



HAL
open science

Effects of salinity on the growth rate and morphology of ten *Skeletonema* strains

Sergio Balzano, Wiebe Kooistra, Diana Sarno

► **To cite this version:**

Sergio Balzano, Wiebe Kooistra, Diana Sarno. Effects of salinity on the growth rate and morphology of ten *Skeletonema* strains. *Journal of Plankton Research*, 2010, 10.1093/plankt/FBQ150 . hal-00648048

HAL Id: hal-00648048

<https://hal.science/hal-00648048>

Submitted on 5 Dec 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



**Effects of salinity on the growth rate and morphology of ten
Skeletonema strains**

Journal:	<i>Journal of Plankton Research</i>
Manuscript ID:	JPR-2010-232.R1
Manuscript Type:	Original Article
Date Submitted by the Author:	11-Oct-2010
Complete List of Authors:	Balzano, Sergio; Station biologique de Roscoff, Diversité du Plancton océanique Kooistra, Wiebe; Stazione Zoologica A. Dohrn, Marine Botany Sarno, Diana; Stazione Zoologica A. Dohrn, Marine Botany
Keywords:	Skeletonema, salinity, growth response curve, euryhaline

SCHOLARONE™
Manuscripts

1
2
3
4
5 **Effects of salinity on the growth rate and morphology**
6 **of ten *Skeletonema* strains¹**
7
8
9

10
11
12
13
14 **SERGIO BALZANO^{12*}, DIANA SARNO¹ AND WIEBE H.C.F. KOOISTRA¹**

15
16 ¹STAZIONE ZOOLOGICA A. DOHRN, VILLA COMUNALE? 80121 NAPLES? ITALY.

17
18 ² PRESENT ADDRESS: UPMC (PARIS 06) ET CNRS, UMR7144, DIVERSITE DU PLANCTON
19 OCEANIQUE , STATION BIOLOGIQUE DE ROSCOFF, FRANCE.
20

21
22
23
24 *CORRESPONDING AUTHOR: balzano@sb-roscoff.fr.
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

ABSTRACT

Skeletonema comprises a planktonic genus of centric diatoms occurring in coastal and brackish waters worldwide. The most commonly reported species, *S. costatum*, was believed to be ubiquitous, morphologically plastic and euryhaline. Yet, recent studies showed that this perceived taxon consists of a series of species, including a more narrowly defined *S. costatum sensu stricto*. Here we investigated the effect of salinity on growth rate and cell morphology. Ten strains belonging to six *Skeletonema* species were incubated at salinities between zero and 35, their growth response was measured and their cell morphology observed using light and electron microscopy. All strains grew well between salinities of 10 and 35, whereas the lowest salinity at which growth was observed differed among species and even within the same species: *Skeletonema marinoi* strains deriving from distinct environments (Baltic and North Sea) showed different lower tolerance limits. Two species, *Skeletonema costatum* and *S. subsalsum*, showed growth between salinities of zero and 35 and exhibited very short intercellular processes when cultured in freshwater. *Skeletonema subsalsum* underwent cell enlargement when exposed to a salinity of 35.

Key Index words: *Skeletonema*, salinity, growth response curve, euryhaline

INTRODUCTION

Marine phytoplankton species often show broad salinity tolerance with many species capable of growing within a wide range (Brand, 1984). Coastal species are usually more euryhaline compared to oceanic species, as salinity is less prone to radical fluctuations offshore than in coastal and estuarine waters. Nevertheless, even typical offshore species exhibit broad salinity tolerance ranges (Brand, 1984). Recent studies in coastal environments have revealed the occurrence of multiple species in the genera *Cyclotella* (Buric *et al.*, 2007), *Pyrodinium* (Phlips *et al.*, 2006) and *Skeletonema* (Castillo *et al.*, 1995, Bergesch *et al.*, 2009) along salinity gradients from the deep open sea into estuaries. Brown (Brown *et al.*, 2006) compared distribution patterns of *Karenia* species along a salinity gradient with results of laboratory growth experiments under different salinities. Despite the large overlap in distribution patterns and growth response curves, the *Karenia* strain isolated from the most brackish environment showed the lowest limit of salinity tolerance (Brown *et al.*, 2006).

The genus *Skeletonema* contributes to phytoplankton blooms in coastal environments all over the globe except in the Antarctic (Karentz & Smayda, 1984, Cloern *et al.*, 1985, Estrada *et al.*, 1985). The most commonly known taxon, *Skeletonema costatum sensu lato* (Greville) Cleve has been shown to be well adapted to salinity changes (Rijstenbil, 1988, Rijstenbil *et al.*, 1989) and is considered ubiquitous, morphologically plastic, ecologically versatile and genetically variable (Gallagher, 1980). However, morphological and molecular phylogenetic appraisals of *Skeletonema* biodiversity have shown that *S. costatum sensu lato* consists of at least eight morphologically and genetically distinct species (Medlin *et al.*, 1991, Sarno *et al.*, 2005, Zingone *et al.*, 2005, Sarno *et al.*, 2007) including a more narrowly defined *S. costatum sensu stricto* (Zingone *et al.*, 2005, Sarno *et al.*, 2007).

Each of the newly described *Skeletonema* species might have broad salinity ranges as *S. costatum sensu lato* was believed to have. A recent study reported the presence of *S. costatum sensu stricto* (denoted in this paper as *S. costatum*) in the natural environment over a wide (1-30) salinity range (Bergesch *et al.*, 2009). In contrast in a tropical coastal lagoon in the Gulf of Mexico *S. tropicum* was found near the mouth of the lagoon but not at lower salinities (Castillo *et al.*, 1995). Moreover, recent laboratory experiments highlighted the ability for two marine *Skeletonema* species (Shikata *et al.*, 2008a, Shikata *et al.*, 2008b), later identified as *S. japonicum* and *S. pseudocostatum* (Yamada M and Shikata T, unpublished data) to grow at salinities as low as 5.

1
2
3 Salinity also influences chain morphology: *Skeletonema costatum* and *S. subsalsum*
4 cultured in the laboratory were found to decrease the distance between the sibling cells at low
5 (0-1) salinities (Paasche *et al.*, 1975, Sarno *et al.*, 2007). Similarly *S. potamos* specimens
6
7 sampled from zero salinities also showed shortened strutted processes (Torgan *et al.*, 2009).
8
9

10 Here we obtain salinity response curves for ten strains belonging to six *Skeletonema*
11 species (*S. ardens*, *S. costatum*, *S. grevillei*, *S. marinoi*, *S. subsalsum*, and *S. tropicum*) to
12 assess if these strains respond differently, if differences are species specific or merely strain
13 specific, and if the optima in the response curves are related to the conditions at sites where
14 and when the strains were sampled. Light and Electron Microscopy observations were also
15 carried out to assess the effects of salinity on cell morphology.
16
17
18
19
20
21

22 METHOD

23
24
25
26 Ten strains were used in the present study (Table 1); their taxonomic classification has
27 been documented in previous studies (Sarno *et al.*, 2005, Sarno *et al.*, 2007, Kooistra *et al.*,
28 2008). The three strains of *S. marinoi*: 7A3, 14A4, and CCMP781, were tested at both 15°C
29 and 20°C to evaluate the effect of temperature in salinity tolerance. Strain CCAP-1077/8 (*S.*
30 *subsalsum*) was tested only at 15°C, and all other strains only at 20°C (Table 1). These
31 temperatures were chosen because they reflect conditions under which these strains were
32 sampled, or because previous workers (Sarno *et al.*, 2005, Sarno *et al.*, 2007) demonstrated
33 rapid growth under these conditions. All strains were maintained in 10 mL medium in 30 mL
34 sterile glass tubes at 100 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ on a 12 h light-12 h dark routine and transferred
35 weekly to new medium. The strain CCAP-1077/8 was cultured at a salinity of 5 in a medium
36 prepared as described below. All other strains were maintained in *f/2* medium (Guillard, 1975)
37 at a salinity of 35.
38
39
40
41
42
43
44
45
46
47

48 Media with different salinities were obtained through mixing autoclaved and
49 subsequently *f/2* enriched seawater with distilled water enriched with autoclaved soil extract
50 (ratio of distilled water to soil extract is 9:1). Soil extract was prepared by autoclaving a mix of
51 1 kg soil from the Naples Botanical Gardens and 1 L of distilled water, filtering the resulting
52 broth and re-autoclaving the liquid after two days.
53
54
55
56

57 Strains were pre-adapted to different salinities by inoculating ca 100 μL culture
58 aliquots to 30 mL media with salinity 0.5 to 5 higher or lower than that in which the strains
59 were growing. The strains were inoculated in media at lower or higher salinities every three
60

1
2
3 days, until the strains reached salinities where cells failed to grow. Growth experiments were
4 carried out within the salinity range at which the cells were able to grow. The strains were
5 maintained at least for one week under the new salinity conditions before the start of the
6 experiments.
7
8
9

10 Experiments commenced by inoculating 30 mL medium with ca. 100 μ L culture
11 aliquots containing pre-adapted cells. Growth experiments were carried out in duplicate
12 cultures for each strain and for all the salinities measured. Cell concentration was assumed to
13 be proportional to fluorescence emitted. Fluorescence was measured over a 5-day period at ca.
14 24-h-intervals using a 10-005R Fluorometer (Turner Designs, Inc. Sunnyvale, CA, USA) and
15 the values obtained were plotted semi-logarithmically against time. For each culture only the
16 fluorescence values which fit within the exponential phase of the growth curve were used to
17 calculate the growth rate. Values recorded at very low and very high algal concentrations were
18 found to be imprecise and were omitted. The growth rate (GR) was calculated as follows:
19
20
21
22
23
24
25

$$26 \quad \text{GR} = (\ln F(t_2) - \ln F(t_1)) / (t_2 - t_1)$$

27
28 where $F(t_2)$ and $F(t_1)$ are the fluorescence at time t_2 and t_1 ; t_2 and t_1 are the time of the last and
29 the first measurement, respectively, on the exponential growth phase. Growth rates were
30 plotted against salinity. Values reported here (Figs 1-3) correspond to average growth rates
31 calculated from two replicate cultures. The error bars indicate the difference between the
32 growth rates measured in the two replicate cultures for each salinity condition.
33
34
35
36

37 Cell chains from exponentially growing cultures were photographed using light
38 microscopy (LM) and prepared for scanning electron microscopy (SEM) observations as
39 described in Sarno *et al.* (Sarno *et al.*, 2005): cells were immersed briefly in a mixture of
40 HNO_3 and H_2SO_4 and rinsed with distilled water. The acid-cleaned material was mounted on
41 stubs, coated with gold-palladium and observed using a Philips 505 scanning electron
42 microscope (Philips, Eindhoven, The Netherlands). Morphological observations were carried
43 out on cells grown under three or four salinity conditions: at 35, at one or two values within the
44 optimal salinity range for growth, and near their minimum salinity tolerance limit for growth.
45 We included in our observations overall length of connected pairs of intercalary strutted
46 processes (as a proxy for the distance between adjacent cells), average number of cells per
47 chain, valvar diameter (width), and transapical diameter (height). These parameters were
48 measured on at least 40 different cell colonies for each culture, to obtain representative mean
49 values for each strain at each salinity condition.
50
51
52
53
54
55
56
57
58
59
60

RESULTS

Growth response curves as a function of salinity for the ten *Skeletonema* strains are shown in Figs 1-3. All strains grew well between salinities of 10 and 25, that is, their growth rates exceeded 0.5 divisions per day. In all strains except *S. tropicum* growth rates decreased slightly at salinities of 30 and 35 (Figs 1-3). Maximum growth rates typically ranged between 0.7 and 1.5 divisions per day, though higher values were recorded occasionally for *S. costatum* and *S. subsalsum*. Differences among the growth response curves of the strains were most pronounced at low (0-10) salinities.

The three strains of *S. marinoi* included in this study were remarkably euryhaline, though differences in their lower salinity tolerance limits were evident. Strain CCMP781 from the North Sea failed to grow below salinities of 7.5 whereas the strains 7A3 and 14A4 from the Bothnian Gulf grew well at a salinity of 5 and showed diminishing growth rates only at salinities as low as 2.5 or 3.5. None of these three strains showed growth at a salinity of zero. Results obtained at 15°C (Fig. 1A) were comparable to those at 20°C (Fig. 1B).

The two strains of *S. costatum* and the one of *S. subsalsum* (Fig. 2) were also remarkably euryhaline. The two strains grew fast between salinities of 10 and 35. The growth rate of strain SZN B211 decreased from salinities of 8 to 3, and growth ceased at a salinity of 2. However, the strain survived that condition for at least 7 days because cell chains transferred subsequently into a salinity of 5 recommenced growth immediately (data not shown). Strain SZN B202 was even more euryhaline; it grew rapidly at salinities of 1 and 2 and even showed some growth at a salinity of 0 (Fig. 2). *Skeletonema subsalsum* strain CCAP1077/8 grew well anywhere between salinities of 1 and 35, though showing slightly depressed rates above 25 and below 3.5. This strain exhibited remarkably high growth rates between salinities of 3.5 and 5.

The strains of *S. ardens*, *S. grevillei* and *S. tropicum* were less euryhaline than those of *S. subsalsum* and *S. costatum* (Fig. 3). *Skeletonema tropicum* strain SZN B141 grew well between salinities of 7.5 and 35, showed marginal growth at 6 and did not grow at 5. *Skeletonema grevillei* strain CCMP1685 grew only between salinities of 10 and 35 (Fig. 3). The growth curves of *Skeletonema ardens* strains CS-347 and CCMP794 were similar, the strains grew well between salinities of 8 and 35. For both strains a slight decrease in the growth rate was found at high (32-35) salinities and growth did not occur at salinities <8. Table 2 lists the effects of salinity on four morphometric traits measured in LM and SEM. The average length of the strutted processes, and hence the distance between adjacent cells

1
2
3 decreased with decreasing salinity for *S. costatum*, *S. grevillei*, *S. subsalsum* and to a lesser
4 extent for *S. tropicum*, whereas no such an effect was evident in the strains of *S. ardens* and *S.*
5 *marinoi* (Table 2). At the lowermost salinity levels at which growth was still observed,
6 adjacent cells in a chain were packed together (Figs 4-5). In the strains of *S. costatum* and *S.*
7 *subsalsum*, the average number of cells per chain decreased markedly with decreasing salinity
8 whereas the strains of *S. marinoi*, on the contrary, showed an increase of this average with
9 decreasing salinity. The remaining strains did not show any clear trend for this parameter.
10 Salinity did not markedly affect valve diameter and cell height in any of the strains (Table 2).

11
12 At a salinity of 35, *S. subsalsum* showed cell enlargement resulting in relatively short
13 chains of cells with a markedly enlarged valve diameter (Figs 4E, 5C, 5D), each cell
14 containing three to four chloroplasts instead on the normal one or two (Fig. 4E), and showed
15 relatively short strutted processes (Table 2, Figs 4-5). The newly established large cells rapidly
16 outgrew the small ones in the culture grown at a salinity of 35. Cell enlargement was not
17 observed in this strain at any other salinity. Neither was cell enlargement shown in any other
18 strain over the duration of the experiments.

31 32 33 34 **DISCUSSION**

35
36
37 Growth experiments revealed euryhaline responses for all strains of *Skeletonema* but
38 also differences among species as well as among strains within species. The differences have a
39 bearing on the distribution of *Skeletonema* species along salinity gradients in the field, though
40 the salinity tolerance ranges observed in the lab are much broader than the field conditions in
41 which the species are recorded.

42
43 Strains showing no growth below a salinity of 7.5 tend to be sampled in plain seawater
44 or mildly brackish seawater. All strains of *S. ardens*, *S. grevillei* and *S. tropicum* known to date
45 were collected in the open sea (Castillo *et al.*, 1995, Kooistra *et al.*, 2008, Bergesch *et al.*,
46 2009, Gonzalez-Piana & Ferrari, 2009). Yet, the distribution patterns and ecological
47 preferences of *S. ardens* and *S. grevillei* are still poorly known whereas those of *S. tropicum*
48 are better known because the defining trait of this species, multiple plastids per cell, makes it
49 easily recognizable using light microscopy. Thus, *S. ardens*, *S. grevillei* and *S. tropicum* appear
50 to be representatives of coastal, open seawater populations *sensu* Brand's ecological
51
52
53
54
55
56
57
58
59
60

1
2
3 classification of plankton species (Brand, 1984). The results are also in line with Brand's
4 (Brand, 1984) observation that even offshore species exhibit broad salinity tolerance ranges.

5
6 Strains that show growth all the way from seawater to a salinity below 7.5 have been
7 raised from cells usually sampled from low salinity environments or under field conditions in
8 which the salinity can change rapidly. All strains of *S. costatum* and *S. subsalsum* known to
9 date have been collected in estuaries and lagoons (Devercelli, 2006, Kooistra *et al.*, 2008,
10 Friedrich & Pohlmann, 2009, Jung *et al.*, 2009). *Skeletonema costatum* and *S. subsalsum* thus
11 appear to be, along with *S. potamos* (Hasle and Evensen 1976, Torgan *et al.*, 2009),
12 representatives of estuarine populations *sensu* Brand's ecological classification of plankton
13 species (Brand, 1984).
14
15
16
17
18
19
20

21 Apparently, the realized niche in the field is much narrower than the physiologically
22 possible niche if only salinity is regarded and strains are grown under nutrient replete and ideal
23 physical conditions, without competitors, grazers and parasites. The realized niche of low
24 salinity waters of *S. costatum* and *S. subsalsum* corroborates results of previous studies in
25 estuarine (Bergesch *et al.*, 2009, Torgan *et al.*, 2009) and riverine environments (Devercelli,
26 2006, Friedrich & Pohlmann, 2009). Especially the estuarine species need to cope with rapid
27 salinity changes. In our study the strains were adapted gently to low salinities and we did not
28 measure their capacity to survive drastic, rapid salinity changes over short periods. But
29 apparently, strains of some species can; a strain of *S. pseudocostatum* grew after being
30 transferred from a salinity of 32 to 5 (Shikata *et al.*, 2008a). Nonetheless, different
31 *Skeletonema* species appear to have partially overlapping distribution ranges along salinity
32 gradients, supporting the view of estuarine ecosystems as ecoclines rather than ecotopes
33 (Attrill & Rundle, 2002, Quinlan & Philips, 2007).
34
35
36
37
38
39
40
41
42
43

44 The intra-specific differences in growth responses at low salinities as observed among
45 the *S. marinoi* strains have been described in other diatoms (Snoeijs & Potapova, 1998) and in
46 seaweeds (Bolton, 1979, Rietema, 1991, Rietema, 1993, Rietema, 1995, Kristiansen *et al.*,
47 1994) from the North Sea and the Baltic Sea. In many of these cases however, low salinity
48 strains from the eastern part of the Baltic Sea died when incubated under North Sea salinity
49 conditions and vice versa, whereas the Umeå (Baltic) strains of *S. marinoi* can grow in North
50 Sea salinity conditions.
51
52
53
54
55

56 Growth responses of strains that have been maintained in culture for extended time
57 might change, especially if the culture conditions differ radically from those in the field. The
58 reason is that the genetic makeup of a strain changes because those changes that foster rapid
59 growth under the culture conditions are selected (Lakeman *et al.*, 2009).
60

1
2
3 The difference in the growth response curves between two strains of *S. marinoi*
4 sampled from the same site (Fig. 1) suggests the existence of population genetic variation in
5 the ability to cope with extremely low salinity and corroborates genetic and physiological
6 heterogeneity documented within *S. marinoi* (Ellegaard *et al.*, 2008, Kooistra *et al.*, 2008,
7 Saravanan & Godhe, 2010). Remarkable physiological diversity has been shown for several
8 strains of *S. marinoi* isolated from Gullmar Fjord (Sweden) during different seasons: although
9 all the strains grew well at the temperatures and salinities tested, different growth rates were
10 measured for the different strains incubated under the same conditions (Saravanan & Godhe,
11 2010).

12
13
14
15
16
17
18
19
20 The capacity of the strains of *S. costatum* and *S. subsalsum* to grow at low salinities, or
21 even in freshwater, and their shared morphological changes observed under these conditions
22 corroborate findings in Sarno *et al.* (Sarno *et al.*, 2007) and Kooistra *et al.* (Kooistra *et al.*,
23 2008) that these species are close relatives. Growth at extremely low salinities is not a derived
24 trait, as genetically not particularly closely related *S. marinoi* is tolerant to low salinities as
25 well. Neither is it a conserved trait because such tolerance can differ among strains within the
26 same species (*S. marinoi*). Nevertheless, the euryhaline response of our strains of *S. subsalsum*
27 and *S. costatum* is probably a characteristic of the two species, as suggested by reports of these
28 species in brackish environments (Devercelli, 2006, Kooistra *et al.* 2008, Friedrich &
29 Pohlmann, 2009, Jung *et al.*, 2009) and because our results on *S. subsalsum* strain CCAP-
30 1077/8 corroborate previous observations (Paasche, 1975, Paasche *et al.*, 1975) on another
31 strain of this species.

32
33
34
35
36
37
38
39
40 The shortening of intercellular processes observed in our strains of *S. costatum* and *S.*
41 *subsalsum* at a salinity of 1 (Figs. 4A, 4C, 5A, 5E) has also been reported in previous studies
42 on *S. costatum* (Sarno *et al.*, 2007) and *S. subsalsum* (Hasle & Evensen, 1975, Paasche *et al.*,
43 1975). This phenotypic change may have adaptive advantages at low salinities, but it might
44 result just as well from problems affecting the formation of “normal” processes at low salinity.

45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
The observation of cell enlargement (Gallagher, 1983) in *S. subsalsum* at a salinity of
35 suggests that high salinity triggers this process. We do not think that the enlargement
resulted from intra-strain sexual reproduction because we did not observe flagellated
microgametes as illustrated in Migita, (1967). The fact that large cells of *S. subsalsum* outgrew
genetically identical small ones in our culture conditions may have some bearing on ecology
because in coastal eutrophic ecosystems, larger phytoplankton cells sustain higher carbon
specific photosynthesis rates than smaller ones (Cermeno *et al.*, 2005). However, the higher
growth rate of the larger cells may also simply reflect the fact that the cells in the culture we

1
2
3 received have become unnaturally small due to several years of ongoing vegetative growth and
4 that small size impairs growth. However, we did not test these possibilities.
5
6
7

8 9 **ACKNOWLEDGEMENTS**

10
11
12
13 The authors thank Adriana Zingone and Isabella Percopo for help with experimental
14 design, Gandi Forlani for help in EM preparations, Gennaro Iamunno and Franco Iamunno for
15 their assistance with SEM and Carmen Minucci for help with culture maintenance. Silvia
16 Méndez graciously provided us with a net sample from the mouth of the Rio de la Plata,
17 Uruguay. The authors thank also Tomoyuki Shikata and Machiko Yamada for providing
18 information on the phylogenetic affiliation of the *Skeletonema* strains investigated in their
19 articles (Shikata *et al.*, 2008a, Shikata *et al.*, 2008b). The present study is a contribution to the
20 EU NoE MARBEF, Marine Biodiversity and Ecosystem Functioning.
21
22
23
24
25
26
27
28
29
30
31
32
33

34 **REFERENCES**

- 35
36
37
38
39 Attrill, M. J. and Rundle, S. D. (2002) Ecotone or ecocline: ecological boundaries in estuaries.
40 *Estuarine, Coastal and Shelf Science*, **55**, 929-936.
41
42 Bergesch, M., Garcia, M. and Odebrecht, C. (2009) Diversity and morphology of
43 *Skeletonema* species in Southern Brazil, Southwestern Atlantic Ocean. *Journal of*
44 *Phycology*, **45**, 1348-1352.
45
46 Bolton, J. J. (1979) Estuarine adaptation in populations of *Pilayella littoralis* (L.) Kjellm.
47 (Phaeophyta, Ectocarpales) *Estuarine and Coastal Marine Science*, **9**, 273-280.
48
49 Brand, L. E. (1984) The salinity tolerance of 46 marine-phytoplankton isolates. *Estuarine*
50 *Coastal and Shelf Science*, **18**, 543-556.
51
52 Brown, A. F. M., Dortch, Q., Van Dolah, F. M., Leighfield, T. A., Morrison, W., Thessen, A.
53 E., Steidinger, K., Richardson, B., Moncreiff, C. A. and Pennock, J. R. (2006) Effect
54 of salinity on the distribution, growth, and toxicity of *Karenia* spp. *Harmful Algae*, **5**,
55 199-212.
56
57
58
59
60

- 1
2
3 Buric, Z., Kiss, K. T., Acs, E., Vilicic, D., Mihalic, K. C. and Caric, M. (2007) The
4 occurrence and ecology of the centric diatom *Cyclotella choctawhatcheeana* Prasad in
5 a Croatian estuary. *Nova Hedwigia*, **84**, 135-153.
6
7
8 Castillo, J. A., Delcastillo, M. E. M. and Hernandez Becerril, D. U. (1995) Morphology and
9 distribution of species of the diatom genus *Skeletonema* in a tropical coastal lagoon.
10 *European Journal of Phycology*, **30**, 107-115.
11
12
13 Cermeno, P., Maranon, E., Rodriguez, J. and Fernandez, E. (2005) Large-sized phytoplankton
14 sustain higher carbon specific photosynthesis than smaller cells in a coastal eutrophic
15 ecosystem. *Marine Ecology-Progress Series*, **297**, 51-60.
16
17
18 Cloern, J. E., Cole, B. E., Wong, R. L. J. and Alpine, A. E. (1985) Temporal dynamics of
19 estuarine phytoplankton. A case-study of San Francisco Bay. *Hydrobiologia*, **129**,
20 153-176.
21
22
23 Devercelli, M. (2006) Phytoplankton of the Middle Parana River during an anomalous
24 hydrological period: a morphological and functional approach. *Hydrobiologia*, **563**,
25 465-478.
26
27
28 Ellegaard, M., Godhe, A., Harnstrom, K. and Mcquoid, M. (2008) The species concept in a
29 marine diatom: LSU rDNA-based phylogenetic differentiation in *Skeletonema*
30 *marinoi/dohrnii* (Bacillariophyceae) is not reflected in morphology. *Phycologia*, **47**,
31 156-167.
32
33
34 Estrada, M., Vives, F. and Alcaraz, M. (1985) Life and the productivity of the open sea. In: R.
35 Margalef (ed) *Key environments: Western Mediterranean*. Pergamon Press Ltd.,
36 Oxford, UK, pp. 148-197.
37
38
39 Friedrich, G. and Pohlmann, M. (2009) Long-term plankton studies at the Lower
40 Rhine/Germany. *Limnologica*, **39**, 14-39.
41
42
43 Gallagher, J. C. (1980) Population genetics of *Skeletonema costatum* (Bacillariophyceae) in
44 Narragansett Bay. *Journal of Phycology*, **16**, 464-474.
45
46
47 Gallagher, J. C. (1983). Cell enlargement in *Skeletonema costatum* (Bacillariophyceae).
48 *Journal of Phycology*, **19**, 539-542.
49
50
51 Gonzalez-Piana, M. and Ferrari, G. (2009) *Skeletonema tropicum* (Bacillariophyceae) present
52 in Uruguayan southern coastal waters. *Iheringia Serie Botanica*, **64**, 145-149.
53
54
55 Guillard, R. (1975) Culture of phytoplankton for feeding marine invertebrates. In: W. L.
56 Smith and M. H. Chanley (eds) *Culture of marine invertebrate animals*. Plenum Press,
57 New York, pp. 26-60.
58
59
60

- 1
2
3 Hasle, G. R. and Evensen, D. L. (1975) Brackish water and freshwater species of diatom
4 genus *Skeletonema*. I. *Skeletonema subsalsum* (Cleve) Bethge. *Phycologia*, **14**, 283-
5 297.
6
7
8
9 Hasle, G. R. and Evensen, D. L. (1976) Brackish water and freshwater species of diatom
10 genus *Skeletonema*. II. *Skeletonema potamos* comb. nov. *Journal of Phycology*, **12**,
11 73-82.
12
13
14 Jung, S. W., Yun, S. M., Lee, S. D., Kim, Y.-O. and Lee, J. H. (2009) Morphological
15 characteristics of four species in the genus *Skeletonema* in coastal waters of South
16 Korea. *Algae*, **24**, 195-203.
17
18
19 Karentz, D. and Smayda, T. J. (1984) Temperature and seasonal occurrence patterns of 30
20 dominant phytoplankton species in Narragansett Bay over a 22-year period (1959-
21 1980). *Marine Ecology-Progress Series*, **18**, 277-293.
22
23
24 Kooistra, W., Sarno, D., Balzano, S., Gu, H. F., Andersen, R. A. and Zingone, A. (2008)
25 Global diversity and biogeography of *Skeletonema* species (Bacillariophyta). *Protist*,
26 **159**, 177-193.
27
28
29
30 Kristiansen, A. A., Pedersen, P. M. and Moseholm, L. (1994) Salinity-temperature effects on
31 growth and reproduction of *Scytosiphon lomentaria* (Fucophyceae) along the salinity
32 gradient in Danish water. *Phycologia*, **33**, 444-454.
33
34
35 Lakeman, M. B., von Dassow P., Cattolico R.A. (2009) The strain concept in phytoplankton
36 ecology. *Harmful Algae*, **8**, 746-758
37
38
39 Medlin, L. K., Elwood, H. J., Stickel, S. and Sogin, M. L. (1991) Morphological and genetic
40 variation within the diatom *Skeletonema costatum* (Bacillariophyta). Evidence for a
41 new species, *Skeletonema pseudocostatum*. *Journal of Phycology*, **27**, 514-524.
42
43
44 Migita, S. (1967) Sexual reproduction of centric diatom *Skeletonema costatum*. *Bulletin of the*
45 *Japanese Society of Scientific Fisheries*, **33**, 392-398.
46
47
48 Paasche, E. (1975) The influence of salinity on the growth of some plankton diatoms from
49 brackish water. *Norwegian Journal of Botany* **22**, 209-215.
50
51
52 Paasche, E., Johansson, S. and Evensen, D. L. (1975) An effect of osmotic pressure on the
53 valve morphology of the diatom *Skeletonema subsalsum* (A. Cleve) Bethge.
54 *Phycologia* **14**, 205-211.
55
56
57 Philips, E. J., Badylak, S., Bledsoe, E. and Cichra, M. (2006) Factors affecting the distribution
58 of *Pyrodinium bahamense* var. *Bahamense* in coastal waters of Florida. *Marine*
59 *Ecology-Progress Series*, **322**, 99-115.
60

- 1
2
3 Quinlan, E. L. and Philips, E. J. (2007) Phytoplankton assemblages across the marine to low-
4 salinity transition zone in a blackwater dominated estuary. *J. Plankton Res.*, **29**, 401-
5 416.
6
7
8 Rietema, H. (1991) Evidence for ecotypic divergence between *Phycodryis rubens* populations
9 from the Baltic Sea and North Sea *Botanica Marina*, **34**, 375-381.
10
11 Rietema, H. (1993) Ecotypic differences between Baltic and North Sea populations of
12 *Delesseria sanguinea* and *Membranoptera alata*. *Botanica Marina*, **36**, 15-21.
13
14 Rietema, H. (1995) Ecoclinal variation in *Rhodomela confervoides* along a salinity gradient in
15 the North Sea and Baltic Sea. *Botanica Marina*, **38**, 475-479.
16
17 Rijnstabil, J. W. (1988) Selection of phytoplankton species in culture by gradual salinity
18 changes. *Netherlands Journal of Sea Research*, **22**, 291-300.
19
20 Rijnstabil, J. W., Mur, L. R., Wijnholds, J. A. and Sinke, J. J. (1989) Impact of a temporal
21 salinity decrease on growth and nitrogen-metabolism of the marine diatom
22 *Skeletonema costatum* in continuous cultures. *Marine Biology*, **101**, 121-129.
23
24 Saravanan, V. and Godhe, A. (2010) Genetic heterogeneity and physiological variation
25 among seasonally separated clones of *Skeletonema marinoi* (Bacillariophyceae) in the
26 Gullmar Fjord, Sweden. *European Journal of Phycology*, **45**, 177-190.
27
28 Sarno, D., Kooistra, W., Medlin, L. K., Percopo, I. and Zingone, A. (2005) Diversity in the
29 genus *Skeletonema* (Bacillariophyceae). II. An assessment of the taxonomy of *S.*
30 *costatum*-like species with the description of four new species. *Journal of Phycology*,
31 **41**, 151-176.
32
33 Sarno, D., Kooistra, W., Balzano, S., Hargraves, P. E. and Zingone, A. (2007) Diversity in the
34 genus *Skeletonema* (Bacillariophyceae): III. Phylogenetic position and morphological
35 variability of *Skeletonema costatum* and *Skeletonema grevillei*, with the description of
36 *Skeletonema ardens* sp. nov. *Journal of Phycology*, **43**, 156-170.
37
38 Shikata, T., Nagasoe, S., Oh, S. J., Matsubara, T., Yamasaki, Y., Shimasaki, Y., Oshima, Y.
39 and Honjo, T. (2008a) Effects of down- and up-shocks from rapid changes of salinity
40 on survival and growth of estuarine phytoplankters. *Journal of the Faculty of*
41 *Agriculture Kyushu University*, **53**, 81-87.
42
43 Shikata, T., Sakurada, K., Jomoto, Y., Onji, M., Yoshida, M. and Ohwada, K. (2008b) Effects
44 of temperature, salinity and light irradiance on phytoplankton growth in the Yatsushiro
45 Sea. *Nippon Suisan Gakkaishi*, **76**, 34-45.
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
- Snoeijs, P. and Potapova, M. (1998) Ecotypes or endemic species? - a hypothesis on the evolution of *Diatoma* taxa (Bacillariophyta) in the northern Baltic Sea. *Nova Hedwigia*, **67**, 303-348.
- Torgan, L. C., Becker, V. and Dos Santos, C. B. (2009) *Skeletonema potamos* (Bacillariophyta) in Patos Lagoon, southern Brazil: Taxonomy and distribution. *Revista Peruana de Biologia*, **16**, 93-96.
- Xu, J., Yin, K. D., Lee, J. H. W., Liu, H. B., Ho, A. Y. T., Yuan, X. C. and Harrison, P. J. (2010) Long-term and seasonal changes in nutrients, phytoplankton biomass, and dissolved oxygen in Deep Bay, Hong Kong. *Estuaries and Coasts*, **33**, 399-416.
- Zingone, A., Percopo, I., Sims, P. A. and Sarno, D. (2005) Diversity in the genus *Skeletonema* (Bacillariophyceae). I. A reexamination of the type material of *S. costatum* with the description of *S. grevillei* sp. nov. *Journal of Phycology*, **41**, 140-150.

42 TABLE AN FIGURE LEGEND

43
44
45
46
47
48 *Table 1: List of Skeletonema strains used and lowest salinity (Low S)*
49 *at which each strain was able to grow*

50
51
52
53
54
55
56
57 *Table 2. Morphometric parameters and standard deviations associated with the Skeletonema*
58 *strains used in the present study.*
59
60

1
2
3
4
5 **Fig. 1.** Growth response curves as a function of salinity of three strains belonging to
6 *Skeletonema marinoi*. Experiments were carried out at (A) 15°C and (B) 20°C.
7
8
9

10 **Fig. 2.** Growth response curves as a function of salinity of (A) two strains of *S. costatum*
11 and (B) a strain of *S. subsalsum*. Growth experiments were carried out at 20°C.
12
13
14

15
16
17 **Fig. 3.** Growth response curves as a function of salinity of four *Skeletonema* strains: (A) *S.*
18 *tropicum* strain SZN B-141 and *S. grevillei* strain CCMP1685, (B) and two strains of *S.*
19 *ardens*. Growth experiments were carried out at 20°C.
20
21
22
23
24
25

26 **Fig. 4.** Morphology of two *Skeletonema* species grown at different conditions of salinity: *S.*
27 *costatum* strain SZN B202 grown at a salinity of (A) 1 and (B) 35. *Skeletonema subsalsum*
28 strain CCAP1077/8 grown at a salinity of (C) 1, (D) 10 and (E) 35.
29
30
31
32

33 **Fig. 5.** Electron micrographs of cleaned silica cell walls of two *Skeletonema* species grown
34 at different conditions of salinity: *S. subsalsum* strain CCAP1077/8 grown at a salinity of (A)
35 1, (B) 10 and (C, D) 35, and *S. costatum* strain SZN B202 grown at a salinity of (E) 1 (F) 35.
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Table 1

Species/clone	Geographical origin	Isolation date	Low S
<i>S. ardens</i>			
CCMP794	Singapore	December 1973	7.5
CS-347	Gulf of Carpentaria, Australia	1981	7.5
<i>S. costatum</i>			
SZN B202	Indian River Lagoon, Fl, USA	January 2003	0
SZN B211	Montevideo, Uruguay	April 2004	3
<i>S. grevillei</i>			
CCMP1685	Arabian Sea, Muscat, Oman	October 1993	10
<i>S. marinoi</i>			
CCMP781	Fladenground, North Sea	April 1976	7.5
7A3	Umeå, Sweden	June 2003	2.5
14A4	Umeå, Sweden	June 2003	2.5
<i>S. subsalsum</i>			
CCAP1077/8	Lake Erne, Ireland	August 1993	0
<i>S. tropicum</i>			
SZN B141	Gulf of Naples, Italy	October 2002	6

Table II

Salinity	Distance ^a	Cells ^b	Diameter ^c	Height ^d
<i>Skeletonema ardens</i> (CCMP794)				
7.5	0.9 ± 0.4	3.9 ± 2.8	4.3 ± 1.8	5 ± 1.6
20	1.1 ± 0.4	3.8 ± 3.5	5 ± 1.5	6.3 ± 1.5
35	1 ± 0.3	4.1 ± 3.3	3.8 ± 1.2	5.8 ± 1.9
<i>Skeletonema ardens</i> (CS-347)				
7.5	1.1 ± 0.5	5.2 ± 4.0	3.3 ± 1.1	5.7 ± 1.6
20	1.1 ± 0.3	5.5 ± 3.9	2.7 ± 1.2	6.1 ± 1.8
35	1.2 ± 0.6	6.5 ± 3.3	2.9 ± 0.8	6.2 ± 2.2
<i>Skeletonema costatum</i> (SZN B202)				
1	0.5 ± 0.4	14 ± 8.9	8 ± 0.9	8.4 ± 2.8
10	1.5 ± 0.3	21 ± 5.8	10 ± 1.3	5.5 ± 1.8
20	3 ± 0.8	25 ± 4.3	8 ± 1.1	6 ± 2.5
35	3 ± 0.6	34 ± 5.5	8.2 ± 0.8	9 ± 2.1
<i>Skeletonema costatum</i> (SZN B211)				
3.5	0.6 ± 0.4	15 ± 9.4	9 ± 1.7	6.2 ± 2.5
10	1.4 ± 0.4	24 ± 6.7	8.4 ± 0.9	7.3 ± 1.6
20	2.7 ± 0.5	27 ± 7.4	9.9 ± 1.4	7.8 ± 1.4
35	3 ± 0.6	41 ± 5.9	11 ± 1.1	8.5 ± 1.9
<i>Skeletonema grevillei</i> (CCMP1685)				
7.5	0.5 ± 0.2	4 ± 2.4	4 ± 1.1	10 ± 2.8
20	3 ± 0.4	3.5 ± 1.8	3 ± 0.8	8 ± 3.1
35	4 ± 0.7	2.2 ± 1.3	3.2 ± 0.6	11.1 ± 3.4
<i>Skeletonema marinoi</i> (CCMP781)				
7.5	6.5 ± 1.0	9 ± 4.4	6 ± 0.4	5 ± 2.7
20	6.6 ± 1.4	10 ± 3.4	5.1 ± 0.8	11 ± 4.4
35	6 ± 1.1	5.1 ± 2.8	6.6 ± 1.5	7.2 ± 2.2
<i>Skeletonema marinoi</i> (7A3)				
5	3.5 ± 0.8	5.4 ± 2.5	3.4 ± 0.5	10 ± 3.3
20	7.6 ± 1.9	5.1 ± 3.3	6.3 ± 0.8	6.7 ± 2.4
35	4.8 ± 1.4	4.9 ± 2.8	4.3 ± 1.1	6.5 ± 3.4
<i>Skeletonema marinoi</i> (14A4)				
5	6.4 ± 1.2	14 ± 6.4	7.6 ± 0.5	5.9 ± 2.3
20	6.7 ± 1.4	9.5 ± 5.8	5.5 ± 0.3	7.2 ± 3.4
35	2.9 ± 1.5	5.1 ± 3.4	5.4 ± 0.9	7 ± 2.8
<i>Skeletonema subsalsum</i> (CCAP1077/8)				
1	0.5 ± 0.4	11 ± 7.4	3 ± 0.5	5.5 ± 2.4
5	1.5 ± 0.5	56 ± 18	7.2 ± 0.7	5.6 ± 1.8
20	3 ± 0.8	26 ± 11	5.7 ± 0.3	4.8 ± 1.8
35	0.5 ± 0.4	10 ± 6.4	10 ± 0.8	6.1 ± 2.1
<i>Skeletonema tropicum</i> (SZN B141)				
10	3 ± 0.6	10 ± 5.4	4.2 ± 1.1	6.4 ± 2.2
20	6 ± 0.3	4.2 ± 2.1	3.2 ± 0.7	7.5 ± 3.1
35	5 ± 0.3	10.3 ± 7.1	6.8 ± 0.6	7.6 ± 2.8

^a Average length of strutted processes (intercellular distance) in μm .

1
2
3 ^b Average number of cells per chain.

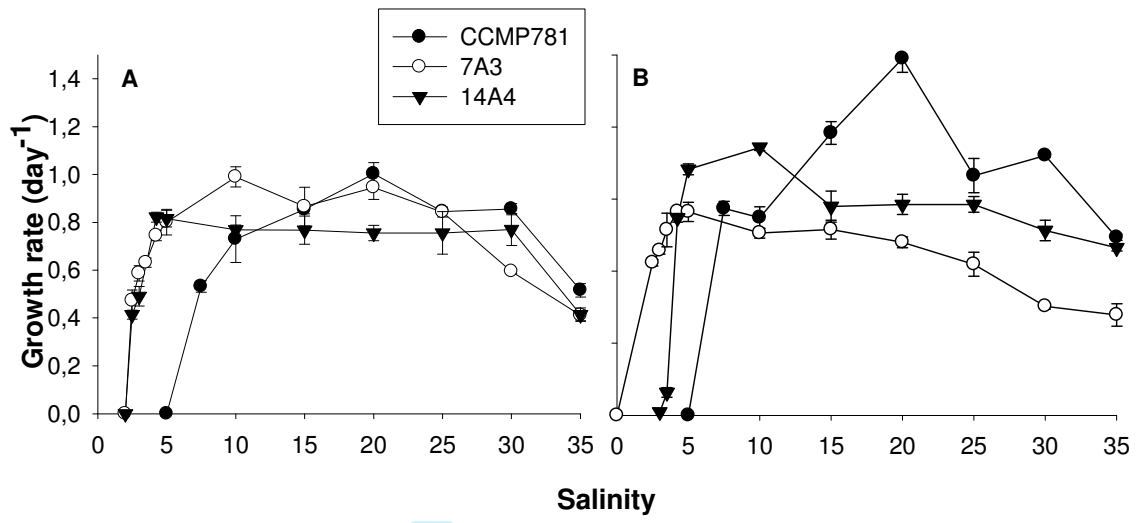
4 ^c Average valve diameter in μm .

5 ^d Average length of the mantle in μm .

6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review

Figure 1



Peer Review

Figure 2

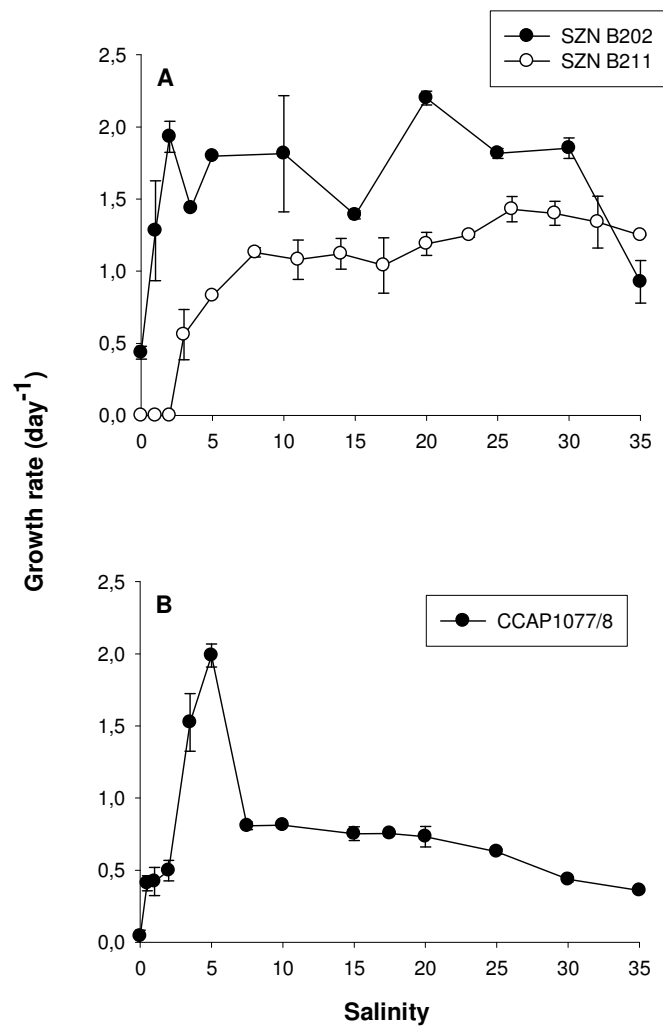


Figure 3

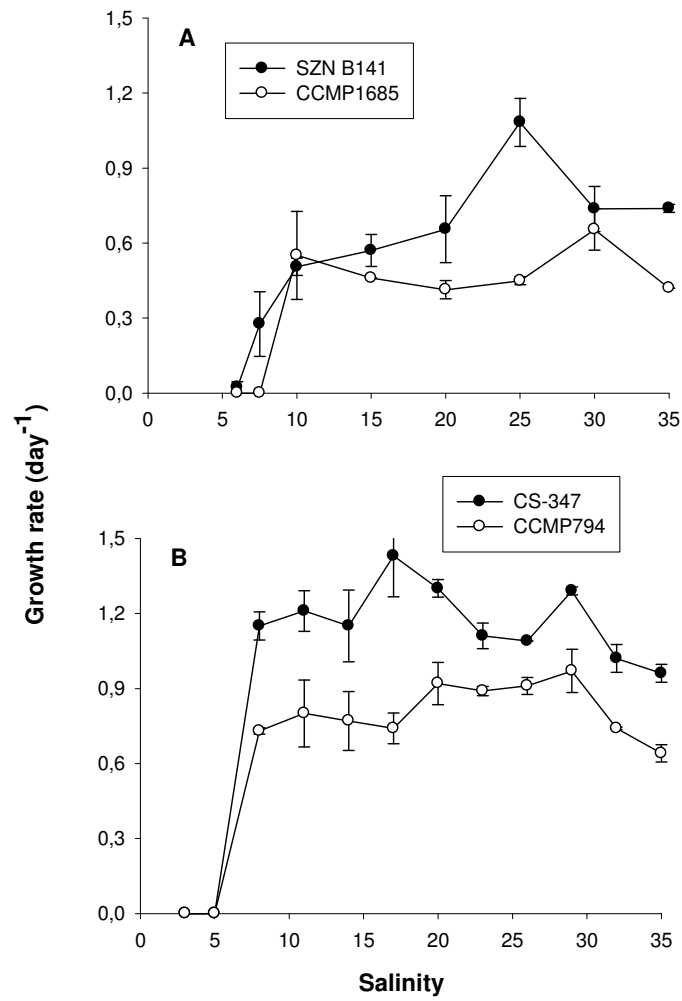
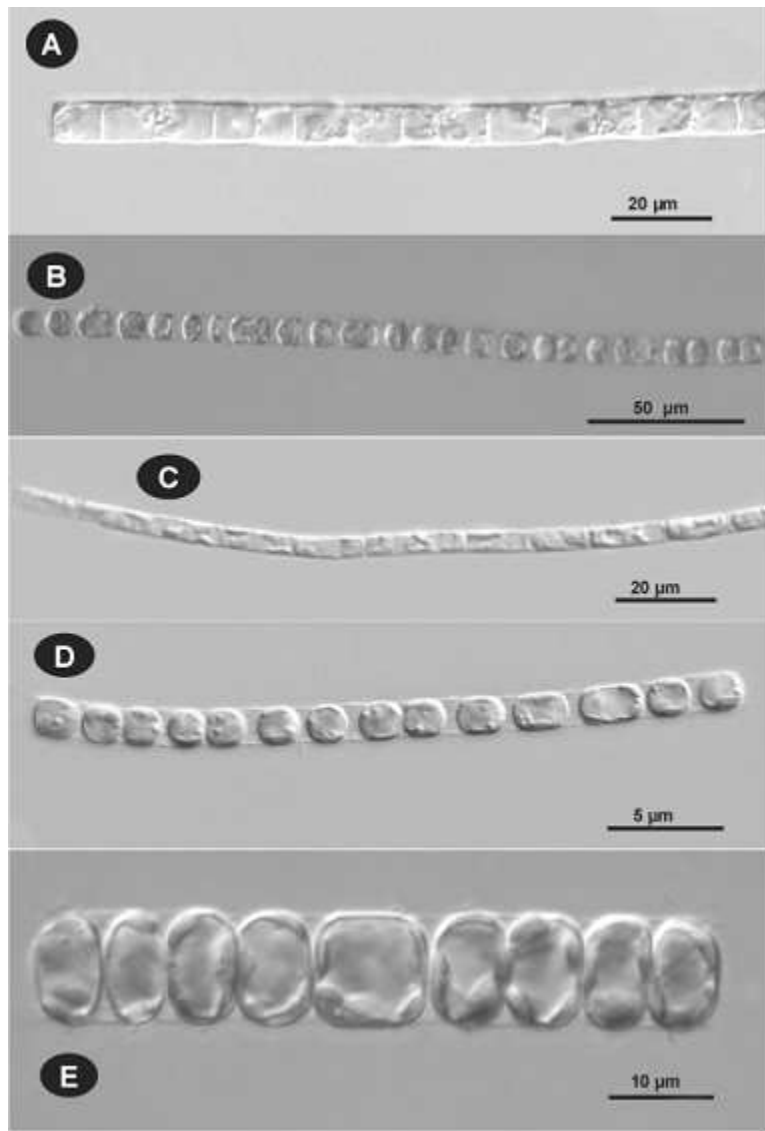


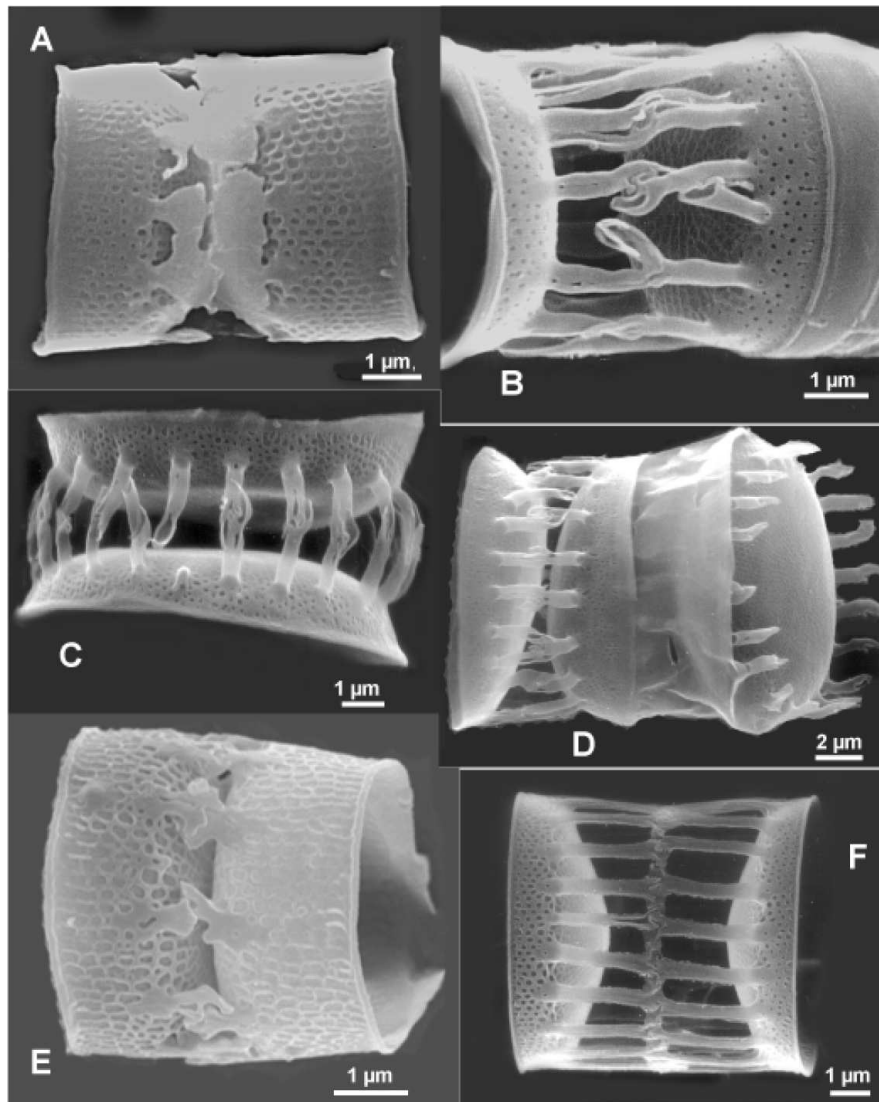
Figure 4



NEW

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Figure 5



ew