



*Supplement of*

## **Assessing the impacts of simulated ocean alkalinity enhancement on viability and growth of nearshore species of phytoplankton**

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# Supplementary 1

Table S1:

Summary of phytoplankton isolates included in the analysis in Section 2. Medium is the growth medium: B1 (Hansen, 1989) modified with addition of a vitamin solution; f/2 (Guillard, 1975); L1 (Guillard and Hargraves, 1993); MWC (Wright, 1964). L:D is the photoperiod (hours:hours).  $E_{\mu}$  is the growth irradiance ( $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ). T is the growth temperature ( $^{\circ}\text{C}$ ).

Division (Class)	Species, Strain	Isolation Site (Year)	Medium	L:D	$E_{\mu}$	T	Source
Chlorophyta	<i>Chlamydomonas</i> sp. CCMP 2294	Baffin Bay, Canada (2003)	L1	16:8	50	3 ± 2	Søgaard et al. (2011)
Cryptophyta	<i>Cryptomonas</i> sp. SAG 26.80	Unknown (Unknown)	MWC	14:10	10-30	15 ± 0.2	Weisse et al. (2006)
	<i>Rhodomonas marina</i> K-0435	Kattegat, Denmark (1990)	mod. B1	16:8	65	15 ± 2	Møgelhøj et al. (2006)
	<i>Rhodomonas marina</i> K-0435	Kattegat, Denmark (1990)	f/2	14:10	150	15	Berge et al. (2010)
	<i>Rhodomonas salina</i> K-0294	Øresund, Denmark (1989)	mod. B1	16:8	65	15 ± 2	Møgelhøj et al. (2006)
	<i>Teleaulax amphioxiae</i>	Øresund, Denmark (2009)	f/2	14:10	150	15	Berge et al. (2010)
Heterokontophyta (Bacillariophyceae)	<i>Coscinodiscus granii</i> K-1048	USA (1994)	f/2	14:10	150	15	Berge et al. (2010)
	<i>Fragilariaopsis nana</i> SCCAP K-0637	Labrador Sea (Unknown)	L1	16:8	50	3 ± 2	Søgaard et al. (2011)
	<i>Fragilariaopsis</i> sp. CCMP 2297	Baffin Bay (1998)	L1	16:8	50	3 ± 2	Søgaard et al. (2011)

Division (Class)	Species, Strain	Isolation Site (Year)	Medium	L:D	$E_{\mu}$	T	Source
Heterokontophyta (Bacillariophyceae)	<i>Nitzschia navis-varingica</i> VHL985	Unknown	f/2 w/ 3x Si	16:8	20, 35, 80, 250	16.5 ± 0.5	Nielsen et al. (2007)
	<i>Nitzschia navis-varingica</i> VHL987	Baiona, Ría Vigo, Spain (2001)	L1	16:8	100	15 ± 1	Lundholm et al. (2004)
	<i>Phaeodactylum tricornutum</i>	Unknown	mod. B1	16:8	65	15 ± 2	Møgelhøj et al. (2006)
	<i>Pseudo-nitzschia australis</i> PS11V	Baiona, Ría Vigo, Spain (2001)	L1	16:8	100	15 ± 1	Lundholm et al. (2004)
	<i>Pseudo-nitzschia calliantha</i> CL-190	Baie-Sainte-Anne, New Brunswick, Canada (2002)	L1	16:8	100	15 ± 1	Lundholm et al. (2004)
	<i>Pseudo-nitzschia cf. turgidula</i> PT	Ocean Station Papa, NE Pacific (2002)	L1	16:8	100	15 ± 1	Lundholm et al. (2004)
	<i>Pseudo-nitzschia delicatissima</i> Tasm10	Hobart Harbour, Tasmania (2000)	L1	16:8	100	15 ± 1	Lundholm et al. (2004)
	<i>Pseudo-nitzschia fraudulenta</i> CL-192	Deadmans Harbour, Bay of Fundy, Canada (2002)	L1	16:8	100	15 ± 1	Lundholm et al. (2004)
	<i>Pseudo-nitzschia granii</i> PG	Ocean Station Papa, NE Pacific (2000)	L1	16:8	100	15 ± 1	Lundholm et al. (2004)
	<i>Pseudo-nitzschia multiseries</i> CL-195	Okkiray Bay, Iwate Prefecture, Japan (2001)	L1	16:8	100	15 ± 1	Lundholm et al. (2004)
	<i>Pseudo-nitzschia multiseries</i> OKPm013-2	Ha Long Bay, Vietnam (1998)	L1	16:8	100	15 ± 1	Lundholm et al. (2004)
	<i>Pseudo-nitzschia pungens</i> CL-193	Deadmans Harbour, Bay of Fundy, Canada (2002)	L1	16:8	100	15 ± 1	Lundholm et al. (2004)
	<i>Pseudo-nitzschia seriata</i> CL-150	Tracadie Harbour, PEI, Canada (2002)	L1	16:8	100	15 ± 1	Lundholm et al. (2004)

Division (Class)	Species, Strain	Isolation Site (Year)	Medium	L:D	$E_{\mu}$	T	Source
Heterokontophyta (Bacillariophyceae)	<i>Pseudo-nitzschia</i> sp. NWFSC095	Sequim Bay, Washington, USA (2002)	L1	16:8	100	15 ± 1	Lundholm et al. (2004)
Heterokontophyta (Raphidophyceae)	<i>Chattonella marina</i> NIES-3	Osaka Bay, Japan (1982)	K	12:12	c. 42	22-24	Liu et al. (2007)
Haptophyta	<i>Chrysochromulina polylepis</i> K-0259	Øresund, Denmark (1988)	f/2	16:8	60	15 ± 1	Schmidt & Hansen (2001)
	<i>Chrysochromulina simplex</i> K-0272	Victoria, Australia (1988)	f/2	16:8	60	15 ± 1	Schmidt & Hansen (2001)
	<i>Prymnesium parvum</i> K-0623	Unknown	f/2	14:10	150	15	Berge et al. (2010)
Dinoflagellata	<i>Ceratium furca</i>	Øresund, Denmark (2003)	f/2	16:8	25, 100, 200	15 ± 1	Søderberg et al. (2007)
	<i>Ceratium fusus</i>	Øresund, Denmark (2003)	f/2	16:8	100	15 ± 1	Søderberg et al. (2007)
	<i>Ceratium lineatum</i>	Øresund, Denmark (1995)	f/2	16:8	60	15 ± 1	Hansen (2002); Hansen et al. (2007)
	<i>Ceratium tripos</i>	Øresund, Denmark (2003)	f/2	16:8	100	15 ± 1	Søderberg & Hansen (2007)
	<i>Heterocapsa triquetra</i>	Unknown	f/2 w/ 3x Si	16:8	20, 35, 80, 250	16.5 ± 0.5	Nielsen et al. (2007)
	<i>Heterocapsa triquetra</i> 2-1, K-1127	Stege Harbour, Denmark (2007)	L1	16:8	150	15	Berge et al. (2012)
	<i>Heterocapsa triquetra</i> 2-3	Stege Harbour, Denmark (2007)	L1	16:8	150	15	Berge et al. (2012)
	<i>Heterocapsa triquetra</i> 2-5	Stege Harbour, Denmark (2007)	L1	16:8	150	15	Berge et al. (2012)

Division (Class)	Species, Strain	Isolation Site (Year)	Medium	L:D	$E_{\mu}$	T	Source
Dinoflagellata	<i>Heterocapsa triquetra</i> 2-6	Stege Harbour, Denmark (2007)	L1	16:8	150	15	Berge et al. (2012)
	<i>Heterocapsa triquetra</i> A2b, K-1131	Copenhagen, Denmark (2007)	L1	16:8	150	15	Berge et al. (2012)
	<i>Heterocapsa triquetra</i> A4a	Copenhagen, Denmark (2007)	L1	16:8	150	15	Berge et al. (2012)
	<i>Heterocapsa triquetra</i> A4c	Copenhagen, Denmark (2007)	L1	16:8	150	15	Berge et al. (2012)
	<i>Heterocapsa triquetra</i> A5c, K-1134	Copenhagen, Denmark (2007)	L1	16:8	150	15	Berge et al. (2012)
	<i>Heterocapsa triquetra</i> CCMP 449	Lawrence Estuary, Canada (1960)	L1	16:8	150	15	Berge et al. (2012)
	<i>Heterocapsa triquetra</i> F1a, K-1124	Gedser Harbour, Denmark (2007)	L1	16:8	150	15	Berge et al. (2012)
	<i>Heterocapsa triquetra</i> F1c, K-1125	Gedser Harbour, Denmark (2007)	L1	16:8	150	15	Berge et al. (2012)
	<i>Heterocapsa triquetra</i> F1f	Gedser Harbour, Denmark (2007)	L1	16:8	150	15	Berge et al. (2012)
	<i>Heterocapsa triquetra</i> F2e	Gedser Harbour, Denmark (2007)	L1	16:8	150	15	Berge et al. (2012)
+	<i>Heterocapsa triquetra</i> HTMS0402	Masan Bay Korea (2004)	L1	16:8	150	15	Berge et al. (2012)
	<i>Heterocapsa triquetra</i> K-0447	The Sound, Denmark (1984)	L1	16:8	150	15	Berge et al. (2012)
	<i>Heterocapsa triquetra</i> K-0481	Øresund, Denmark (1988)	f/2	16:8	60	15 ± 1	Hansen (2002)
	<i>Heterocapsa triquetra</i> K-0481	Øresund, Denmark (1988)	f/2	16:8	60	15 ± 1	Hansen et al. (2007)

Division (Class)	Species, Strain	Isolation Site (Year)	Medium	L:D	$E_\mu$	T	Source
Dinoflagellata	<i>Heterocapsa triquetra</i> K-0481	Øresund, Denmark (1988)	mod. B1	16:8	65	15 ± 2	Møgelhøj et al. (2006)
	<i>Heterocapsa triquetra</i> K-0482	Kattegat, Denmark (1988)	L1	16:8	150	15	Berge et al. (2012)
	<i>Heterocapsa triquetra</i> K-1133	Baltic (2007)	f/2	14:10	150	15	Berge et al. (2010)
	<i>Heterocapsa triquetra</i> KAC 26	Kalmarsund, Sweden (2000)	L1	16:8	150	15	Berge et al. (2012)
	<i>Heterocapsa triquetra</i> KAC 27	Kalmarsund, Sweden (2000)	L1	16:8	150	15	Berge et al. (2012)
	<i>Heterocapsa triquetra</i> KAC 49	West Coast, Sweden (1986)	L1	16:8	150	15	Berge et al. (2012)
	<i>Heterocapsa triquetra</i> NC 98	West Coast, Norway (1998)	L1	16:8	150	15	Berge et al. (2012)
	<i>Heterocapsa triquetra</i> NIES 235	Osaka Bay, Japan (1982)	L1	16:8	150	15	Berge et al. (2012)
	<i>Heterocapsa triquetra</i> NIES 7	Farima-Nada, Japan (1981)	L1	16:8	150	15	Berge et al. (2012)
	<i>Heterocapsa triquetra</i> PLY 169	Tamar Estuary, England (1957)	L1	16:8	150	15	Berge et al. (2012)
<i>K. veneficum</i>	<i>Karlodinium veneficum</i> K-1413	North Sea (2007)	f/2	14:10	150	15	Berge et al. (2010)
	<i>Prorocentrum micans</i> K-0335	Kattegat, Denmark (1989)	mod. B1	16:8	65	15 ± 2	Møgelhøj et al. (2006)
	<i>Prorocentrum micans</i> K-1137	Skagerrak (2008)	f/2	14:10	150	15	Berge et al. (2010)

Division (Class)	Species, Strain	Isolation Site (Year)	Medium	L:D	$E_{\mu}$	T	Source
Dinoflagellata	<i>Prorocentrum minimum</i> K-0295	Kattegat, Denmark (1989)	f/2	16:8	60	15 ± 1	Hansen (2002); Hansen et al. (2007)
	<i>Prorocentrum minimum</i> K-0295	Kattegat, Denmark (1989)	mod. B1	16:8	65	15 ± 2	Møgelhøj et al. (2006)
	<i>Prorocentrum minimum</i> K-1138	Skagerrak (2008)	f/2	14:10	150	15	Berge et al. (2010)

Berge, T., Daugbjerg, N., Balling Andersen, B., and Hansen, P.J.: Effect of lowered pH on marine phytoplankton growth rates, Mar Ecol Prog Ser, 416, 79–91, doi:10.3354/meps08780, 2010.

Berge, T., Daugbjerg, N., and Hansen, P. J.: Isolation and cultivation of microalgae select for low growth rate and tolerance to high pH, Harmful Algae, 20, 101–110, doi:10.1016/j.hal.2012.08.006, 2012.

Guillard, R. R. L.: Culture of phytoplankton for feeding marine invertebrates, in: Culture of marine invertebrate animals, edited by:Smith, W. L. and Chanley, M. H., Plenum, New York, 108–132, [https://doi.org/10.1007/978-1-4615-8714-9\\_3](https://doi.org/10.1007/978-1-4615-8714-9_3), 1975

Guillard, R. R. L. and Hargraves, P. E.: *Stichochrysis immobilis* is a diatom, not a chrysophyte., Phycologia, 32, 234-236, 1993.

Hansen, P. J.: The red tide dinoflagellate *Alexandrium tamarensense*: effects on behaviour and growth of a tintinnid ciliate, Marine Ecology Progress Series, 53, 105-116, 1989.

Hansen, P. J.: Effect of high pH on the growth and survival of marine phytoplankton: Implications for species succession, Aquat Microb Ecol, 28, 179-288, doi:10.3354/ame028279, 2002.

Hansen, P. J., Lundholm, N., and Rost, B.: Growth limitation in marine red-tide dinoflagellates: Effects of pH versus inorganic carbon availability, Mar Ecol Prog Ser, 334, 63–71, doi:10.3354/meps334063, 2007.

Liu, W., Au, D. W. T., Anderson, D. M., Lam, P. K. S., and Wu, R. S. S.: Effects of nutrients, salinity, pH and light:dark cycle on the production of reactive oxygen species in the alga *Chattonella marina*, J Exp Mar Biol Ecol, 346, 76–86, doi:10.1016/j.jembe.2007.03.007, 2007.

Lundholm, N., Hansen, P. J., and Kotaki, Y.: Effect of pH on growth and domoic acid production by potentially toxic diatoms of the genera *Pseudo-nitzschia* and *Nitzschia*, Mar Ecol Prog, 273, 1-15, doi:10.3354/meps273001, 2004.

Møgelhøj, M., Hansen, P. J., Henriksen, P., and Lundholm, N.: High pH and not allelopathy may be responsible for negative effects of *Nodularia spumigena* on other algae, Aquat Microb Ecol, 43, 43–54, doi:10.3354/ame043043, 2006.

Nielsen, L. T., Lundholm, N., and Hansen, P. J.: Does irradiance influence the tolerance of marine phytoplankton to high pH? Mar Bio Res, 3(6), 446-453, doi:10.1080/17451000701711820, 2007.

Schmidt, L. E. and Hansen, P. J.: Allelopathy in the prymnesiophyte *Chrysochromulina polylepis*: effect of cell concentration, growth phase and pH doi:10.3354/meps216067, Mar Ecol Prog Ser, 216, 67–81, 2001.

Søderberg, L., and Hansen, P.: Growth limitation due to high pH and low inorganic carbon concentrations in temperate species of the dinoflagellate genus *Ceratium*, Mar Ecol Prog Ser, 351, 103–112, doi:10.3354/meps07146, 2007.

Søgaard, D.H., Hansen, P.J., Rysgaard, S., and Glud, R.N.: Growth limitation of three Arctic sea ice algal species: Effects of salinity, pH, and inorganic carbon availability, Polar Biol, 34(8), 1157–1165, doi:10.1007/s00300-011-0976-3, 2011.

Weisse, T., and Stadler, P.: Effect of pH on growth, cell volume, and production of freshwater ciliates, and implications for their distribution, Limnol Oceanogr, 51, 1708–1715, doi:10.4319/lo.2006.51.4.1708, 2006.

## Supplementary 2

### Comparison of Aerated and pH-drift Cultures of *Thalassiosira pseudonana* CCMP1335

In both cultures, there was a rapid increase in particles with chlorophyll a autofluorescence in the first 48 hours of the experiment (Figure S1 a, b).

- 5 Thereafter, the concentration of particles decreased but their size, as inferred from the median side-scatter, increased (Figure S1 a, b). Both the initial peak in concentration and the subsequent rise in side scatter were more pronounced in the aerated culture. The most parsimonious explanation for this is that the cells aggregated in stationary phase. A biomass proxy (Figure S1 a, b) was calculated as the product of the two terms. This demonstrated the expected biomass response of exponential growth followed by stability in early stationary phase.
- 10 The changes in the biomass proxy were coincident with a draw-down in CO<sub>2</sub> and bicarbonate and a rise in carbonate (Figure S1 c, d). The drawdown was persistent in the pH-drift (sealed) culture but was reversed in the aerated culture after active biomass accumulation ceased. The lower pH in the latter favoured bicarbonate over carbonate, while carbonate dominated in the sealed culture.

There was a decline in photosynthetic efficiency ( $F_v/F_m$ ) in both cultures, which was more pronounced in the pH-drift culture (Figure S1 e, f). In

- 15 contrast, there was relatively little variation in the photosynthetic cross-section ( $\sigma$ ) in either.

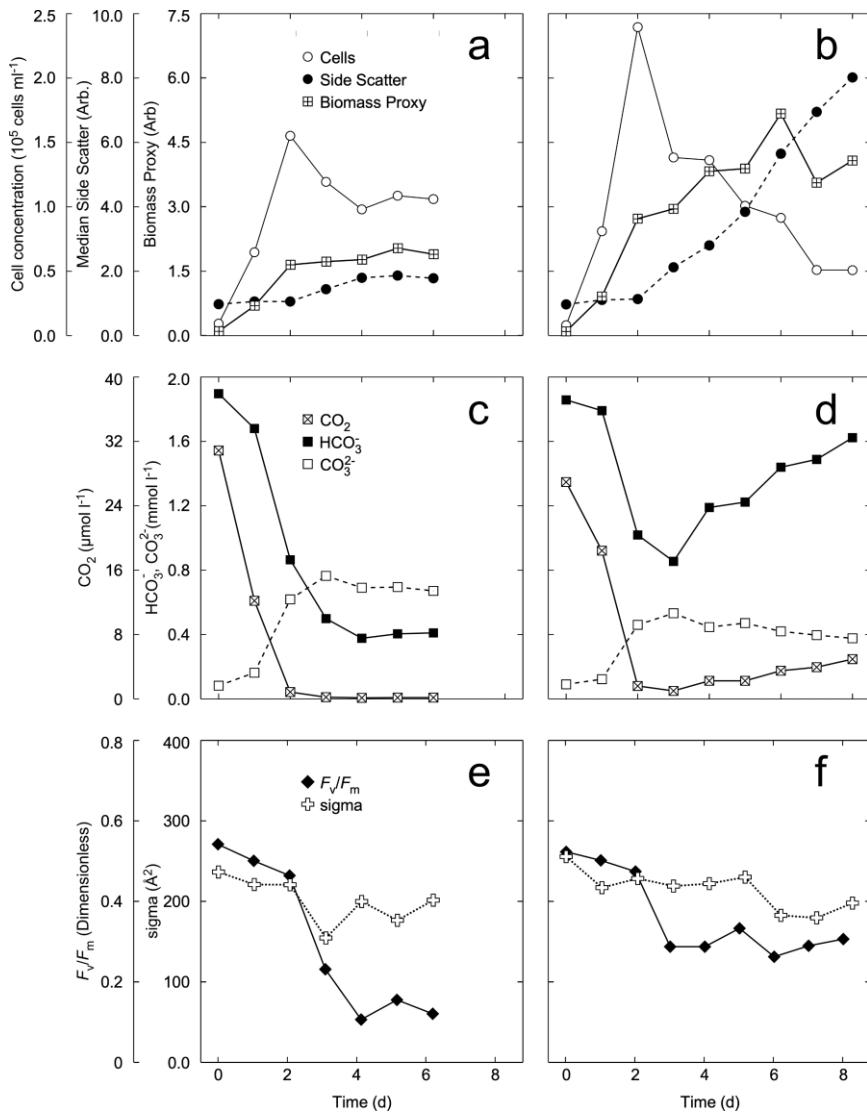


Figure S1: Time courses of biomass parameters (a, b), concentrations of carbon species (c, d), and photosynthetic response variables (e, f) in sealed (a, c, e) and aerated (b, d, f) cultures of *Thalassiosira pseudonana*. Concentrations of carbon species were calculated from pH and DIC (see Methods for details).