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10, 7579–7615, 2013

**Deformities in larvae
and juvenile
European lobster**

A.-L. Agnalt et al.

Deformities in larvae and juvenile European lobster (*Homarus gammarus*) exposed to lower pH at two different temperatures

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Received: 31 March 2013 – Accepted: 9 April 2013 – Published: 2 May 2013

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Published by Copernicus Publications on behalf of the European Geosciences Union.

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Abstract

Trends of increasing temperatures and ocean acidification are expected to influence benthic marine resources, especially calcifying organisms. The European lobster (*Homarus gammarus*) is among those species at risk. A project was initiated in 2011 aiming to investigate long-term synergistic effects of temperature and projected increases in ocean acidification on the life cycle of lobster. Larvae were exposed to $p\text{CO}_2$ levels of ambient water (water intake at 90 m depth, tentatively of $380 \mu\text{atm } p\text{CO}_2$), 727 and $1217 \mu\text{atm } p\text{CO}_2$, at temperatures 10 and 18°C . Long-term exposure lasted until 5 months of age. Thereafter the surviving juveniles were transferred to ambient water at 14°C . At 18°C the development from Stage 1 to 4 lasted from 14 to 16 days, as predicted under normal pH values. Growth was very slow at 10°C and resulted in only two larvae reaching Stage 4 in the ambient treatment. There were no significant differences in carapace length at the various larval stages between the different treatments, but there were differences in total length and dry weight at Stage 1 at 10°C , Stage 2 at both temperatures, producing larvae slightly larger in size and lighter by dry weight in the exposed treatments. Stage 3 larvae raised in 18°C and $1217 \mu\text{atm } p\text{CO}_2$ were also larger in size and heavier by dry weight compared with $727 \mu\text{atm } p\text{CO}_2$. Unfortunate circumstances precluded a full comparison across stages and treatment. Deformities were however observed in both larvae and juveniles. At 10°C , about 20% of the larvae exposed to elevated $p\text{CO}_2$ were deformed, compared with 0% in larvae raised in pH above 8.0. At 18°C and in high $p\text{CO}_2$ treatment, 31.5% of the larvae were deformed. Occurrence of deformities after 5 months of exposure was 33 and 44% in juveniles raised in ambient and low $p\text{CO}_2$, respectively, and 20% in juveniles exposed to high $p\text{CO}_2$. Some of the deformities will possibly affect the ability to find food, sexual partner (walking legs, claw and antenna), respiration (carapace), and ability to swim (tail-fan damages).

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1 Introduction

The world's atmosphere is increasingly becoming more saturated with the concentration of CO₂ as carbon emissions from burning fossil fuels keep increasing (IPCC, 2007; Caldeira et al., 2005). Atmospheric CO₂ is currently around 380 ppmv, but is predicted to increase to 780 ppmv and 1200 ppmv by 2100 and 2200 respectively. Increased absorption of atmospheric CO₂ into the marine environment leads to an increase in total dissolved inorganic carbon (DIC), which changes the chemistry and acid-base balance resulting in a decreased seawater pH (Dickson et al., 2007). Currently the global average sea water pH is about 8.05 units and is associated with a DIC of 2026 (Fabry et al., 2008). It is predicted to drop by 0.3 to 0.4 pH units by 2100 (Feely et al., 2009). At the same time, ocean temperature is predicted to increase by 2–4 °C by 2100. The absorption of CO₂ by the ocean and ocean acidification (OA) occurs mostly in the upper 100 m, and varies with latitude and temperature (Orr et al., 2005; Fabry et al., 2008). Regional monitoring of DIC at Station M in the eastern Norwegian Sea, shows a value of 2140 (Skjelvan et al., 2008), which is above the global average of 2026. This agrees with recent modeling that CO₂ uptake is higher and pH lower (7.84) in the eastern Norwegian Sea which has cold waters of Atlantic origin (Olsen et al., 2006). Low pH levels will most likely decrease the depth at which calcium carbonate becomes undersaturated, from ~100 m to 50 m by 2100 at high latitudes (Fabry et al., 2008). The Arctic region is already warming up and the warming is predicted to increase further. Marine organisms in colder regions are therefore at a greater risk of being affected by the effects of both warming and OA, especially calcifying marine organisms. One such species is the European lobster *Homarus gammarus*.

H. gammarus is found along the continental shelf in the northeast Atlantic, extending from the warm waters off Morocco to the colder areas near the Arctic Circle i.e. Tysfjord and Nordfolda (68° N) (Agnalt et al., 2009). This distribution covers a large latitudinal and temperature range. With CO₂ absorption varying greatly with latitude and temperature, the effects of acidification may vary greatly for subpopulations across the

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distribution range. The life cycle of *Homarus* consists of 4 larval stages, a juvenile stage, a sub-adult stage (~ 50 mm carapace length CL) and adult of > 60 mm CL (Factor, 1995). Larvae are pelagic in the three first stages (stage 1–3), after which they settle during larval stage 4. Little is known about the benthic stages of *H. gammarus* juveniles less than 40 mm CL in the wild (Linnane et al., 2001). The European lobster is esteemed as a valuable marine resource, and has supported the coastal fishery in northern Europe for several centuries (Agnalt, 2008). In the 1960s, lobster populations in Norway were depleted below sustainable levels and due to low recruitment the recovery has been slow. Any additional factors that reduce recruitment and population size further may push this species to the brink of extinction in these areas.

Only one study has investigated effects of OA ($p\text{CO}_2$) in *H. gammarus*, focusing on larvae at the predicted future scenario of $p\text{CO}_2$ 1200 μatm (Arnold et al., 2009). Calcification (Ca and Mg) was significantly reduced in Stages 3 and 4, with no direct effect on growth observed. The decrease in calcification observed in Stage 4 at a $p\text{CO}_2$ of 1200 μatm may have been due to energy being channelled towards growth and possibly acid-base regulation (Arnold et al., 2009). Growth in lobsters takes place through the process of moulting, occurring more frequently in juveniles compared with adults. Moulting is highly temperature dependent, making juveniles highly vulnerable to temperature changes (Waddy et al., 1995). Moulting also involves depositing of CaCO_3 to harden the shell which is energetically costly and therefore puts great physiological stress on the animal. Low pH resulting from OA increases physiological stress and may be devastating to moulting juveniles already under metabolic stress. Warmer temperatures, predicted to co-occur with increased OA, may have its added metabolic stress on juvenile lobsters, as seen in the crab *Hyas araneus*, if thermal tolerance limits are exceeded (Walther et al., 2010). *Homarus americanus* juveniles increased calcification by 600 % under very high $p\text{CO}_2$ levels ($p\text{CO}_2$ 2800 μatm) for 60 days but at a very high cost to survival (Ries et al., 2009). The increase in calcification are thought to be initiated by actively increasing pH at the calcifying centres, therefore reducing H^+ and converting bicarbonate (HCO_3^-) to carbonate (CO_3^{2-}). CO_2 induced acidosis in

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crustaceans is usually compensated for by increasing bicarbonate production (Truchot, 1978; Pörtner et al., 2004; Spicer et al., 2007). Bicarbonate production is energetically costly and may be reduced over the long term if energy reserves are low. Trends in climate change indicate that warming and OA will be most pronounced in colder regions. The one study on OA in *H. gammarus* and studies concerning other crustacean species suggest different impacts occurring in different stages of the life cycle. The synergistic effects of warming and OA on the life cycle of *H. gammarus* (and other lobster species) are unknown and urgently needs to be studied. A project was therefore initiated in 2011 aiming to investigate long-term synergistic effects of temperature and projected increases in ocean acidification on the early life cycle of lobster, i.e. the larval and juvenile phase of *H. gammarus*.

2 Material and methods

Experiments combining OA with temperature were conducted at IMR-Matre (60°52' N, 05°35' E) over a period of five months, lasting from 28 September 2011 to 22 March 2012. Raw water was pumped from 90 m depth, water intake located close to the field station, thus representing ambient water. Each experimental unit consisted of a 400 L tank of 0.87 × 0.87 × 0.53 m. Six of these units were used for the ambient, medium and high $p\text{CO}_2$ treatment run at two temperatures. For the larval rearing, two 40 L incubators (Hughes kreisel) were placed in each unit (Fig. 1a). For on-growing, the juveniles were kept individually in trays, placed in the experimental units (Fig. 1b). From 5 months of age, the surviving juveniles were continued monitored but under ambient conditions at 14 °C, until 25 October 2012 of which they were about 1 year old. The larvae and juveniles were all kept in 8 to 10 hours light and 16 to 18 h dark.

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2.1 Water quality

The salinity during the course of the experiment was on average 33.7 ± 0.2 ppt. Temperature was run at 10°C and 18°C , to simulate a lower limit and an optimum threshold for homarid lobsters, respectively (Wickins and Lee, 2002; Kristiansen et al., 2004). Initially a middle temperature of 14°C was also included, but due to a limited number of larvae this had to be excluded. The experiments were run at ambient water, believed to be at current $p\text{CO}_2$ of $380 \mu\text{atm}$ occurring in the natural oceanic environment, and treatments with medium $p\text{CO}_2$ of approximately $750 \mu\text{atm}$ and with high $p\text{CO}_2$ aiming to be $1200 \mu\text{atm}$, to simulate low and medium OA scenarios predicted for 2100 and 2200, respectively. In the medium $p\text{CO}_2$ treatment the actual values were $727 \pm 12 \mu\text{atm}$, and correspondingly $1217 \pm 134 \mu\text{atm}$ in the high $p\text{CO}_2$ treatment (Table 1), combined for both temperatures. CO_2 was bubbled into two enclosures, using 2×2 AGA 25 kg gas bottles, providing a mixture of ambient air and pure CO_2 . The flow rates were regulated by controllers to be able to reach the desired seawater pH (pH = 8.06 in ambient treatment, pH = 7.79 in medium $p\text{CO}_2$ treatment and pH = 7.62 in high $p\text{CO}_2$ treatment). Water from the enclosures were supplied to and circulated through the experimental tanks. Temperature, pH and oxygen were monitored continuously in each experimental tank by probes connected to a computer. The pH was measured every minute using Orbisint CPS11D from Endress+Hausser electrodes, using the National Bureau of Standards (NBS) scale. To calibrate the pH, a spectrophometry Hitachi U-2900 connected to Refrigerated Heating Circulator Julabo F12 combined with temperature sensors TD301A from SAIV A/S was used. Samples for analysis of seawater content CO_2 in μatm , total alkalinity (A_T) and total dissolved inorganic carbon (C_T) were collected in 350 mL amber glass bottles with minimal headspace. 300 μL of saturated H_9Cl_2 solution was added to preserve the sample. Aragonite and calcite saturation state (Ω) and pH on the total hydrogen ion scale (pH_T) were then calculated.

Unfortunately, the water quality in ambient water, i.e. intake at 90 m depth, did not stay stable. At 10°C the pH in the larval experiments was on average 8.01 from 28

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September until 11 November then dropped to 7.92 and even down to 7.84 by the end of the larval period (Fig. 2). In ambient water at 18° the pH in the larval phase was relatively stable (7.80 ± 0.02). In other words, pH in ambient water was low and did not account as a control in any of the larval experiments, except in the early phase of the larval period at 10 °C. pH was stable in the experiments run at medium and high $p\text{CO}_2$, with only a few outliers (Fig. 2). In the juvenile experiments, pH in ambient water was also lower than expected, corresponding to $706 \pm 7 \mu\text{atm } p\text{CO}_2$ (Fig. 3). In these experiments there was no true control either, since the water quality in the ambient water was at the same level as in the medium $p\text{CO}_2$ treatment. Despite this, the term ambient has been kept throughout the paper. In the experiments monitoring the surviving juveniles from the exposed treatments grown in ambient water until 8 months of age, pH varied around 7.95 to 7.96 (data not shown).

2.2 Brood stock

Ovigerous females were collected from the *H. gammarus* population in Øygarden (60°35' N, 4°50' E) during the commercial fishing season September to October 2011, and transported to IMR-Matre. They were acclimatized at 6 °C in individual 75 L tanks (52 × 52 × 28 cm) in a CT room with the lighting set to a 12 h dark: 12 h light cycle. Lobsters were fed frozen shrimps and fish twice a week. The CO_2 -control system was operative from early October 2011, and the hatching was postponed accordingly. In early September the temperature was slowly increased to 18 °C, to induce hatching that commenced 28 September 2011. Of a total of 14 females, only 4 had eggs that hatched during the experimental period. The sizes of the ovigerous females were 91, 110, 113 and 135 mm carapace length (CL; measured as the distance from the posterior rim of the eye socket to the posterior edge of the carapace). The females hatched 15 000 larvae in total.

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2.3 Larval rearing and sampling

Each of the individual tanks with the ovigerous females had an overflow through a 20 mm water hose leading to separate containers equipped with a filter to retain the hatched larvae. Larvae normally hatch during late night/dawn and were collected from the outflow containers each morning, counted and transferred to the 40 L upstream incubators (plankton Kreisler; Hughes et al., 1974). The incubators were supplied with 11 L per minute sea water. The larvae were fed daily with frozen *Artemia* sp. Larvae hatching over a period of 3 days were mixed in the same incubator. Larvae with larger difference in age were not mixed due to increased risk of cannibalism. Maximum density for each incubator was set to 1000 larvae, or 25 larvae per litre. Each treatment i.e. temperature and CO₂ had two replicates. Every third day the incubators were treated with Chloramid-T to control growth of the bacterium *Leucothrix mucor*, as previously experienced in other lobster rearing systems (D. Boothroyd, National Lobster Hatchery, Padstow UK, personal communication 2009). The larvae were staged (1 to 4), according to Sars (1875) and Herrick (1909). A total of 10 larvae at each development stage (1 to 4) from each incubator (parallels) in each temperature and pCO₂ treatment were collected for measurement of CL, total length (TL; measured from the tip of rostrum to the end of telson) and dry weight. The stage 1 larvae had been exposed for a minimum of three days before sampling. All measurements of CL and TL were recorded using a dissecting microscope. Dry weights of individual larvae were recorded after 3 days of drying in Termaks dry oven at 60 °C, and recorded to closest microgram (µg) using Mettler Toredon scale (AG204 Delta Range). Care was taken to look for intermediate stages, as this has been observed in American lobster *H. americanus*, especially between stage 2 and 3 and between stage 3 and 4 (Templeman, 1936; Wells and Sprague, 1976; Charmantier and Aiken, 1987). This has also been observed in hybrids i.e. offspring from female American lobster and male European lobster (A. L. Agnalt, personal communication, 2010). Unfortunately, the sampling program did not go as scheduled. The freezer containing many of the later larval stages

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broke at a point, and the samples decayed and could not be processed further. This resulted in a much lower sampling number for each larval stage than first planned. In total 409 larvae have size recordings, 185 for the experiments run at 10 °C and 224 at 18 °C giving on average 20 larvae for each treatment.

2.4 Long-term exposure of juveniles; five months

While still pelagic, stage 4 larvae were collected one by one and transferred to trays consisting of single-cell compartments, made of black PVC plastic. Each tray consisted of 30 to 40 individual compartments with perforated bottoms (1 mm × 1 mm holes) to ensure water flow. Three to four trays were placed together in 400 litres units (87 × 87 × 53 cm). Each unit was given water quality according to ambient, medium $p\text{CO}_2$ or high $p\text{CO}_2$ treatment. Water flow was set to 18 L per minute. The lobster juveniles were fed special commercially produced pellets (2 mm), patented by Norwegian Lobster Farm (<http://www.norwegian-lobster-farm.com/no>) produced by Nofima (<http://www.nofima.no/>). On 22 March, TL and CL were recorded for each of the surviving juveniles.

2.5 Continued monitoring of surviving juveniles in ambient water until 1 yr old

From five months, the juveniles were all kept in ambient water i.e. the water quality in the raw water intake, at 14 °C from 22 March to 25 October 2012. The purpose was to verify if the deformities were retained or lost through moulting when kept in water quality of higher pH, as described in 2.1 pH varied between 7.95 and 7.96. The lobster juveniles were fed special commercially produced pellets (5 mm), patented by Norwegian Lobster Farm (<http://www.norwegian-lobster-farm.com/no>) produced by Nofima (<http://www.nofima.no/>). At the end of the experiment, CL and TL were recorded for each surviving juvenile.

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2.6 Statistics

To determine any significant difference between lobsters undergoing the different $p\text{CO}_2$ and temperature treatments, two-way analysis of variance (ANOVA) was used.

3 Results

3.1 Growth

Irrespective of $p\text{CO}_2$ levels, growth was very slow at 10°C , and after 5 weeks none of the larvae moulted into Stage 4. Eventually, only two larvae reached Stage 4, in ambient treatment, but died within the following two days. At 18°C , development from Stage 1 to 4 lasted from 14 to 16 days independent of $p\text{CO}_2$ treatment, as predicted under normal pH values. Of the 450 larvae investigated, none were found in intermediate stages.

There were no significant differences in CL in larvae in the same development stage between different $p\text{CO}_2$ and temperature treatments (all gave $p > 0.01$, ANOVA, Fig. 4). There were significant differences in TL between $p\text{CO}_2$ treatments at Stage 1 and Stage 2 at 10°C ($p < 0.05$, $F = 6.35$ and $F = 5.27$ respectively ANOVA), and high $p\text{CO}_2$ treatments produced slightly larger larvae. Dry weight also differed significantly between $p\text{CO}_2$ treatments at these stages ($p < 0.05$, $F = 4.6308$ and $F = 6.123$, respectively, ANOVA). Larvae raised in ambient water were lighter in weight at stage 1 and heavier at stage 2 compared with the treated larvae (Fig. 4). At 18°C , TL and dry weight differed significantly at stage 3 comparing high $p\text{CO}_2$ treatment with medium and ambient ($p < 0.05$, $F = 11.38$ for TL and $F = 13.44$ for dry weight ANOVA), also producing slightly larger and heavier larvae. There were no significant differences in TL, or dry weight comparing stage 3 from medium $p\text{CO}_2$ treatment with ambient ($p > 0.05$, ANOVA).

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As only larvae raised at 18 °C successfully moulted into Stage 4, this was the only temperature where long-term exposure to $p\text{CO}_2$ could be conducted. After 5 months of exposure 148 juveniles had survived. There were significant differences in CL as a result of $p\text{CO}_2$ treatment ($p < 0.05$, $F = 18.4$ ANOVA, Fig. 5), but the sample size was however low at the medium $p\text{CO}_2$ treatment ($N = 16$), accounting for the difference since there were no significant differences between ambient and high $p\text{CO}_2$ treatment ($p = 0.109$, $F = 1.38$ ANOVA).

3.2 Deformities

Deformities i.e. a difference in the shape of a body part or organ compared to the average or normal shape was found in both the larvae (Table 2) and the juveniles (Table 3). The morphological abnormalities in the larvae were classified as curled carapace (Fig. 6), damages to the tail fan or that the rostrum was bent or curved (Table 2). No larvae suffered multiple deformities. The most affected part was curled carapace, occurring in 59 % of the deformed larvae when combining all treatments. A bent rostrum was found in 27 %, and damages to the tail fan in 14 % of all the deformed larvae.

In the ambient treatment at 10 °C (Fig. 7), two Stage 2 larvae had developed deformities (tail-fan damages and bent rostrum). However, these larvae had been hatched 9 November and sampled 22 November, i.e. at the period when pH dropped. In the medium $p\text{CO}_2$ treatment, at 10 °C, 19 % of the larvae were deformed. Curled carapace represented 45 % of the deformations, followed by damages to the tail (33 %) and a bent rostrum (22 %). Concurrently, approximately 20 % of the larvae raised in high $p\text{CO}_2$ were misshaped, with curled carapace as the only deformity. At 18 °C, 12 % of the larvae raised in the ambient treatment were deformed (Fig. 7), with a bent rostrum (33.3 %), curled carapace (33.3 %) or damages to the tail fan (33.3 %). Concurrently, at medium $p\text{CO}_2$, 22 % of the larvae were misshaped. Half of the deformed larvae had a bent rostrum, 38 % with a curled carapace and 12 % had damages to the tail fan. As much as 31.5 % of the larvae exposed to high $p\text{CO}_2$ were misshaped, all with a curled carapace.

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Of the 148 juveniles that survived after five months, 40 were classified as morphologically deformed (see Table 2). In overall, 33 and 44 % of the juveniles in ambient (i.e. medium $p\text{CO}_2$) and medium $p\text{CO}_2$ treatments respectively, were deformed compared with 21 % in juveniles exposed to high $p\text{CO}_2$ (Fig. 8). In ambient and medium $p\text{CO}_2$ treatment, deformed claws were most often found (56 %), followed by stiff/twisted walking legs (39 %) and puffy carapace (39 %). When the juveniles from ambient and medium $p\text{CO}_2$ treatment had deformed claw(s) about 54 % had also developed stiff/twisted walking legs. In comparison, at high $p\text{CO}_2$, 71 % of the deformed juveniles had developed a puffy carapace. Of these, 24 % had also developed deformed claws. In overall, about 50 % of the juveniles from the ambient and medium $p\text{CO}_2$ treatment had two or three different abnormalities (Fig. 10). At high $p\text{CO}_2$ treatment 70 % of the juveniles had multiple deformities.

At one year age 76 of the 148 juveniles had survived. Mortality was equally high for those juveniles that had been exposed until 5 months in ambient or in high $p\text{CO}_2$. Overall, 28 % of the juveniles were deformed. The most common occurring deformities (28 %) were puffy carapace with stiff/twisted walking leg as illustrated in Fig. 10. Of the 40 deformed juveniles found 22 March 2012, only 12 had survived another seven months. Of these, six were still deformed (four of the juveniles even developed at least one additional deformity). In other words, 50 % of the survivors had managed to recuperate, most likely through moulting. However, of the 108 juveniles there were classified as normal at five months of age, 15 had seven months later developed deformities.

4 Discussion

Deformities were observed both in the larval and juvenile phase when exposed to higher $p\text{CO}_2$. As much as 31 % of the larvae were deformed when raised in 18 °C and exposed for high level $p\text{CO}_2$ of around 1217 μatm . At 10 °C only 20 % were deformed in the same $p\text{CO}_2$ treatment. After five months of exposure, 44 % of the juveniles at medium $p\text{CO}_2$ were deformed, compared with 20 % in high $p\text{CO}_2$. At high exposure,

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70% of the deformed juveniles had developed multiple deformities. Deformities in lobster larvae and juveniles have not previously been reported, neither in European or American lobster, although larvae and juveniles have been produced for several experimental studies, including ocean acidification and stock enhancement (Gruffydd et al., 1975; Capuzzo and Lancaster, 1979; Latrouite and Lorec, 1991; Addison and Banister, 1994; Uglem et al., 1995; Agnalt et al., 1999, 2001; Nicosia and Lavalli, 1999; Linnane et al., 2000; Jørstad et al., 2001, 2005; Wickins and Lee, 2002; Kristiansen et al., 2004; Agnalt, 2008; Arnold et al., 2009, Ries et al., 2009, Schmalenbach et al., 2009).

A major concern with the present study is the lack of control. It was only in the first part of the larval phase of the 10 °C that had a true control in place. No larvae raised in sea water with pH above 8.0 developed deformities. On the other hand, the entire juvenile phase of the experiment was without a true control. In other words, why even bother trying to publish this work, and still, why are we so sure that the deformities are due to high exposure $p\text{CO}_2$ and not something that normally occurs in this species? The authors of this paper have since the early 1990's hatched and produced larvae and juvenile European lobster for various studies as e.g stock enhancement (Agnalt et al., 1999, 2001, Agnalt 2008), fitness and genetic studies (Jørstad et al., 2001, 2005), carrying capacity (Agnalt, 2013) and conditioning juveniles for release purposes (Agnalt, 2013; Aspaas, 2012; Trengereid, 2012). The hatching experiments were made in open areas at Kvitsøy and Øygarden. We have only in a few occasions found that the first pair of the periopods, or claws, could be misshaped, but this trait was lost after one or two moults. None of these misshapes looked like the claw deformities found in the experiments with elevated $p\text{CO}_2$.

Deformities have not been reported in the two ocean acidification studies conducted on lobster, Arnold et al. (2009) with *H. gammarus* and Ries et al. (2009) with *H. americanus*. This could however be due to less experience with larvae and juveniles of Homarus sp. under normal conditions. In the present study, deformities in the larval phase were found in the carapace, tail fan or in the rostrum. Inexperienced personnel

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might miss these traits. In this study, all measurements and classifications were made by one experienced person only (main author), and as well, the individuals were classified blindly i.e. without knowing the treatment. Traits as the curled carapace was originally assigned as “seems to be in a moulting process, but a bit odd since I have not seen this before”, but later this showed to be related to exposed larvae only. The rostrum in newly-hatched larvae is bent when braking out of the egg shell, but will be erected within a few hours. The trait “bent rostrum” was found across all larval stages, even in the juvenile stages, and again only in exposed individuals.

Our study shows that deformities occur in lobster larvae from $p\text{CO}_2$ levels of around $727 \mu\text{atm}$ at 10°C , but does not explain why the degree of deformation is similar between 727 and $1217 \mu\text{atm}$. Whiteley (2011) stated that the exoskeleton of planktonic decapod larvae is unmineralised and elevated $p\text{CO}_2$ should therefore not affect larval conditions. However, Arnold et al. (2009) analysed the magnesium concentrations of the carapace of *H. gammarus* larvae with concentrations varying from 0.01 to $0.02 \mu\text{g mm}^{-2}$, and found a significant reduction at elevated $p\text{CO}_2$ of $1200 \mu\text{atm}$. Mg-CaCO_3 is more soluble than pure calcite or aragonite (Andersson et al., 2008). Even though $\Omega_{\text{Aragonite}}$ was at the lowest 1.12 in this study, but above the threshold limit of 1.0 thus carbonate ions were not under saturated, elevated $p\text{CO}_2$ could potentially still have an effect on e.g. formation of the new exoskeleton. The higher % of deformities observed in larvae at 18°C was surprising since we expected a greater effect at 10°C . Assuming that 10°C is below the lower optimal temperature limit (metabolic range), 10°C would pose greater metabolic stress on the larvae than at the optimal temperature of 18°C . One reason for the higher prevalence of deformities at 18°C may be explained by the water chemistry parameters such as under saturation of the carbonate ions (total alkalinity, HCO_3^- , CO_3^{2+}) essential for the calcification process. The increased deformities found in the 18°C treatment could also be a result of increased growth due to elevated $p\text{CO}_2$ levels. Growth above normal may result in thinning of the shell as reported in blue mussels *Mytilus edulis* and Pacific Oyster *Crassostrea gigas* (Gazeau et al., 2007; Melzner et al., 2011). A thinner shell in lobster larvae may not

be strong enough to keep its shape and especially when covering organs like the gills where water flow continuously makes a pressure on the exoskeleton.

In the juveniles, the deformities were affecting the carapace (puffy or swollen thus often leaving part of the gills exposed), stiff walking legs, twisted claws, stiff joints in the abdomen, tail fan damages and even stiff antenna. The latter is of vital importance in communication in lobster, among others finding a partner (Johnson and Atema, 2005). Growth in crustaceans requires replacement of the old exoskeleton by a new and larger exoskeleton, in a process called moulting. The lobster exoskeleton can be divided into three layers epi-, exo- and endocuticle (e.g. Sachs et al., 2006; Boßelmann et al., 2007; Romano et al., 2007; Al-Sawalmih et al., 2008; Sachs et al., 2008; Fabritius et al., 2009). The exoskeleton of *Homarus* sp. consists of chitin, protein, calcium, magnesium, phosphate and a few other compounds (Ba, Mn, Sr), although variable in composition in the different layers (Al-Sawalmih et al., 2008; Kunkel et al., 2012). The epicuticle is particularly rich in calcium, magnesium and phosphate. The outermost exocuticle consists of a thin calcite-containing layer while the rest is fully mineralized, mostly with amorphous calcium carbonate that is highly soluble and acts as a transient source of calcium. The endocuticle consists of crystalline calcite. Moulting is a complicated process (Greenaway, 1985; Dillaman et al., 2005; Politi et al., 2010), and for instance, reabsorption of calcium occurs from the old exoskeleton before it is shed. The new is uncalcified and rapidly needs to harden by deposition of calcium carbonate. In other words, elevated $p\text{CO}_2$ can affect various cuticle layers and various parts in the moulting cycle. Thus, the deformities may have been caused by irregularities in the depositing of Mg-CaCO_3 in the shell. So that too high deposits, thought to be a compensation for low haemolymph pH (acidosis), resulted in the stiff walking legs observed during our study. Whereas too little depositing of CaCO_3 in the shell results in the incomplete formation of certain structures in the exoskeleton. This could be related to depleted energy resources (to maintain homeostasis) required for converting HCO_3^- to CO_3^{2+} and therefore the depositing of enough CaCO_3 .

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The high % of deformities observed in juvenile lobsters in the ambient treatment at 18 °C were most likely due to the low pH values prevalent in the ambient water in Masfjorden. Compared to pH in ambient water at IMR's research stations Austevoll (pH 7.98, Andersen et al., 2013) and Parisvatn (pH 8.11, A. L. Agnalt, personal communication, 2013) located in outer coastal areas in the same region, the water in Masfjorden seems to have quite a large natural variation and may drop to levels well below average global pH of 8.05 (Fabry et al., 2008). In fact, ambient pH values were similar to that in the 727 µatm treatment, though a bit higher in some instances, which may explain the slightly lower % of deformities in this treatment. That fewer deformities occurred in juveniles exposed to 1217 µatm was unexpected, but the ones that were affected had also developed multiple deformities i.e. more severe damages.

Returning juveniles to “control” water at 14 °C may have caused severe metabolic stress which led to thermal shock. The juveniles may not be able to maintain energy resources high enough to regulate their metabolism (below their metabolic scope), thus resulting in metabolic exhaustion and therefore death. The pH at 14 °C may also have varied dramatically, with water taken directly from 90 m without regulating pH or CO₂ levels. This may have affected the depositing of CaCO₃, therefore resulting in the high % of deformities observed in the surviving juveniles. We found that some of the deformities as e.g. damages to the tail fan could not be repaired through moulting, but the walking legs would eventually be replaced by normal walking legs, and even swollen carapace would become normal after several moultings.

Deformities due to OA has been documented on the embryonic stages for a few species, often resulting in low or even no hatching success (Parker et al., 2009; Kawaguchi et al., 2010; Byrne, 2011), but also in the larval stages in a few species (Kurihara, 2008; Byrne et al., 2011; and references therein). In aquaculture however, hatchery-induced changes have been described for a number of species (Olla et al., 1998; Svåsand et al., 1998; Tsukamoto et al., 1999; and references therein). In shellfish, most changes documented are morphological as e.g. lower shell strength in greater scallop (*Pecten maximus*) and queen conch (*Strombus gigas*), lack of spines

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in topshell (*Trochus niloticus*) or lack of differentiation in the claws in lobster *Homarus* spp. (Govind and Pearce, 1986; Stoner and Davis, 1994; Purcell 2002; Grefsrud and Strand, 2006). To overcome the problem with lack of claw differentiation in lobster, shell sand should be added as substrate to stimulate normal development of a crusher and a scissor claw (Wickins, 1986; Govind and Pearce, 1986). Deformities of 40 to 58 % have also been found in wild populations of shrimps of the genus *Palaemon* in the Gironde estuary in France (Béguer et al., 2008, 2010). The deformities were reported to affect adult individual mortality and egg production. It is not sure the causes for these deformities, stress or environmental contamination. However, a possible response to lower $p\text{CO}_2$ has not yet been investigated. This study shows deformities in the larval and juvenile phase of European lobster. Some of the deformities will possibly affect the ability to find food and partner (walking legs, claw and antenna), respiration (carapace), ability to swim (tail-fan damages), and the real long-term effect of this has not yet been investigated.

5 Conclusions and future work

Although the present study lack a true control throughout most of the experimental period, the high ratio of deformed lobster larvae and juveniles strongly indicates that there is a negative effect of elevated $p\text{CO}_2$ on *H. gammarus* from hatching to one year of age. Working with early stages of this species on a yearly basis during the last 20–25 yr, no deformities described in the present study has ever been observed. However, to confirm these findings the experiment has to be repeated with a true control and within a temperature range that is within the species optimal range.

The variation in pH measured in the ambient water used in the present study shows that the water quality may change dramatically within a short period of time, even at depths of 90 m. Also Barton et al. (2012) showed that the carbon chemistry in the ambient water on the Oregon coast was variable, pH varied from 7.6 to 8.2 in the early summer of 2009. Knowledge about the pH in the Norwegian fjord systems and how

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it fluctuates between seasons and years is scarce. Deep water in fjords may be old (i.e. no exchange of water for a period of days, weeks or months) depending on the topography and hydrology of the fjord. When water from outside the fjord is flushed into the system, the change in water quality may be dramatic. More effort needs to be put into monitoring fluctuation in water quality throughout the water column and how this affects the ecosystem and life processes in the fjords.

Acknowledgements. We thank the staff at the Institute of Marine Research – Matre Research Station for helpful assistance during the project period. This study was supported by the Institute of Marine Research through the project number 13193-01, Ocean Acidification – Lobster.

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Table 1. Seawater parameters measured and calculated during the OA experiment. A_T – total alkalinity, C_T – total dissolved inorganic carbon. Analysis of nutrition gave estimates of nitrate ($12.3 \mu\text{mol kg}^{-1}$), phosphate ($1.3 \mu\text{mol kg}^{-1}$) and silicate ($7.1 \mu\text{mol kg}^{-1}$) used in the calculations.

	Medium $p\text{CO}_2$ treatment	High $p\text{CO}_2$ treatment
$p\text{CO}_2$ (μatm)	727 ± 12	1217 ± 134
Salinity (ppt)	33.37 ± 0.12	33.37 ± 0.12
A_T ($\mu\text{mol kg}^{-1}$)	2310.0 ± 1.1	2309 ± 2.3
C_T ($\mu\text{mol kg}^{-1}$)	2171.0 ± 1.5	2245.0 ± 7.0
pH_T	7.82 ± 0.01	7.61 ± 0.04
CO_3^{-2}	112.0 ± 1.5	72.2 ± 6.7
Ω_{calcite}	2.71 ± 0.09	1.74 ± 0.16
$\Omega_{\text{aragonite}}$	1.70 ± 1.12	1.12 ± 0.10

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Table 2. Classification of deformities found in larval stages 1 to 4 in European lobster (*Homarus gammarus*) exposed to elevated $p\text{CO}_2$ levels.

Category	Organ affected	Description
Curled carapace	Carapace	The carapace was curled at the edge, often forming ridges penetrating the side of the carapace.
Tail-fan damages	Uropod	Damages to parts of the tail fan, or even lacking one or both of the tail fans.
Bent rostrum	Rostrum	The rostrum was bent, as if not yet straighten out after hatching.

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Table 3. Classification of deformities found in juvenile stages of European lobster (*Homarus gammarus*) exposed to elevated $p\text{CO}_2$ levels.

Category	Organ affected	Description
Puffy carapace	Carapace	Carapace puffy/swollen, or up folded on one side often leaving some parts of the gills exposed.
Stiff/twisted walking legs	2nd–5th pereopod	The joints were fused together as if the joints were over calcified. The entire pereopod leg was like one stiff piece, sometimes “frozen” in an arbitrary/twisted position.
Miss-shaped claw	1st pereopod/ cheliped	Various shapes of the cheliped, but most often twisted, deviating from normal.
Bent rostrum	Rostrum	The rostrum was bent, as if not yet erected after hatching.
Tail-fan damages	Uropod	Damages to parts of the tail fan, or even lacking one or both of the tail fans.
Twisted abdomen	Abdomen	Abnormal shape of the abdomen as if some of the segments were once broken and then grown back in a wrong shape.
Stiff antenna	2nd antenna	Segments of the antenna were fused, as if the joints were over calcified. Felt “stiff” when touching. Difficult to observe when animal was out of water.

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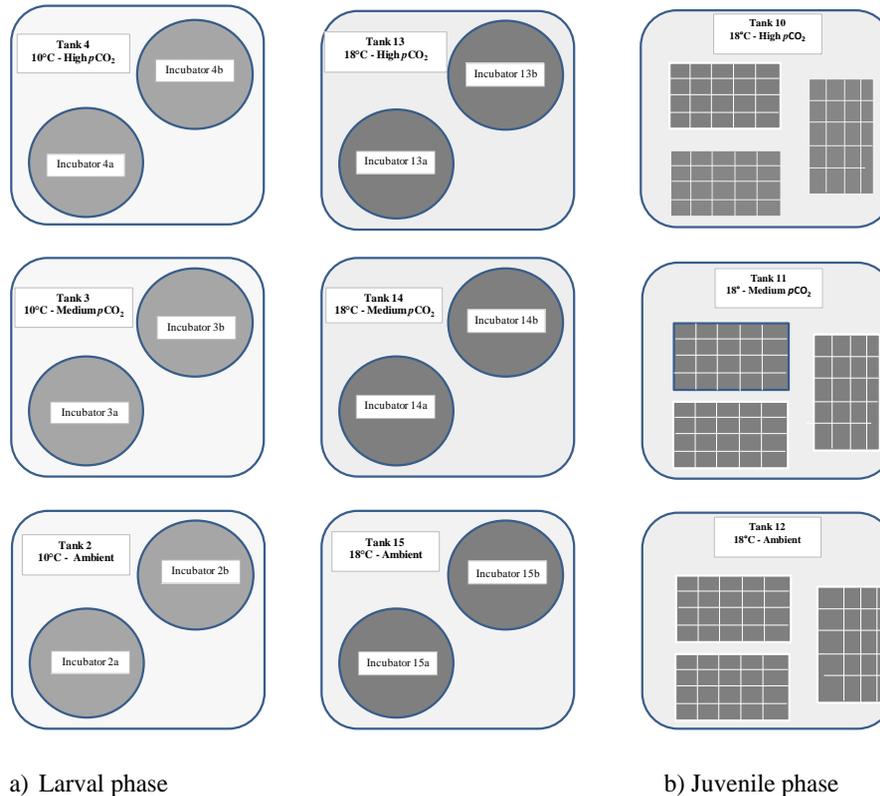


Fig. 1. Overview of the experimental setup during the **(a)** larval (two incubators as parallels in each tank unit) and in the **(b)** juvenile phase with trays with individual compartments (several trays in each tank unit).

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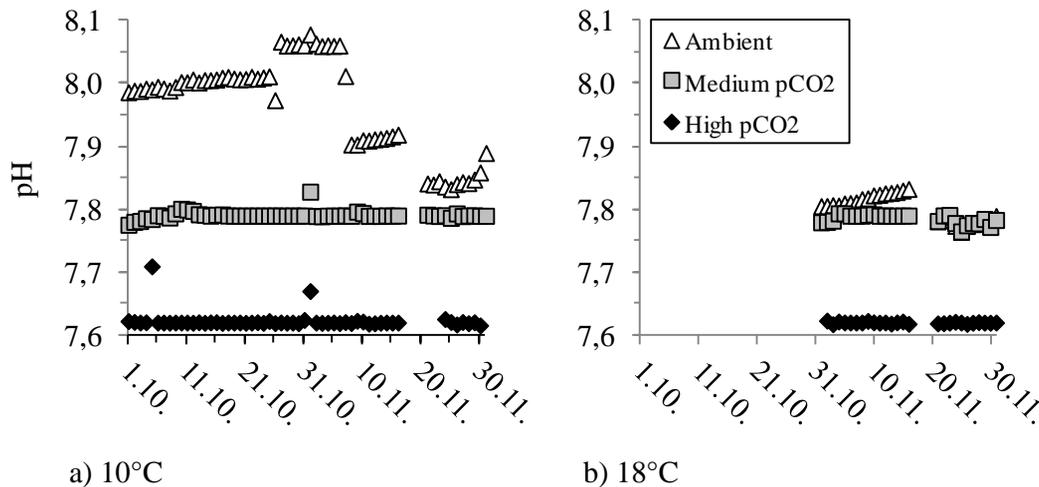


Fig. 2. Variation in pH_{NBS} in the larval phase run at **(a)** 10°C and **(b)** 18°C in 2011.

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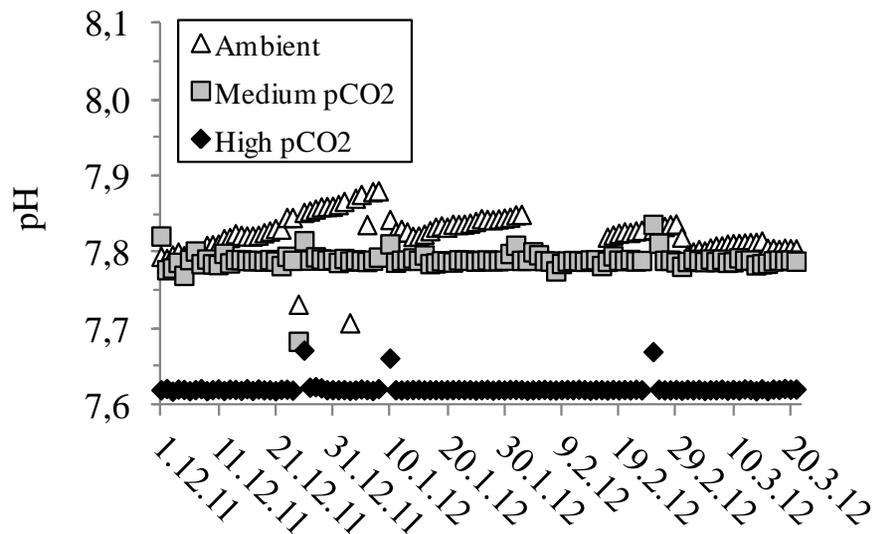


Fig. 3. Variation in pH_{NBS} in the juvenile phase until 22 March 2012 in the experiments run at 18°C. Note that only juveniles in 18°C were monitored, as non reached stage 4 in 10°C.

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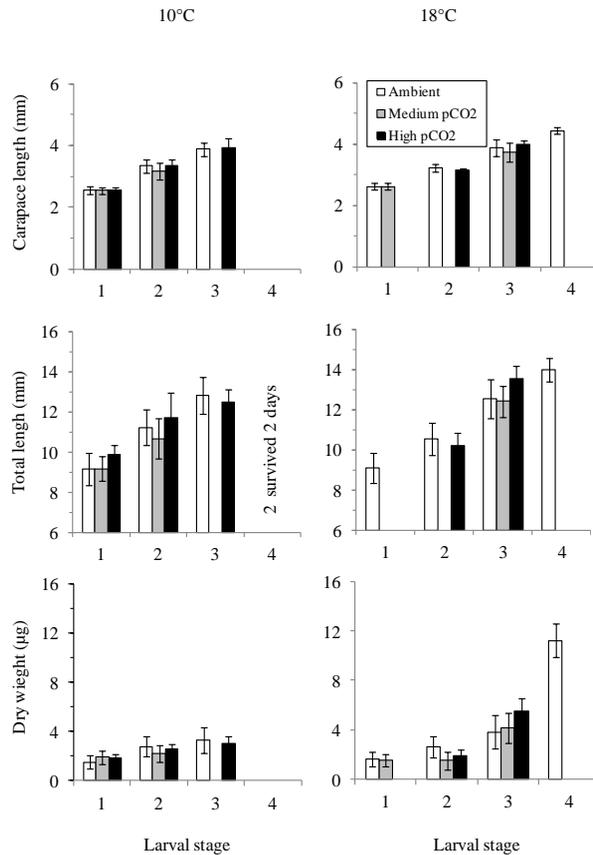


Fig. 4. Carapace length (mm), total length (mm) and dry weight (µg) at each larval stage of *Homarus gammarus* for 10° and 18°C for the various $p\text{CO}_2$ treatments. Ambient is raw water intake at 90 m depth. Note that lacking bars are due to a freezer broke and the samples decayed.

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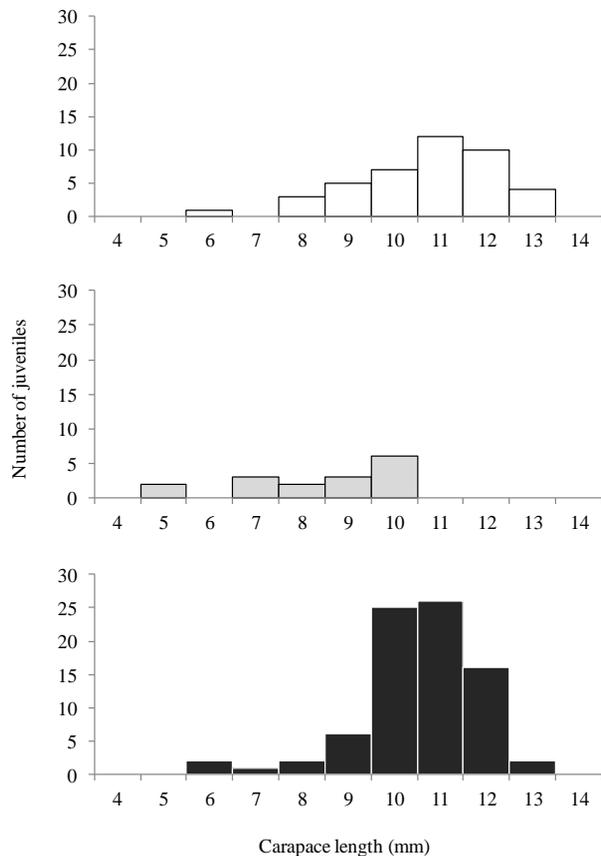


Fig. 5. Carapace length(mm) of juvenile *Homarus gammarus* raised in 18°C from newly hatched larvae until 5 months of age in (a) ambient water, (b) medium $p\text{CO}_2$ treatment and (c) high $p\text{CO}_2$ treatment. Size recordings were made 22 March 2012.



Fig. 6. A Stage 3 *Homarus gammarus* larvae raised in environment with lower pH_{NBS} than 8.1. The carapace is curled, leaving “curls” on the side (indicated with the arrow). This is classified as a deformity.

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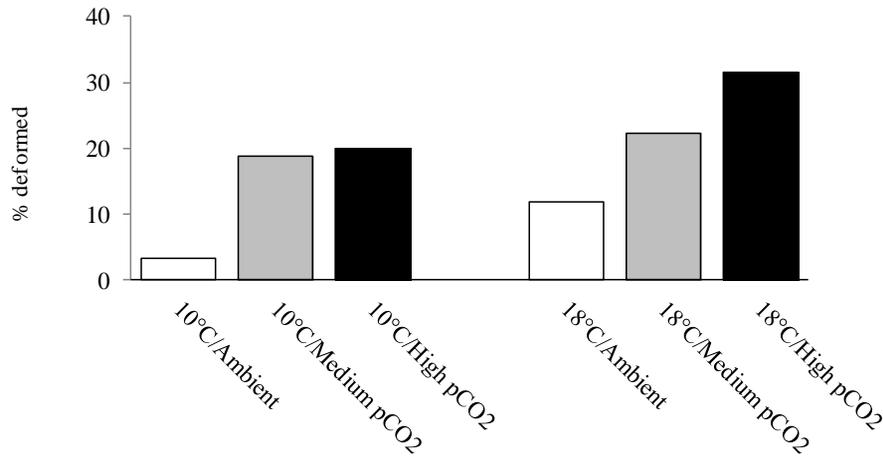


Fig. 7. Percentage of *Homarus gammarus* with deformities in the larval phase.

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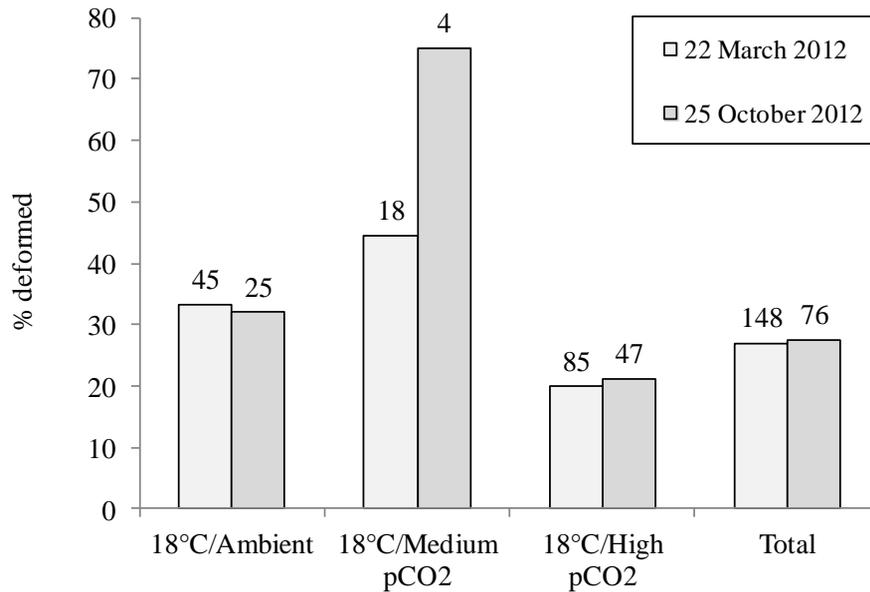


Fig. 8. The percentage of juveniles with deformities. Light grey bar represents juveniles surviving 5 months of exposure and darker grey bar the juveniles after another 7 months in ambient water only. Total number of juveniles in each category is given above each column.

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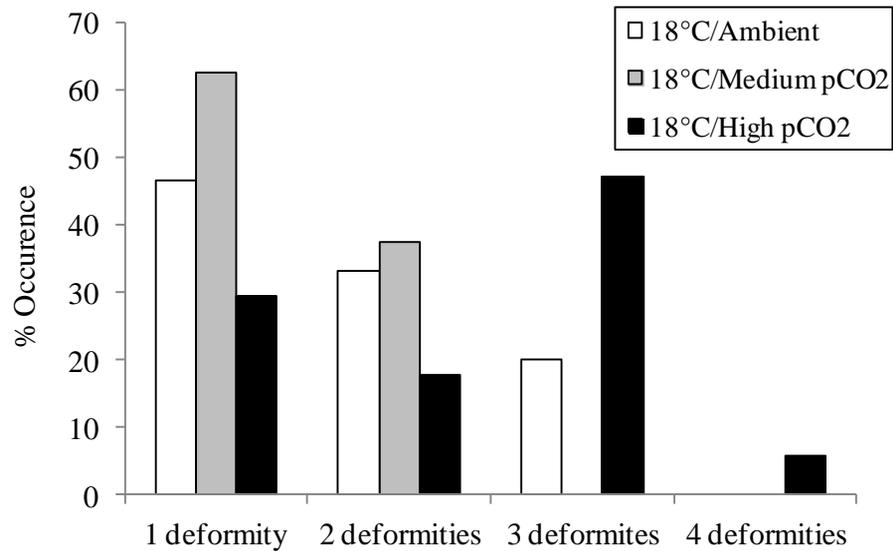


Fig. 9. The percentage of lobster juveniles with single or multiple occurrence of deformity when exposed to elevated levels of $p\text{CO}_2$ for five months.

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Fig. 10. *Homarus gammarus* juvenile, one year of age, exposed to pH_{NBS} lower than 8.1 since birth displaying two deformities, puffy carapace and stiff/twisted walking legs.

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