Long-term effects of predicted future seawater CO_2 conditions on the survival and growth of the marine shrimp *Palaemon pacificus.*

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Abstract: The increasing atmospheric concentration of carbon dioxide (CO_2) has been driving all marine organisms to live in increasingly acidic environments. In the present study, we evaluated the long-term effects of increased seawater CO_2 on survival, growth, feeding and moulting of the marine shrimp *Palaemon pacificus*. The shrimps were reared in seawater equilibrated with air containing 1,000 ppmv (parts per million by volume, seawater pH 7.89 ± 0.05) or 1,900 ppmv (pH 7.64 ± 0.09) CO_2, the atmospheric CO_2 concentrations predicted for the year 2100 and 2300, for 30 and 15 weeks, respectively. Survival was significantly suppressed in both experimental groups compared to respective controls; final survival rates were 55% (experimental) vs. 90% (control) in the 1,000 ppmv experiment, and 65% (experimental) vs. 95% (control) in the 1,900 ppmv experiment. Growth was unaffected in the 1,000 ppmv experiment but significantly depressed compared to the control after 6 weeks in the 1,900 ppmv experiment. Feeding was unaffected by either treatment. Moulting frequency was significantly affected in both 1,000 ppmv (experimental > control) and 1,900 ppmv (experimental < control) experiments. Egg production was suppressed in the 1,000 ppmv shrimps compared with the control (no observation was made in the 1,900 ppmv experiment). In addition, the second antennae determined at the end of the experiment

were significantly shorter in the 1,000 ppm v shrimps than in the control. The present results demonstrate for the first time that the predicted future seawater CO_2 conditions would potentially reduce shrimp, and possibly other crustacean, populations through negatively affecting mortality, growth, and reproduction. This could threaten entire marine ecosystem through disrupting marine food web.

Key words: CO_2, long-term effect, marine shrimp, ocean acidification, pH

Introduction

The atmospheric carbon dioxide (CO_2) concentration has increased from 280 ppmv (parts per million by volume) of the pre-industrial revolution period to the present level of 380 ppmv within the last 250 years due largely to the burning of fossil fuels (IPCC, 2007). Continuously increasing CO_2 emission is projected to raise the atmospheric CO_2 concentration to 540 (based on SRES B1 scenario) - 970 (SRES A1F1 scenario) ppmv by the year 2100 (IPCC, 2001), and possibly to as high as 1,900 ppmv by the year 2300 (Caldeira and Wickett, 2003). Of the annual emission of the anthropogenic CO_2 (6.0 Gt C yr^{-1}), roughly 1.6 Gt C yr^{-1} is estimated to dissolve in the ocean (Takahashi et al., 1997). Dissolution of CO_2 in seawater shifts carbonate equilibria such that it increases H^+ ion concentration (i.e. decreasing pH) and decreases CO_3^{2-} concentration. It is generally held that the surface ocean pH has already decreased by 0.1 units since the industrial revolution and possibly by 0.77 units by the year 2300 (Caldeira and Wickett, 2003). Recent studies reported that the projected future rises of seawater pCO_2 and accompanying reductions of pH will lead to decreases of $CaCO_3$ saturation state (Feely et al., 2004), to the extent that several marine calcifying

organisms suffer from a reduction of calci fication rate and an i ncrease in $CaCO_3$ dissolution rate (Riebesell et al., 2000; Orr et al., 2005; Kleypas et al., 2006; Gazeau et al., 2007). Hence, calcifying m arine organisms are probably one of the earliest organisms to be impacted by the ocean acidification due to ever-increasing atmospheric CO_2 concentrations (Kurihara and Shirayama, 2004; Raven et al., 2005; Kleypas et al., 2006).

The concern over the biological im pacts of ocean acidification d ue to increasing CO_2 concentration has been growing rapi dly. Recently, Michealidis et al. (2005) showed that hemolymph pH and metabolic rate remained depressed for a bivalve *Mytilus galloprovincialis* reared under hypercapnia (seawat er pH adjusted to 7.3 by bubbling CO_2) for 3 m onths, with a si gnificant decrease of growth rate. Ber ge et al. (2006) also reported gr owth reductions of *M. edulis* reared for 44 days under elevate d CO_2 conditions (pH < 7.1). Similarly, the survival and growth of sea urchins *Hemicentrotus pulcherrimus* and *Echinometra mathaei* were reduced by 6- month exposure to only 200 ppmv above the am bient level (Shirayama and Thornton, 2005). Knowledge of the i mpacts of long-term exposure of marine animals to future seawater CO_2 conditions is critically im portant to understand possible future alteration of the

marine ecosystem due to increasing CO_2. However, to our knowledge, the above studies are the only examples that have addressed this issue experimentally.

The aim of the present study was therefore to evaluate the long-term effects of increased CO_2 on the marine shrimps *Palaemon pacificus*. Crustaceans are an essential constituent in the marine ecosystem, and many are also commercially important. The shrimps were reared in seawater equilibrated with air containing 1,000 and 1,900 ppmv CO_2 (concentrations projected to occur by year 2100 and 2300) for 30 and 15 weeks respectively, to study survival, growth, feeding and moulting rates of the exposed animals.

1. Material and Methods

Test animals

The common Indo-West Pacific rocky-shore shrimp *P. pacificus* were collected from tidal pools of a rocky shore near the Teguma Harbour, Nagasaki, Japan, in August 2004 (total length 19.8 ± 2.0 (S.D.) mm, n = 40) and September 2005 (10.5 ±

0.9 mm, n = 40). The specimens were kept in 30 L aquaria supplied with natural seawater for several weeks prior to use. The shrimps were fed daily with a commercially available shrimp feed.

Experimental conditions

The shrimps were reared in seawater equilibrated with air containing either 1,900 ppmv of CO_2 for 15 weeks (from 24 September 2004 to 7 January 2005) or 1,000 ppmv of CO_2 for 30 weeks (from 29 September 2005 to 28 April 2006). The experimental conditions were identical between the two experiments except for the CO_2 concentration. Forty specimens were chosen randomly, and divided into two groups. The experimental setup consisted of an aquarium (capacity 36 L), an equilibration column (capacity 3 L, diameter 10 cm, length 37 cm), and a filter tank (capacity 9.5 L). Total seawater volume of the setup was 40 L. The shrimps were kept individually in plastic bottles (capacity 500 mL), which had their sides cut away and wrapped with a net (mesh size 1 mm) to allow unrestricted flow through the bottles. Twenty bottles were placed in each of two aquaria (control and experimental). Seawater was

recirculated through each setup at a flow rate of 4 L min^{-1}. To avoid the accumulation of metabolic wastes, fresh seawater was added continually to the setup at a flow rate of 300 mL min^{-1}. The seawater in the setup was bubbled through the equilibration column at a gas flow rate of 3 L min^{-1} with either CO_2-enriched air or out-door air (CO_2 concentration 380 ppmv). The CO_2-enriched air was prepared with a mass-flow gas blender (Kofloc, GB-2C, Japan) by mixing pure CO_2 and outdoor air. The seawater temperature was adjusted to 25 °C. The seawater salinity (measured with a refractometer Atago, 100-S, Japan) and the alkalinity (determined with a PHM290 pH meter and an ABU901 autoburette Radiometer, Denmark) were 36 ± 0.6 and 2.26 mEq L^{-1}, respectively, throughout the experiments. The seawater pH was measured daily with a pH meter (Mettler Toledo, MP125, USA). The seawater pH was 8.17 ± 0.05 (control) and 7.89 ± 0.05 (experimental) in the 1,000 ppmv experiment, and 8.15 ± 0.04 (control) and 7.64 ± 0.09 (experimental) in the 1,900 ppmv experiment. The shrimps were fed daily with a shrimp feed till satiation.

The survivorship and occurrence of moulting was checked every day. Shed exoskeletons, when found, were removed immediately. The moulting frequency was

calculated by dividing the incidence of moult by the number of surviving shrimps every 3 weeks. The daily feeding rate was estimated by weighing the amount of dried residual feed 24 h after the shrimps were fed. The data were calculated only for those individuals which survived at the end of each 3-week period. Lateral images of the shrimps were photographed every 3 weeks to determine total length using image analysing software (Scion Image, USA). At the end of the 1,000 ppmv experiment, the oxygen consumption rate of shrimps was measured by closed respirometry. Shrimps were acclimated for 12 h to a respiratory chamber (capacity 350 mL), to which control or 1,000 ppmv CO_2 seawater was continuously supplied at a flow rate of 60 mL min^{-1}. The chamber was closed and seawater (0.5 mL) was sampled from the chamber at 0, 1.5 and 3 h to determine seawater pO_2 with an oxygen electrode (Model 1302 StrathKelvin, USA) and a meter (Model 1782, StrathKelvin, USA).

Following the determinations of oxygen consumption rate, dorsal images of the shrimps were photographed to determine antenna length. Sex of the shrimps was checked at the end of the 1,000 ppmv experiment on the basis of the 2nd pleopod morphology (no observations were made for the 1,900 ppmv experiment).

Statistical analysis

Statistical analyses were performed using JMP software package, version 7 (SAA Institute Inc., 2007). Log-rank test was used to compare survival of the control and CO_2 shrimps in the two experiments. GMANOVA (Generalized Multivariate Analysis of Variance) was performed to compare growth, feeding and moulting rates of the control and CO_2 shrimps through the experiment. Where a significant effect was found by GMANOVA, Mann-Whitney's *U*-test was run to detect a significant difference at a given time. Student *t*-test was used to compare oxygen consumption rate and the ratio of antenna length / total length measured of the control and CO_2 shrimps at the end of the 1,000 ppmv experiment.

2. Results

Survival

Survival was significantly suppressed in both experimental groups compared

with the respective controls (Log-rank test, $p < 0.05$, Fig. 1a, b). Shrim ps of the 1,000 ppmv group started to die 18 weeks after th e onset of the exposure with the final survival rates of 55 % (9 of 20 i ndividuals died) as co mpared to 90% (2 of 20 individuals died, of which 1 died due to ha ndling error) in the control group (Fig. 1a). When analyzed separately for sex, the final survival rates of the males and females were 53% (8 of 15 individuals died) and 20% (1 of 5 individual s died), respectively. The 1,900 ppmv shrimps started to die in 7 weeks with an exception of one specimen, which had died in 13 days. The final survival rates were 65 % (7 of 20 individuals died) for the 1,900 ppmv group and 95% (1 of 20 individuals died) in the control group (Fig. 1b).

Growth, feeding and moulting

The growth was unaf fected in the 1,000 ppmv experim ent (Fig. 2a), but significantly reduced in th e 1,900 ppm v experiment compared with the control (GMANOVA $F = 15.858$, $p < 0.001$, Fig. 2b). The final total length of the 1,000 ppmv shrimps (27.5 ± 3.0 mm, n = 1 1) did not significantl y differ from the value of the controls (29.6 ± 3.7 mm, n = 18). The total leng th of the 1,900 ppm v shrimps became significantly smaller than c ontrol 6 weeks after exposure, resulting in significantly

smaller total length for the experim ental shrimps (24.2 ± 1.8 mm, n = 13) than for the control (26.4 ± 2.3 mm, n = 19, Mann-W hitney's *U*-test, $p < 0.01$) at the end of the experiment. When the data in the 1,000 ppm v experiment were analyzed separately for sex, the females in the CO_2 group tended to becom e smaller than in the control as the exposure prolonged, though the dif ferences were not statistically si gnificant (Fig. 3b. GMANOVA). No such trend was detected for males (Fig. 3a).

Feeding was unaf fected in both 1,000 and 1,900 ppm v experiments throughout the experiments (Fig. 4, GMANOVA). Significant differences were detected for the moulting frequency in both experiments (GMANOVA, $F = 4.33$, $p < 0.05$ for the 1,000 ppmv experiment, and $F = 20.71$, $p < 0.0001$ for the 1,900 ppmv experiment), although the patterns were dif ferent (Fig. 5). The m oulting frequency became significantly higher in the 1,000 ppm v shrimps than in the control after 21 weeks (Mann-Whitney's *U*-test, $p < 0.05$, Fig. 5a), while the frequency was significantly low er for the period of 10-12 weeks in the 1,900 exposure experiment (Mann-Whitney's *U*-test, $p < 0.05$, Fig. 5b). Oxygen consum ption was unaffected by the 30-week exposure to 1,000 ppm v (control 0.389 ± 0.056 mm ol min^{-1} g^{-1} (n = 6), CO_2 0.351 ± 0.088 (n = 6), t-test, $p > 0.05$). These values are com parable to the reported oxygen

consumption rates for other marine shrimps of similar size and at the same temperature (Emmerson, 1985).

The second antennae were shorter in the 1,000 ppmv shrimps than in the control as determined at the end of the exposure (Fig. 6a, b), and the ratio of antenna length / total length of the 1,000 ppmv shrimps (54.0 ± 21.9%) was significantly smaller than that of the control (164.9 ± 14.2%, *t*-test, $p < 0.001$, Fig. 6c) (no observations were made for the 1,900 ppmv experiment). In addition, of the five females in the 1,000 ppmv group, only one female bore eggs twice compared with 2 to 3 times of egg production in 5 of the 6 control females.

3. Discussion

The present study demonstrated that the common rocky-shore shrimp *Palaemon pacificus* could be lethally affected by exposure to seawater equilibrated with the CO_2 concentrations of 1,000 ppmv and 1,900 ppmv, which are predicted to occur in the year 2100 and 2300, respectively (Caldeira and Wickett, 2003). Even though some of the observed responses to elevated CO_2 appear to be CO_2-concentration dependent

(e.g. earlier mortality in the 1,900 ppmv than in the 1,000 ppmv experiment, and significant growth reduction in the 1,900 ppmv but not in the 1,000 ppmv experiment), it may not be appropriate to quantitatively compare the data of the two experiments due to the difference in initial body size (ca. 10 mm in the 1,000 ppmv experiment vs. ca. 20 mm in the 1,900 experiment). Rather, what is significant is the fact that many of the CO_2 impacts appeared only after at least several weeks of exposure to elevated CO_2 conditions (Figs. 1, 2, and 5), which stresses the critical importance of long-term exposure experiments. Characteristically, CO_2 mortality of the shrimp became manifest only after 18 and 7 weeks of exposure to 1,000 and 1,900 ppmv CO_2 respectively (Fig. 1). A similar, delayed mortality was reported by Shirayama and Thornton (2005) for the sea urchins (*Hemicentrotus pulcherrimus* and *Echinometra mathaei*) and the snail (*Strombus luhuanus*), where mortality was first recorded 12-18 weeks of exposure to 560 ppmv CO_2. The reason for this delayed mortality is unclear, but limited capacity of marine invertebrates for acid-base regulation may be involved. Recently, Miles et al. (2007) found that the sea urchin *Psammechinus miliaris* exposed to hypercapnia (pH 7.44, CO_2 2,000 ppmv) could compensate acid-base balance of the coelomic fluid for only 1.5 days, followed by the development of significant acidosis. A lack of acid-base

compensation of the extracellular fluid has also been reported for the mussel *Mytilus galloprovincialis* exposed to seawater acidified by elevated CO_2 (pH 7.3, Michaelidis et al., 2005). Aquatic crustaceans generally have a higher capacity for extracellular acid-base regulation than other marine invertebrates such as echinoderms and mollusks, due to ion exchange mechanisms residing mainly in the branchial epithelium (Wheatly and Henry, 1992). However, the capacity for extracellular acid-base regulation in marine crustaceans appears to be species-specific, and also influenced by environmental conditions such as temperature and dissolved oxygen (Pane and Barry, 2007). Furthermore, acid-base status during CO_2 exposure spanning several moulting cycles has never been investigated. Thus, we hypothesize that *P. pacificus* has a relatively low capacity for extracellular acid-base regulation, and resultant chronic acidosis led to mortality and reduced growth (see below) during the long-term CO_2 exposures.

Decreased intracellular pH disrupts a number of cell properties (e.g. membrane permeability) and processes (e.g. epithelial transport and metabolism), which are important in normal development and growth (Roos and Boron, 1981). However, under relatively small changes in ambient CO_2 and pH relevant to ocean acidification, intracellular pH may be protected by high buffer capacities of intracellular

fluids and acid-base regulatory machinery across cell membrane (Roos and Boron, 1981). In fact, measuring by the homogenate method developed by Pörtner et al. (1990), Michaelidis et al. (2005) found that intracellular pH of selected tissues was kept unchanged in the mussel, exposed to seawater pH of 7.3. In this context, it is worth noting that Reipschläger and Pörtner (1996) found that depressed metabolism can only be related to extracellular, not intracellular, pH in the non-perfused body wall preparation of the sipunculid worm *Sipunculus nudus* subjected to normocapnic or hypercapnic conditions. If this observation holds true for other marine animals, then low capacities for extracellular acid-base regulation of marine invertebrates are expected to depress metabolism of CO_2-exposed animals, which would lead to reduced growth. In fact, the present as well as earlier studies have demonstrated reduced growth of several marine invertebrates by CO_2 exposure. In *P. pacificus*, the growth reduction became significant 6 weeks of exposure to 1,900 ppmv CO_2, before the shrimps started to die (Fig. 2b). A similar, though not statistically significant, growth reduction was observed only for females toward the end of 30-week exposure in the 1,000 ppmv experiment (Fig. 3b). Decreased growth was reported for sea urchins reared in seawater acidified by CO_2 to only 560 ppmv (Shirayama and Thronton, 2005), and for bivalves kept in

seawater acidified by the addition of CO_2 (seawater pH 7.3, Michaelidis et al., 2005) or mineral acids (Bamber, 1987; 1990).

The possible causes for growth reductions in crustaceans include: (1) metabolic depression, (2) reduced feeding and/or food assimilation efficiency, and (3) disrupted endocrinological control of moulting. Firstly, metabolism of *P. pacificus* appears to have been unaffected by the 30-week exposure to 1,000 ppmv CO_2, judging from the insignificant change in oxygen uptake determined at the end of the exposure period. However, because living in a high CO_2 environment is thought to entail additional costs for acid-base regulation and respiratory ventilation (Ishimatsu et al., in press), the unchanged oxygen uptake of *P. pacificus* may imply a reduction of energy allocated to house-keeping metabolism involved in animal growth. The mussel and the sipunclid worm were shown to reduce oxygen uptake during hypercapnic exposure (Michaelidis et al., 2005; Pörtner et al., 1998). Secondly, feeding was not significantly affected in both 1,000 and 1,900 ppmv experiments (Fig. 4), but it is possible that a part or entire processes of digestion, nutrient absorption from the gut, and nutrient assimilation were depressed in our shrimps, which should be addressed in further experiments. Bamber (1990) reported a significant positive correlation between the

feeding activity and ambient seawater pH for marine bivalves, *Crassostrea gigas* and *M. edulis,* with a significant reduction of feeding at pH < 7.2. Thirdly, the significantly higher moulting frequency observed after 21 weeks of 1,000 ppmv CO_2 exposure (Fig. 5a) may be a result of endocrinological disruption, although no such trend was seen in the 1,900 ppmv experiment. It is widely known that moulting and regeneration of appendages are interrelated in crustaceans (Madhavan and Madhavan, 1981). Loss of the antenna affected the subsequent moulting interval in the terrestrial isopod, *Armadilium vulgare*, although the effect on moulting varied depending on the timing of antennal ablation (Madhavan and Madhavan, 1981). Hence, the shortening of the second antenna observed in the 1,000 ppmv shrimps (Fig. 6) possibly had some influence on the higher moulting frequency of the animals (Fig. 5a).

The second antenna provides much of the tactile information and plays an important role in forming an image of the environment in decapod crustaceans, and hence its partial loss interferes with normal behaviour (Harrison et al., 2001; Koch et al., 2006). The shortening of the second antennae observed in our 1,000 ppmv shrimps may be due to lowered calcification rate and/or increased dissolution of $CaCO_3$ exoskeletons, as known for several calcifying organisms during CO_2 exposure (Riebesell et al., 2000;

Orr et al., 2005; Kleypas et al., 2006; Gazeau et al., 2007) and might affect the shrimp's behaviour.

Female body size and fecundity are strongly correlated in *P. pacificus* (Ito et al., 1991) as in other crustaceans (Somers, 1991), and therefore growth reductions by long-term CO_2 exposure will likely lead to decreased egg production. The present results of the 1,000 ppmv experiment suggest this possibility, although the sample size is too small to make any decisive conclusion on this point. Combined with negative effects on early developmental (Kurihara et al., 2004; 2007; Kurihara, in press), CO_2 may have profound impacts on recruitment and population size of the affected animals.

Recent research has revealed that organisms could evolve on contemporary timescales (within few decades) in response to environmental changes (Stockwell et al., 2003). It is therefore possible for the shrimps to adapt to the acidified marine environment expected for the coming centuries. The present experiment protocol cannot test this possibility and hence may have overestimated the impacts. However, the relatively long generation length of *P. pacificus* (12-15 months, Ito *et al*. 1991), which is an important factor for the evolutionary potential of a species, makes "rapid evolution" of the shrimps unlikely in response to the changes in ocean environment (Berteaux et al.,

2004).

In conclusion, the present results demonstrated that long-term exposure to the CO_2 concentrations predicted to occur during the next few centuries could reduce survivorship, growth, and possibly reproductivity of the common rocky-shore shrimp *P. pacificus*. Considering that *P. pacificus* inhabits rocky coastal areas including tidal pools where temperature, CO_2 and O_2 concentrations display large diurnal fluctuations, the shrimp might have higher environmental tolerance than more pelagic shrimps. If other crustaceans are similarly or more severely affected by ocean acidification due to increasing CO_2, marine ecosystems and productivity may face substantial risk.

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Figure captions

Fig. 1 Effects of long-term CO_2 exposure on the survival of the marine shrimp *Palaemon pacificus*. Survival was significantly suppressed (Log-rank test, $p < 0.05$) when shrimps were individually reared under **a** 1,000 ppmv and **b** 1,900 ppmv CO_2 conditions for 30 and 15 weeks, respectively.

Fig. 2 Effects of long-term CO_2 exposure on the growth of the marine shrimp *Palaemon pacificus* (data pooled for both sexes). The initial total lengths of the shrimps used in the 1,000 and 1,900 ppmv experiments were 10.5 ± 0.9 (S.D.) mm (n = 40) and 19.8 ± 2.0 mm (n = 40), respectively. Growth was not significantly affected when shrimps were reared under **a** 1,000 ppmv for 30 weeks, but was significantly reduced when reared under **b** 1,900 ppmv CO_2 conditions for 15 weeks (GMANOVA $F = 15.858$, $p < 0.001$). The number of individuals at each observation time decreased from 20 at the onset of exposure due to the mortality as given in Fig. 1. *Significantly different from the corresponding control values (Mann-Whitney *U*-test, $p < 0.05$).

Fig. 3 Effects of 30-week exposure to 1,000 ppmv CO_2 on sex-specific growth of **a** male and **b** female marine shrimp *Palaemon pacificus*. The number of individuals at each observation time decreased from 20 at the onset of exposure due to the mortality as given in Fig. 1.

Fig. 4 Effects of long-term CO_2 exposure on the feeding of the marine shrimp *Palaemon pacificus*. Feeding was unaffected by both **a** 1,000 ppmv and **b** 1,900 ppmv CO_2 exposures of 30 and 15 weeks, respectively. The feeding rate of the week 21 for 1,000 ppmv was not included due to low number of observations for that week. The number of individuals at each observation time decreased from 20 at the onset of exposure due to the mortality as given in Fig. 1. 0

Fig. 5 Effects of long-term CO_2 exposure on the moulting frequency of the marine shrimp *Palaemon pacificus*. The moulting frequency was significantly affected in both **a** the 1,000 ppmv (GMANOVA, $F = 4.33$, $p < 0.05$) and **b** 1,900 ppmv CO_2 experiments (GMANOVA, $F = 20.71$, $p < 0.00\,01$). The moulting frequency became significantly higher (*t*-test, $p < 0.05$) 21 weeks after exposure to **a** 1,000 ppmv CO_2, whereas

moulting was significantly suppressed (*t*-test, $p < 0.05$) for the period of 10-12 weeks in the shrimps reared under **b** 1,900 ppmv CO_2 conditions. The number of individuals at each observation time decreased from 20 at the onset of exposure due to the mortality as given in Fig. 1.

Fig. 6 Effects of 30-week exposure to 1,000 ppm v CO_2 on the antenna length of the marine shrimp *Palaemon pacificus* (**a** control, **b** experimental). The ratio of antenna/total length was significantly smaller in experimental shrimps (54.0 ± 21.9%, n = 9, 2 individuals not measured) than in controls (164.9 ± 14.2%, n = 18, *t*-test, $p < 0.001$, **c**). Bar = 1 cm.

FIG. 1

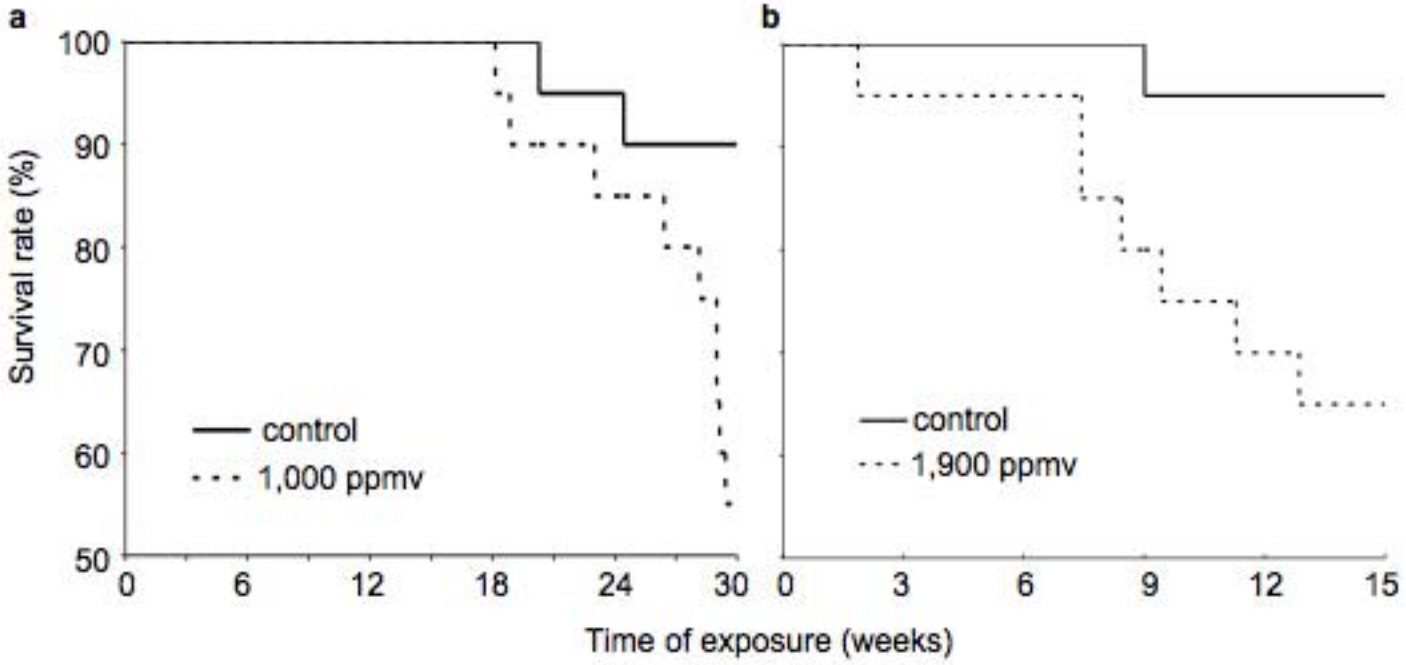

a
b
Survival rate (%)
100
90
80
70
60
50
0
6
12
18
24
30
3
9
15
control
1,000 ppmv
1,900 ppmv
Time of exposure (weeks)

FIG. 2

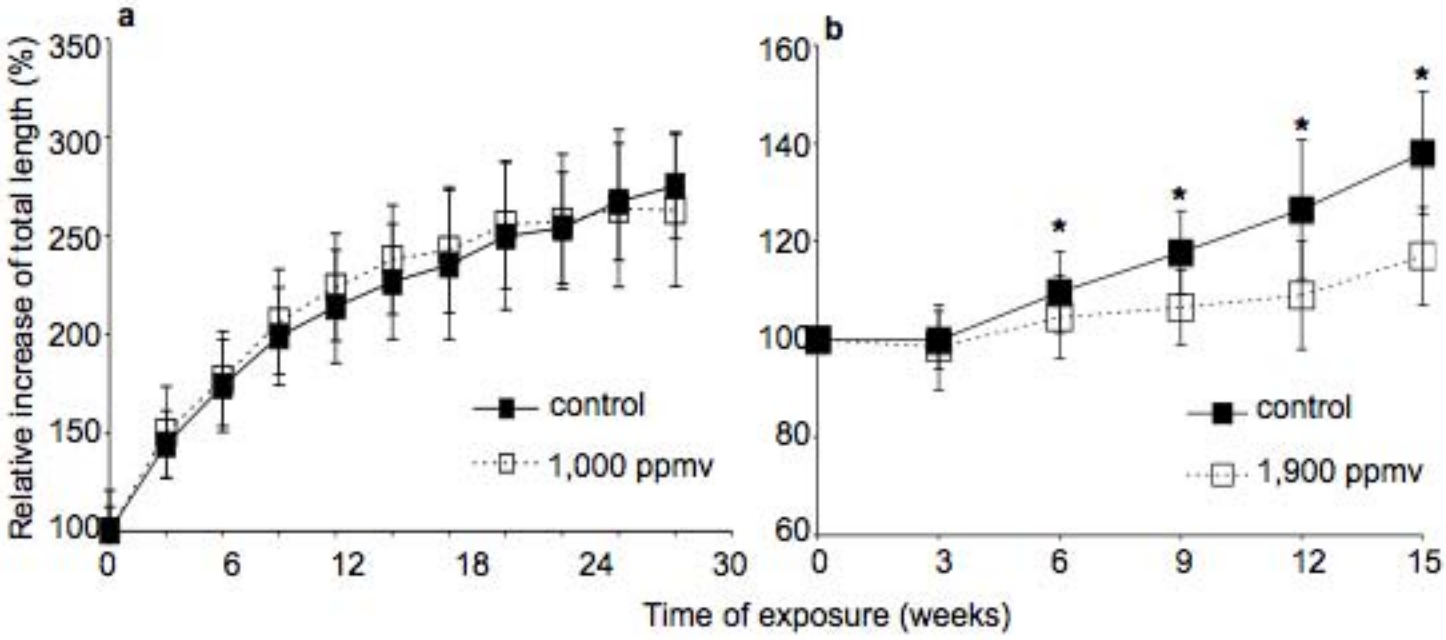

a
b
Relative increase of total length (%)
350
300
250
200
150
100
160
140
120
100
80
60
0
6
12
18
24
30
0
3
6
9
12
15
control
1,000 ppmv
control
1,900 ppmv
*
Time of exposure (weeks)

FIG. 3

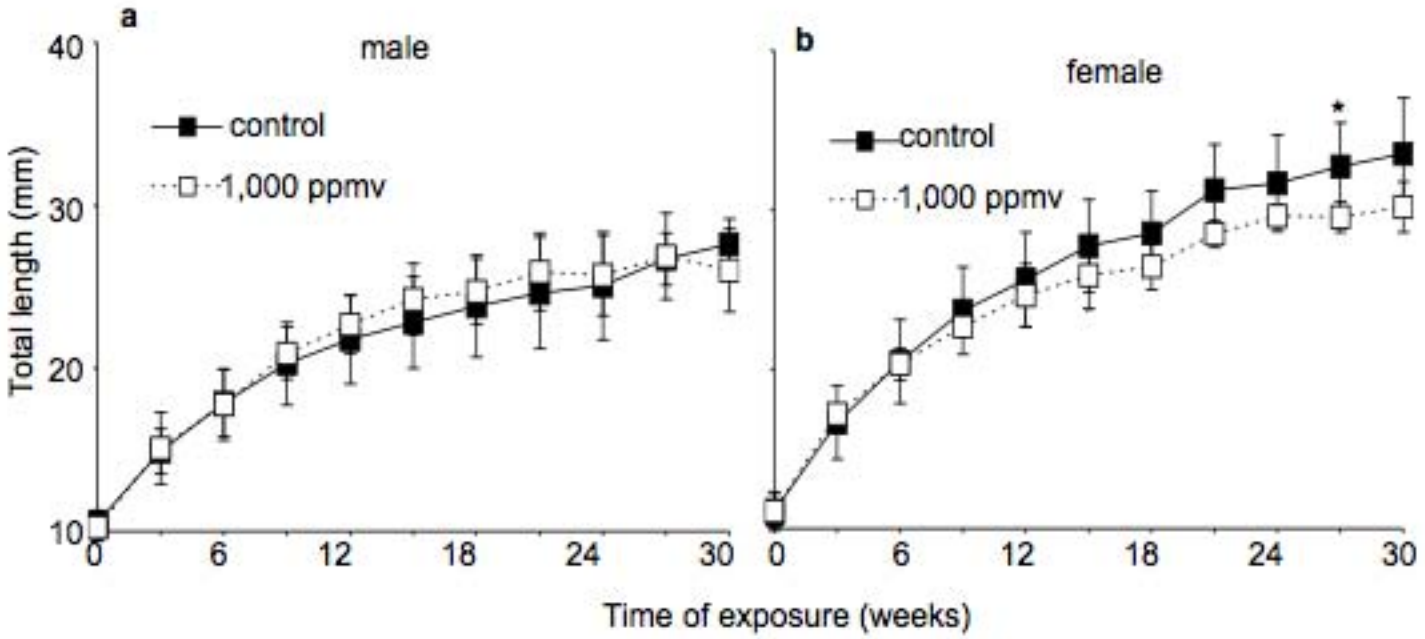

a
male
control
1,000 ppmv
Total length (mm)
40
30
20
10
0
6
12
18
24
30
b
female
control
1,000 ppmv
*
0
6
12
18
24
30
Time of exposure (weeks)

Fig. 4

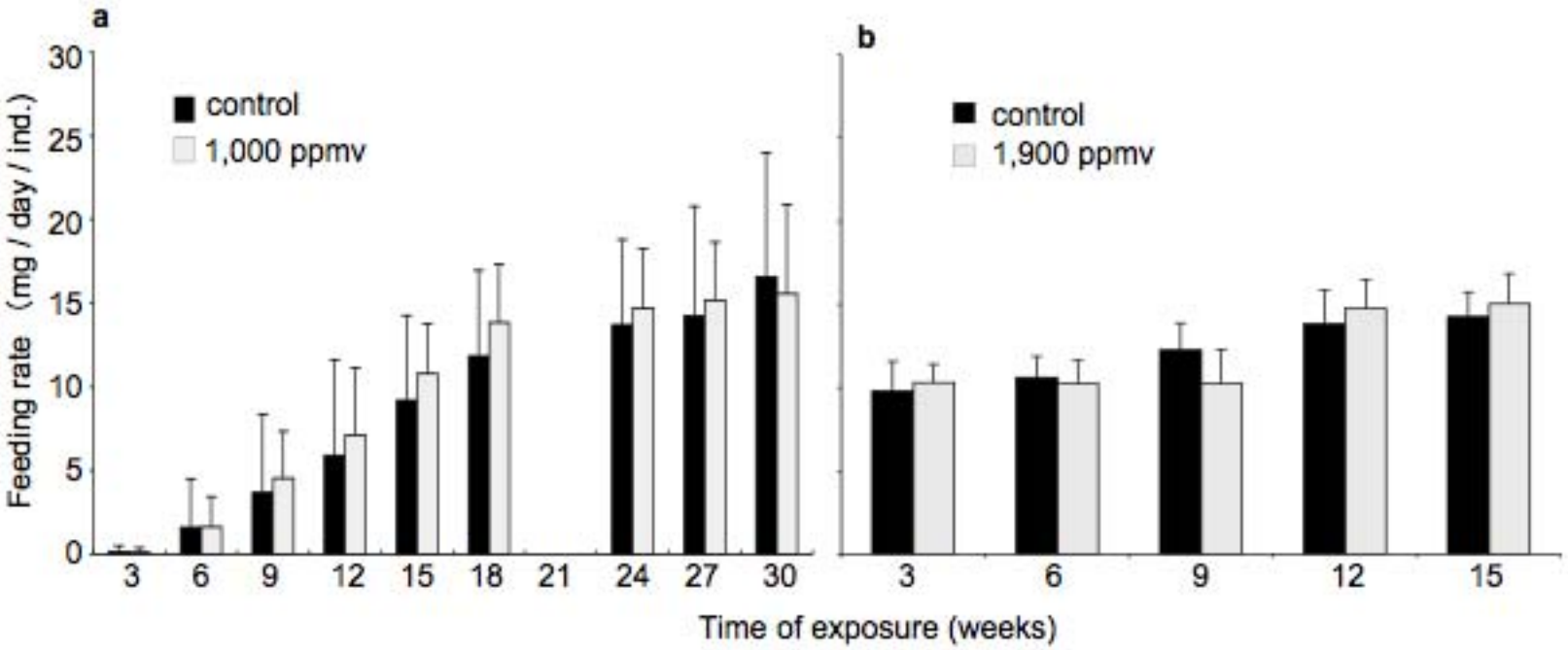

a
b
control
1,000 ppmv
control
1,900 ppmv
Feeding rate (mg / day / ind.)
0
5
10
15
20
25
30
3
6
9
12
15
18
21
24
27
30
Time of exposure (weeks)

FIG. 5

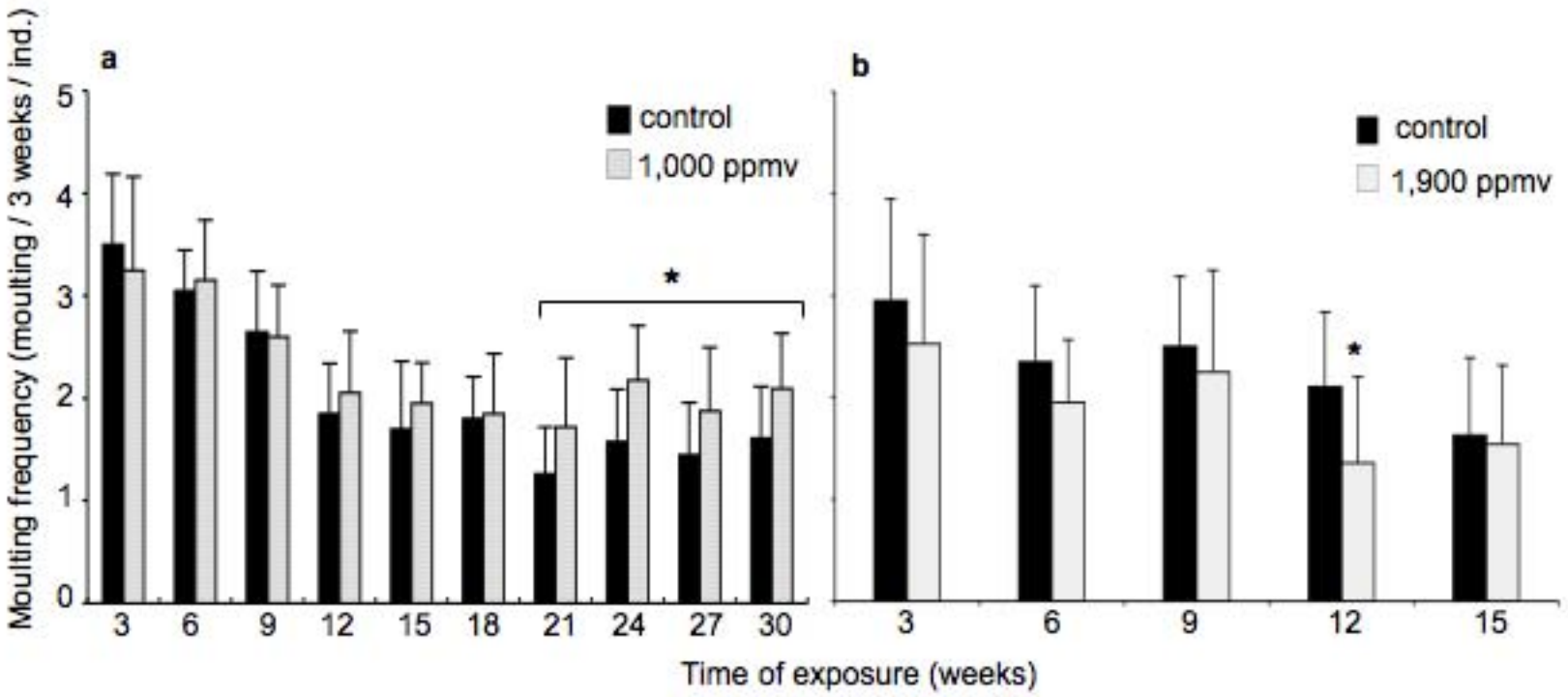
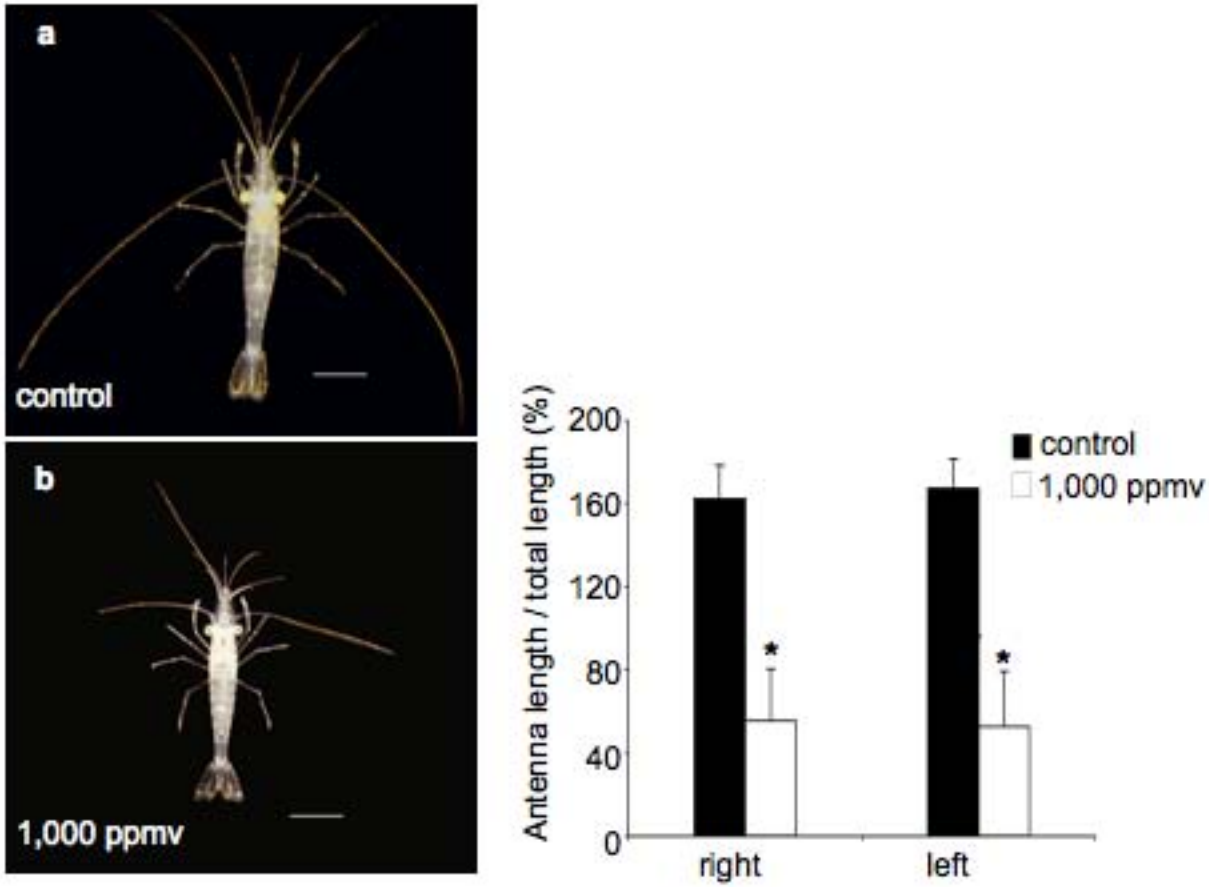

a
b
control
1,000 ppmv
1,900 ppmv
Moulting frequency (moulting / 3 weeks / ind.)
0
1
2
3
4
5
*
*
3
6
9
12
15
18
21
24
27
30
Time of exposure (weeks)

FIG. 6


a
control
b
1,000 ppmv
Antenna length / total length (%)
200
160
120
80
40
0
control
1,000 ppmv
*
*
right
left