Plankton Biol. Ecol. 46 (1): 62–67, 1999

plankton
biology & ecology

Acute toxicity of lowered pH to some oceanic zooplankton

YUICHIRO YAMADA & TSUTOMU IKEDA

Biological Oceanography Laboratory, Faculty of Fisheries, Hokkaido University, Hakodate 041–0821, Japan

Received 11 November 1997; accepted 10 July 1998

Abstract: Acute toxicity of lowered 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 (LC_{50}) and zero mortality (LC_0) were estimated from the mortality–log_{10}[pH] relationship every 24 h for up to 168 h. The sensitivity of zooplankton to lowered pH was species specific; 24-h LC_{50} ranged from pH 4.7 to 6.2, 48-h LC_{50} from pH 5.0 to 6.4 and 72-h LC_{50} from 5.0 to 6.7, and 96-h LC_{50} from 5.8 to 6.6, whereas 24-h LC_0 ranged from pH 5.1 to 7.0, 48-h LC_0 from 5.3 to 7.2, 72-h LC_0 from 5.4 to 7.8, and 96-h LC_0 from 5.6 to 7.8. For all species tested, both LC_{50} and LC_0 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 CO_2 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 CO_2 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*

Corresponding author: Tsutomu Ikeda; e-mail, tom@pop.fish.hokudai.ac.jp

cristatus, *Eucalanus bungii bungii*, *Pseudocalanus minutus*, *Metridia pacifica*, *Paraeuchaeta 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 (42–43°N, 140–145°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 species were preserved in 10% formalin-seawater solution. For dry weight determination, a batch of 4 to 10 preserved specimens was rinsed with distilled water, dried at 60°C for 4 h, and weighed.

pH adjusted seawater

Seawater was collected concurrently with net tows at each zooplankton sampling site using a plastic bucket (for surface water), and Van Dorn- or Niskin samplers (for water below 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 CO_2 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, 5.0, 4.8, 4.5 and 4.2.

Lethal effect of lowered pH

To determine the lethal effect of lowered pH of seawater, 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

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

Animal taxa	Species, stage	Sampling date	Food habit type	Behavior pattern	Dry weight (mg/indiv.)	Nos. of Indiv. ×replicates	Container vol (ml)	pH levels established
Crustacea								
Ostracoda	*Conchoecia* sp.	21 Oct. '96	NC	I	0.027	10×1	50	10
Copepoda	*Calanus pacificus*, V	29 Aug. '96	NC	I	0.0457	10×2	100	6
	Neocalanus cristatus, V	8 Apr. '97	NC	I	3.80	10×1	200	9
	Eucalanus bungii bungii, IV–V	21 Oct '96	NC	S	0.116	10×2	200	10
	Pseudoclanus minutus, V	29 Aug. '96	NC	C	0.0227	10×2	100	6
	Metridia pacifica, III–V	8 Aug. '96	NC	C	0.0698	10×2	50	6
	Paraeuchaeta elongata, VI	8 Apr. '97	C	C	3.09	10×1	200	9
Amphipoda	*Themisto japonica*	24 Jul. '96	C	C	0.0967	5×2	100	6
Euphausiacea	*Euphausia pacifica*, nauplii	21 Oct. '96	NC	S	0.0051	10×2	2.5	10
	Euphausia pacifica, juvenile	24 Jul. '96	NC	C	0.417	5×1	100	6
Chaetognatha	*Saitta elegans*	10 Apr. '97	C	S	0.940	10×1	500	8

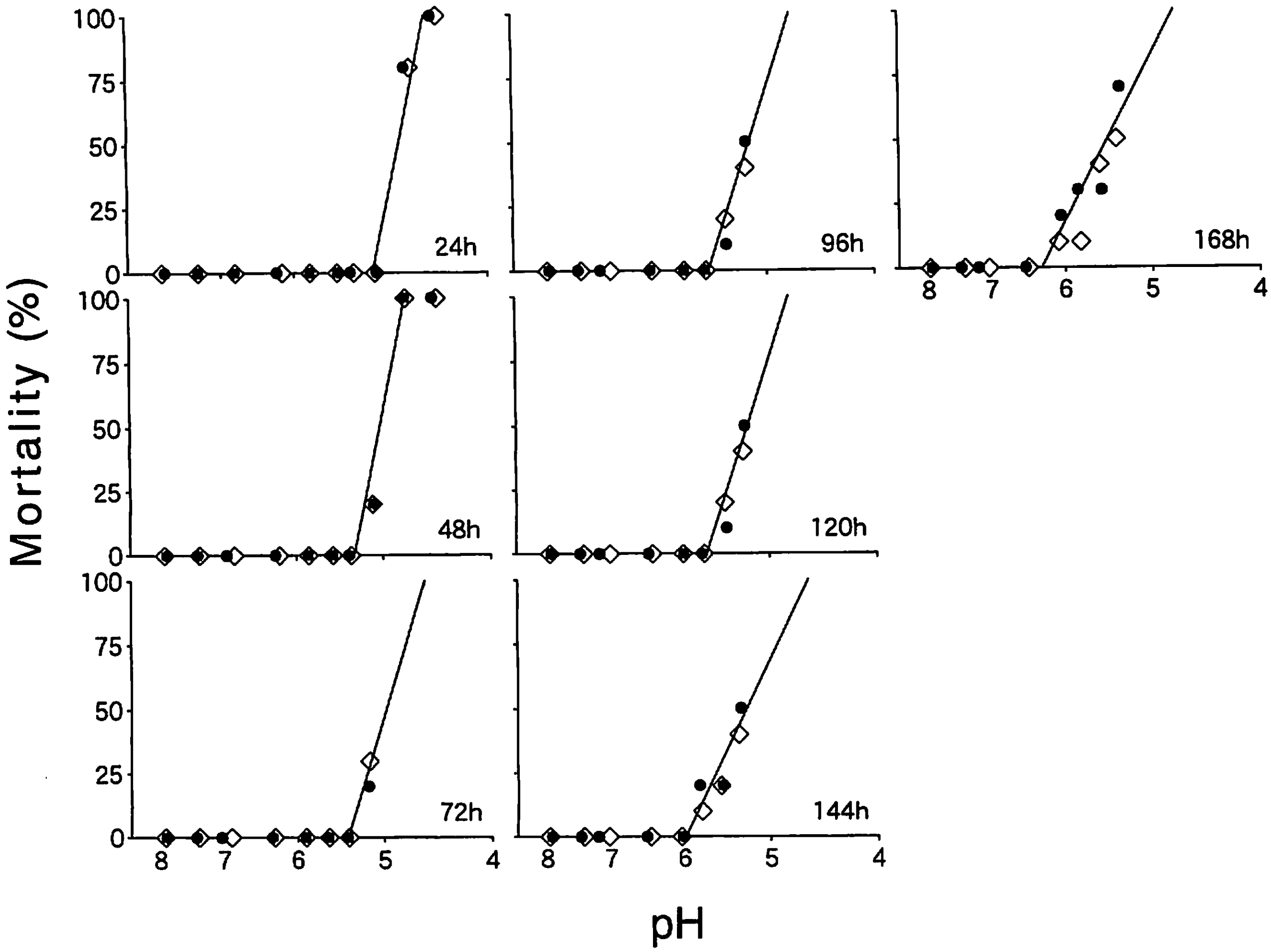

Fig. 1. A model experiment on the mortality–$\log_{10}$pH relationship as a function of exposure time for every 24 h up to 168 h using *Eucalanus bungii bungii* as the test animal. The results from duplicated experiments are shown by open and closed symbols. The pH levels causing 50% mortality (LC_{50}) and 0% mortality (LC_0) were computed from the regression line.

(Table 1). For the amphipod *T. japonica* and the euphausiid *E. pacifica* juveniles, five specimens were incubated per container to avoid cannibalism by crowding for the former and due to limited availability of specimens for the latter. The experiments were run in the dark at 5°C, which is within the natural temperature range of all the species used in this study. Once every 24 h for up to a total of 168 h, the pH of each container was measured and dead specimens were removed. The experiment ended when individuals in the control container showed signs of either anomalous swimming behavior or death. Specimens were defined as "dead" when they exhibited faded body color, no movement of appendages, and/or no response to touching with a glass pipette. Dead specimens often sank to the bottom of the container. Because initially adjusted pHs were observed to change during the course of the experiments (0.3 pH unit at most over 169 h), pH for LC_{50} or LC_0 was expressed as an average of the previous 24 hourly readings, including that at the start of the experiment (0 h).

Results

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

Table 2. LC_{50} and LC_0 of zooplankton exposed to low pH seawater as a function of exposure time. Blanks denote no data as the experiment was ended due to the onset of death in control animals. The increase in LC_{50} or LC_0 with increasing exposure time was tested by using a linear regression model.

Species		Exposure time (h)							H_0: slope>0
		24	48	72	96	120	144	168	p
Conchoecia sp.	LC_{50}	5.40	5.95	6.11	6.25	6.44			<0.05
	LC_0	5.98	6.43	6.63	7.79	8.38			<0.01
Calanus pacificus	LC_{50}	5.83	6.02	6.02	6.14	6.14			<0.05
	LC_0	6.33	6.32	6.33	6.79	6.79			NS
Neocalanus cristatus	LC_{50}	5.39	5.67	5.82	5.95	6.07	6.16		<0.01
	LC_0	5.92	6.21	6.22	6.16	6.24	6.38		<0.05
Eucalanus bungii bungii	LC_{50}	4.79	5.02	4.96	5.16	5.12	5.23	5.53	<0.01
	LC_0	5.07	5.30	5.39	5.63	5.69	5.97	6.35	<0.01
Pseudocalanus minutus	LC_{50}	5.57	(5.64)*	(5.70)*	5.77	5.78	6.02		<0.01
	LC_0	5.89	(6.14)*	(6.38)*	6.63	6.65	6.65		<0.01
Metridia pacifica	LC_{50}	5.07	5.40	5.68					<0.05
	LC_0	6.00	5.92	6.39					NS
Paraeuchaeta elongata	LC_{50}	6.23	6.36	6.50	6.57	6.64	6.83		<0.01
	LC_0	6.95	7.16	7.21	7.24	7.24	7.41		<0.01
Themisto japonica	LC_{50}	4.74	5.33	5.11	5.63	6.25	6.44	6.70	<0.01
	LC_0	5.07	5.77	6.18	6.26	6.81	7.15	7.28	<0.01
Euphausia pacifica, nauplii	LC_{50}	5.22	5.44	5.73	5.93	6.10			<0.01
	LC_0	5.45	6.28	6.57	6.80	6.90			<0.05
Euphausia pacifica, juvenile	LC_{50}	5.93	$(6.03)^{+}$	$(6.14)^{+}$	$(6.24)^{+}$	6.34	6.61	6.84	<0.01
	LC_0	6.51	$(6.65)^{+}$	$(6.80)^{+}$	$(6.94)^{+}$	7.08	7.58	7.54	<0.01
Sagitta elegans	LC_{50}	5.91	6.24	6.73					~0.05
	LC_0	6.70	6.93	7.76					NS

* Interpolated from 24 and 96 h data. $^{+}$ Interpolated from 24 and 120 h data.

swimming behavior and death of specimens in control seawater was observed after 72 h for *Metridia pacifica* and *Sagitta elegans*, and after 168 h for *E. bungii bungii*, *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 LC_{50}, *T. japonica* and *E. bungii bungii* were the most tolerant of a decrease in pH (4.7 or 4.8), while *Paraeuchaeta elongata* was the most sensitive to lowered pH (6.2). For the 10 species examined, the pH levels for 48-h LC_{50}, 72-h LC_{50} and 96-h LC_{50} ranged from 5.0 to 6.4, 5.0 to 6.7 and 5.8 to 6.6, respectively, showing a gradual increase with increasing exposure time. This increase of LC_{50} with increasing exposure time was significant in 9 out of 10 species (linear regression model, $p<0.05$), and its pattern was highly species-specific (covariance test; $F=11.81$, df=10,33, $p<0.005$). In terms of 24-h LC_0, the highest pH (7.0) and lowest pH (5.1) 24-h LC_0 were also found in *P. eleongata*, and *T. japonica* and *E. bungii bungii*, respectively (Table 2). As for LC_{50}, the pH levels of the 10 species for 48-h LC_0 (5.3 to 7.2), 72-h LC_0 (5.4 to 7.8) and 96-h LC_0 (5.6 to 7.8) increased species-specifically with increasing exposure time (covariance test; $F=11.89$, df=10,33, $p<0.005$). In trials lasting 48 and 72 h with *Pseudocalanus minutus*, and 48 and 96 h with *E. pacifica* juveniles, mortality was too low to calculate a regression line, so LC_{50} and LC_0 were estimated by linear interpolation from 24 and 96 h data for the former and from 24 and 120 h data for the latter.

The relationships between LC_{50} (or LC_0) and 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 LC_{50} or LC_0 data for 10 oceanic zooplankton species including *Euphausia pacifica* nauplii. A scatter diagram for the relationship between 24-h LC_{50} (or LC_0) 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 LC_{50} or LC_0.

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

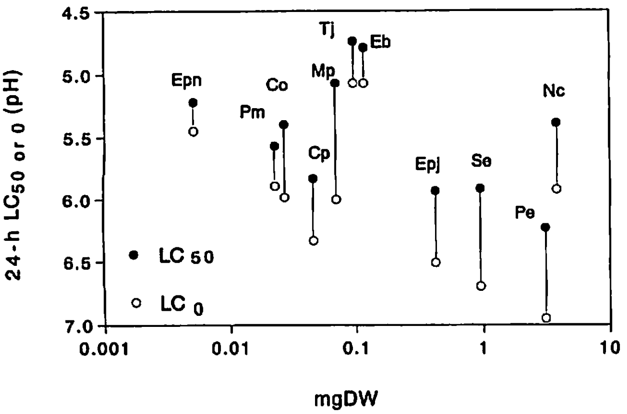

Fig. 2. The relationships between sensitivity to lowered pH (expressed as 24-h LC_{50} and 24-h LC_0) and dry weight (mgDW) of 10 oceanic zooplankton species. Both relationships are not significant ($p>0.05$). Species abbreviations are: Co=*Conchoecia* sp., Cp=*Calanus pacificus*, Nc=*Neocalanus cristatus*, Eb=*Eucalanus bungii bungii*, Pm=*Pseudocalanus minutus*, Mp=*Metridia pacifica*, Pe=*Paraeuchaeta elongata*, Tj=*Themisto japonica*, Epn=*Euphausia pacifica* nauplii, Epj=*Euphausia pacifica* juvenile, and Se=*Sagitta elegans*.

was apparently unharmed (i.e. 48-h LC_0 >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 LC_{50} (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 LC_{50} 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). Mickel & Childress (1978) examined the effects of pH on oxygen consumption and pleopod movement in a mysid, *Gnathophausia 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, Mickel & 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 (LC_{50} and LC_0) 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 LC_{50} or LC_0 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, *Paraeuchaeta elongata*, exhibited the greatest sensitivity (the highest 24-h LC_{50} and LC_0) 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 LC_{50} and LC_0 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*, *Branchinecta paludosa*) survive at pH >4.5 and higher up to 400 h, and insect larvae (*Chironomus riparius*, *Orthocladius consobrinus*, *Limnephilus pallens*) survive at pH ≥3.5 for 50 h to 20 d. Walton et al. (1982) also have observed that *Daphinia 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, CO_2 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 LC_0) 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 marine environments is presently lacking and, as a result, an explanation as to why marine zooplankton are more sensitive to pH change than freshwater zooplankton is not possible. From the viewpoint of seawater chemistry, a reduction in pH affects the carbonate system, the chemical form of metals and the characteristics of substances dissolved in it. Thus, a drop in pH can reduce or increase the toxicity and availability for uptake of many substances, in particular weak acids and bases (Knutzen 1981).

While LC_{50} and LC_0 data gained in the present study are useful when assessing acute (lethal) effects of lowered pH on marine zooplankton, information about the levels that induce chronic (sublethal) effects would be a more appropriate measure for estimating the long-term consequences for a given zooplankton population, i.e. zooplankton that suffer sublethal effects due to low pH can survive but may not be able to produce normal offspring. For freshwater zooplankton, such sublethal stress due to low pH can reduce clutch size and delay maturation (Walton et al. 1982). Grice et al. (1973) observed that the marine copepod *Temora longicornis* failed to produce nauplii at pH 6.6, and only a few nauplii hatched at pH 6.9. A pH around 7 has been suggested to be sublethal to marine lamellibranch molluscs; below this level, reduced feeding, growth suppression, reduced shell size and shell dissolution occur (Knutzen 1981; Bamber 1987, 1990). Sublethal effects are not limited only to reproduction and growth, but may also alter behavior, but no information about the behavioral response of marine zooplankton to lowered pH is presently available (cf. Omori et al. 1998). For marine fishes, behavioral responses have been observed to occur at pH <6.5 in sand smelt (*Atherina boyeri*) (Davies 1991) and at pH 5 in Gulf killifish (*Fundulus grandis*) (MacFarlane & Livingston 1983).

In conclusion, since our present knowledge of pH tolerance in marine organisms, including zooplankton, is limited as such, estimating the possible effects of local or global anthropogenic acidification in the ocean is not possible. To help in efforts to conserve natural marine systems, research on the toxicity of lowered pH now deserves immediate attention from biological oceanographers.

Acknowledgments

We are grateful to C. B. Miller of Oregon State University for his critical reading of the early manuscript and valuable comments. J. R. Bower and two anonymous reviewers provided comments which improved the text.

Literature Cited

Bamber, R. N. 1987. The effects of acidic seawater on young carpet-shell clams *Venerupis decussata* (L.) (Mollusca: Veneracea). *J. Exp.Mar. Biol. Ecol.* **108**: 241–260.

Bamber, R. N. 1990. The effects of acidic seawater on three species of lamellibranch mollusc. *J. Exp. Mar. Biol. Ecol.* **143**: 181–191.

Chester, R. 1990. *Marine Geochemistry*. Chapman & Hall, London, 698 pp.

Davies, J. K. 1991. Reactions of sand smelt to low pH sea-water. *Mar. Pollut. Bull.* **22**: 74–77.

Freel, R. W. 1978. Oxygen affinity of the hemolymph of the mesopelagic mysidacean *Gnathophausia ingens*. *J. Exp. Zool.* **204**: 267–274.

Grice, G. D., P. H. Wiebe & E. Hoagland 1973. Acid-iron waste as a factor affecting the distribution and abundance of zooplankton in the New York Bight. I. Laboratory studies on the effects of acid waste on copepods. *Estur. Coast. Mar. Sci.* **1**: 45–50.

Havas, M. & T. C. Hutchinson 1982. Aquatic invertebrates from the Smoking Hills, N.W.T.: Effect of pH and metal on mortality. *Can. J. Fish. Aquat. Sci.* **39**: 890–903.

Heath, A. G. 1995. *Water Pollution and Fish Physiology*. CRC Press, Inc., Boca Raton, 375 pp.

Ikeda, T. 1985. Metabolic rates of epipelagic marine zooplankton as a function of body mass and temperature. *Mar. Biol.* **85**: 1–11.

Jeffries, M. & D. Mills 1990. *Freshwater Ecology*. Belhaven Press, London, 285 pp.

Knutzen, J. 1981. Effects of decreased pH on marine organisms. *Mar. Pollut. Bull.* **12**: 25–29.

MacFarlane, R. B. & R. L. Livingston 1983. Effects of acidified water on the locomotor behaviour of the Gulf Killifish, *Fundulus grandis*: a time series approach. *Arch. Env. Toxicol.* **12**: 163–168.

Mangum, C. P. 1983. Oxygen transport in the blood, p. 373–429. In *The Biology of Crustacea*, vol. 5 (ed. Mantel, L.H.). Academic Press, New York.

Marshall, S. M., A. G. Nicholls & A. P. Orr 1935. On the biology of *Calanus finmarchicus*. Part VI. Oxygen consumption in relation to environmental conditions. *J. Mar. Biol. Ass. U.K.* **20**: 1–27.

Mickel, T. J. & J. J. Childress 1978. The effect of pH on oxygen consumption and activity in the bathypelagic mysid *Gnathophausia ingens*. *Biol. Bull.* **154**: 138–147.

Omori, M., C. P. Norman & T. Ikeda Oceanic disposal of CO_2: Potential effects on deep-sea plankton and micronekton—A review. *Plankton Biol. Ecol.* **45**: 87–99.

Prosser, C. L. & F. A. Brown, Jr. 1962. *Comparative Animal Physiology*. W.B. Saunders, Philadelphia & London, 688 pp.

Rose, C. D., W. G. Williams, T. A. Hollister & P. R. Parrish 1977. Method for determining acute toxicity of an acid waste and limiting permissible concentration at boundaries of an oceanic mixing zone. *Environ. Sci. Technol.* **11**: 367–371.

Teal, J. M. & F. G. Carey 1967. Respiration of a euphausid from the oxygen minimum layer. *Limnol. Oceanogr.* **12**: 548–550.

Vaccaro, R. F., G. Grice, G. Rowe & P. H. Wiebe 1972. Acid-iron waste as a factor affecting the distribution and abundance of zooplankton in the New York Bight. *Water Res.* **6**: 231–256.

Vangenechten, J. H. D., Witters, H. and Vanderborght, O. L. J. 1989. Laboratory studies on invertebrate survival and physiology in acid waters, p. 153–169. In *Acid Toxicity and Aquatic Animals* (eds. Morris, R., E. W. Taylor, D. J. A. Brown & J. A. Brown). Cambridge University Press, Cambridge.

Walton, W. E., S. M. Compton, J. D. Allan & R. E. Daniels 1982. The effect of acid stress on survivorship and reproduction of *Daphnia pulex* (Crustacea: Cladocera). *Can. J. Zool.* **60**: 573–579.