Reduced pH increases mortality and genotoxicity in an Arctic coastal copepod, Acartia longiremis

Halsband, Claudia; Dix, Mascha F; Sperre, Kristine Hopland; Reinardy, Helena C

Published in:
Aquatic Toxicology

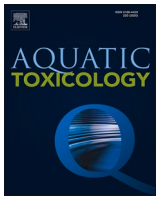
Publication date:
2021

Aquatic Toxicology 239 (2021) 105961

Contents lists available at ScienceDirect

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aqtox



Reduced pH increases mortality and genotoxicity in an Arctic coastal copepod, *Acartia longiremis*

Claudia Halsband [+,a], Mascha F. Dix [+,b], Kristine Hopland Sperre [a], Helena C. Reinardy [*,b,c]

[a] *Akvaplan-niva, Fram Centre, Tromsø, Norway*
[b] *Scottish Association for Marine Science, PA37 1QA, Dunstaffnage, UK*
[c] *University Centre in Svalbard, P.O. Box 156, Longyearbyen, Svalbard, Norway*

ARTICLE INFO

Editor: Willie Peijnenburg

Keywords:
DNA damage
fast micromethod
oxidative stress
zooplankton
Crustacea

SUMMARY

This study investigates DNA damage and mortality in an Arctic marine copepod after long-term exposure to lowered pH. *Acartia longiremis* were collected from northern Norway and incubated in ambient pH 8.1, and reduced pH 7.6 and 7.2 over 3-4 weeks. Cumulative mortality was significantly elevated in the lowered pH treatments in all exposures. The fluorescence-based fast micromethod for analysis of DNA strand breaks and alkali-labile sites was modified for use on crustaceous zooplankton. DNA damage initially increased in the lowered pH treatments, decreasing after >14 days, and DNA damage was significantly higher in lowered pH conditions. This method is ideal for investigating oxidative stress and genotoxicity response to low pH in Arctic marine copepods exposed to future ocean acidification conditions.

1. Main

DNA damage can stunt development, reduce reproductive success, and inherited compromised DNA can adversely impact individual survival and population resilience (Depledge, 1998). In response to environmental stress, intracellular reactive oxygen species (ROS) may increase, leading to oxidative damage to DNA (Silva *et al.*, 2016). Low seawater pH from rising atmospheric CO_2 emissions represents such an environmental stressor, especially in Arctic regions (Steinacher *et al.*, 2009). Sea surface pH is projected to decrease by up to 0.5 pH units by 2100 (Ciais *et al.*, 2013). Marine organisms have varying responses to reduced seawater pH (Kroeker *et al.*, 2010), and copepods have demonstrated both inter- and intra-specific variability in acid-base regulation capacity (Zhang *et al.*, 2011; McConville *et al.*, 2013). Transcriptional changes in redox and DNA repair genes suggest a shift in oxidative balance and induction of DNA repair mechanisms induced by lowered pH in Arctic *Calanus glacialis* (Bailey *et al.*, 2017). Negative sub-lethal effects may impact long-term fitness of copepod populations, and elevated mortality may affect species distribution and abundance patterns.

Here, mortality and DNA damage were analysed in the Arctic copepod *Acartia longiremis* during long-term exposure to ambient and reduced pH seawater (Table 1). The fast micromethod for analysis of DNA strand breaks and alkali-labile sites by fluorescence detection was used (Batel *et al.*, 1999; Schröder *et al.*, 2006; El-Bibany *et al.*, 2014). It allows more rapid, precise, and equally sensitive genotoxicity analysis in comparison to the widely used comet assay (Bihari *et al.*, 2002), and has been successfully applied to detect DNA damage in cells, whole larvae, tissues, and extracted DNA of several marine species (Reinardy and Bodnar, 2015, Reinardy *et al.*, 2016), but not copepods. The method was modified to allow break-down of the chitinous crustacean exoskeleton by adding a homogenisation step, and the DNA denaturation pH was elevated to initiate alkaline unwinding of copepod DNA. This method facilitates genotoxicity analysis in non-model species and is well-suited to copepods preserved after experimentation.

A. longiremis were collected from Håkøybotn, Norway (69°40'19.6''N, 18°47'07.0''E). Seawater from an intake pipe at 60m depth was filtered (10μm) and pumped into a 1000L header-tank where CO_2 was injected by pH controller (Aquamedic). Untreated (pH 8.0-8.1) and low pH water was filled into twelve replicate 1L glass bottles (n=24); pH was measured before and after water replacement (3-4 days) using five (Exp1) or three (Exp2 and Exp3) electrodes (AquaMedic, total scale). Mean pH was calculated for each exposure treatment. *A. longiremis* (late copepodite stages C5 and adults) were transferred into

* Corresponding author.
E-mail address: helena.reinardy@sams.ac.uk (H.C. Reinardy).
[+] joint first authors.

https://doi.org/10.1016/j.aquatox.2021.105961
Received 23 July 2021; Received in revised form 29 August 2021; Accepted 30 August 2021
Available online 4 September 2021

Table 1
Experimental exposure details, including dates, sampling days, salinity and mean ambient temperature, mean measured pH (n=56 in 2018, n=117 in 2019), pCO_2 calculated in CO2sys_v2.1 (Pierrot *et al.*, 2006), means (±standard deviation), and approximate counts of copepods per bottle at the start of each exposure.

Exposure	Collection date	Start – end dates	Sampling (day)	Copepods per bottle	S (PSU)	T (°C)	Incubation pH		Incubation pCO_2 (µatm)	
							Control	Low	Control	Low
Exp1	11/07/2018	17/07/ - 07/08/2018	7, 14, 17, 21	20	35	7.4 (±0.5)	8.1 (±0.1)	7.2 (±0.1)	366	3319
Exp2	20/06/2019	25/06/ - 23/07/2019	7, 14, 21, 28	70		6.4 (±0.5)	8.1 (±0.1)	7.5 (±0.2)	329	1437
Exp3	19/07/2019	23/07/ - 20/08/2019		100		6.8 (±0.5)	8.1 (±0.0)	7.7 (±0.1)	304	1002

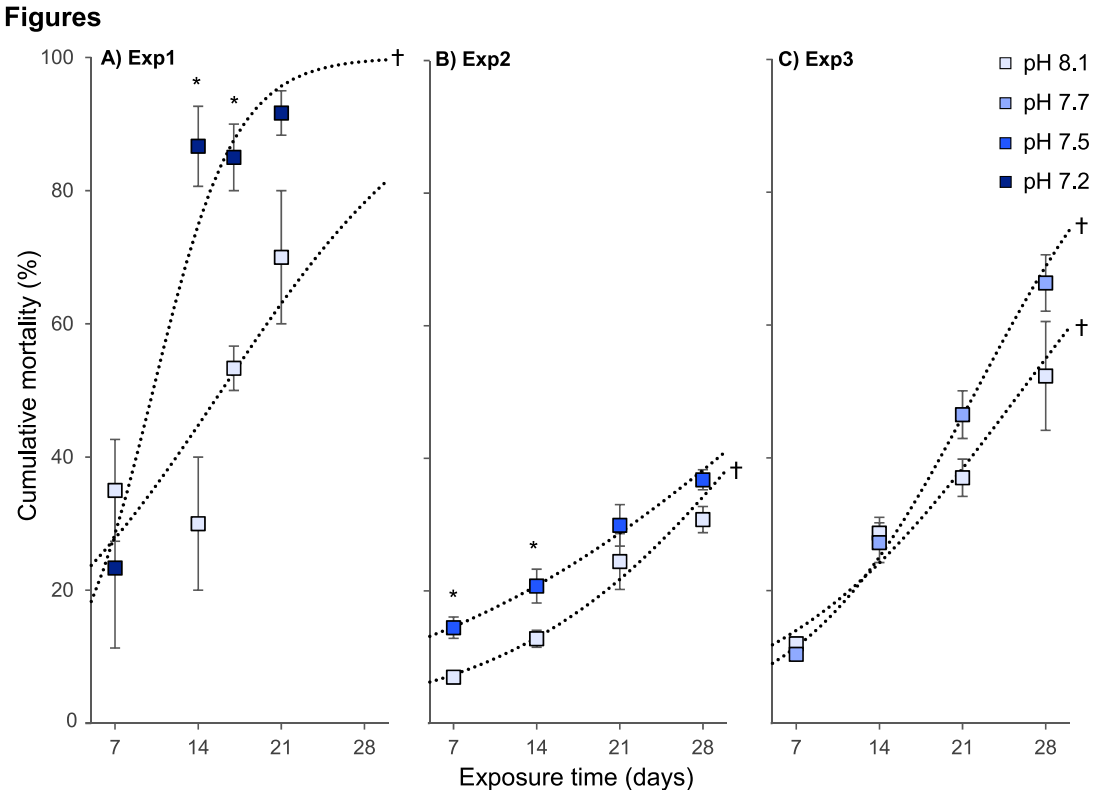


Fig. 1. Cumulative mortality in percent in July 2018 (**A**), July 2019 (**B**), and August 2019 (**C**). Data are mean±s.e.m. *significant within-timepoint difference from control (one-way ANOVA or Kruskal-Wallis test, $p<0.05$). Dotted line logistic regression, † significant fit ($p<0.05$).

bottles and *Rhodomonas baltica* algal food (strain NIVA-5191) was added *ad libitum* and refreshed with the water changes. Bottles were placed on an immersed plankton wheel (0.26rpm) to maintain *in situ* temperature. Dead individuals were counted and discarded weekly. Relative and cumulative mortalities were determined from initial total and subsequent dead individual counts. Three bottles of each treatment were removed every seven days, live copepods were counted and preserved in RNAlater (Sigma Aldrich) for DNA damage analysis.

Three exposures to nominal pH 7.2 (Exp1) and 7.6 (Exp2 and Exp3) were conducted; measured mean pH fluctuated between water changes (Table 1). The mean pH difference in controls was -0.01±0.06 (Exp1), -0.01±0.04 (Exp2), and 0.00±0.01 (Exp3); the mean pH difference in the lowered pH treatments was 0.10±0.07 (Exp1), 0.07±0.07 (Exp2), and 0.07±0.02 (Exp3) (mean±s.d., n=6 Exp1, n=8 Exp2&3). Number of copepods per bottle was increased between exposures (Table 1) to yield enough survivors for DNA damage analysis. Exp1 was terminated after 21 days due to high mortality in the low pH treatment. Tissue samples from Exp1 were lost during transport and thus not available for DNA damage analysis.

For fast micromethod analysis, 16-21 individuals were pooled per bottle, and gently homogenised (4µl/individual, 20mM EDTA, 10% DMSO) with a hand-pestle to breakdown the chitinous exoskeleton. 20µl homogenate was loaded into triplicate or quadruplicate wells (equalling 5 individuals/well) of a 96-well black-walled plate. Lysis buffer (20µl 9M urea, 0.1% SDS, 0.2M EDTA, 2% Quant-iT™ PicoGreen®) was added for 40min lysis, in the dark and on ice. Homogenisation buffer and lysis solution were used for volume-matched blanks. Unwinding solution (200 µl, 20mM EDTA, 1M NaOH, pH adjusted to 13.74±0.02 with NaOH or EDTA) was added to each well to initiate unwinding. Fluorescence was recorded immediately on initiation of unwinding and every 5min (gain 1500, excitation 485nm, emission 520nm POLARstar® Omega). Strand scission factor (SSF) was calculated after 20min: SSF=log(% dsDNAsample/%dsDNAcontrol); dsDNAsample is double-stranded (ds) DNA in lowered and ambient pH samples, and dsDNAcontrol is ambient pH samples only. Negative SSFs for DNA from lowered pH treatment wells indicated no DNA damage and were set to 0 (30% of lowered pH samples). Increasing fluorescence over the kinetic period of detection may indicate crosslinking of DNA (Schröder *et al.*, 2006), and were excluded from the analysis, reducing technical replication from quadruplicate to triplicate or duplicate (5.6% of all wells).

Low pH treatments resulted in significantly higher cumulative mortality in all exposures (GLM, $p<0.05$, Figure 1). Cumulative mortality fit significantly with a sigmoidal increase over time (logistic regression, $p<0.05$, Figure 1). Relative mortality was below 20% at all time points in both Exp2 and Exp3 (not available for Exp1), generally higher in lowered pH treatments than controls, and significantly higher

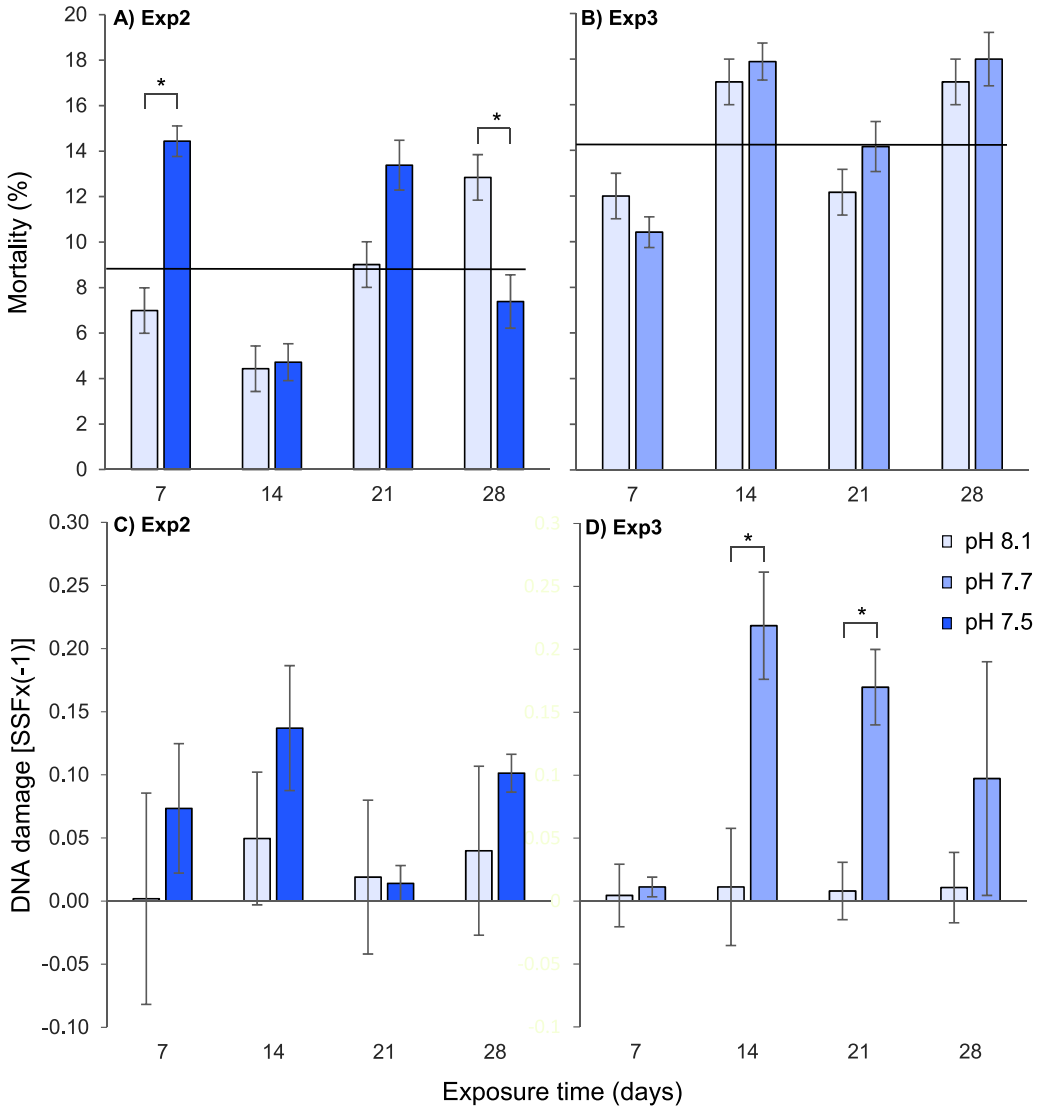


Fig. 2. Mortality in percent (**A, B**) with total mean mortality (horizontal line, n=60 measurements), and DNA damage (strand scission factor, SSF, **C, D**) in *Acartia longiremis* following 28-day exposure to control and low pH treatments. Data are mean±s.e.m. *significant within-timepoint difference from control (one-way ANOVA or Kruskal-Wallis test, $p<0.05$).

in Exp3 than Exp2 (Figure 2A&B), despite similar pH regime (mean mortalities of 14.1±0.7% and 8.9±0.7%, respectively; one-way ANOVA, $p<0.0001$, n=120 measurements, Statgraphics 18×64). Mortality was highest in Exp2 on day 7, likely caused by low pH in the first four days. *Acartia* congeners show interspecific variability in susceptibility to lowered pH, with negative or negligible effects on survival at similar treatment levels. The cosmopolitan estuarine *A. tonsa* showed no effects on daily mortality at 1000µatm (pH 7.8) and 2000µatm (pH 7.6); only extreme conditions of 3000µatm (pH 7.4) and 6000µatm (pH 7.2) negatively affected survival (Cripps *et al.*, 2014), similar to *A. spinicauda* (Zhang *et al.*, 2011). *Acartia pacifica* and *Acartia erythrea* showed no increased mortality after exposure to 5000ppm (pH 7.0) and 10000ppm (pH 6.8) pCO_2, while survival of *Acartia steueri* was not affected up to 10000ppm (pH 6.8) pCO_2 (Zhang *et al.*, 2011; Kurihara *et al.*, 2004). The survival of the Arctic *A. longiremis* was affected at pH 7.6 and thus appears to be more sensitive to lowered pH than that of its temperate counterparts.

DNA damage was elevated in low pH treatments in Exp2 and Exp3 with a peak in low pH treatments on day 14 in both exposures (Figure 2C&D); DNA damage in Exp3 was significantly higher in the treatment than in the controls (GLM, p=0.01, n=24 bottles). Similar pH levels and exposure durations induced DNA damage in other marine organisms, including marine amphipods (Roberts *et al.*, 2013), clams (Conradi *et al.*, 2016), and lobster larvae (Rato *et al.*, 2017). Additionallly, increased oxidative stress was measured in copepods exposed to lowered pH (Zhang *et al.*, 2012), suggesting that this could be the underlying mechanism behind genotoxicity. DNA damage reduced over time, possibly indicating upregulation of DNA repair. Our modified method for detection of DNA damage in crustacean tissue homogenates is well-suited to investigate the dynamic processes of oxidative stress, DNA damage, and induction of DNA repair.

Both mortality and DNA damage were higher in Exp3 compared to Exp2. Mean DNA damage in controls of Exp2 showed higher variation between replicate bottles and time points than controls in Exp3. Relative mortality also varied more between treatments and time points in Exp2, with significant difference on day 28 (one-way ANOVA, p=0.04, n=6). The observed differences between Exp2 (July) and Exp3 (August) were possibly due to seasonal differences in physiology (e.g. senescence).

In conclusion, *A. longiremis* mortality and sub-lethal DNA damage were elevated in lowered pH conditions of 7.7, 7.5, and 7.2 (mortality only). This may have important implications on vulnerability of Arctic copepod populations to changing environmental conditions such as

ocean acidification. The fast micromethod is adaptable to pooled tissue homogenates of crustaceous zooplankton and has potential for further modification to assess genotoxic susceptibility and DNA repair capacity in individual copepods. Our preliminary results provide data for further hypotheses testing, including clarifying the full oxidative stress and genotoxicity response capacity of Arctic marine organisms to lowered pH.

We thank Iris Hendriks, Helene Lødemel, Melissa Chierici, and staff from FISK, Akvaplan-niva. Project funding: Fram Centre Ocean Acidification Flagship.

CRediT authorship contribution statement

Claudia Halsband: Conceptualization, Methodology, Investigation, Resources, Writing – review & editing, Project administration, Funding acquisition. **Mascha F. Dix:** Methodology, Formal analysis, Investigation, Writing – original draft, Visualization. **Kristine Hopland Sperre:** Data curation, Resources, Writing – review & editing. **Helena C. Reinardy:** Conceptualization, Methodology, Formal analysis, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Bailey, A., De Wit, P., Thor, P., Browman, H.I., Bjelland, R., Shema, S., Fields, D.M., et al., 2017. Regulation of gene expression is associated with tolerance of the Arctic copepod *Calanus glacialis* to CO_2-acidified sea water. Ecol. Evol. 7, 7145–7160 doi: 10.002/ece3.3063.

Batel, R., Jakšić., Ž., Bihari, N., Hamer, B., Fafanđel, M., Chauvin, C., Schrőder, et al., 1999. A microplate assay for DNA damage determination (*Fast Micromethod*) in cell suspensions and solid tissues. Anal. Biochem. 270, 195–200. https://doi.org/10.1006/abio.1999.4098.

Bihari, N., Batel, R., Jakšić., Ž, Müller, W.E.G., Waldmann, P., Zahn, R.K, 2002. Comparison between the comet assay and fast micromethod for measuring DNA damage in HeLa cells. Croatia Chemica Acta 75 (3), 793–804.

Ed. by Ciais, P., Sabine, C., Bala, G., Bopp, L., Brovkin, V., Canadell, J., Chabra, A., et al., 2013. Carbon and other biogeochemical cycles. In: Stocker, T.F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., et al. (Eds.), Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 465–570. Ed. by.

Conradi, M., Riba, I., Almagro-Pastor, V., DelValls, T.A, 2016. Lethal and sublethal responses in the clam *Scrobicularia plana* exposed to different CO_2-acidic sediments. Environ. Res. 151, 642–652. https://doi.org/10.1016/j.envres.2016.08.032.

Cripps, G., Lindeque, P., Flynn, K.J, 2014. Have we been underestimating the effects of ocean acidification in zooplankton? Global Change Biol. 20, 3377–3385. https://doi.org/10.1111/gcb.12582.

Depledge, M.H., 1998. The ecotoxicological significance of genotoxicity in marine invertebrates. Mutat. Res. 399, 109–122. https://doi.org/10.1016/S0027-5107(97)00270-4.

El-Bibany, A.H., Bodnar, A.G., Reinardy, H.C, 2014. Comparative DNA damage and repair in echinoderm coelomocytes exposed to genotoxicants. PLoS One 9, e107815. https://doi.org/10.1371/journal.pone.0107815.

Kurihara, H., Shimode, S., Shirayama, Y., 2004. Effects of raised CO_2 concentration on the egg production rate and early development of two marine copepods (*Acartia steueri* and *Acartia erythraea*). Mar. Pollut. Bull. 49, 721–727. https://doi.org/10.1016/j.marpolbul.2004.05.005.

Kroeker, K.J., Kordas, R.L., Crim, R.N., Singh, G.G, 2010. Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. Ecol. Lett. 13 (11), 1419–1434. https://doi.org/10.1111/j.1461-0248.2010.01518.x.

McConville, K., Halsband, C., Fileman, E.S., Somerfield, P.J., Findlay, H.S., Spicer, J.I., 2013. Effects of elevated CO2 on the reproduction of two calanoid copepods. Mar. Pollut. Bull. 73 (2), 428–434. https://doi.org/10.1016/j.marpolbul.2013.02.010.

Pierrot, D., Lewis, E., Wallace, D.W.R, 2006. MS Excel program developed for CO2 system calculations. *Carbon Dioxide Information Analysis*. Center Oak Ridge National Laboratory, United States of America.

Rato, L.D., Novais, S.C., Lemos, M.F.L., Alves, L.M.F., Leandro, S.M, 2017. *Hommarus gammarus* (Crustacea: Decapoda) larvae under an ocean acidification scenario: responses across different levels of biological organization. Compar. Biochem. Physiol. Part C 203, 29–38. https://doi.org/10.1016/j.cbpc.2017.09.002.

Reinardy, H.C., Bodnar, A.G, 2015. Profiling DNA damage and repair capacity in sea urchin larvae and coelomocytes exposed to genotoxicants. Mutagenesis 30, 829–839. https://doi.org/10.1093/mutage/gev052.

Reinardy, H.C., Chapman, J., Bodnar, A.G., 2016. Induction of innate immune gene expression following methyl methanesulfonate-induced DNA damage in sea urchins. Biol. Lett. 12, 20151057 https://doi.org/10.1098/rsbl.2015.1057.

Roberts, D.A., Birchenough, S.B., Lewis, C., Sanders, M.B., Bolam, T., Sheahan, D., 2013. Ocean acidification increases the toxicity of contaminated sediments. Global Change Biol. 19, 340–351. https://doi.org/10.1111/gcb.12048.

Ed. by Schröder, H.C., Batel, R., Schwertner, H., Boreiko, O., Müller, W.E.G., 2006. Fast micromethod DNA single-strand-break assay. In: Henderson, D.S (Ed.), DNA Repair Protocols. Methods in Molecular Biology™ Volume, 314. Humana Press, Totowa, NJ, USA, pp. 287–305. https://doi.org/10.1385/1-59259-973-7:287. Ed. by.

Silva, C.S.E., Novais, S.C., Lemos, M.F.L., Mendes, S., Oliveira, A.P., Gonçalves, E.J., Faria, A.M, 2016. Effects of ocean acidification on the swimming ability, development and biochemical responses of sand smelt larvae. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2016.04.091, 563-564:89-98.

Steinacher, M., Joos, F., Frölicher, T.L., Plattner, G.-K., Doney, S.C, 2009. Imminent ocean acidification in the Arctic projected with the NCAR global coupled carbon cycle-climate model. Biogeosciences 6, 515–533. https://doi.org/10.5194/bg-6-515-2009.

Zhang, D., Li, S., Wang, G., Guo, D., 2011. Impacts of CO_2-driven seawater acidification on survival, egg production rate and hatching success of four marine copepods. Acta Oceanolog. Sin. 30, 86–94. https://doi.org/10.1007/s13131-011-0165-9.

Zhang, D., Li, S., Wang, G., Guo, D., Xing, K., Zhang, S., 2012. Biochemical responses of the copepod *Centropages tenuiremis* to CO_2-driven acidified seawater. Water Sci. Technol. 65, 30–37. https://doi.org/10.2166/wst.2011.821.