.01^{C}	8.06 ± 0.01^{A}	7.71 ± 0.01^{B}	6.88 ± 0.01^{C}
Salinity	34.1 ± 0.06	34.0 ± 0.03	34.1 ± 0.05	34.0 ± 0.04	34.0 ± 0.03	34.1 ± 0.05
A_T	2198 ± 11	2195 ± 11	2195 ± 7	2194 ± 17	2170 ± 14	2210 ± 21
$^{1}TCO_2$	2023 ± 10^{A}	2153 ± 14^{B}	2497 ± 10^{C}	2019 ± 16^{A}	2120 ± 13^{B}	2508 ± 26^{C}
$^{1}pCO_2$	430 ± 6^{A}	1156 ± 38^{B}	7974 ± 139^{C}	517 ± 11^{D}	1247 ± 23^{B}	9025 ± 181^{E}
$^{1}[HCO_3^-]$	1877 ± 9^{A}	2049 ± 14^{B}	2172 ± 7^{C}	1871 ± 16^{A}	2014 ± 12^{B}	2185 ± 21^{C}
$^{1}[CO_3^{2-}]$	128 ± 2.1^{A}	58 ± 1.7^{B}	9.3 ± 0.2^{C}	130.1 ± 1.8^{A}	62.8 ± 1.2^{B}	10.2 ± 0.2^{C}
$^{1}\Omega_{cal}$	3.08 ± 0.05^{A}	1.39 ± 0.04^{B}	0.22 ± 0.01^{C}	3.13 ± 0.04^{A}	1.51 ± 0.03^{B}	0.25 ± 0.01^{C}
$^{1}\Omega_{ara}$	1.97 ± 0.03^{A}	0.89 ± 0.03^{B}	0.14 ± 0.01^{C}	2.02 ± 0.03^{A}	0.97 ± 0.02^{B}	0.16 ± 0.01^{C}

5.2.6 Statistical analysis

All data were assessed for normal and homogenous distribution, using a Kolmogorov – Smirnov Test for Normality, followed by a Levene's test for equal variances. A two-way ANOVA was performed to analyse the effects of temperature, pCO_2, and their interactions on all proxies at each inter-moult stage. For oxygen consumption, a two-way ANCOVA was performed with WBM as a covariant. Tank effects were analysed as a random factor and if not significant, were discarded from analysis.

5.3 Results

5.3.1 Haemolymph acid-base status

The acid-base status of juvenile *H. gammarus* exposed to elevated temperature and pCO_2 is presented in Figure 5.1.

There was a significant interaction between elevated temperature and pCO_2 on haemolymph pH ($F_{2, 49} = 10.314$, $p < 0.001$, Fig 5.1a) along with significant effects of elevated temperature ($F_{1, 49} = 10.407$, $p < 0.001$, Fig 5.1a) and pCO_2 ($F_{2, 49} = 7.691$, $p < 0.001$, Fig 5.1a). This resulted in a decrease in haemolymph pH due to elevated pCO_2 at 17 °C compared with no changes in haemolymph pH at 13 °C (Fig. 5.1a).

There was a significant interaction between elevated temperature and pCO_2 on haemolymph pCO_2 ($F_{2, 49} = 6.559$, $p < 0.001$, Fig. 5.1b) along with a significant effect of elevated temperature ($F_{1, 49} = 4.083$, $p < 0.005$, Fig 5.1b) and elevated pCO_2 ($F_{2, 49} = 20.273$, $p < 0.001$, Fig. 5.1b). This resulted in an increase in haemolymph pCO_2 due to elevated seawater pCO_2, which was stronger at elevated temperature (Fig. 5.1b).

There was a significant interaction between elevated temperature and pCO_2 on haemolymph [HCO_3^-] ($F_{2, 49} = 9.618$, $p < 0.001$, Fig 5.1c) along with a significant effect of elevated temperature ($F_{1, 49} = 15.103$, $p < 0.001$, Fig 5.1c) and pCO_2 ($F_{1, 49} = 11.299$,

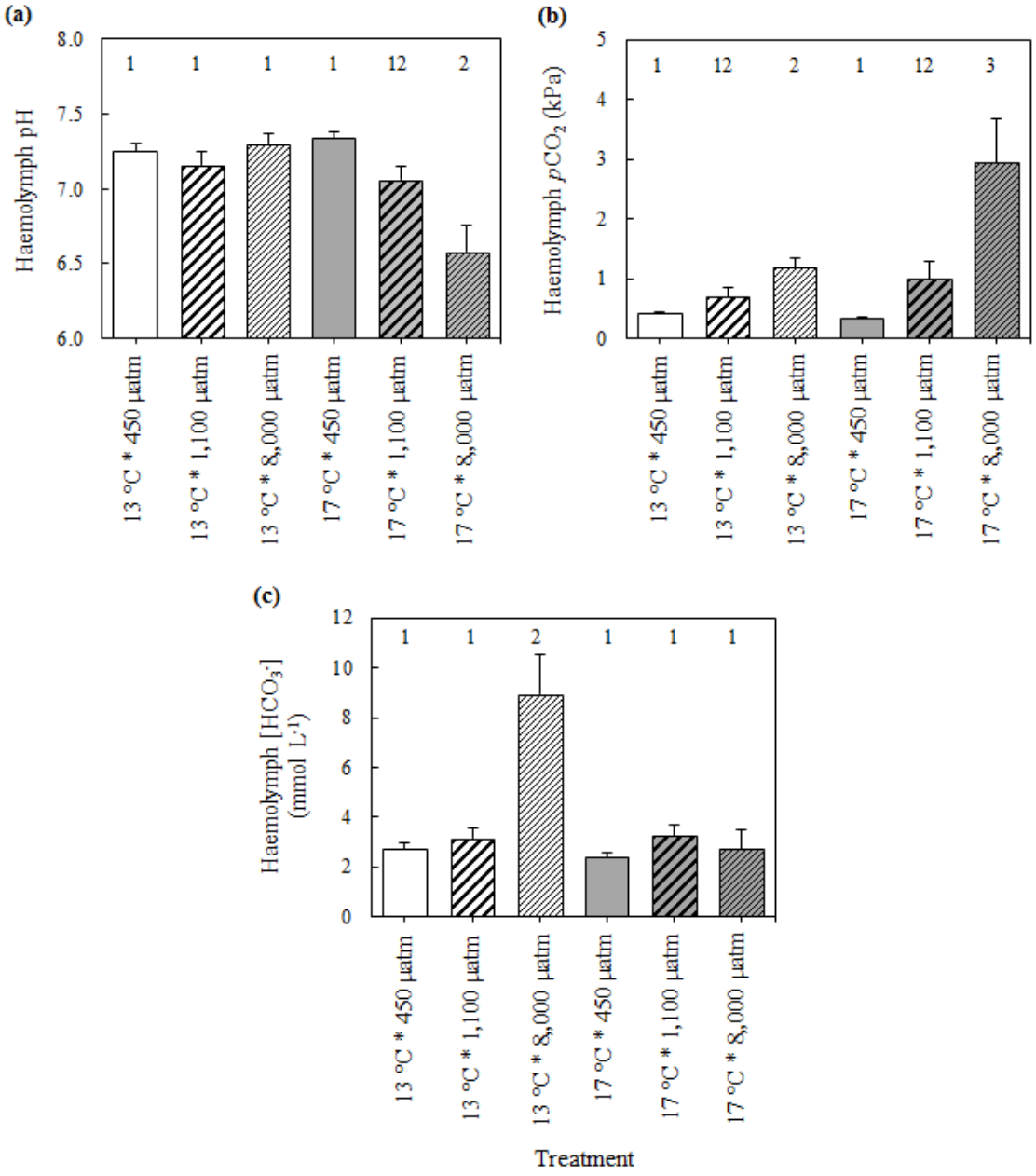

Figure 5.1 Haemolymph acid–base status of 1 year old juvenile *Homarus gammarus* after two week exposure to elevated temperature and pCO_2 (Means ± S.E., d.f = 2, N = 49): A) Haemolymph pH (NBS scale), B) haemolymph carbon dioxide partial pressure (pCO_2 - kPa), and C) haemolymph bicarbonate concentration ($[HCO_3^-]$ - mmol L^{-1}). White bars indicate 13 °C, grey bars indicate 17 °C. Clear bars indicate 450 µatm pCO_2, thick striped bars indicate 1,100 µatm pCO_2, and thin striped bars indicate 8,000 µatm pCO_2. Numbers indicate significant differences between treatments.

$p < 0.001$, Fig 5.1c). This resulted in an increase in haemolymph [HCO_3^-] due to elevated pCO_2 at 13 °C, but no change in haemolymph [HCO_3^-] at 17 °C (Fig 5.1c).

5.3.2 Rates of oxygen and food consumption

Rates of oxygen and food consumption of juvenile *H. gammarus* exposed to elevated temperature and pCO_2 are displayed in Figure 5.2. There was no significant effect of pCO_2 and temperature, in isolation or combined, on the rate of oxygen consumption of juvenile *H. gammarus* ($p \geq 0.05$).

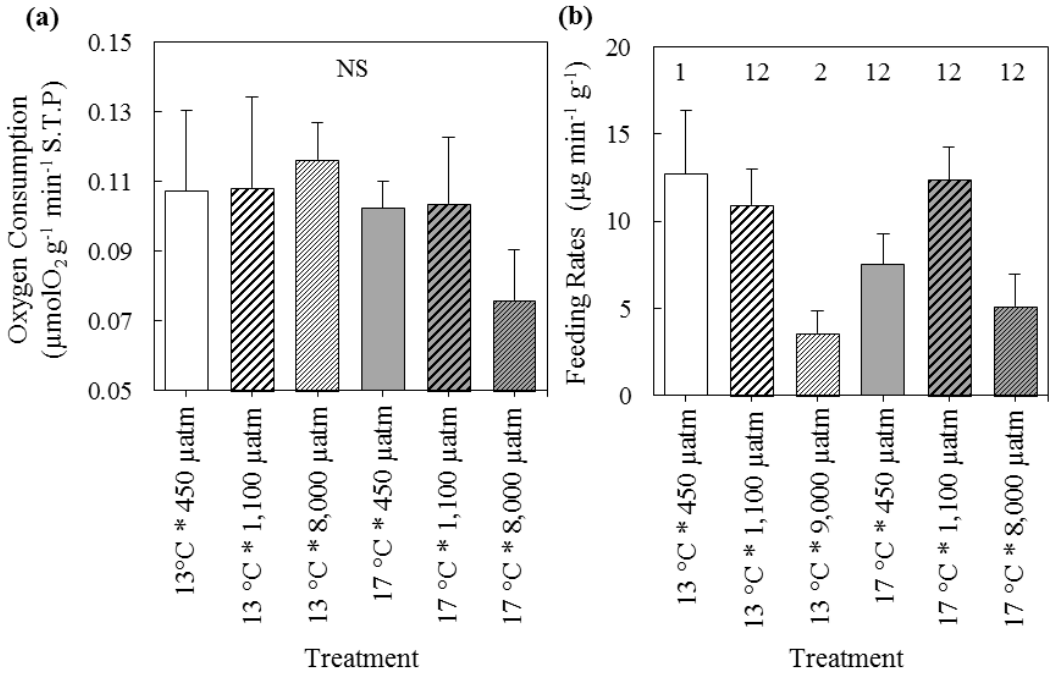

Figure 5.2 Oxygen consumption and feeding rates of 1 year old juvenile *H. gammarus* after two week exposure to elevated temperature and pCO_2 (Means ± S.E., d.f = 2, N = 50): (a) Oxygen consumption (µmol O_2 min^{-1} g^{-1} S.T.P.) and (b) food consumption (mg g^{-1} h^{-1}). White bars indicate 13 °C, grey bars indicate 17 °C. Clear bars indicate 450 µatm pCO_2, thick striped bars indicate 1,100 µatm pCO_2, and thin striped bars indicate 8,000 µatm pCO_2. Numbers indicate significant differences between treatments.

There was no significant interaction between elevated temperature and pCO_2 on juvenile food consumption, nor a significant effect of elevated temperature. Food consumption was significantly affected by pCO_2 ($F_{2, 50} = 5.732$, $p = 0.01$) which was driven by the decrease in food consumption between 450 µatm pCO_2 and 8,000 µatm pCO_2 at 13 °C.

5.3.3 Epipodite Na^+/K^+-ATPase activity

Na^+/K^+-ATPase activity in the epipodites of juvenile *H. gammarus* after two week exposure to elevated temperature and pCO_2 are displayed in Figure 5.3. There were no significant effects of elevated temperature and pCO_2, neither individually or together,

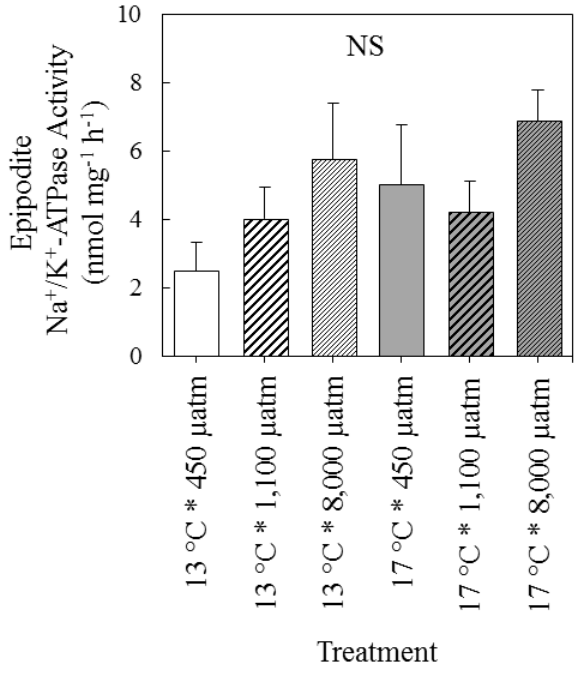

Figure 5.3 Epipodite activity of 1 year old juvenile *H. gammarus* after two week exposure to elevated temperature and pCO_2 (Means ± S.E., d.f = 2, N = 49): Epipodite Na^+/K^+-ATPase activity (nmol mg^{-1} h^{-1}). White bars indicate 13 °C, grey bars indicate 17 °C. Clear bars indicate 450 µatm pCO_2, thick striped bars indicate 1,100 µatm pCO_2, and thin striped bars indicate 8,000 µatm pCO_2. Numbers indicate significant differences between treatments.

on Na^+/K^+-ATPase activity, despite an apparent increase due to elevated pCO_2 at 13 °C ($p = 0.089$, Fig. 5.3).

5.3.4 Haemolymph ions and biochemistry

Concentrations of juvenile haemolymph protein, *L*-lactate, and ions after 2 week exposure to elevated temperature and pCO_2 are displayed in Table 5.2. There were no significant interctions between elevated temperature and pCO_2 on any aspect of juvenile haemolymph ions or biochemistry, however there were significant effects of elevated temperature and pCO_2 in isolation. In detail, haemolymph protein levels significantly increased due to elevated temperature ($F_{1, 47} = 5.144$, $p = 0.029$). There was no significant effect of elevated temperature on haemolymph *L*-lactate ($p \geq 0.05$). There was no significant effect of elevated temperature on haemolymph $[Ca^{2+}]$, $[Mg^{2+}]$, or $[K^+]$, however there was a significant effect of elevated temperature on haemolymph $[Cu^{2+}]$ ($F_{1, 48} = 4.057$, $p = 0.049$). Finally, there was a significant decrease in haemolymph $[Na^+]$ due to elevated pCO_2 ($F_{2, 48} = 4.626$, $p = 0.015$) at 17 °C.

5.4 Discussion

5.4.1 Overview

The present study investigated the haemolymph buffering capacity of early juvenile *H. gammarus* exposed to elevated pCO_2 conditions representing Ocean Acidification (OA – pH 7.7, 1,100 µatm pCO_2) and Carbon Capture and Storage leakage (CCS – pH 6.9, 8,000 µatm pCO_2) scenarios under current and future predictions of elevated temperature (13 °C *vs* 17 °C).

Table 5.2 Haemolymph biochemistry of juvenile *Homarus gammarus* after two week exposure to elevated temperature and pCO_2 (Means ± S.E., d.f = 2, N = 48): Haemolymph calcium ([Ca^{2+}] - µmol L^{-1}), magnesium ([Mg^{2+}] - µmol L^{-1}), copper ([Cu^{2+}] - µmol L^{-1}), sodium ([Na^{+}] - µmol L^{-1}), potassium ([K^{+}] - µmol L^{-1}), protein ([protein] – mg mL^{-1}), and *L*-lactate ([*L*-lactate] – mmol L^{-1}) concentrations. Superscript capital letters indicate significant differences between treatments.

	13 °C			17 °C		
	450 µatm	1,100 µatm	8,000 µatm	450 µatm	1,100 µatm	8,000 µatm
[Ca^{2+}]	11.27 ± 0.3	11.12 ± 0.5	11.14 ± 0.4	10.95 ± 0.6	12.39 ± 0.4	12.13 ± 0.2
[Mg^{2+}]	33.34 ± 2.8	29.70 ± 3.2	35.54 ± 2.0	29.10 ± 3.0	27.90 ± 2.3	34.16 ± 2.8
[Cu^{2+}]	0.10 ± 0.03^{A}	0.11 ± 0.03^{A}	0.10 ± 0.02^{A}	0.19 ± 0.06^{B}	0.15 ± 0.03^{B}	0.13 ± 0.02^{B}
[Na^{+}]	385 ± 6	373 ± 3	383 ± 5	395 ± 9	374 ± 5	384 ± 5
[K^{+}]	13.0 ± 1.9	14.8 ± 2.1	15.0 ± 3.1	18.2 ± 2.7	16.0 ± 2.4	18.9 ± 2.3
[Protein]	3.93 ± 0.96^{A}	5.46 ± 1.58^{A}	4.75 ± 0.75^{A}	9.21 ± 2.79^{B}	6.31 ± 0.95^{B}	6.92 ± 1.49^{B}
[*L*-lactate]	1.91 ± 0.66	1.28 ± 0.22	1.64 ± 0.40	2.67 ± 1.36	1.82 ± 1.17	189 ± 0.76

Juvenile *H. gammarus* can successfully buffer haemolymph pH despite increases in haemolymph pCO_2 at 13 °C by increasing haemolymph [HCO_3^-], however at 17 °C this buffering capacity is lost. Despite successful buffering capacity at 13 °C, lobsters are energetically limited due to the absence of an increase in oxygen consumption and a decrease in feed ingestion despite a presumed increase in energetic demands of increased haemolymph buffering. This may be partially explained by high haemolymph *L*-lactate levels, representing recourse to anaerobic metabolism, and low haemolymph protein levels, which represents minimal non-bicarbonate buffering and oxygen transport capacity, under control conditions. These haemolymph characteristics also lead to an inability to increase aerobic scope with increasing temperature, resulting in severe energy limitation under future predicted temperature conditions and no ability to regulate internal acid–base balance.

5.4.2 The haemolymph buffering capacity of juvenile lobsters

Juvenile (12 month old) *H. gammarus* exposed to 13 °C possessed an effective haemolymph buffering ability under all levels of elevated pCO_2. The increase in haemolymph pCO_2 due to increases in water pCO_2 under simulated OA and CCS leakage conditions was accompanied by an increase in haemolymph [HCO_3^-], resulting in no differences in haemolymph pH. However, at 17 °C the bicarbonate buffering of haemolymph pH is lost, resulting in elevated haemolymph pCO_2 and reduced haemolymph pH under both OA and CCS conditions. This has also been shown for *H. araneus* (Zittier *et al.* 2012) which experienced acidosis under elevated pCO_2 conditions at elevated temperature. The buffering of acid-base disturbances by means of increasing haemolymph HCO_3^-, as shown by juveniles exposed to 13 °C, is a known response of crustaceans to hypercapnic environments (Cameron 1978), and has been demonstrated

with adult *H. gammarus* when exposed to air (Taylor and Whiteley 1989, Whiteley and Taylor 1990). Both Taylor and Whiteley (1989) and Whiteley and Taylor (1990) observed an increase in haemolymph [Ca^{2+}] and [Mg^{2+}] accompanying the increase in haemolymph [HCO_3^-] which suggests that HCO_3^- for haemolymph buffering is derived from the dissolution of the mineralised exoskeleton. However, in the present study there is no change in haemolymph [Ca^{2+}] or [Mg^{2+}] despite an increase in [HCO_3^-]. This could be due to the availability of water, which is absent when exposed to air, in which to secrete such ions and prevent build up in the haemolymph and maintain diffusion gradients. Internal stores of HCO_3^- only appear to be utilised by certain species during aerial exposure (Defur *et al*. 1980, Henry *et al*. 1981, Taylor and Whiteley 1989, Whiteley and Taylor 1990, Whiteley *et al.* 2001) as species studied to date in hypercapnic water seem to utilise external water as an HCO_3^- source with minimal shell dissolution (Truchot 1979, Cameron 1985, Spicer *et al*. 2007, Small *et al*. 2010). However, the possibility that lobsters are able to utilise external stores when submerged and internal stores when immersed is not supported here, as there was no significant change in Na^+/K^+-ATPase activity in epipodites or haemolymph [K^+], despite an increasing trend with elevated pCO_2 at 13 °C, indicating that this pump is not the primary driver in HCO_3^- regulation under the conditions tested here. Na^+/H^+ and Cl^-/HCO_3^- exchange is a key mechanism in acid-base regulation with a key role of Na^+/K^+-ATPase (Wheatly and Henry 1992, Freire *et al.* 2008) and therefore acid- base buffering capabilities have been linked to ionic and osmotic regulatory capabilities (Widdicombe and Spicer 2008). Lobsters possess limited osmo-regulatory abilities with Na^+/K^+ ATPase localised in epipodites and branchiostegites (Lucu and Devescovi 1999, Charmantier *et al*. 2001), which explains the limited up-regulation of Na^+/K^{+-}ATPase under elevated pCO_2 observed here, indicating that when exposed to hypercapnic water,

H. gammarus relies on internal stores of HCO_3^- for internal acid–base regulation indicating efficient internal ionic regulatory capabilities despite poor osmo-regulatory capabilities.

Haemolymph acid-base regulation, whether *via* carapace dissolution or *via* acquisition from the surrounding water, is an energetically expensive process, and there for may have energetic repercussions even in species which possess good regulatory capabilities.

5.4.3 The energetic consequences of haemolymph regulation

Haemolymph [HCO_3^-] regulation, whether achieved using external water sources or internal mineralised structures, is an energetically expensive processes dependant on the regulation of many ion exchange channels. Under 13 °C, lobsters effectively buffer haemolymph pH under both OA and CCS conditions, however there is no change in rates of oxygen consumption. Maintenance of internal homeostasis and processes under unfavourable conditions often results in costs associated with other aspects of organism performance and fitness (Calow and Forbes 1998). In this case, the demand for energy increases due to elevated buffering activity, however the supply of energy does not change, therefore energy used for bicarbonate buffering is being likely diverted from other processes (Whiteley 2011) such as activity and maintenance (Dissanayake *et al.* 2010, Dissanayake and Ishimatsu 2011), growth (Wickins 1984a) and reproduction (Kurihara *et al.* 2004), as seen in other phyla (Michaelidis *et al.* 2005, Wood *et al*. 2008, Beniash *et al.* 2010, Stumpp *et al.* 2012). Furthermore, feeding rates in juvenile lobsters exposed to OA and CCS conditions at 13 °C decreases, this indicates a decrease in energy intake and indicates that under conditions of elevated pCO_2 at current temperatures, lobsters are energetically limited.

Lobsters also appear to have a limited aerobic scope when it comes to temperature, which may explain the lack of buffering capacity at 17 °C. Rates of oxygen consumption of juveniles exposed to 17 °C were not different to rates of oxygen consumption measured in juveniles exposed to 13 °C. When crustaceans are above their thermal optimum, oxygen consumption is known to level off or even decrease as temperature increases. This pattern, which is observed here, indicates that 17 °C is above the optimum temperature for juvenile *H. gammarus* (Dehnel 1960, Schatzlein and Costlow 1978, Vernberg *et al.* 1981, Anger 1987, Storch *et al*. 2009a, b). The energetic demands of increasing temperature, and the lack of a response in oxygen consumption, indicates that juvenile *H. gammarus* are energy limited at 17 °C, and so have less available energy for acid–base regulation. Juvenile and adult life cycle phases are predicted to be more tolerant to environmental changes associated with climate change due to them having a wide aerobic scope (Pörtner and Farrell 2008) however here we show that this may not hold true for *H. gammarus*, as juveniles stages appear to be energetically limited under normal conditions, and have a limited aerobic scope with increased temperature.

To understand the basis for energy limitation mentioned here we can look at the general haemolymph physiology in terms or ionic content and biochemistry of juvenile lobsters compared to other crustaceans and adults.

5.4.4 Juvenile haemolymph physiology and biochemistry

The haemolymph physiology and biochemistry of juvenile lobsters assessed in the present chapter can partially explain the energy limitation with regards to exposure to elevated temperature and pCO_2. Compared to adult lobsters under normocapnic conditions, juvenile lobsters (wet body mass = 0.678 ± 0.02 g) exposed in the present

study to control conditions (13 °C, 450 µatm pCO_2, pH 8.1) exhibit substantially higher levels of haemolymph *L*-lactate and Mg^{2+}, but much lower levels of haemolymph pH, HCO_3^-, and Cu^{2+} (Taylor and Whiteley 1989, Whiteley and Taylor 1990). Compared to later stage juveniles (wet body mass = 40 – 50 g) haemolymph protein levels were severely reduced (Hagerman 1983). Despite low haemolymph pH, non-bicarbonate buffer lines were similar to those of adult lobsters (Taylor and Whiteley 1989), indicating that under normocapnic conditions juveniles are experiencing a severe metabolic acidosis (Fig. 5.4). This acidosis is most probably caused by a volatile metabolic end product which is not present in haemolymph once stored for later analysis of non–bicarbonate buffer lines, such as ammonia. Protein catabolism is the major source of energy for early juvenile lobsters (Capuzzo and Lancaster 1979) and therefore high levels of protein catabolism would result in high levels of haemolymph ammonia. Juvenile crustaceans do not possess effective regulatory capabilities of ions such as Mg^{2+} as seen in adults (Brown and Terwilliger 1992), and therefore may not possess effective ammonia excretion capabilities resulting in a metabolic acidosis of haemolymph. This notion is supported by the fact that juveniles exposed to the high pCO_2 levels associated with CCS leakage conditions feed less, and experience no metabolic acidosis as blood chemistry is on the non-bicarbonate buffer line (Fig. 5.4). Haemolymph *L*-lactate and Mg^{2+} levels are in comparison with those of less active, benthic crustaceans such as *C. pagurus* and the spiny spider crab, *Maja squinado* (Herbst 1788) (Watt *et al*. 1990). Watt *et al.* (1990) state that *L*-lactate levels of 1.5 – 2.5 mmol L^{-1} in *C. pagurus* and *M. squinado* respectively, compared to those of 1.1 mmol L^{-1} in *N. puber* are attributable to a higher anaerobic metabolism, coupled with low oxygen consumption and ventilation rates in *M. squinado*. Therefore, we assume that the high *L*-lactate levels in juvenile lobster described in this study, when compared

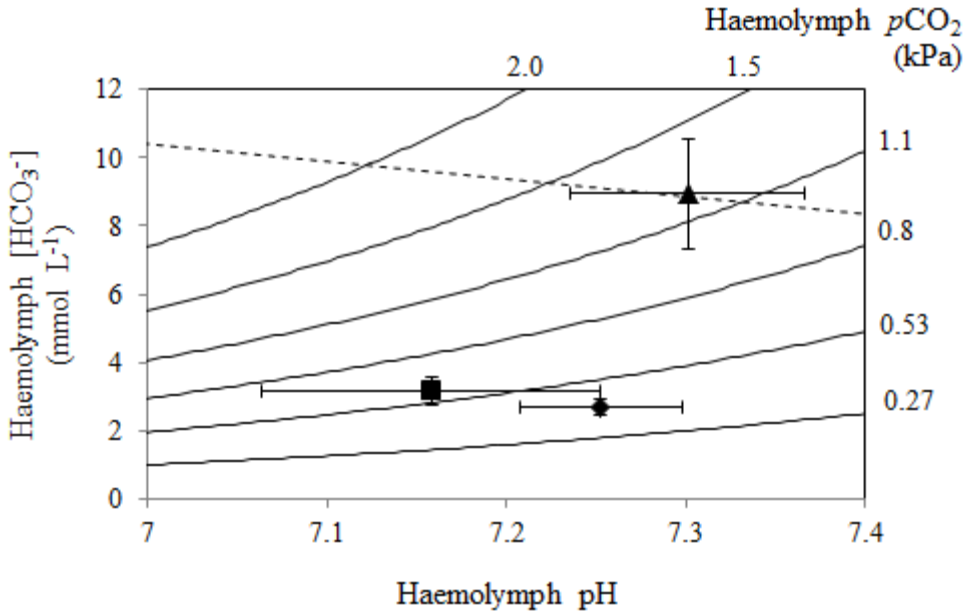

Figure 5.4 Davenport diagram of 1 year old juvenile *H. gammarus* after two week exposure to elevated pCO_2 at 13 °C (Means ± S.E., N = 24): Diamond indicates 450 µatm pCO_2, square indicates 1,100 µatm pCO_2, and triangle indicates 8,000 µatm pCO_2. Isopleths indicate haemolymph pCO_2 (kPa). Dashed line indicates haemolymph non bicarbonate buffering capacity.

to these crabs and to adult lobsters (Taylor and Whiteley 1989, Whiteley and Taylor 1990), may indicate that they possess higher levels of anaerobic metabolism. Interestingly, *L*-lactate levels were negatively correlated with feeding rates, indicating that individuals under control conditions, which feed well, have higher anaerobic metabolism than those under elevated pCO_2 levels where feeding is reduced, and therefore energy expenditure is also reduced.

This indicates that early juvenile *H. gammarus* were sensitive to environmental perpetrations related to elevated pCO_2 due to OA and CCS, elevated temperature due to ocean warming, and combinations of the two. Partially, this may be due to the fact that early juvenile marine crustaceans are still developing adult type regulatory mechanisms, and with limited energetic capacity they constitute a long lasting, sensitive transition

between early development and later juveniles/adults and therefore another potential bottleneck.

5.4.5 Conclusion

I conclude that juvenile stages of the European lobster, *H. gammarus*, are energetically limited under normal conditions due to the incomplete and on-going development of their regulatory systems and abilities, which make them sensitive to elevated oceanic pCO_2-related to OA (Caldeira and Wickett 2003, 2005) and extreme pCO_2 perturbations predicted to occur in situations of CCS leakages (Blackford *et al.* 2009, Kano *et al.* 2010). The energetic limitation of early juvenile lobsters results in decreased feeding rates under elevated pCO_2 at 13 °C due to energy being diverted for maintaining internal homeostasis. In addition, early juvenile lobsters have a limited aerobic scope with increasing temperature, and at 17 °C this results in energy limitation under elevated pCO_2 conditions and no ability to regulate internal acid-base balance. Such limitations will likely have severe consequences for this commercially and ecologically important species as energy limitation due to diverted resources for internal regulation may result in reduced growth, survival, and performance, while the limited aerobic scope with increasing temperature may result in geographic range shifts or restrictions.

Chapter 6

General discussion

6.1 Overview

The aim of the present thesis was to investigate the eco-physiological sensitivities and potential life history bottlenecks during larval and early juvenile development of the European lobster, *Homarus gammarus* (Linnaeus 1758), to elevated temperature and pCO_2 in lines with predictions of anthropogenic climate change. *H. gammarus* was chosen as a model organism due to its economic and ecological importance in northwest European waters, but also as our knowledge on the early larval development and the techniques for rearing large numbers of this species allows the opportunity to study the early development under elevated temperature and pCO_2 in detail.

The first objective of the thesis, addressed in Chapter 2, was to assess the sensitivity of lobster larval development, in terms of the key life history traits (survivorship and growth) together with physiological responses (oxygen consumption and organic content) to elevated temperature and pCO_2 in line with predictions of future ocean warming and OA (Caldeira and Wickett 2003, 2005, Sokolov *et al*. 2009). Chapter 2 then discusses how physiological sensitivities throughout larval development may be linked to changes in life history traits.

The second objective of the thesis, addressed in Chapter 3, was to investigate the morphometric development and carapace mineralisation of larval *H. gammarus* under conditions of elevated temperature and pCO_2, which allowed further discussion on the physiological sensitivities of larval development and the functional trade-offs which occur under unfavourable environmental conditions.

The third objective of the thesis, addressed in chapter 4, was to assess the sensitivity of survival, growth, and underlying physiological and functional biology of early benthic juvenile *H. gammarus* under elevated temperature and pCO_2 conditions. As in Objective 1, Chapter 2, this allowed for discussion of how aspects of physiological and functional biology can be linked to life history traits and potential life history bottlenecks.

The fourth objective of the thesis, addressed in Chapter 5, was to assess the ability of early juvenile *H. gammarus* to regulate internal acid-base balance under elevated temperature and pCO_2, and the energetic consequences of such physiological regulation.

The fifth and final objective of the thesis was to bring together the findings of Objectives 1-4 in order to discuss the developmental eco-physiology of *H. gammarus*, and the sensitivity of development and ontogeny of physiological functions under elevated temperature and pCO_2 conditions in relation to future predictions of ocean warming, OA, and in the case of juveniles, CCS leakage scenarios. This will be addressed in the present Chapter.

Such a detailed investigation into the responses of life history traits throughout early larval and juvenile development in relation to the underlying physiological development and functions allows a thorough overview of the developmental sensitivities of *H. gammarus* to elevated temperature and pCO_2. Using this information, a life history diagram has been constructed (Fig. 6.1) based on original comparisons between developmental stages and ecological phases by Wahle and Steneck (1992) and Cobb and Wahle (1994) to include physiological development in terms of the ontogeny of aerobic scope limitations and underlying haemolymph physiological biochemistry of juvenile stages and associated sensitivities to elevated temperature and pCO_2 in terms of physiological function and life history traits. This diagram will form the basis for the

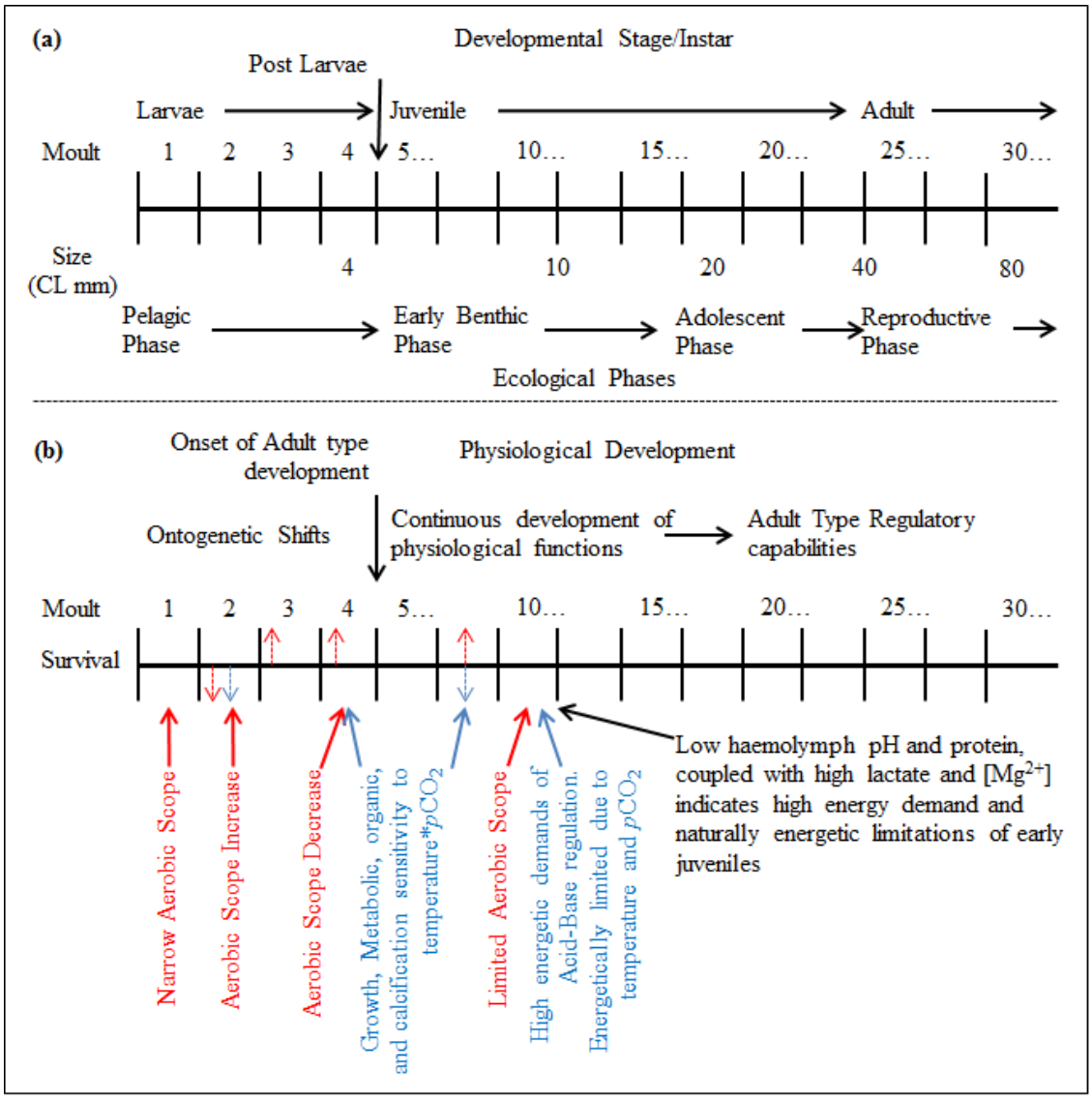

Figure 6.1 Time line of developmental events during the life history of *Homarus gammarus*: (a) Comparison of developmental stages and ecological phases, sourced from Cobb and Wahle (1994). (b) Adaptation to include aspects of physiological development. Red text and lines indicate ontogeny of aerobic scope. Blue text and lines indicate the effects of elevated temperature and pCO_2. Dashed arrows indicate the effects of temperature (Red) and pCO_2 (Blue) on the survivorship of *H. gammarus*. Up pointing arrows indicate positive comparative survival, and down pointing arrows indicate negative comparative survival.

following discussion on the effects of elevated temperature and pCO_2 on the ecophysiological development of *H. gammarus*.

6.2 Larval development of H. gammarus under elevated temperature and pCO_2

The physiological changes during the larval development of *H. gammarus* and their responses to elevated temperature and pCO_2 are complex (Fig. 6.1, Chapters 2 and 3). One of the physiological drivers throughout development is the ontogeny of aerobic scope, and hence the sensitivity of certain stages to elevated temperature and pCO_2. The aerobic scope of Stage I individuals, assessed by comparing the oxygen consumption between individuals reared at 17 °C and 21 °C is relatively narrow, indicated by a decrease in oxygen consumption at elevated temperature. This corresponds with a decrease in survival to Stage II. Between Stages I and II there is an ontogenetic shift in aerobic scope, as Stage II individuals exhibit signs of a wider aerobic thermal window and increased survival to Stage III. This trend continues throughout Stage III, resulting in increased survival to Stage IV under elevated temperature. At Stage IV, there is a further ontogenetic shift in aerobic scope, as aerobic thermal window appears to narrow once more, as temperature has no effect on oxygen consumption (Chapter 2). This is accompanied by temperature-related decreases in body mass (Chapter 2) and size (Chapter 3), representing energetic limitations under elevated temperature at Stage IV. Temperature is the major abiotic driver of crustacean larval development (Mackenzie 1988, Anger 1998, Anger 2001, Weiss *et al*. 2009a, b, 2010, Arnberg *et al*. 2013) and maximum growth and survival is usually found in narrow, optimum mid-range temperatures (Mackenzie 1988, Weiss *et al*. 2009a, b, 2010). Beyond these optimal windows, organisms become oxygen limited and therefore exhibit reduced scope for aerobic activity and increasing dependence on anaerobic metabolism (Frederich and

Pörtner 2000, Pörtner 2002, Pörtner and Farrell 2008). Marine crustacean larvae are known perform within similar limits of thermal aerobic scope (Storch *et al*. 2009a, b). As in Chapter 2, similar ontogenetic shifts in optimal temperature conditions for survival have been identified for a range of crustaceans, especially in combination with salinity (Costlow *et al.* 1960, 1962, 1966, Anger 1991, Agard 1999) and have been linked through exposure to elevated temperature and pCO_2 to changes in the aerobic thermal window of individual larval stages (Walther *et al.* 2010). Here, the ontogeny of physiological function, in this case aerobic scope, directly underlies stage specific survivorship and growth responses of *H. gammarus* larvae.

Changes in aerobic thermal windows can be seen to 'bridge the gap' between the effects of elevated temperature and pCO_2 on the larval development of *H. gammarus* in the present study. While there were no major significant interactions between elevated temperature and pCO_2, the two are linked through physiological processes. Pörtner and Farrell (2008) hypothesis that additional drivers such as hypoxia, or in this case hypercapnia, will force a narrowing of an organisms aerobic thermal window. This has been demonstrated in adults of the edible crab, *Cancer pagurus* (Linnaeus 1758) (Metzger *et al.* 2007) and has been proposed as the reason to why the great spider crab, *Hyas araneus* (Linnaeus 1758) megalopa appear particularly sensitive to elevated temperature and pCO_2 (Walther *et al*. 2010, 2011). In the present study, similar conclusions can be drawn to the sensitivity of Stages I and IV *H. gammarus*. The narrow aerobic scope exhibited by Stage I individuals is accompanied by decreased survival due to elevated pCO_2. Likewise, the narrow aerobic scope of Stage IV individuals is coupled by the increased oxygen consumption, alterations of organic content, and reductions in mineralisation due to elevated pCO_2 at elevated temperatures (Chapters 2 and 3). As in Walther *et al*. (2010, 2011) such responses indicate that Stage

IV, or the Megalopa stage, is a potential population bottleneck in relation to elevated temperature and pCO_2. However, we can expand upon this by including Stage I due to the decreased survival to Stage II being coupled with a narrow aerobic scope.

The response of *H. gammarus* to elevated temperature and pCO_2 during larval development seen in the present thesis can provide insight into the possible population effects of global change. Temperature-dependant limitations of aerobic scope will have consequences to species' geographical distributions (Pörtner 2002) and in relation to ocean warming, will have impacts on the southward distribution, and ability to disperse, of *H. gammarus* Stages I and IV larvae. Furthermore, Stage I is also susceptible to elevated pCO_2 with decreased survival at current temperatures, again potentially restricting the range of larval dispersal. Indeed, it is known that increased ocean temperature is causes a northward shift in decapod larvae (Lindley 1998) and associated benthic distributions (Hawkins *et al*. 2009).

6.3 On the transition between life stages

One recurring aspect of the responses of marine crustaceans to elevated pCO_2, whether alone or in concert with elevated temperature, is that the more sensitive stages of development is the transitory megalopa stage (Arnold *et al*. 2009, Walther *et al.* 2010, 2011). The megalopa stage represents a transition between pelagic larvae and benthic juveniles, and is characterised by large changes in behaviour, morphology, habitat, chemical composition, and physiology (Anger 1998, 2001, Fig. 6.1). The development of new physiological milestones has been identified as a potential cause of ontogenetic changes in environmental sensitivities during larval development (Agard 1999) and in the crustaceans the megalopa stage involves the development of new physiological structures and functions in relation to, for example, osmo- and oxy-regulation

(Charmantier *et al*. 1988, 1998, 2001, 2002, Thuet *et al*. 1988, Charmantier and Charmantier-Daures 1991, Charmantier 1998, Tankersley and Wieber 2000, Spicer and Eriksson 2003, Cieluch *et al*. 2004). Physiological changes and the energetic requirements of undergoing such changes, therefore, may be one reason as to why the megalopa stage of crustacean development has a narrow aerobic thermal window and is a potential developmental bottleneck under future predictions of ocean warming and OA (Nasrolahi *et al*. 2013). The temperature-related decrease in body size and aerobic performance of Stage IV *H. gammarus*, along with the changes in organic content ratios and mineralisation due to elevated pCO_2 as shown in the present study will have implications for future populations as the physiological conditions along with survivorship, body mass, body size, and time of settlement, have profound effects not only on recruitment to juvenile populations but the performance, growth, and survival of later juveniles (Incze and Wahle 1991, Wahle and Steneck 1991, 1992, Wahle 1992, Cobb and Wahle 1994, Jarrett and Pechenik 1997, Qiu and Qian 1999, Beckerman *et al*. 2002, Pechenik *et al*. 2002, Jarrett 2003, Gimenez *et al*. 2004, Nasrolahi *et al*. 2012).

6.4 The physiological development of early benthic juveniles under elevated temperature and pCO_2

The third objective of the present thesis, in Chapter 4, was to assess the sensitivity of early benthic juvenile *H. gammarus* to elevated temperature and pCO_2 in terms of life history traits and the development/response of underlying physiological processes, while the fourth objective, in Chapter 5, was to assess the energetic consequences of HCO_3^- regulation of early benthic juveniles exposed to elevated temperature and pCO_2. Figure 6.1 highlights the ecological distinction between early and late juvenile marine invertebrates (Wahle and Steneck 1991, Cobb and Wahle 1994) which appears to be

coupled with a physiological distinction due to the continuous development of physiological functions during juvenile phases of marine crustaceans (Incze and Wahle 1991, Brown and Terwilliger 1992, Newton and Potts 1993, Terwilliger and Brown 1993, Charmantier 1998, Charmantier *et al.* 1998, 2001, 2002, Terwilliger and Dumler 2001, Terwilliger and Ryan 2001, Spicer and Eriksson 2003;, Cieluch *et al*. 2004). This distinction has yet to be made with regards to the effects of elevated temperature and pCO_2 due to future predictions of ocean warming and OA, however Green *et al*. (2004) distinguished the differences between mortality due to shell dissolution in under-saturated sediments of early and late juvenile hard clams, *Mercenaria mercenaria* (Linnaeus 1758). This indicates that assessing the sensitivities of early juvenile stages, rather than the juveniles as a whole will provide a more holistic insight into the effects of elevated temperature and pCO_2 on future populations.

In Chapter 4, after 5 weeks exposure, elevated temperature increased the growth, oxygen consumption, and feeding rates in 6 month old early benthic lobsters. The growth and metabolism of marine invertebrates' increases with temperature until threshold levels are reached (Mackenzie 1988, Frederich and Pörtner 2000, Pörtner 2001, 2002, 2010, Pörtner and Farrell 2008). Therefore the increases in growth and metabolism due to elevated temperature in Chapter 4 would be expected, as individuals were acclimated to winter temperatures, and so elevated temperature is still within the tolerance range of this species. However, 'mild' elevated pCO_2 in relation to OA resulted in a decrease in oxygen consumption and feeding rates at both temperatures. Similar decreased oxygen consumption, and so metabolism, is a response to unfavourable environmental conditions including elevated pCO_2 possibly to preserve ATP (Reipschlager and Pörtner 1996, Langenbuch and Pörtner 2002, Small *et al*. 2010, Melatunan *et al*. 2011). Such decreased metabolism will come at a cost of reduced

aerobic scope and energy availability for routine activity (Dissanayake and Ishimatsu 2011) as seen by the decrease in feeding in Chapter 4. For early benthic juvenile *H. gammarus* there are increased energetic demands due to elevated pCO_2, which ultimately resulted in a slight increase in mortalities due to moult complications. The mineralisation response was more complicated. Under control temperatures there was no change in carapace mineral content due to elevated pCO_2 in line with OA predictions, however there were decreases in carapace [Ca^{2+}], [Mg^{2+}], and [Sr^{2+}] under elevated temperature and pCO_2. Interestingly, individuals who moulted under such conditions were able to hyper-calcify, and regain 'lost' minerals. Increased mineralisation under elevated pCO_2 has been documented in a range of marine crustaceans (Wickins 1984b, Ries *et al*. 2009, Ries 2011) and may be due to the increased availability of CO_2 for post moult exoskeleton regeneration (Cameron and Wood 1985b, Wood and Cameron 1985) however may come with additional costs in terms of growth and maintenance (Wood *et al.* 2008, Findlay *et al*. 2010b, Melatunan *et al*. 2011). Elevated pCO_2 in line with CCS predictions, on the other hand, resulted in no growth, high mortality rates, and severe shell dissolution possibly due to HCO_3^- regulation (Henry *et al.* 1981, Taylor and Whiteley 1989, Whiteley and Taylor 1990, Whiteley 1999). The findings from Chapter 4 indicate a range of complex responses to elevated temperature and pCO_2 of early benthic juvenile *H. gammarus*; however all of these responses are related to aerobic performance and internal acid–base regulation/disturbances, which were assessed in Chapter 5.

Early benthic juvenile *H. gammarus* individuals were able to effectively regulate internal acid-base balance under control temperatures of 13 °C when exposed to both mild and extreme hypercapnia. Such regulation involved increased haemolymph [HCO_3^-] to buffer decreased pH due to elevated pCO_2, as is common amongst marine

crustaceans (Cameron 1978, 1985, Truchot 1979, Henry *et al*. 1981, Wood and Cameron 1985, Taylor and Whiteley 1989, Whiteley and Taylor 1990, Whiteley 1999, Spicer *et al.* 2007, Dissanayake *et al.* 2010, Small *et al*. 2010, Whiteley 2011). There were, however, energetic consequences of such regulation as there was no response in terms of oxygen consumption and a decrease in feeding rates suggesting a miss-match in energy supply and increased demand, and so energy is diverted from activity (Dissanayake and Ishimatsu 2011). Under elevated temperature, 17 °C, individuals were not able to regulate internal acid-base balance due to elevated pCO_2 and experienced internal acidosis. The breakdown of buffering capacity due to elevated temperature can be explained by the apparent limited aerobic scope of early benthic juveniles, as oxygen consumption does not increase with increasing temperature, suggesting that there is once more a mismatch between oxygen supply and demand due to elevated temperature, resulting in the in ability to compensate for internal acidosis. Such elevated temperature and pCO_2 responses in energetics and acid-base regulation shown in Chapter 5 can explain the acid-base and energetic responses seen in Chapter 4, highlighting the energetic demands of juvenile development. The reason for this energetic state in juvenile *H. gammarus* can be seen through the assessment of haemolymph biochemistry in Chapter 5, which provides an insight into the ontogeny of physiological functions during early juvenile development. Early benthic juvenile *H. gammarus* haemolymph biochemistry is defined, in comparison with similar data from adult lobsters (Taylor and Whiteley 1989, Whiteley *et al.* 1990, Whiteley and Taylor 1990), by high levels of Mg^{2+}, but low levels of protein and Cu^{2+} (indicating haemocyanin). This indicates that the physiological development of haemocyanin structure and function and Mg^{2+} regulation is not complete in early benthic juvenile *H. gammarus* as is also seen in the dungeness crab, *Cancer magister* (Dana 1852) (Brown and Terwilliger 1992,

Terwilliger and Brown 1993, Terwilliger and Dumler 2001, Terwilliger and Ryan 2001). Furthermore, high levels of haemolymph *L*-lactate and low haemolymph pH in control individuals can indicate high levels of uncompensated anaerobic metabolism (Watt *et al*. 1990), once more highlighting the incomplete development of physiological functions in early benthic juvenile *H. gammarus* compared to adults (Taylor and Whiteley 1989, Whiteley *et al*. 1990, Whiteley and Taylor 1990) and the energetic demands of this developmental stage seen in Chapters 4 and 5. Overall, the findings from Chapters 4 and 5 indicate that the early benthic phase of juvenile development in *H. gammarus* is particularly sensitive to abiotic factors such as elevated temperature and pCO_2 due to global change. Coupled with the high mortality of early benthic invertebrates (Gosselin and Qian 1997) this may prove to be a further life history bottleneck under global change.

6.5 Conclusions and future perspectives

This thesis has assessed the developmental eco-physiology of *H. gammarus* throughout larval and early juvenile life stages, and has characterised the responses of developmental stages to elevated temperature and pCO_2 through linking physiology to life history traits. The early development of *H. gammarus* is sensitive to elevated temperature and pCO_2, and the main driver appears to be the ontogeny of aerobic scope throughout development. As aerobic windows narrow at specific stages, individuals become sensitive to elevated pCO_2 especially at elevated temperature. Similar insights have been documented for *H. araneus* larvae (Walther *et al*. 2010, 2011) suggesting that the underlying physiology of individual larval stages during crustacean development must be considered in order to gain a holistic view into the effects of elevated

temperature and pCO_2 on larval dynamics and potential life history bottlenecks and population responses to global change drivers.

Furthermore, this study also highlighted the sensitivity to elevated temperature and pCO_2 of early juvenile stages of *H. gammarus* development. It documents how the development of physiological processes during and energetic demands of juvenile development result in increased sensitivity to abiotic factors through energy limitation and narrow aerobic thermal windows. This provides a link between the high mortality experienced by early juvenile populations (Gosselin and Qian 1997) and the ecological distinctions between early and late juvenile individuals (Wahle and Steneck 1991, Gosselin 1997) with the continuous development of physiological functions.

Future studies into the development of marine invertebrates under elevated pCO_2 and temperature need to take into account stage specific changes in the ontogeny of physiological functions, and how these can translate into changing sensitivity in terms of survival and growth between not only life history stages, but individual aspects of these stages such as individual larval stages or the distinction between early and late juveniles. Utilising such linkages between physiological development of marine invertebrates and life history traits will allow for more holistic predictions to be made regarding population responses to global change drivers such as ocean warming and ocean acidification, and will highlight potential developmental bottlenecks which will have profound effects on the success of future populations.

6.6 Critique

Reflecting upon the experimental methods and procedures I used during my PhD, there are a few points which can be highlighted for improvement which may be useful for

future invetigations in this field. This reflection was prompted largely by discussions that took place during my viva voca examination, and so have been added to the thesis in making minor corrections to the text.

Rearing a large number of lobster larvae to ensure aqequate survival is not a trivial task. Although numberous trials were conducted on the larval experimental system, higher overall survival rates could have been obtained with further adjustments to the experimental system such larger rearing cones which would allow more individuals to be introduced at the start of the experiments and more space per individual, as larval success in hatchery environments can be space dependant. With such improvements to survival would come increased scope for more indepth analysis of metabolic and energetic effects of climate change drivers, e.g. the production of more replicates for additional proxies such as energetic content (e.g. bomb calorimetry techniques) and ontogeny of aerobic scope related enzyme activity (e.g. citrate synthase). A similar argument can be made for increased numbers of individuals during juvenile trials, which would also provide higher replicates for additional proxies.

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