



High sensitivity to chronically elevated CO₂ levels in a eurybathic marine sipunculid

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Abstract

CO₂ levels are expected to rise (a) in surface waters of the oceans as atmospheric accumulation continues or (b) in the deep sea, once industrial CO₂ dumping is implemented. These scenarios suggest that CO₂ will become a general stress factor in aquatic environments. The mechanisms of sensitivity to CO₂ as well as adaptation capacity of marine animals are insufficiently understood. Here, we present data obtained in *Sipunculus nudus*, a sediment-dwelling marine worm that is able to undergo drastic metabolic depression to survive regular exposure to elevated CO₂ levels within its natural habitat. We investigated animal survival and the proximate biochemical body composition during long-term CO₂ exposure. Results indicate an unexpected and pronounced sensitivity characterized by the delayed onset of enhanced mortality at CO₂ levels within the natural range of concentrations. Therefore, the present study contrasts the previously assumed high-CO₂ tolerance of animals adapted to temporary hypercapnia. As a consequence, we expect future loss of species and, thereby, detrimental effects on marine benthic ecosystems with as yet poorly defined critical thresholds of long-term tolerance to CO₂.

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Currently rising concentrations of CO₂ in atmosphere and surface waters (Brewer, 1997; Wolf-Gladrow et al., 1999) as well as anticipated scenarios of anthropogenic CO₂ disposal in the deep sea (Auerbach et al., 1996) indicate that this abiotic factor will progressively become a general stress factor in aquatic environ-

ments. An increase in atmospheric CO₂ concentrations to 1900 ppm (0.19 kPa PCO₂) unprecedented since 300 Mio years is expected from business as usual scenarios of anthropogenic CO₂ production for the next 300 years, resulting in a decrease of pH values in surface ocean waters by up to 0.77 units (Caldeira and Wickett, 2003). Even larger pH excursions are expected locally during CO₂ disposal scenarios (Adams et al., 1997).

A suitable animal model to investigate medium to long-term CO₂ effects arising from both scenarios is the invertebrate *Sipunculus nudus*, a eurybathic

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invertebrate living at water depths of up to 2300 m (Stephen and Edmonds, 1972). In intertidal sediments, this species is regularly exposed to CO₂ oscillations (Diaz and Rosenberg, 1995). Under CO₂-induced acidosis, *S. nudus* displays a reduction in energy turnover (Pörtner et al., 1998), a strategy typically observed in animals under environmental stress (see Hand and Hardewig, 1996 for review). Suppression of aerobic energy turnover is partly realized by the slowing of acid–base regulation (Pörtner et al., 2000) and by the accumulation of the neurotransmitter adenosine, that causes a reduction of neuronal and motor activity (Reipschläger et al., 1997). In addition, a decrease in N-excretion and a concomitant 60% reduction of protein biosynthesis rates indicate a downregulation of protein turnover under severe respiratory acidosis (Langenbuch and Pörtner, 2002; Langenbuch, unpublished data), similar to observations during oxygen limitation in rat liver (Surks and Berkowitz, 1971) or in crustacean tissues (Mente et al., 2003). As protein biosynthesis is a key prerequisite for growth and reproduction, processes that support long-term survival, its downregulation is likely only tolerated temporarily. In fact, all of the above mentioned strategies may only have evolved to enhance survival of temporary CO₂ exposure. Considering the non-transient character of future increases in ocean CO₂ levels, the question arises, if and at which point in time-limited cellular energy budgets and rates of protein synthesis (due to metabolic depression) may restrict survival and growth under CO₂.

We therefore incubated three groups of 25–30 specimens of *S. nudus* in three sealed gastight aquaria with a bottom layer of sediment to allow animals to remain burrowed during the experiment. The aquaria were constantly aerated under control conditions, which simulated those of long-term aquarium maintenance in our laboratory, where specimens thrive for several years with very low mortality. During experimentation, recirculating seawater was equilibrated with gas mixtures containing rising concentrations of CO₂ (control group at 0.03 kPa PCO₂, two experimental groups at 1.01 and 3.03 kPa PCO₂, respectively).

Apart from observing behavioural patterns, body fresh weight of the animals was monitored. All specimens were extracted from the sediment in regular intervals of 2–3 weeks to determine their fresh weight. Finally, animals were sacrificed and whole animal

dry weight, ash free dry weight and CHN (carbon, hydrogen and nitrogen) elemental ratios were analysed in total dry biomass and ash to detect possible changes in body composition (according to Craig et al., 1978; Gnaiger and Bitterlich, 1984). Mortality curves for the different CO₂ treatments depict the number of specimens that perished during the incubation period (dying animals do not burrow any more). Mortality within different experimental groups was statistically analysed comparing the respective linear regressions using two-factorial ANCOVA together with a parallelity test. Significant differences of water/ash content between groups were evaluated using one-way ANOVA followed by a multiple comparison test (Student–Newman–Keuls method). Body composition determined for control and the 1% CO₂ group were compared using the Mann–Whitney rank sum test. In all cases, $P < 0.05$ was accepted to indicate a significant difference.

The present study addressed two main questions: first, sensitivity to permanently elevated levels of water PCO₂ was monitored in an organism previously considered as apparently “CO₂-tolerant” (Pörtner and Reipschläger, 1996). Secondly, we analysed whether continued CO₂ exposure leads to the degradation of body protein *in vivo*, as a consequence of decreased protein synthesis and ongoing use of amino acid substrates (Langenbuch and Pörtner, 2002; Langenbuch, unpublished data).

The control group displayed low but continuous mortality, which can be attributed to animal handling, e.g. the repeated removal from the sediment for weighing. This indicates that all animals had to cope with a slightly increased level of background stress, which should clearly reveal additional harmful effects of elevated CO₂. Parallelity comparison of fitted linear regressions indeed revealed significant differences between animal survival rates in all experimental groups (see Fig. 1). Exposure to 3% CO₂ caused an immediate onset of an elevated death rate (as compared to control animals; $F = 61.806$, $P < 0.001$) indicating the transgression of an upper lethal threshold. Even incubation under 1% CO₂ led to markedly increased mortality ($F = 97.756$, $P < 0.001$), but only after 35 days of CO₂ exposure ($F = 367.489$, $P < 0.001$). This initial delay, reflected in non-significant differences between slopes observed in control animals and those under 1% CO₂ for about 5 weeks ($F = 0.096$, $P = 0.763$), indicates

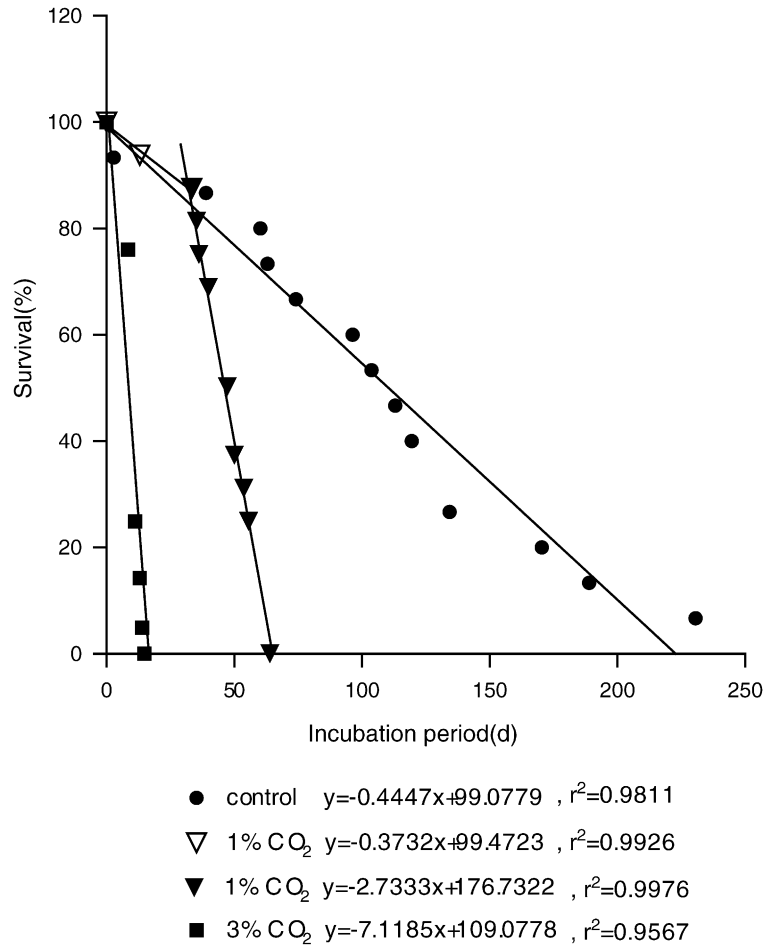


Fig. 1. Percent survival of *Sipunculus nudus* over time during long-term incubation under control conditions (bubbling with 100% air) or continued exposure to elevated levels of PCO₂ (99% air/1% CO₂ or 97% air/3% CO₂).

that, for a limited time period, *S. nudus* is able to fully cope with moderately elevated PCO₂ conditions (1%). Overall, time to 100% mortality decreased from more than 220 days under control conditions (seawater pH 8.10 ± 0.10) to 64 days under 1% CO₂ (pH 7.05 ± 0.10) and 15 days under 3% CO₂ (pH 6.55 ± 0.05).

Interestingly, a change in animal behaviour occurred concomitant to the CO₂ induced increase in mortality. Control animals burrowed immediately after placing them on the sediment surface and dwelling times in their submerged burrow were rarely but regularly interrupted by new burrowing activities. In contrast, *S. nudus* incubated under 1% CO₂ reacted more slowly and after about 4 weeks of incubation some of the ani-

mals were too weak to reburrow, remained on the sediment surface and most of them perished within several days. At high-CO₂ levels (3%), animals seemed apathetic from the beginning of the incubation period. Specimens left their sediment burrows and remained on the surface without displaying any burrowing activities.

Continued starvation was hypothesized to be a consequence of the observed reduction in motor activities (animals are ingesting sediment while burrowing; Hyman, 1959). Due to the fact that energy production in *S. nudus* is almost exclusively fuelled by protein or amino acids (Langenbuch and Pörtner, 2002), we expected a depletion of nutrient stores, especially mus-

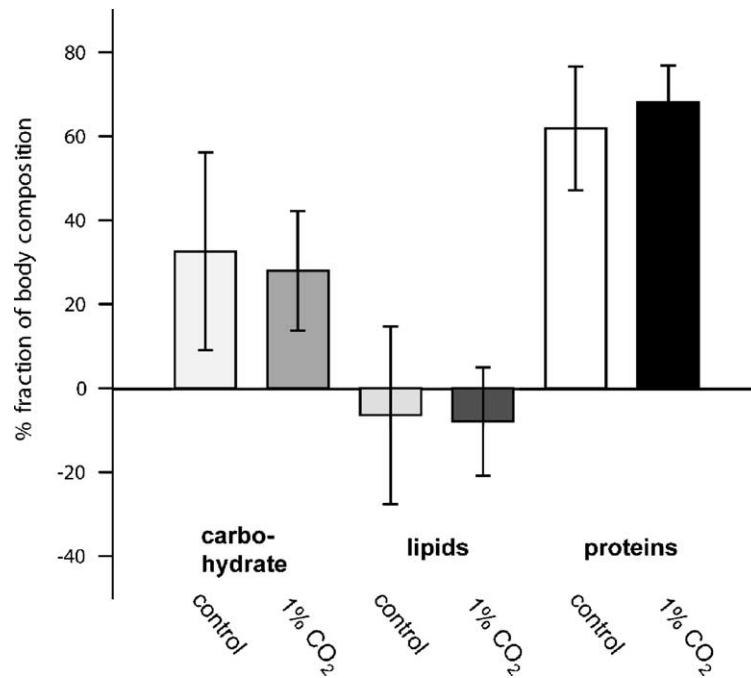


Fig. 2. Proximate biochemical body composition of *Sipunculus nudus* after 67 days of exposure either to control conditions (0.03% CO₂) or to water equilibrated with 1% CO₂ in air. Data are given as mean \pm S.D., $N = 5$. Analyses of CHN ratios of total dry biomass and ash revealed no significant differences in the fractions of body stores between control and CO₂-exposed groups. In contrast to high-protein levels, whole body lipid content was found minimal with small-negative computed values and high-standard deviations. This slight underestimation seems to be an intrinsic problem of the stoichiometric model (Gnaiger and Bitterlich, 1984) also reported from other studies (Anger and Harms, 1990).

cle protein. However, body nutrient classes calculated from CHN (carbon, hydrogen and nitrogen) elemental ratios determined in total dry mass as well as in ash (according to Gnaiger and Bitterlich, 1984) indicated a stable body composition of *S. nudus* under CO₂ stress (see Fig. 2) for the carbohydrate and lipid fraction ($P = 1.0$ in both cases) as well as for proteins ($P = 0.7$). A moderate decrease in body dry weight was found in groups exposed to 1 and 3% CO₂, mirrored by a significant decline in body ash content (Fig. 3B) from a control value of $38.45 \pm 8.86\%$ to a value as low as $21.36 \pm 6.92\%$ under 3% CO₂ ($P = 0.009$). As *S. nudus* ingests sediment and feeds on the organic compounds (Hyman, 1959), an intestinal tract filled with sand would thus explain high-ash contents in control animals and its decrease upon reduced feeding under CO₂. A concomitant increase in body water content by 3–4% (Fig. 3A; $P = 0.028$ for the 1% CO₂ group, $P = 0.032$ for the 3% CO₂ group) was sufficient to compensate for the loss in dry weight thus maintaining constant

animal freshweight. In conclusion, chronic CO₂ exposure neither led to a reduction in the animals' body mass (data not shown; $F = 0.214$; $P = 0.811$) nor a decline in the fraction of body protein or other body stores implying that no detrimental reduction in organic body compounds occurred at reduced metabolic rates prior to mortality (Fig. 2).

As a corollary, environmental stress conditions of equal magnitude as temporarily experienced in the natural habitat (values of up to 3.3 kPa PCO₂ were measured in the coelomic fluid of the intertidal polychaete *Arenicola marina* during low tide; Giebels, 1993) had fatal consequences during long-term CO₂ exposure (1%, pH 7.05). Clearly, beneficial effects seen during short-term transient exposures need to be distinguished from harmful long-term effects. One of the effective parameters is water pH and tolerance periods likely depend on the level of acidosis. A significant influence of large-scale ambient pH disturbances on short-term survival rates of other marine invertebrates

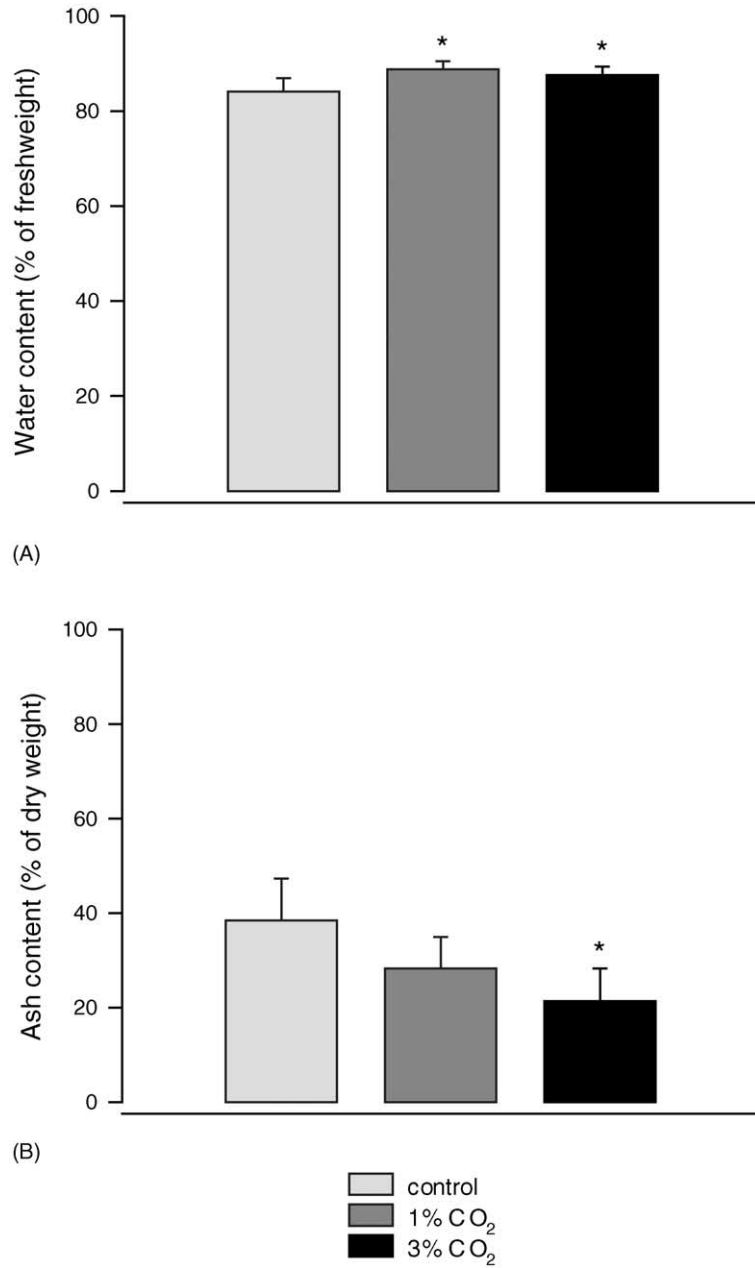


Fig. 3. Body water (A) and ash content (B) of *Sipunculus nudus* subjected to 67 days under 0.03 and 1% or 15 days under 3% CO₂. (*) Indicates values significantly different from the control group at 0.03% CO₂. Values are mean ± S.D., N = 5–6.

has been found in a variety of studies. Critical levels for significant mortality, for instance, ranged from pH 6.6 for *Mytilus edulis* to pH 6.0 for *Crassostrea gigas* after 30 days (Bamber, 1990). Other organisms, like the mesopelagic copepod *Paraeuchaeta elongata*, displayed much higher sensitivity to water pH. A reduction by just 0.2 units below that typically found at mid-depths off California was sufficient to cause 50% mortality after 6 days of exposure (Yamada and Ikeda, 1999). In addition to pH, CO₂ and associated changes in bicarbonate levels may exert specific effects and enhance sensitivity (Pörtner and Reipschläger, 1996).

Present data clearly show that CO₂-exposed animals did not die from a depletion of body stores. Animals were obviously able to effectively reduce ATP-consuming processes and to sustain maintenance metabolism although nutrient provision was diminished. Therefore, the identification of lethal mechanisms under moderately increased CO₂ concentrations awaits further investigation. Other factors than starvation are undoubtedly involved and mortality may, in fact, result from an integration of several effects. The inhibitory neurotransmitter adenosine that accumulates under CO₂ (Reipschläger et al., 1997) exerts its inhibitory effects via depressing neuronal excitability (seen e.g. in mammalian brain; Rudolphi et al., 1992) as well as the excitability at neuromuscular endplates (Robitaille et al., 1999; Thomas and Robitaille, 2001). As shown previously, the accumulation of adenosine causes metabolic depression and is beneficial to survive temporary CO₂ fluctuations (Reipschläger et al., 1997) but is possibly getting harmful under long-term exposure. Toxic consequences of hypercapnia may not only involve shifting pH and ion equilibria (Cameron and Iwama, 1989) but as a consequence of reduced protein biosynthesis rates under CO₂ (Langenbuch and Pörtner, 2002; Langenbuch, unpublished data) may also include disturbance of the continual replacement of damaged functional proteins essential in homeostatic regulation.

Future studies will have to clarify the dose- and time-dependent influence of CO₂-induced disturbances of cellular ion homeostasis and central neurotransmitter patterns on animal survival. In addition to some progress in the development of a mechanistic understanding of CO₂-induced mortality, our experimental approach and results emphasize the utmost importance of establishing in vivo critical

thresholds for the limitation of growth, scope for activity and reproductive performance in various marine animal groups. Long-term exposure to lower sublethal CO₂ concentrations may, nevertheless, exert changes in body composition and metabolic equilibria and thus may shorten survival only on a very long time scale. This issue remains to be examined by a larger series of titration experiments to study the long-term effects of progressively increasing levels of hypercapnia. Recent experiments, for instance, showed that in animals with calcite shells, like sea urchins or gastropods, reduced growth and enhanced mortality may set in at CO₂ concentrations only 200 ppm above present levels (Shirayama, 2002). Other organisms may transiently survive under chronic CO₂ stress but may not live long enough to produce viable offspring. Long-term consequences for species density and success result, which will most probably affect a much higher number of marine species than previously thought, even those adapted to regular CO₂ fluctuations. Such changes may become effective long before the future rise in ocean CO₂ will reach acutely lethal concentrations.

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