

Influence of salinity on the growth of marine and estuarine benthic diatoms

Makoto Mizuno

Department of Bioproduction, Faculty of Bioindustry, Tokyo University of Agriculture, Abashiri, Hokkaido 099-24, Japan

Mizuno, M. 1992. Influence of salinity on the growth of marine and estuarine benthic diatoms. Jpn. J. Phycol. 40: 35–39.

Growth responses to salinity were investigated in twenty-one marine and estuarine benthic diatoms. Estuarine diatoms tended to tolerate a wider salinity range than marine diatoms. Most marine diatoms did not grow or their growth rate was reduced to less than a half of the maximum in the media with a salinity of 15‰ or less, although an optimum salinity and a suitable salinity range were not uniform. Growth responses to salinity in marine benthic diatoms examined here were little different from those in planktonic diatoms called typically marine species. Four of six estuarine diatoms were euryhaline but other two grew well only in a narrow range from 15 to 29‰ S. Growth response of the latter suggested that salinity was an important factor in their distribution.

Key Index Words: benthic diatoms—estuarine—growth—marine—salinity.

Salinity is one of factors limiting the distribution of algae. Growth responses to salinity have been studied in a number of marine and estuarine diatoms (Guillard and Ryther 1962, Guillard 1963, Lewin and Guillard 1963, Williams 1964, Paasche 1975, Eppley 1977). In general, estuarine benthic and planktonic diatoms tend to be euryhaline, while marine planktonic species are stenohaline (Eppley 1977). Since the information about growth responses to salinity in marine benthic diatoms is limited, it is unclear whether or not they are stenohaline. The salinity in the marine littoral zone where marine benthic diatoms live is variable, which is not the case in the open sea. In the intertidal zone rain and desiccation at low tide cause a low and high salinity, respectively. The present study reports the growth response of 21 marine and estuarine diatoms at various salinities.

Materials and Methods

Twenty-one clonal diatoms were examined. Two clonal axenic diatoms were added to 19 diatoms of my previous paper (Mizuno 1991). One was *Fragilaria fasciculata* (Ag.) L.-

Bert. var. *fasciculata* collected from Lake Komuke-ko, Mombetsu, Hokkaido and another was *Navicula directa* (W. Sm.) Ralfs in Pritchard var. *directa* collected from Minamigaoka, Mombetsu. All were benthic pennate diatoms except *Coscinodiscus* sp. which is centric and seems to be planktonic habit.

Seawater concentrated by heating at 70–80°C or diluted with distilled water was enriched in the same way as modified BSW-2 (Mizuno 1989). Chlorinities of the media were measured by titration with AgNO₃. Salinity (S) was estimated by the following equation (Japan Meteorological Agency 1970):

$$\text{Salinity (‰)} = 1.80655 \times \text{Chlorinity (‰)}$$

The salinities of experimental media ranged from 7 to 43‰ S. The stock culture was grown in media of various salinities for 4–16 days under experimental conditions prior to initiation of the experiment. The experiments were carried out at 18°C using a 16 : 8 h LD cycle. The photon flux density was 180 μE·m⁻²·s⁻¹. After preculture, cells were inoculated into media with the same salinity and were cultured under the experimental conditions. Each experiment was run in

triplicate. Some diatoms stick to the culture vessel wall or grow in a gelatinous sheet. Consequently, to obtain a good estimate of algal abundance, cells were freed from culture vessel wall or from the gelatinous sheet with 0.2–0.5 ml of HNO_3 and heat. Three tubes were prepared per replicate, and these tubes were acid-treated as growth progressed.

The cells in 0.01–0.04 ml of algal suspension were counted using a ruled counting plate under a light microscope. The growth rate in exponential growth phase was calculated by the following equation:

$$\mu = (\ln N_t - \ln N_0) / t,$$

where N_t and N_0 are the cell number at times t and zero, respectively, and μ is the growth rate (day^{-1}) (Guillard 1973).

Statistical analyses followed Sokal and Rohlf (1973).

Results

Mean growth rates (μ ; day^{-1}) of 21 diatoms at various salinities were shown in Table 1. In the present study, the salinity where the maximum growth rate occurred was considered as an optimum salinity. Furthermore, the salinity range where the growth rate being not significantly different from the maximum growth rate ($P > 0.05$; by a posterior comparison of one-way ANOVA) occurred was considered as a suitable salinity range.

Marine diatoms. Fifteen diatoms were examined. Optimum salinity of each diatom was different from diatom to diatom and ranged from 24 to 39‰ S. Suitable salinity range of each diatom was different from diatom to diatom as well as the optimum salinity. A wide suitable range of 20‰ S was observed in *Gyrosigma prolongatum* var. *closterioides*, *Navicula complanatula* and *Navicula directa* var. *cuneata*. On the other hand, a narrow suitable range of 5‰ S was observed in *Coscinodiscus* sp. and Muroran strain of *Pleurosigma intermedium* var. *nubecula*. The lower limit of the suitable salinity range was 19–20 or 24‰ S in most diatoms examined. Most diatoms did not grow or their growth

rate was reduced to less than a half of the maximum at 15‰ S or less. Growth rate at 38–39‰ S was significantly smaller than the maximum ($P < 0.05$) in a half of diatoms examined, although two diatoms (*Amphora* sp. and *Navicula* sp.) grew optimally at this salinity level. In *Pleurosigma intermedium* var. *nubecula*, different growth responses were observed in two strains collected from different localities.

Estuarine diatoms. Six diatoms were examined. Optimum salinity of each diatom occurred between 15–24‰ S. A wide suitable salinity range was observed in *Achnanthes brevipes* var. *intermedia*, *Fragilaria fasciculata* var. *fasciculata*, *Navicula cryptocephala* var. *cryptocephala* and *Surirella ovata*. *Navicula elegans* and *Pleurosigma elongatum* showed a narrow suitable salinity range and a poor growth at 7–8‰ S and 33–34‰ S which is the same strength as a natural seawater, or more.

Discussion

The present study has demonstrated that there are diatoms with various optimum salinities and suitable salinity ranges in the marine littoral zone and in the estuarine region, and shown that estuarine diatoms tend to tolerate a wider salinity range than marine diatoms, as Eppley (1977) pointed out. Although marine benthic diatoms in the present study were not uniform in the optimum salinity and the suitable salinity range, most of them were suppressed to grow at 15‰ S or less and grew well at more than 19–20‰ S or more than 24‰ S. Furthermore, their growth reduced at a higher salinity than that of a natural seawater in a half of them. Ohgai and his colleagues showed that four marine benthic diatoms were suppressed to grow below 9‰ of chlorinity (16‰ S) and grew well in the range from 11 to 18‰ Cl (20–33‰ S) (Ohgai et al. 1984, Ohgai et al. 1984). There is not much difference in growth response to salinity between the present diatoms and the diatoms reported by Ohgai and his colleagues.

Oceanic planktonic clone (13-1) of *Cyclotella nana* (now named *Thalassiosira pseudonana*) iso-

Table 1. Mean growth rate (μ ; day⁻¹) and SD (in parentheses) of diatoms in media of various salinities at 18°C and a 16 : 8 h LD cycle with 180 $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1\text{a}}$.

Diatom	Salinity (‰)								
	7-8	15	19-20	24	29	33-34	38-39		43
Marine diatom									
<i>Amphora</i> sp. (MIZ-13)	—	—	0.44 (0.02)	0.55 (0.04)	<u>0.61</u> (0.07)	<u>0.67</u> (0.08)	<u>0.74</u> (0.04)	<u>0.66</u> (0.06)	
<i>Caloneis linearis</i> (Grun.) Boyer (MIZ-16)	NG ^b	NG	0.28 (0.02)	<u>0.37</u> (0.04)	<u>0.43</u> (0.01)	<u>0.41</u> (0.04)	0.31 (0.03)	—	
<i>Coscinodiscus</i> sp. (MIZ-27)	—	NG	0.86 (0.02)	<u>1.46</u> (0.01)	<u>1.40</u> (0.02)	1.21 (0.08)	1.05 (0.04)	—	
<i>Gyrosigma prolongatum</i> var. <i>closterioides</i> Grun. (MIZ-29)	NG	NG	0.86 (0.12)	0.99 (0.03)	1.03 (0.09)	<u>1.06</u> (0.03)	<u>0.99</u> (0.02)	—	
<i>Gyrosigma tenuissimum</i> (W. Sm.) Cl. var. <i>tenuissimum</i> (MIZ-26)	NG	NG	0.58 (0.03)	<u>0.68</u> (0.06)	<u>0.73</u> (0.02)	<u>0.73</u> (0.05)	0.53 (0.02)	—	
<i>Licmophora abbreviata</i> var. <i>pappeana</i> (Grun.) Hust. (MIZ-22)	NG	NG	NG	<u>0.97</u> (0.04)	<u>0.97</u> (0.02)	<u>1.04</u> (0.02)	0.85 (0.02)	—	
<i>Licmophora gracilis</i> var. <i>anglica</i> (Kütz.) Perag. (MIZ-3)	—	—	0.33 (0.05)	0.82 (0.05)	<u>1.18</u> (0.01)	<u>1.12</u> (0.04)	<u>1.08</u> (0.01)	0.86 (0.01)	
<i>Navicula complanatulula</i> Hust. (MIZ-11)	—	0.17 (0.20)	<u>1.00</u> (0.05)	<u>1.07</u> (0.12)	0.76 (0.07)	<u>0.84</u> (0.10)	<u>0.81</u> (0.15)	—	
<i>Navicula directa</i> (W. Sm.) Ralfs in Pritchard var. <i>directa</i> (MIZ-20) ^c	NG	NG	0.12 (0.03)	0.34 (0.01)	<u>0.49</u> (0.04)	<u>0.50^d</u> (0.01)	0.32 ^d (0.07)	—	
<i>Navicula directa</i> var. <i>cuneata</i> Östr. (MIZ-14)	—	0.68 (0.15)	<u>1.24</u> (0.06)	<u>1.24</u> (0.02)	<u>1.40</u> (0.02)	<u>1.33</u> (0.06)	<u>1.18</u> (0.01)	—	
<i>Navicula</i> sp. (MIZ-21) ^d	—	NG	0.08 (0)	<u>0.62</u> (0.05)	<u>0.64</u> (0.03)	<u>0.68</u> (0.01)	<u>0.68</u> (0)	—	
<i>Nitzschia bilobata</i> var. <i>minor</i> Grun. (MIZ-24)	NG	0.78 (0.03)	<u>1.16</u> (0.07)	<u>1.21</u> (0.03)	<u>1.27</u> (0.08)	<u>1.15</u> (0.13)	0.88 (0.08)	—	
<i>Nitzschia</i> sp. (MIZ-23)	—	NG	NG	<u>0.31</u> (0.07)	<u>0.52</u> (0.10)	<u>0.53</u> (0.09)	<u>0.42</u> (0.11)	—	
<i>Pleurosigma intermedium</i> var. <i>nubecula</i> W. Sm. (Muroran) (MIZ-15)	—	0.23 (0.04)	<u>0.48</u> (0.02)	<u>0.52</u> (0)	0.39 (0.02)	0.35 (0.02)	0.34 (0.05)	—	
<i>Pleurosigma intermedium</i> var. <i>nubecula</i> W. Sm. (Mombetsu) (MIZ-28) ^d	—	NG	0.25 (0.02)	<u>0.51</u> (0.03)	<u>0.51</u> (0.01)	<u>0.44</u> (0.02)	0.28 (0.04)	—	
Estuarine diatom									
<i>Achnanthes brevipes</i> var. <i>intermedia</i> (Kütz.) Cl. (MIZ-32-1)		0.73 (0.17)	<u>1.15</u> (0.04)	1.08 (0.14)	1.06 (0.11)	1.01 (0.22)	0.98 (0.06)	0.98 (0.09)	—
<i>Fragilaria fasciculata</i> (Ag.) L.-Bert. var. <i>fasciculata</i> (MIZ-30)		NG	<u>1.00</u> (0.04)	<u>1.09</u> (0.02)	0.97 (0.03)	<u>1.02</u> (0.02)	<u>1.01</u> (0.02)	0.95 (0.04)	—
<i>Navicula cryptocephala</i> Kütz. var. <i>cryptocephala</i> (MIZ-33)		<u>0.77</u> (0.02)	0.83 (0.08)	<u>0.90</u> (0.02)	<u>0.90</u> (0.06)	0.88 (0.06)	<u>0.87</u> (0.09)	<u>0.73</u> (0.04)	—
<i>Navicula elegans</i> W. Sm. (MIZ-34)		0.10 (0.03)	<u>0.23</u> (0.01)	<u>0.28</u> (0.05)	0.23 (0.01)	0.23 (0.01)	0.11 (0.01)	NG	—
<i>Pleurosigma elongatum</i> W. Sm. (MIZ-36)		NG	<u>0.25</u> (0.01)	<u>0.25</u> (0.01)	<u>0.22</u> (0.04)	<u>0.17^d</u> (0.04)	0.03 ^d (0)	0.07 (0.05)	—
<i>Surirella ovata</i> Kütz. (xenic) (MIZ-35)		<u>1.22</u> (0.07)	<u>1.37</u> (0.10)	<u>1.37</u> (0.06)	<u>1.33</u> (0.05)	<u>1.19</u> (0.05)	1.13 (0.01)	<u>1.17</u> (0.06)	—

^a Mean of 3 replicates. A double underline shows the maximum and a single underline shows the value which is not significantly different from the maximum ($P > 0.05$, by a posterior comparison of one-way ANOVA).

^b No growth in pre- or experimental culture.

^c Temperature dropped to 14°C during the culture experiment.

^d N=2.

lated from Sargasso Sea did not even survive at salinities below half-strength seawater (17‰ S, when a natural seawater is 34‰ S) and grew well only in media above 24‰ S (Guillard and Ryther 1962, Lewin and Guillard 1963). The similar growth response to salinity was observed in planktonic diatoms examined by Ryther (1954) and Kain and Fogg (1958), which were called "typically marine species" by Lewin and Guillard (1963), and other oceanic planktonic diatoms (Guillard 1963). It is found that the growth response to salinity of marine benthic diatoms is not much different from that of planktonic diatoms called typically marine species.

It is probable that on a heavily rainy day the littoral zone are exposed to the salinity of 15‰ S where most of the present marine diatoms are suppressed to grow (Edelstein and McLachlan 1975, Mizuno 1984). Fischer (1963) reported that 50% of cells of the marine littoral diatom *Gyrosigma balticum* died within 2 hours after transferring to 0.4 strength natural seawater (14‰ S, when a natural seawater is 34‰ S). Exposure to a low salinity (14‰ S) for one hour per day completely suppressed the growth of the marine tube-dwelling diatom (Mizuno 1989). A low salinity occurring inshore will damage the marine benthic diatoms which live there. There is a probability of a higher salinity than that of a natural seawater occurring in the upper-supra littoral zone (Edelstein and McLachlan 1975, Mizuno 1984). The present study has shown that a half of marine diatoms examined was suppressed to grow at 38–39‰ S. A high salinity in the upper-supra littoral zone will prevent these diatoms from growing there.

Most estuarine diatoms are known to be euryhaline (Guillard and Ryther 1962, Williams 1964, Admiraal 1977, Eppley 1977). Four of six estuarine diatoms examined here were euryhaline. On the other hand, it is confirmed by the culture experiments that there are diatoms with stenohaline character in estuarine ones (*Thalassiosira baltica* and *Skeletonema subsalsum* in Paasche 1975). Two of the present estuarine diatoms showed the similar behavior to the stenohaline estuarine diatoms of Paasche

(1975) and grew well only in a narrow range from 15 to 29‰ S. Since the growth of these diatoms was strongly suppressed in the salinity of a natural seawater, they will be unable to immigrate to the marine region. Salinity below 15‰ S damaging them is likely to occur in the estuarine region. The salinity may be an important factor in the distribution of these diatoms.

Acknowledgments

I thank the National Institute for Environmental Studies for support and Prof. R. W. Holmes of the University of California for critically reviewing the draft of the manuscript. I also thank anonymous reviewers for valuable comments.

References

- Admiraal, W. 1977. Salinity tolerance of benthic estuarine diatoms as tested with a rapid polarographic measurement of photosynthesis. *Mar. Biol. (Berl.)* 39: 11–18.
- Edelstein, T. and McLachlan, J. 1975. Autecology of *Fucus distichus* ssp. *distichus* (Phaeophyceae: Fucales) in Nova Scotia, Canada. *Mar. Biol. (Berl.)* 30: 305–324.
- Eppley, R. W. 1977. The growth and culture of diatoms. p. 24–64. *In* D. Werner (ed.), *The biology of diatoms*. Blackwell Sci. Publ., Oxford.
- Fischer, H. 1963. Zur osmotischen Resistenz von Diatomeen der Gezeitenzone. *Protoplasma* 57: 344–353.
- Guillard, R. R. L. 1963. Organic sources of the nitrogen for marine centric diatoms. p. 93–104. *In* C. H. Oppenheimer (ed.), *Symposium on Marine Microbiology*. C. C. Thomas Publ., Springfield.
- Guillard, R. R. L. 1973. Division rates. p. 289–311. *In* J. R. Stein (ed.), *Handbook of Phycological Methods*. Cambridge University Press, Cambridge.
- Guillard, R. R. L. and Ryther, J. H. 1962. Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* (Cleve) Gran. *Can. J. Microbiol.* 8: 229–239.
- Japan Meteorological Agency 1970. *Kaiyokansokushishin*. Oceanogr. Soc. Jap., Tokyo. (in Japanese).
- Kain, J. M. and Fogg, G. E. 1958. Studies on the growth of marine phytoplankton I. *Asterionella japonica* Gran. *J. mar. biol. Ass. U.K.* 37: 397–413.
- Lewin, J. and Guillard, R. R. L. 1963. Diatoms. *Ann. Rev. Microbiol.* 17: 373–417.
- Mizuno, M. 1984. Environment at the front shore of

- the Institute of Algological Research of Hokkaido University. Sci. Pap. Inst. Algal. Res. Fac. Sci., Hokkaido Univ. 7: 263-292.
- Mizuno, M. 1989. Autecological studies on the marine tube-dwelling diatom *Berkeleya obtusa* (Grev.) Grunow. Sci. Pap. Inst. Algal. Res. Fac. Sci., Hokkaido Univ. 8: 63-115+4 pls.
- Mizuno, M. 1991. Influence of cell volume on the growth and size reduction of marine and estuarine diatoms. J. Phycol. 27: 473-478.
- Ohgai, M., Matsui, T. and Ishida, Y. 1984. The effect of the environmental factors on the growth of two attached diatoms, *Melosira nummuloides* (Dillwyn) Agardh and *Achnanthes longipes* Agardh, *in vitro*. J. Simonoseki Univ. Fish. 32: 83-89. (in Japanese).
- Ohgai, M., Tsukahara, H., Matsui, T. and Nakajima, K. 1984. The effect of the environmental factors on the growth of two epiphytic diatoms *Licmophora abbreviata* Agardh and *L. paradoxa* (Lyngbye) Agardh *in vitro*. Bull. Jap. Soc. Sci. Fish. 50: 1157-1163. (in Japanese).
- Paasche, E. 1975. The influence of salinity on the growth of some plankton diatoms from brackish water. Norw. J. Bot. 22: 209-215.
- Ryther, J. H. 1954. The ecology of phytoplankton blooms in Moriches Bay and Great South Bay, Long Island, New York. Biol. Bull. 106: 198-209.
- Sokal, R. R. and Rohlf, F. J. 1973. Introduction to Biostatistics, translated into Japanese by K. Fujii. Kyoritsu Shuppan, Tokyo.
- Williams, R. B. 1964. Division rates of salt marsh diatoms in relation to salinity and cell size. Ecology 45: 877-880.

水野 真：海産及び河口産底生珪藻の増殖におよぼす塩分濃度の影響

21株の海産及び河口産底生珪藻の塩分濃度に対する増殖特性を室内培養実験によって調べた。河口産珪藻は海産珪藻より広い塩分濃度範囲に耐える傾向が認められた。海産珪藻各株の最適濃度と増殖に好適な濃度範囲は一樣ではなかったが、多くのものは15‰以下の塩分濃度で増殖しなかったり、最高増殖速度の1/2以下の速さでしか増殖しなかった。今回調べた海産底生珪藻の塩分濃度に対する増殖特性は海産プランクトン珪藻の中で典型的な海産種とみなされているものほとんど差はみられなかった。河口産珪藻のうち4株は広塩性を示したが、他の2株は15‰から29‰の狭い範囲でのみよく増殖した。塩分濃度が後者の分布を規定する重要な要因となることを示唆した。(099-24 網走市字八坂196 東京農業大学生物産業学部生物生産学科)

