Acute toxicity of lowered pH to some oceanic zooplankton

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Abstract: Acute toxicity of lowered pH (pH≤4) was tested on 10 oceanic zooplankton species, including Conchoecia sp., Calanus pacificus, Neocalanus cristatus, Eucalanus bungii bungii, Pseudocalanus minutus, Metridia pacifica, Paraeuchaeta elongata, Themisto japonica, Euphausia pacifica (nauplii and juveniles) and Sagitta elegans. As indices of pH sensitivity in the zooplankton, the pH levels causing 50% mortality (LC₅₀) and zero mortality (LC₀) were estimated from the mortality-log₁₀[pH] relationship every 24 h for up to 168 h. The sensitivity of zooplankton to lowered pH was species specific; 24-h LC₅₀ ranged from pH 4.7 to 6.2, 48-h LC₅₀ from pH 5.0 to 6.4 and 72-h LC₅₀ from 5.0 to 6.7, and 96-h LC₅₀ from 5.0 to 6.7, whereas 24-h LC₀ ranged from pH 5.1 to 7.0, 48-h LC₀ from 5.3 to 7.2, 72-h LC₀ from 5.4 to 7.8, and 96-h LC₀ from 5.6 to 7.8. For all species tested, both LC₅₀ and LC₀ increased with increasing exposure time. Differences in swimming behavior, food habit, size and presence of gills in the zooplankton were not significantly related to sensitivity to lowered pH. The present results suggest that marine zooplankton are much more sensitive than freshwater zooplankton to acidic pH.

Key words: zooplankton, acute toxicity, pH

Introduction

The environmental impact of low pH is well known in freshwater ecosystems in North America and Europe since the detrimental effects of acid precipitation and acid mine discharge became evident (Jeffries & Mills 1990; Heath 1995). Studies have shown that decreases in the diversity of phytoplankton, zooplankton and fish have occurred in recently acidified freshwater systems, and critical low pH levels causing significant loss in species have been established for various types of organisms (Jeffries & Mills 1990). In contrast, low pH has been an environmental issue only in local marine systems, such as nearshore waters receiving acid effluents from coastal power plants (Knutzen 1981; Bamber 1990) and acid-waste dumping sites (Vaccaro et al. 1972). Currently available information about the effect of reduced pH on marine organisms is limited to several species of algae, benthic molluscs and fishes, most of which are from nearshore waters (Knutzen 1981; Bamber 1987, 1990; Davies 1991).

The pH range of oceanic waters is 7.5 to 8.4 (Chester 1990). Because it is relatively stable in the sea, pH has long been neglected as an environmental parameter in the physiological study of marine zooplankton. To date, the acute effect of lowered pH on marine zooplankton has been studied only incidentally in a few neritic copepods (Marshall et al. 1935; Grice et al. 1973; Rose et al. 1977). As a tentative measure to mitigate global warming, Omori et al. (1998) discussed the potential effects of ocean CO₂ disposal on planktonic and nektonic animals and noted our complete lack of knowledge about the tolerance of these pelagic animals to the co-occurring decrease in pH and the increase in partial pressure of CO₂ in the seawater. In light of the increasing threat of anthropogenic acidification, study of pH stress on marine biota is becoming more important today than thought previously.

The present study aimed at establishing the acidic pH levels which are lethal to various marine zooplankton, most of which live in the epipelagic realm of the ocean. Relationships were examined between pH sensitivity and behavioral, nutritional, dimensional and morphological attributes of the species tested. The present results were then compared with those reported on freshwater zooplankton.

Materials and Methods

Zooplankton

Ten zooplankton species, including an ostracod (Conchoecia sp.), six copepods (Calanus pacificus, Neocalanus
cristatus, Eucalanus bungii bungii, Pseudocalanus minutus, Metridia pacifica, Paraeruchaeta elongata), an amphipod (Themisto japonica), a euphausiid (Euphausia pacifica nauplii and juveniles) and a chaetognath ( Sagitta elegans) were collected from several sites offshore of southern Hokkaido, Japan (42°30'N, 140°15'E) during July 1996 to April 1997 (Table 1). Except for P. elongata, which is mesopelagic, all other species are epipelagic. E. pacifica juveniles and T. japonica have respiratory gills, while the others lack such organs (i.e. exchange of respiratory gases occurs by diffusion through the body integument). The 10 species were grouped into three swimming behavior types (continuous swimming, intermittent swimming, and mostly suspension within the water column), and two food-habit types (carnivores, and non-carnivores) (Table 1). Non-carnivores include primary herbivores (C. pacificus, N. cristatus, E. bungii bungii, P. minutus, M. pacifica, E. pacifica juveniles) and non-feeding larvae (E. pacifica nauplii).

Live zooplankton were collected using NORPAC standard nets or 80-cm ring nets towed through various bathymetric ranges between the surface and 500 m. After retrieval, the contents of the cod-end were immediately transferred to a large container filled with chilled seawater (5°C), and undamaged specimens were sorted out within 4 h after the collection. Sorted specimens were maintained in unfiltered natural seawater at 5°C, brought back to the land laboratory and used in the following experiments 0 to 3 d after collection. Subsamples (ca. 10 specimens) of each zooplankton species were placed in 10% formalin-seawater solution. A typical pH series with 10 grade steps was 8.2 (control), 7.2, 6.5, 5.8, 5.5, 5.2, 4.8, 4.5 and 4.2.

To determine the lethal effect of lowered pH, ten specimens of each zooplankton species were placed in individual 50- to 500-ml air-tight glass containers or 2.5 ml plastic multi-well plates, depending on the size of the animal, after filling with control or pH-adjusted seawater (for water below the surface), and Van Dorn- or Niskin samplers (for water above the surface) depending on the zooplankton species. Seawater was filtered through GF/F filters, autoclaved and well oxygenated at 5°C for use in experiments. Non-autoclaved seawater was also used for experiments that required a large volume of seawater (>200 ml containers). The present use of filtered seawater for experiments is advantageous because the pH of seawater will not be affected by the respiratory CO2 output by prey organisms, but the lack of prey limits zooplankton survival time. Salinity of seawater ranged from 32.2 to 34.2 PSU.

Concentrated hydrochloric acid (Wako Pure Chemical Industries, Ltd., Super Special Grade), diluted to 1 N with pure water (Milli RX 12 Plus), was used to adjust the pH of seawater. Seawater without hydrochloric acid (pH: 8.0 to 8.3) served as a control. For one series of experiments, seawater of 6 to 10 graded pH levels between 8 (control seawater) and 4 (hydrochloric acid added) was prepared. pH levels were determined with needle (#6069-10C, Horiba) or standard pH electrodes (#6366-10D), depending on the container size, connected to a pH meter (Horiba, Lab pH meter F-21). The pH meter was standardized using two pH standard solutions (4.01 and 6.95) prior to every use. Since the pH of acid-treated seawater varied to a great extent during the first 24 h (perhaps due to an exchange of carbon dioxide via the air-seawater interface), pH-adjusted seawater was left for 24 h prior to the incubation of zooplankton. A typical pH series with 10 grade steps was 8.2 (control), 7.2, 6.5, 5.8, 5.5, 5.2, 4.8, 4.5 and 4.2.

Lethal effect of lowered pH

Table 1. Zooplankton species, food habits (C: carnivores, NC: non-carnivores), swimming behavior patterns (C: continuous swimming, I: intermittent swimming, S: mostly suspended) and experimental details

<table>
<thead>
<tr>
<th>Animal taxa</th>
<th>Species, stage</th>
<th>Sampling date</th>
<th>Food habit type</th>
<th>Dry weight (mg/indiv.)</th>
<th>Nos. of Indiv. x replicates</th>
<th>Container vol (ml)</th>
<th>pH levels established</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crustacea</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ostracoda</td>
<td>Conchoecia sp.</td>
<td>21 Oct. '96</td>
<td>NC</td>
<td></td>
<td>0.027</td>
<td>10X1</td>
<td>50</td>
</tr>
<tr>
<td>Copepoda</td>
<td>Calanus pacificus, V</td>
<td>29 Aug. '96</td>
<td>NC</td>
<td></td>
<td>0.0457</td>
<td>10X2</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Neocalanus cristatus, V</td>
<td>8 Apr. '97</td>
<td>NC</td>
<td></td>
<td>3.80</td>
<td>10X1</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>Eucalanus bungii bungii, IV–V</td>
<td>21 Oct. '96</td>
<td>S</td>
<td>0.116</td>
<td>10X2</td>
<td>200</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Pseudocalanus minutus, V</td>
<td>29 Aug. '96</td>
<td>NC</td>
<td></td>
<td>0.0227</td>
<td>10X2</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Metridia pacifica, III–V</td>
<td>8 Aug. '96</td>
<td>C</td>
<td>0.0698</td>
<td>10X2</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Paraeruchaeta elongata, VI</td>
<td>8 Apr. '97</td>
<td>C</td>
<td>3.09</td>
<td>10X1</td>
<td>200</td>
<td>9</td>
</tr>
<tr>
<td>Amphipoda</td>
<td>Themisto japonica</td>
<td>24 Jul. '96</td>
<td>C</td>
<td>0.0967</td>
<td>5X2</td>
<td>100</td>
<td>6</td>
</tr>
<tr>
<td>Euphausiacea</td>
<td>Euphausia pacifica, nauplii</td>
<td>21 Oct. '96</td>
<td>S</td>
<td>0.0051</td>
<td>10X2</td>
<td>2.5</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Euphausia pacifica, juvenile</td>
<td>24 Jul. '96</td>
<td>C</td>
<td>0.417</td>
<td>5X1</td>
<td>100</td>
<td>6</td>
</tr>
<tr>
<td>Chaetognatha</td>
<td>Sagitta elegans</td>
<td>10 Apr. '97</td>
<td>C</td>
<td>0.940</td>
<td>10X1</td>
<td>500</td>
<td>8</td>
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</tbody>
</table>
Fig. 1. A model experiment on the mortality–log_{10}pH relationship as a function of exposure time for every 24 h up to 168 h using *Eucalanus bungii bungii* as the test animal. The results from duplicated experiments are shown by open and closed symbols. The pH levels causing 50% mortality (LC_{50}) and 0% mortality (LC_{0}) were computed from the regression line.

(continued)

Assessment model for lethal and non-lethal effects

To establish an assessment model, the results of the copepod *Eucalanus bungii bungii* were selected as a typical time series data set. Groups of 10 specimens were placed in seawater with pH adjusted to 10 graded levels between 8.0 (control) and 4.4, and the mortality of specimens was examined every 24 h up to 168 h. The experiment was run with two replicates. Plotting data on a semi-log graph indicated that there was a linear relationship between percent mortality and pH (Fig. 1). The pH levels that caused 50% (LC_{50}) and 0% mortality (LC_{0}) of the specimens were calculated every 24 h from the fitted regression line. No death of control specimens was observed up to 168 h, and thus the calculated 24, 48, 72, 96, 120, 144 and 168-h LC_{50} or LC_{0} are summarized in Table 2.

LD_{50} and LD_{0} for lowered pH

Among the 10 zooplankton species tested, abnormal
swimming behavior and death of specimens in control sea-water was observed after 72 h for *Metridia pacifica* and *Sagitta elegans*, and after 168 h for *E. pacifica juveniles*, *Themisto japonica* and *Euphausia pacifica juveniles*. For the other 5 species and *E. pacifica* nauplii, specimens in control seawater exhibited abnormal swimming behavior and death after 72–168 h (Table 2). Judging from 24-h swimming behavior patterns, trophic type, morphology and size (dry weight) of zooplankton were examined using 24 hourly data up to 72 h exposure. The results of statistical tests indicated that swimming pattern (*F*-test, *p* > 0.2), feeding habit (*t*-test, *p* > 0.08) and presence of gills (*t*-test, *p* > 0.5) were all not correlated significantly with the observed variations in 24-h, 48-h and 72-h LC50 or LC0 data for 10 oceanic zooplankton species including *Euphausia pacifica* nauplii. A scatter diagram for the relationship between 24-h LC50 (or LC0) and size (mg dry weight) of zooplankton is shown in Fig. 2. The correlation coefficient between these two parameters was not significant (*p* > 0.05), nor was there significant correlation in 48-h and 72-h data sets of LC50 or LC0.

### Discussion

In the first report on the effect of lowered pH on marine zooplankton, Marshall et al. (1935) noted that the copepod *Calanus finmarchicus* exposed to pH 6.7 for 48 h at 12°C
pressed as 24-h LC50 and 24-h LC0) and dry weight (mgDW) of Calanus pacificus, Neocalanus cristatus, Eucalanns bungii, Pseudocalamts minutus, Metridia pacifica, Sagitta elegans.

was apparently unharmed (i.e. 48-h LC0 > pH 6.7). As part of a study evaluating the effect of acid-iron waste, Grice et al. (1973) reared the marine copepod Temora longicornis in seawater with pH lowered by the addition of sulphuric acid and found the 48-h LC50 (pH) to be about 5.5 at 17–18°C. Rose et al. (1977) tested the acute toxicity of low pH seawater (adjusted by the addition of hydrochloric acid) on the marine copepod Acartia tonsa and observed that the 96-h LC50 of this copepod was pH 6.31 at 21°C. Taking into account the exposure time, all results by previous workers fall well within the range of the present results. However, direct comparison of the sensitivity data gained at dissimilar temperatures may not be valid, since the effect of temperature on the tolerance of zooplankton toward lowered pH is currently unknown. An increase in pH sensitivity with increasing temperature has been postulated to occur in marine benthic molluscs (Bamber 1987, 1990).

From the viewpoint of biology at low pH, inhabitants of the oxygen minimum layer, which develops at 700–800 m depth in the Pacific off the coasts of North and South America, are of special interest, since pHs in this layer are 7.5 or less (Park 1968). Michel & Childress (1978) examined the effects of pH on oxygen consumption and pleopod movement in a mysid, Gnaithophausia ingens, collected from the oxygen minimum layer off the coast of southern California and brought to a land laboratory. Comparing the results obtained at pHs 7.1 and 7.9, Michel & Childress (1978) found no appreciable difference in oxygen consumption rates, pleopod movements nor any relationship between the two. G. ingens is different from most other zooplankton in that it has an extreme capacity to withdraw oxygen from a low oxygen environment (Teal & Carey 1967) and uses hemocyanin as an oxygen carrier (Freel 1978). Many marine planktonic crustaceans and other groups are known to lack oxygen carriers in their body fluids (Prosser & Brown 1961; Mangum 1983). Because of these differences, direct application of pH sensitivity of G. ingens to other marine zooplankton cannot be made.

The sizes of the 10 oceanic zooplankton species used in this study ranged over three orders of magnitude (0.0051 to 3.80 mg dry weight, cf. Table 1). A correlation analysis indicated no significant relationship between pH sensitivity (LC50 and LC0) and zooplankton size (Fig. 2). This suggests that the metabolic activity of zooplankton, which is expressed as a function of body size (Ikeda 1985), is not an important parameter affecting the sensitivity to lowered pH in the present results. Similarly, swimming pattern, food habit, and presence of a gill organ in the zooplankton were all found to be unrelated to the observed LC50 or LC0 levels of pH in this study. These results suggest that the ability to tolerate lowered pH is highly variable between and possibly within species (as in the case of nauplii and juveniles of Euphausia pacifica, Table 2), as was noted in the study of Bamber (1987, 1990) on several marine benthic molluscs. A mesopelagic copepod, Paracalanus elongata, exhibited the greatest sensitivity (the highest 24-h LC50 and LC0) among the 10 species tested (Table 2). In light of the wide species-specific variation in the epipelagic data and paucity of mesopelagic data, whether or not the bathymetric range of zooplankton habitat affects the sensitivity to lowered pH is difficult to conclude at present.

A common feature seen over the 10 oceanic zooplankton species studied is a progressive increase in LC50 and LC0 with increasing exposure time (Table 2), suggesting that exposure time is also an important parameter when evaluating the tolerance of zooplankton toward lowered pH. According to Havas & Hutchinson (1982), freshwater planktonic crustaceans (Daphnia middendorffiana, Diaptomus arcticus, Lepidurus arcticus, Branchiopoda) survive at pH > 4.5 and higher up to 400 h, and insect larvae (Chironomus riparius, Orthocladiinae consobrinus, Limnephilus pallens) survive at pH ≥3.5 for 50 h to 20 d. Walton et al. (1982) also have observed that Daphnia pulex survive at pH 3.7 for 96 h with no appreciable mortality. Although tolerance toward low pH in freshwater zooplankton differs within species to some extent, depending on the pH and the composition of potentially toxic elements (Ca, Na, CO2 and heavy metals) in the water from which they originated, critical pH levels (the pH below which mortality increases significantly compared to the control) for these freshwater zooplankton are 3.5 to 4.5. All of these reported critical pH levels for freshwater zooplankton are much lower than the 5.0 to 6.7 (24- to 96-h LC0) range for oceanic zooplankton determined in the present study. For freshwater zooplankton, harmful effects of acid pH are considered to be due to a failure in ionic regulation, e.g. exchange and balance of Na+ and Cl− (Vangenecten et al. 1989). However, information about any similarity/dissimilarity in the lethal mechanism of this low pH to zooplankton living in freshwater and
Lowered pH and Zooplankton

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Literature Cited


