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Tolerance of benthic diatoms from temperate aquatic and terrestrial habitats to experimental desiccation and temperature stress

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Soils differ from aquatic sediments in environmental characteristics such as moisture availability and temperature fluctuations, and it is therefore believed that soil-inhabiting diatoms have a broader tolerance range to these stresses than aquatic diatoms. To test this hypothesis, we assessed the survival capacities of vegetative cells of 34 benthic diatom species from terrestrial and aquatic habitats in Belgium when exposed to desiccation and temperature stress. Six different stress conditions were studied: gradual heating up to +30°C and +40°C, abrupt heating to +40°C, freezing to −20°C and desiccation with and without preconditioning at +30°C. All six conditions resulted in a significantly decreased survival of cells compared to control conditions. Desiccation killed all tested strains, freezing was survived by only three species and abrupt heating was significantly more lethal than gradual heating, suggesting a generally high sensitivity of vegetative diatom cells to these three stress factors. While tolerance to temperature extremes (+40°C and −20°C) was to a large extent species-specific, habitat-specific differences in cell survival were also detected. Only terrestrial species survived freezing, and aquatic diatoms were less tolerant to gradual heating to +40°C, both pointing at a higher tolerance of terrestrial diatoms to temperature extremes. Moreover, in two species with both aquatic and terrestrial isolates, only the terrestrial strains survived +40°C. We conclude that vegetative cells of benthic diatoms (1) are very sensitive to desiccation, freezing and abrupt heating and (2) have a habitat-dependent tolerance to temperature extremes. The consequences of these observations for the dispersal capacities and the subsequent biogeographical patterns of diatoms are discussed.

Key Words: aquatic, benthic diatoms, desiccation, dispersal capacity, stress tolerance, temperature extremes, terrestrial

INTRODUCTION

The majority of diatom species are confined to aquatic environments (Patrick 1977). However, various species belonging to several genera can be found in terrestrial habitats such as soils and humid rocks (Patrick 1977), where they grow together with cyanobacteria and green algae (Ettl & Gärtner 1995). Terrestrial microalgal assemblages are characterized by specialized species (Ettl & Gärtner 1995; Johansen 1999; van Kerckvoorde *et al.* 2000; Van de Vijver *et al.* 2004). In unicellular green algae, phylogenetic studies have shown the separate evolution of terrestrial and aquatic lineages (Lewis & Flechtner 2004; Zoe *et al.* 2008). The main reasons for this lineage divergence are probably the specific adaptations required in terrestrial environments (Zoe *et al.* 2008). Diatoms inhabiting soils, for instance, must typically withstand large diurnal fluctuations in temperature, pH and moisture availability in the near-surface layer (Starks *et al.* 1981; Gao *et al.* 2008).

In order to survive locally, terrestrial algae have to be adapted to these extreme fluctuations in environmental conditions. Some terrestrial cyanobacteria and green algae are known for their enhanced tolerance to various habitat-related stress conditions such as desiccation (Dodds & Gudder 1995; Potts 1999; Sabacka & Elster 2006; Gray *et al.* 2007), osmotic stress (Tamaru *et al.* 2005), freezing (Sabacka & Elster 2006) and heating (Renaud *et al.* 2002). This is achieved by diverse protection mechanisms including extracellular polysaccharide layers (Potts 1999; Tamaru *et al.* 2005), thick cell walls (Evans 1958, 1959) and morphologically distinct resting stages (Evans 1958, 1959; Paerl 1988; Hoffmann 1996). For terrestrial diatoms however, data on their tolerance to temperature extremes and drought are scarce. Freezing tolerance has been reported for a single terrestrial species (Hostetter & Hoshaw 1970), and two desiccation experiments on soil samples from temporary ponds revealed both species-specific survival and a decrease in the number of surviving diatom species with decreasing moisture content (Evans 1958, 1959; Hostetter & Hoshaw 1970). In planktonic freshwater diatoms from temperate climates, lethal heating temperatures range from +30°C to +40°C (Suzuki & Takahashi 1995; Butterwick *et al.* 2005).

Stress protection mechanisms in terrestrial diatoms are poorly known. Both the accumulation of oil in the cytoplasm and vertical movements in the sediment layer were observed in benthic diatoms from drying ponds (Evans 1958, 1959). Morphologically discernable resting spores are commonly formed in marine centric species during nutrient depletion and are made to survive cold and dark winter conditions (reviewed in Hargraves & French 1983; McQuoid & Hobson 1996), but only a single experiment gave evidence for a partly enhanced tolerance for desiccation (Hargraves & French 1975). In benthic

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freshwater diatom species resting spores have thus far been observed in only seven species and are all associated with the formation of internal valves (Geitler 1953, 1971; Schmid 1979; von Stosch & Fecher 1979). Interestingly one of these species, *Craticula cuspidata* (Kützing) D.G. Mann, forms resting stages in case of desiccation and recovers after rehydration (Schmid 2009). However, most freshwater species are not known to form similar morphologically distinct spores, and there is no evidence yet that soil diatom communities bridge harsh environmental conditions in this way. Another possibility is the formation of physiological resting cells (McQuoid & Hobson 1996), but no information on their stress tolerance is available.

Apart from the importance of stress tolerance for local population persistence, aerial dispersal success also depends on tolerance for stress factors such as desiccation, UV irradiance and abrupt temperature changes (Isard & Gage 2001; Figuerola & Green 2002; Sharma *et al.* 2007). Of various dispersal mechanisms (reviewed by Kristiansen 1996), waterfowl is considered to be the most important vector for aquatic microalgae (Schlichting 1960; Kristiansen 1996; Figuerola & Green 2002). Survival of the attached cells is limited mainly by desiccation (Figuerola & Green 2002). Schlichting (1960) noted that most of the green algae externally transported on birds formed protection mechanisms such as slime sheets, resting spores or cysts. The dominance of terrestrial cyanobacteria and green algae found in air traps (Van Overeem 1937; Schlichting 1961, 1964; Brown *et al.* 1964; Roy-Ocotla & Carrera 1993; Marshall & Chalmers 1997) suggests that terrestrial microalgae are more easily transported by air currents (Schlichting 1961; Brown *et al.* 1964; Marshall & Chalmers 1997). In both waterfowl- and wind-mediated dispersal pathways, living diatoms are rarely encountered. Although this may be explained by their lower abundance in terrestrial habitats compared to cyanobacteria and green algae (Davey & Clarke 1991; Zancan *et al.* 2006), this could also be due to a lower tolerance of diatoms for the adverse environmental conditions encountered during transport. Regardless, differences in stress tolerance between algal groups or between algae from different habitats [e.g. aquatic vs terrestrial habitats, as seen in green algae (Gray *et al.* 2007)] may result in differential dispersal rates and therefore pronounced differences in population structure and the geographical distribution of species.

A better understanding of the stress tolerance and associated dispersal abilities of diatoms could thus provide insight on their biogeography. Because of undersampling and controversy regarding taxonomic resolution and species delineation, the biogeographical distribution patterns of diatom species remain poorly known (Vanormelingen *et al.* 2008b). In contrast to the hypothesis that diatoms are dispersed on a broad spatial scale (Finlay *et al.* 2002), recent analyses on lacustrine diatoms have demonstrated the importance of historical factors such as isolation (and thus dispersal limitation) for diversity patterns (Telford *et al.* 2006; Vyverman *et al.* 2007). This is also supported by reports of endemism in some regions (Vanormelingen *et al.* 2008b). One of the possible mechanisms behind these restricted distribution patterns is the supposed sensitivity of diatoms to stress factors associated with dispersal. Furthermore, a different tolerance level between terrestrial and aquatic diatoms might have repercussions for their distribution patterns.

In this experimental study, we broaden the knowledge on the stress tolerance of diatoms by investigating in culture experiments (1) to what extent vegetative cells of terrestrial and benthic freshwater diatoms tolerate desiccation and extreme temperatures and (2) whether terrestrial diatoms survive these harsh conditions better than aquatic taxa.

MATERIAL AND METHODS

Sampling

Samples of dry, moist and aquatic sediments were taken at five different locations in Belgium: 'Campus Sterre' (St) in Gent, nature reserve 'De Westhoek' (Wes) in De Panne, nature reserve 'Ter Yde' (Yde) in Oostduinkerke, nature reserve 'De Fonteintjes' (Fon) in Blankenberge and lake 'Kraenepoel' (Kra) in Aalter. St is situated in the centre of Belgium where the soil consists of sandy loam; Wes, Yde and Fon are situated at the Belgian coast and have a sandy soil; and Kra is located slightly further inland and is characterized by a humus-rich sandy soil (Geo-Vlaanderen 2001). Table 1 gives an overview of the different samples from each location with the geographical coordinates taken by GPS and habitat, sediment and vegetation characteristics. The St samples were taken in autumn on 21 November 2007; all other samples on 3 March 2008.

Cultures

A total of 20 samples from soils (15) and ponds (5) was incubated on the day of sampling in liquid WC-medium (Guillard & Lorenzen 1972) in standard conditions of 18°C $\pm$ 2°C, 25–30 µmol ph m^{-2} s^{-1} light intensity and a 12:12-h light:dark period. After 1 wk of incubation, single diatom cells were isolated by micropipette and grown as monoclonal cultures (strains) at the above conditions. We isolated diatom cells from all different sizes and shapes present in the sample, in an attempt to culture all species present, without *a priori* species selection. Where feasible, at least two clones were isolated per species. Cultures were reinoculated in fresh medium when they reached late exponential phase. For morphological analysis, cultures were oxidized in hydrogen peroxide and embedded in Naphrax®. Pictures were taken using a Zeiss Axioplan 2 microscope equipped with an AxioCam Mrm camera. Valve length, width and stria density of 10 valves per strain were measured using ImageJ 1.37v software. Identifications were based on Lange-Bertalot & Krammer (1986, 1988, 1991a, b) and Krammer (2000) for the genus *Pinnularia* Ehrenberg, but the most recent nomenclature was used. For *Hantzschia amphioxys* (Ehrenberg) Grunow, *Rhopalodia gibba* (Ehrenberg) O. Müller and *Cymbella subaequalis* Grunow, clear discontinuities in valve morphology (width, striae density, overall shape) were observed between the isolates. As it has become clear recently that subtle, discontinuous variations in morphology in diatom morphospecies are generally correlated with differences in reproductive, molecular, physiological and ecological char-

Table 1. Characteristics of the environmental samples from which the tested diatom strains were isolated.

Location	Sample	Geographical coordinates	Type[1]	Habitat description	Sediment type	Vegetation type and cover
Sterre	St 1	51°01′28.0″N 03°42′45.2″E	T	soil-filled fissure between two concrete slabs, moist	sandy-silt with humus	moss 5%
Sterre	St 2	51°01′33.3″N 03°42′52.8″E	T	wet car track in meadow with grasses and *Betulus*	silt	none
Sterre	St 3	51°01′38.6″N 03°42′58.4″E	T	bare soil under *Quercus* in meadow, moist	sandy-silt with humus	moss 5%
Sterre	St 4	51°01′40.3″N 03°43′04.6″E	T	bare soil under *Chamaecypares*, moist	sandy-silt with humus	microbial biofilm
Sterre	St 5	51°01′44.5″N 03°43′06.2″E	T	bare soil under *Taxus*, moist	sandy	microbial biofilm, moss 3%
Sterre	St 6	51°01′30.8″N 03°42′55.2″E	T	concrete pavement with biofilm, moist	sandy-silt with humus	moss 5%
Sterre	St 8	51°01′26.9″N 03°42′50.2″E	T	grooves between pavement tiles, moist	sandy-silt	moss 5%
Westhoek	Wes 1	51°05′49.9″N 02°34′26.7″E	T	dry sand and moss on dune	sand	moss 100%
Westhoek	Wes 2	51°04′96.8″N 02°34′43.4″E	T	humus-rich moist soil in dune shrub (*Hippophaea rhamnoides*)	humus-rich sand	*Claytonia perfoliata* 75%
Westhoek	Wes 3	51°05′33.7″N 02°34′65.3″E	T	dry sand and moss on dune	sand	moss 30%
Westhoek	Wes 4	51°05′28.6″N 02°34′59.2″E	A	sandy sediment of shallow lake in dune slack, aquatic 200 m^2	sand	*Phragmites australis* 10%
Westhoek	Wes 5	51°05′27.2″N 02°34′57.3″E	T	wet sand with biofilm in dune slack	sand + biofilm	microbial biofilm, moss 5%, *Carex arenaria* 10%
Westhoek	Wes 6	51°05′43.9″N 02°34′37.5″E	A	sandy sediment of temporary puddle in dune slack, aquatic 40 m^2	sand + fine organic material	*Chara* 5%
Westhoek	Wes 7	51°05′44.4″N 02°34′38.3″E	T	wet sand in dune slack with biofilm	sand	*C. arenaria* 10%
Ter Yde	Yde 1	51°08′18.4″N 02°41′86.1″E	T	moist sample of moss from moss-covered plain in dune slack	none (small amount of sand)	moss 100%
Ter Yde	Yde 2	51°08′18.4″N 02°41′86.1″E	T	moist till wet sample of humid sand from moss-covered plain in dune slack	sand	moss 50%
Ter Yde	Yde 3	51°08′18.4″N 02°41′86.1″E	T	wet sample of wet sand from moss-covered plain in dune slack	sand	moss 30% (none in depression)
Fonteintjes	Fon 1	51°19′31.7″N 03°09′38.7″E	A	sediment sample of fishpond, aquatic 100 m^2	sand + organic material	*Chara*, *Phragmites*, *Ceratophyllum*; total 20%
Fonteintjes	Fon 3	51°19′50.1″N 03°09′93.4″E	A	sediment sample of puddle in meadow, aquatic 5 m^2	sand + organic material	moss, *Carex*, *Luzula*; total 75%
Kraenepoel	Kra 1	51°04′53.8″N 03°28′93.9″E	A	sediment sample of lake, aquatic	organic material, little sand	*Phragmites*, *Luzula*, submersed macrophytes

[1] T = terrestrial samples, A = aquatic samples.

acters (e.g. Mann 1999; Behnke *et al.* 2004; Lundholm *et al.* 2006; Vanormelingen *et al.* 2008a), these isolates were divided in different morphotypes (Table 2) and treated as separate species. Their taxonomy needs further study however. Voucher slides of all natural samples and cultures are kept in the Laboratory of Protistology and Aquatic Ecology and are available upon request. Sixty-nine strains from aquatic or terrestrial habitat were selected for the stress tolerance experiments. Pictures of oxidized material from the different species taken using light microscopy are presented in Figs 1–35.

Experimental setup

Seven different treatments were carried out in triplicate for each strain: (1) control (standard growth conditions), (2) gradual heating to +30°C, (3) gradual heating to +40°C, (4) abrupt heating to +40°C, (5) freezing at −20°C, (6) desiccation during 10 min, and (7) preconditioning by gradual heating to +30°C followed by desiccation during 10 min (Table 3). The temperatures 30°C and 40°C were chosen because the lethal temperature for most diatoms is situated between these two values (Suzuki & Takahashi 1995; Butterwick *et al.* 2005). Gradual heating and preconditioning were applied because acclimatization increases the tolerance to unfavourable conditions in various organisms (e.g. Bayley *et al.* 2001; Bierkens *et al.* 1998; Sung *et al.* 2003; Dunlap *et al.* 2007), including diatoms (after 8 h; Rousch *et al.* 2004). Because periods of dryness coincide with elevated temperatures, we chose to precondition the cells to desiccation by gradual heating.

Table 2. Overview of the examined diatom strains with morphometric features and results of the stress tolerance treatments.

Habitat [1]	Taxon [2]	Origin	Length [3] (µm)	Width [3] (µm)	# striae [3] / 10 µm	Minimal – maximal % of viable cells after treatment [4]						
						Control	+ 30°C	+ 40°C gradual	+ 40°C abrupt	- 20°C	desic. 10 min.	+ 30°C + desic.
T	*Achnanthes coarctata* (Brébisson) Grunow	(Wes3)f	28.7 ± 1.2	7.0 ± 0.5	16.4 ± 1.1	98 – 99	94 – 98	65 – 70	60 – 74	0	0	0
T	*Achnanthidium minutissimum* (Kützing) Czarnecki	(Yde2)d	10.4 ± 0.3	3.0 ± 0.0	28.0 ± 1.2	89 – 93	80 – 90	39 – 48	0	0	0	0
T	*Caloneis molaris* (Grunow) Krammer	(Yde2)a	36.0 ± 0.6	7.3 ± 0.2	20.4 ± 0.5	84 – 93	65 – 79	83 – 91	72 – 87	0	0	0
T	*Caloneis molaris* (Grunow) Krammer	(Yde2)b	35.0 ± 0.8	7.5 ± 0.2	21.2 ± 0.4	88 – 94	65 – 79	54 – 71	72 – 87	0	0	0
T	*Craticula* cf. *halophila* (Grunow in Van Heurck) D.G. Mann	(Wes7)b	35.5 ± 0.7	8.2 ± 0.4	18.0 ± 0.0	97 – 99	96 – 99	65 – 69	64 – 72	0	0	0
T	*Cymbella subaequalis* Grunow morph.1	(Yde2)e	24.9 ± 1.2	7.8 ± 0.3	13.4 ± 0.9	91 – 95	94 – 98	2 – 7	0	0	0	0
T	*Hantzschia amphioxys* (Ehrenberg) Grunow morph.1	(St1)e	42.9 ± 0.4	6.2 ± 0.1	25.2 ± 0.9	98 – 99	99	85 – 88	91 – 97	0	0	0
T	*Hantzschia amphioxys* (Ehrenberg) Grunow morph.1	(St3)a	36.8 ± 0.5	6.6 ± 0.5	23.7 ± 1.0	87 – 94	86 – 91	89 – 93	85 – 90	0	0	0
T	*Hantzschia amphioxys* (Ehrenberg) Grunow morph.1	(St3)c	42.8 ± 0.6	6.4 ± 0.2	24.7 ± 1.1	98 – 99	98	98 – 99	91 – 95	0	0	0
T	*Hantzschia amphioxys* (Ehrenberg) Grunow morph.1	(St4)a	36.0 ± 0.6	6.0 ± 0.4	25.8 ± 0.4	71 – 76	74 – 82	73 – 77	79 – 86	0	0	0
T	*Hantzschia amphioxys* (Ehrenberg) Grunow morph.1	(St4)b	38.1 ± 0.8	6.0 ± 0.3	24.1 ± 0.9	93 – 96	82 – 92	94 – 95	1	0	0	0
T	*Hantzschia amphioxys* (Ehrenberg) Grunow morph.1	(St5)a	40.5 ± 0.5	5.6 ± 0.2	24.0 ± 0.0	98 – 99	98 – 99	99	97 – 98	0	0	0
T	*Hantzschia amphioxys* (Ehrenberg) Grunow morph.1	(Wes2)a	39.5 ± 0.7	6.0 ± 0.3	24.0 ± 0.7	73 – 79	66 – 72	43 – 72	66 – 68	0	0	0
T	*Hantzschia amphioxys* (Ehrenberg) Grunow morph.1	(Wes2)b	40.4 ± 0.9	6.2 ± 0.2	24.6 ± 0.9	96 – 98	96 – 97	96 – 98	91 – 95	0	0	0
T	*Hantzschia amphioxys* (Ehrenberg) Grunow morph.2	(St1)f	48.3 ± 0.9	9.0 ± 0.4	21.6 ± 0.7	97 – 98	97 – 98	47 – 64	41 – 45	0	0	0
T	*Hantzschia amphioxys* (Ehrenberg) Grunow morph.2	(St8)c	46.8 ± 0.7	8.2 ± 0.3	20.3 ± 1.3	62 – 80	55 – 62	85 – 88	30 – 49	0 – 3	0	0
T	*Hantzschia amphioxys* (Ehrenberg) Grunow morph.3	(St6)e	52.3 ± 0.5	7.2 ± 0.4	19.7 ± 0.7	85 – 89	81 – 88	80 – 88	6 – 25	0	0	0
T	*Hantzschia amphioxys* (Ehrenberg) Grunow morph.3	(St6)f	52.3 ± 0.4	7.3 ± 0.4	18.8 ± 1.0	99 – 100	93 – 98	97 – 99	16 – 40	0	0	0
T	*Hantzschia amphioxys* (Ehrenberg) Grunow morph.4	(Wes1)a	56.6 ± 0.8	7.2 ± 0.4	20.0 ± 0.0	89 – 92	81 – 83	5 – 44	1 – 3	0	0	0
T	*Hantzschia amphioxys* (Ehrenberg) Grunow morph.4	(Wes1)b	56.1 ± 0.2	7.1 ± 0.6	20.4 ± 0.5	97 – 98	91 – 96	93 – 96	85 – 90	11 – 64	0 – 5 [6]	0

Table 2. Continued.

Habitat [1]	Taxon [2]	Origin	Length [3] (µm)	Width [3] (µm)	# striae [3] / 10 µm	Minimal – maximal % of viable cells after treatment [4]						
						Control	+ 30°C	+ 40°C gradual	+ 40°C abrupt	- 20°C	desic. 10 min.	+ 30°C + desic.
T	*Hantzschia amphioxys* (Ehrenberg) Grunow morph.4	(Wes3)a	52.6 ± 0.7	7.3 ± 0.4	20.0 ± 0.0	98 – 99	100	98 – 99	76 – 78	0 – 1 [6]	0	0
T	*Hantzschia amphioxys* (Ehrenberg) Grunow morph.4	(Wes3)b	51.8 ± 0.2	7.3 ± 0.3	19.6 ± 0.9	99 – 100	97 – 99	88 – 98	20 – 46	0 – 2 [6]	0	0
T	*Mayamaea atomus* (Kützing) Lange-Bertalot var. *permitis* (Hustedt) Lange-Bertalot	(Wes2)e	8.6 ± 0.3	3.8 ± 0.2	20.4 ± 0.9	100	100	49 – 85	51 – 76	2 – 39	0	0
T	*Mayamaea atomus* (Kützing) Lange-Bertalot var. *permitis* (Hustedt) Lange-Bertalot	(Wes2)f	8.7 ± 0.1	3.9 ± 0.2	22.4 ± 1.9	100	100	31 – 43	19 – 54	10 – 18	0	0
T	*Navicula cryptotenella* Lange-Bertalot	(Wes5)c	22.6 ± 0.4	5.4 ± 0.2	15.0 ± 0.7	82 – 87	77 – 81	75 – 80	74 – 80	0	0	0
T	*Navicula libonensis* Schoeman	(Wes3)e	32.3 ± 0.4	6.6 ± 0.2	13.0 ± 0.0	97 – 98	92 – 93	89 – 91	88 – 92	0	0	0
T	*Navicula radiosa* Kützing	(Wes3)d	45.0 ± 1.1	9.6 ± 0.2	11.3 ± 0.4	100	99 – 100	0	0	0	0	0
T	*Navicula radiosa* Kutzing	(Wes5)a	41.5 ± 1.5	9.7 ± 0.2	12.0 ± 0.0	98 – 99	96 – 97	0	0	0	0	0
T	*Navicula radiosa* Kützing	(Wes5)b	43.0 ± 1.6	9.8 ± 0.2	11.7 ± 0.4	96 – 97	95 – 97	0	0	0	0	0 – 84 [6]
T	*Navicula veneta* Kützing	(Yde3)f	22.2 ± 0.2	22.2 ± 0.2	15.6 ± 0.5	79 – 80	71 – 85	69 – 88	76 – 80	0	0	0
T	*Navicula* cf. *wiesneri* Lange-Bertalot	(Yde1)h	16.2 ± 0.4	4.9 ± 0.1	13.0 ± 0.7	90 – 92	81 – 96	31 – 59	6 – 24	0	0	0
T	*Nitzschia austriaca* Hustedt	(Yde1)c	18.0 ± 0.5	2.9 ± 0.1	28.0 ± 0.7	64 – 73	73 – 78	72 – 78	79 – 85	0	0	0
T	*Nitzschia palea* (Kützing) W. Smith	(Wes7)e	44.0 ± 0.4	4.3 ± 0.5	invisible [5]	91 – 95	92 – 94	71 – 82	86 – 88	0	0	0
T	*Nitzschia perminutum* (Grunow) M. Peragallo	(St8)e	32.7 ± 0.7	2.9 ± 0.2	29.7 ± 0.5	74 – 85	64 – 74	62 – 86	66 – 80	0	0	0
T	*Pinnularia borealis* Ehrenberg	(St1)b	39.6 ± 1.2	7.6 ± 0.3	6.2 ± 0.4	65 – 82	74 – 77	48 – 63	63 – 73	14 – 23	0	0
T	*Pinnularia borealis* Ehrenberg	(St6)c	37.1 ± 0.8	8.7 ± 0.5	6.3 ± 0.4	67 – 75	77 – 82	62 – 70	62 – 70	2 – 5	0	0
T	*Pinnularia borealis* Ehrenberg	(St6)g	28.5 ± 1.4	6.9 ± 0.3	6.6 ± 0.6	81 – 85	85 – 88	91 – 93	86 – 90	3 – 12	0	0

Table 2. Continued.

Habitat [1]	Taxon [2]	Origin	Length [3] (µm)	Width [3] (µm)	# striae [3] / 10 µm	Minimal – maximal % of viable cells after treatment [4]						
						Control	+ 30°C	+ 40°C gradual	+ 40°C abrupt	- 20°C	desic. 10 min.	+ 30°C + desic.
T	*Pinnularia borealis* Ehrenberg	(St8)a	38.6 ± 1.1	7.4 ± 0.3	6.2 ± 0.4	80 – 85	85 – 92	81 – 87	68 – 82	15 – 21	0	0 – 10 [6]
T	*Pinnularia borealis* Ehrenberg	(St8)d	42.4 ± 0.6	8.9 ± 0.4	5.0 ± 0.0	76 – 86	80 – 86	89 – 91	67 – 71	22 – 47	0	0
T	*Pinnularia borealis* Ehrenberg	(St1)d	40.9 ± 0.6	8.1 ± 0.3	6.0 ± 0.2	56 – 75	45 – 48	22 – 31	33 – 47	0	0	0
T	*Pinnularia borealis* Ehrenberg	(St1)i	35.9 ± 0.6	8.2 ± 0.3	5.8 ± 0.4	45 – 52	20 – 37	1 – 8	1 – 12	0	0	0
T	*Pinnularia borealis* Ehrenberg	(St1)k	33.7 ± 0.8	7.6 ± 0.3	6.0 ± 0.0	46 – 55	26 – 28	1 – 20	2 – 8	0	0 – 10 [6]	0
T	*Pinnularia borealis* Ehrenberg	(St5)c	40.7 ± 0.6	7.9 ± 0.5	6.0 ± 0.0	65 – 84	56 – 68	64 – 74	47 – 65	0	0	0
T	*Pinnularia borealis* Ehrenberg	(St6)a	34.6 ± 0.7	7.2 ± 0.4	6.7 ± 0.5	87 – 90	84 – 88	81 – 82	62 – 70	0	0	0
T	*Pinnularia divergentissima* (Grunow) Cleve	(St5)b	23.0 ± 0.7	3.6 ± 0.2	14.1 ± 0.7	89 – 93	62 – 81	1 – 3	0 – 1 [6]	0	0	0
T	*Pinnularia isselana* Krammer	(St2)d	27.1 ± 1.7	7.1 ± 0.4	12.8 ± 0.4	90 – 92	88 – 89	85 – 94	89 – 91	0	0	0
T	*Pinnularia marchica* Ilka Schönfelder	(St2)a	29.0 ± 0.6	5.4 ± 0.3	14.5 ± 0.5	94 – 98	91 – 98	93 – 97	96 – 96	0	0	0
T	*Pinnularia obscura* Krasske	(Wes2)c	18.0 ± 0.4	4.5 ± 0.3	13.2 ± 0.8	46 – 63	100	53 – 66	5 – 32	0	0	0
T	*Pinnularia subcommutata* Krammer var. *nonfasciata* Krammer	(Yde1)a	62.3 ± 2.0	11.4 ± 0.4	10.1 ± 0.2	97 – 98	98 – 99	94 – 98	44 – 60	0	0	0
T	*Pinnularia subcommutata* Krammer var. *nonfasciata* Krammer	(Yde1)f	65.8 ± 0.7	10.9 ± 0.3	10.3 ± 0.3	98 – 99	96 – 98	15 – 38	3 – 16	0	0	0
T	*Rhopalodia gibba* (Ehrenberg) O. Müller morph.1	(Yde1)d	41.4 ± 0.7	8.1 ± 0.6	7.5 ± 0.7 [7]	87 – 92	90 – 92	21 – 33	34 – 61	0	0	0
T	*Rhopalodia gibba* (Ehrenberg) O. Müller morph.1	(Yde1)e	42.2 ± 0.8	8.4 ± 0.5	7.6 ± 0.5 [7]	97 – 98	98 – 99	63 – 94	78 – 83	0	0	0
A	*Amphora pediculus* (Kützing) Grunow	(Fon1)d	16.4 ± 1.7	3.3 ± 0.2	20.2 ± 0.4	95 – 98	96 – 99	0	0	0	0	0
A	*Cymbella subaequalis* Grunow morph.2	(Wes6)c	24.3 ± 0.9	6.4 ± 0.2	13.7 ± 0.4	85 – 91	86 – 91	0	0	0	0	0
A	*Eunotia bilunaris* (Ehrenberg) Schaarschmidt	(Fon3)g	56.3 ± 1.6	4.3 ± 0.5	15.4 ± 0.9	70 – 75	28 – 42	49 – 63	15 – 40	0	0	0
A	*Eunotia bilunaris* (Ehrenberg) Schaarschmidt	(Fon3)i	56.3 ± 1.4	4.5 ± 0.3	16.0 ± 1.6	79 – 82	45 – 66	40 – 61	30 – 45	0	0	0
A	*Eunotia implicata* Nörpel *et al.*	(Kra1)b	26.7 ± 0.6	4.4 ± 0.2	14.4 ± 0.9	96 – 97	89 – 97	87 – 90	46 – 67	0	0	0

Table 2. Continued.

Habitat [1]	Taxon [2]	Origin	Length [3] (µm)	Width [3] (µm)	# striae [3] / 10 µm	Minimal – maximal % of viable cells after treatment [4]						
						Control	+ 30°C	+ 40°C gradual	+ 40°C abrupt	- 20°C	desic. 10 min.	+ 30°C + desic.
A	*Eunotia implicata* Nörpel *et al.*	(Kra1)c	25.5 ± 0.9	4.2 ± 0.3	15.0 ± 1.0	96 – 98	90 – 95	86 – 95	58 – 65	0	0	0
A	*Navicula libonensis* Schoeman	(Wes4)e	32.1 ± 0.1	6.6 ± 0.1	13.0 ± 0.0	88 – 91	84 – 91	84 – 90	86 – 92	0	0	0
A	*Navicula radiosa* Kützing	(Wes4)a	68.9 ± 0.5	10.9 ± 0.2	11.1 ± 0.2	88 – 91	80 – 90	0	0	0	0	0
A	*Navicula radiosa* Kützing	(Wes4)d	44.4 ± 0.7	9.5 ± 0.1	11.8 ± 0.4	94 – 95	92 – 98	0	0	0	0	0
A	*Navicula radiosa* Kützing	(Fon3)e	64.3 ± 1.4	10.3 ± 0.4	11.0 ± 0.6	92 – 96	85 – 91	0	0	0	0 – 74 [6]	0
A	*Navicula radiosa* Kützing	(Fon3)f	66.1 ± 1.0	10.3 ± 0.3	10.9 ± 0.7	91 – 93	85 – 91	0	0	0	0	0
A	*Navicula radiosa* Kützing	(Kra1)a	50.3 ± 0.9	10.3 ± 0.1	11.0 ± 0.0	99 – 100	98	0	0	0	0	0
A	*Navicula veneta* Kützing	(Wes6)d	21.5 ± 0.4	5.7 ± 0.1	16.0 ± 0.0	99	99	98	97 – 97	0	0	0
A	*Pinnularia viridiformis* Krammer	(Fon3)a	86.4 ± 2.1	18.6 ± 0.7	8.9 ± 0.2	77 – 84	65 – 74	81 – 90	59 – 80	0	0	0
A	*Rhopalodia gibba* (Ehrenberg) O. Müller morph.2	(Fon1)a	53.9 ± 0.9	9.1 ± 0.3	8.8 ± 0.8 [7]	71 – 77	69 – 80	0	0	0	0	0
A	*Rhopalodia gibba* (Ehrenberg) O. Müller morph.2	(Fon1)b	52.0 ± 1.6	9.1 ± 0.4	8.6 ± 0.5 [7]	90 – 92	85 – 93	0	0	0	0	0

[1] A stands for aquatic, T for terrestrial habitat of origin.

[2] Morph. means morphotype.

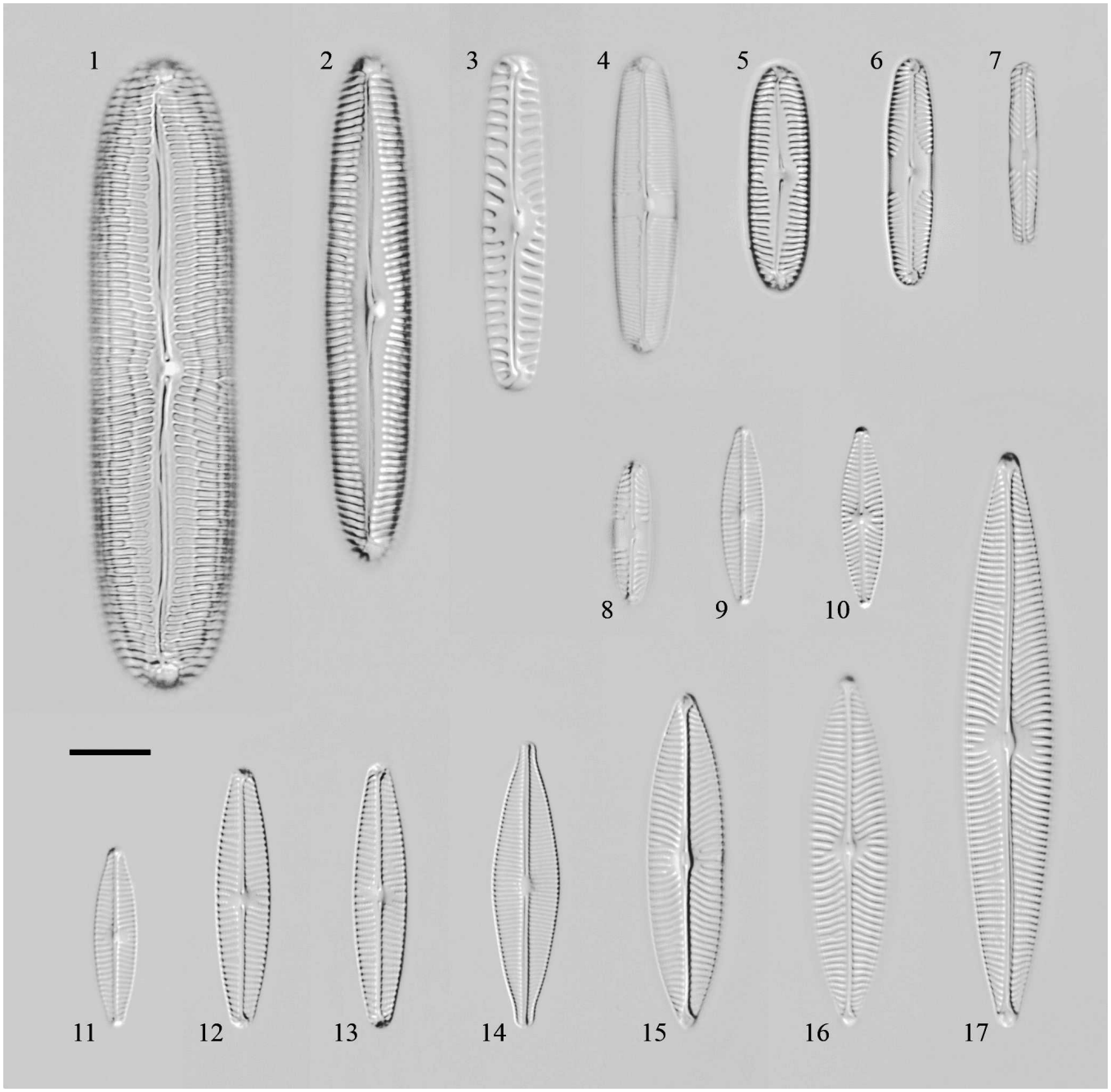
[3] Values are given as average ± standard deviation, N=10.

[4] The range of %VC is given based on triplicate treatments. Cases are shaded in function of the minimum %VC: minimum 0% (no shading), 1-24% (10% shading), 25-49% (25% shading), 50-74% (50% shading) and 75-100% (75% shading). Desic. means desiccation.

[5] Invisible in LM.

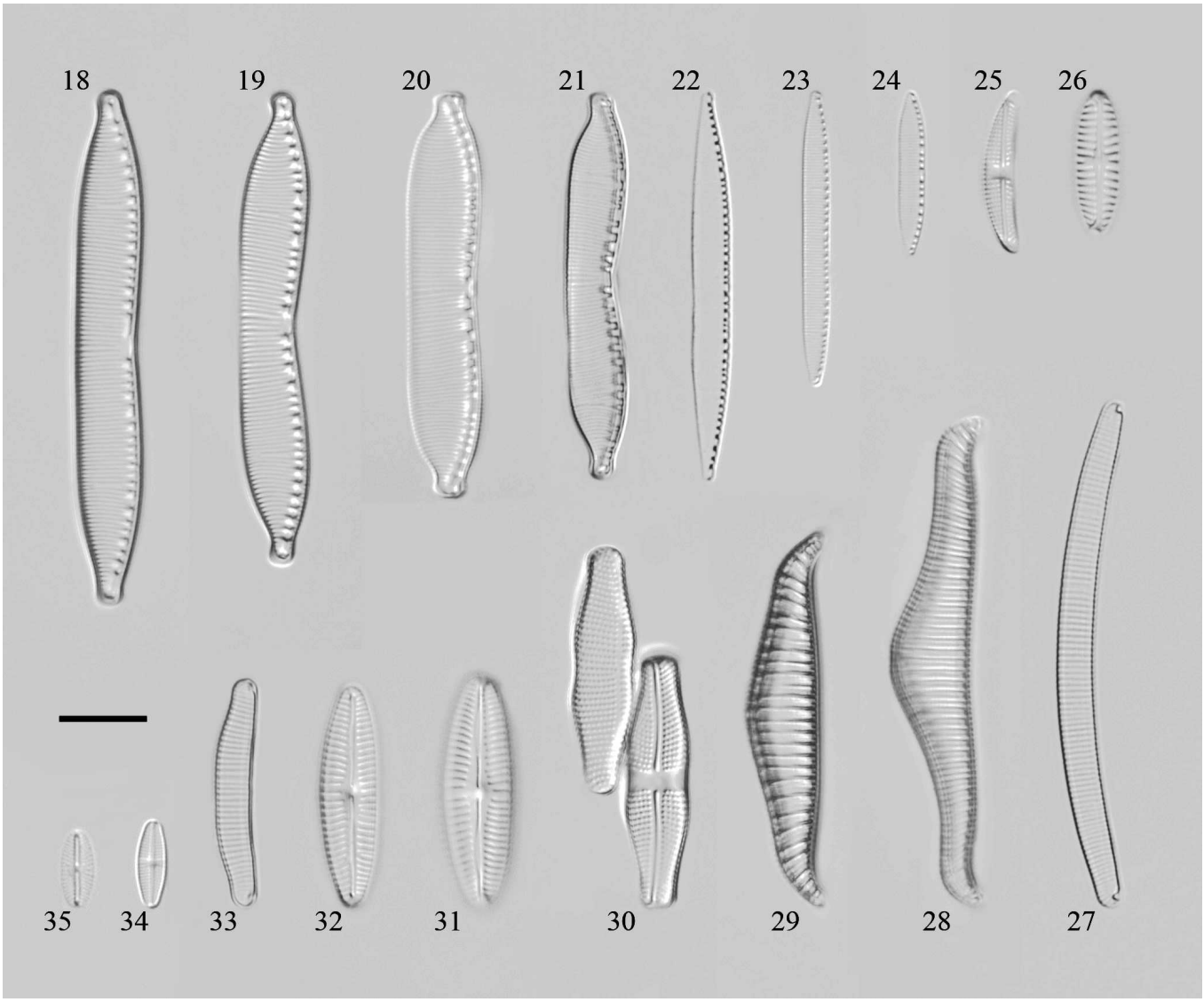
[6] Growth observed in a single replicate.

[7] Number of fibulae.



Figs 1–17. LM pictures from oxidized material of a selection of the strains used in the experiments with the corresponding voucher slide identification and habitat type. Scale bar = 10 μm. A = aquatic habitat types of origin, T = terrestrial habitat types.

Fig. 1. *Pinnularia viridiformis* (Fon3)a; A.
Fig. 2. *Pinnularia subcommutata* var. *nonfasciata* (Yde1)a; T.
Fig. 3. *Pinnularia borealis* (St1)b; T.
Fig. 4. *Caloneis molaris* (Yde2)a; T.
Fig. 5. *Pinnularia isselana* (St2)d; T.
Fig. 6. *Pinnularia marchica* (St2)a; T.
Fig. 7. *Pinnularia divergentissima* (St5)b; T.
Fig. 8. *Pinnularia obscura* (Wes2)c; T.
Fig. 9. *Navicula veneta* (Wes6)c; A.
Fig. 10. *Navicula cryptotenella* (Wes5)c; T.
Fig. 11. *Navicula veneta* (Yde3)f; T.
Fig. 12. *Navicula libonensis* (Wes3)e; T.
Fig. 13. *Navicula libonensis* (Wes4)e; A.
Fig. 14. *Craticula* cf. *halophila* (Wes7)b; T.
Fig. 15. *Navicula radiosa* (Wes5)a; T.
Fig. 16. *Navicula radiosa* (Wes3)d; T.
Fig. 17. *Navicula radiosa* (Wes4)a; A.



Figs 18–35. LM pictures from oxidized material of a selection of the strains used in the experiments with the corresponding voucher slide identification and habitat type. Scale bar = 10 μm. A = aquatic habitat types of origin, T = terrestrial habitat types.

Fig. 18. *Hantzschia amphioxys* morphotype 4 (Wes1)a; T.
Fig. 19. *Hantzschia amphioxys* morphotype 3 (St6)f; T.
Fig. 20. *Hantzschia amphioxys* morphotype 2 (St8)c; T.
Fig. 21. *Hantzschia amphioxys* morphotype 1 (St1)e; T.
Fig. 22. *Nitzschia palea* (Wes7)e; T.
Fig. 23. *Nitzschia perminutum* (St8)e; T.
Fig. 24. *Nitzschia austriaca* (Yde1)c; T.
Fig. 25. *Amphora pediculus* (Fon1)d; A.
Fig. 26. *Navicula* cf. *wiesneri* (Yde1)h; T.
Fig. 27. *Eunotia bilunaris* (Fon3)i; A.
Fig. 28. *Rhopalodia gibba* morphotype 2 (Fon1)a; A.
Fig. 29. *Rhopalodia gibba* morphotype 1 (Yde1)e; T.
Fig. 30. *Achnanthes coarctata* (Wes3)f; T.
Fig. 31. *Cymbella subaequalis* morphotype 1 (Yde2)e; T.
Fig. 32. *Cymbella subaequalis* morphotype 2 (Wes6)c; A.
Fig. 33. *Eunotia implicata* (Kra1)c; A.
Fig. 34. *Achnanthidium minutissimum*(Yde2)d; T.
Fig. 35. *Mayamaea atomus* var. *permitis* (Wes2)e; T.

The experiments were conducted in 24-well plates. A separate well-plate was used for each of the seven treatments, and for every treatment three wells per strain were used as replicates. Not all strains could be tested at the same moment. Every day six or seven randomly chosen strains were subjected to the seven treatments. Each well was filled with 1.5 ml of fresh WC medium, and cells from exponentially growing cultures were inoculated in the

Table 3. Overview of the protocols of the different stress tolerance treatments. Dark conditions occurred at the same temperatures as stated in the middle column, except if further specified between parentheses.

Treatment	Temperature treatment (duration)	Duration of dark condition
Control	18°C	15 h + 3 h
+30°C gradual	25°C (15 h) + 28°C (1 h 30 min) + 30°C (2 h)	15 h + 1 h 30 min + 2 h
+40°C gradual	25°C (15 h) + 28°C (1 h 30 min) + 30°C (2 h) + 40°C (3 h 15 min)	15 h + 1 h 30 min + 2 h + 3 h 15 min
+40°C abrupt	40°C (3 h 15 min)	15 h (18°C) + 3 h 15 min (40°C)
Freezing −20°C	−20°C (4 h 45 min)	15 h (18°C) + 4 h 45 min (−20°C)
Desiccation 10 min	air-dry desiccation (10 min)	15 h (18°C) + 3 h (18°C) + 10 min
+30°C + desiccation 10 min	25°C (15 h) + 28°C (1 h 30 min) + 30°C (2 h) + desiccation (0 h 10 min)	15 h + 1 h 30 min + 2 h + 10 min

experimental wells 3 d before the treatment. We used different starting densities for the different species (ranging from around 120 to 185,000 cells per well) because cell densities varied widely between species due to differences in cell size (valve lengths ranging from 86.4 μm to 8.6 μm).

The control treatment was placed in the dark at 18°C for 15 h overnight plus an additional 3 h to approximate the length of stress treatments. The four heating treatments were conducted in a temperature-controlled incubator, and air temperature was verified with a mercury thermometer. Details of exposure time to the different temperatures are listed in Table 3. For the freezing treatment, the plates were placed in a freezer at −20°C for 4 h 45 min. For the desiccation treatment, the culture medium was removed with a Pasteur pipette, after which each well was carefully observed with a binocular until the whole film of water surrounding the cells and in the edge of the well had evaporated. After an additional 10 min, 1.5 ml of fresh WC medium were added. Preconditioning before desiccation was achieved by gradual heating of the plates to +30°C for 18 h 30 min (Table 3). All treatments were conducted in the dark to avoid photo-oxidative stress. After treatment, the plates were returned to standard conditions.

Immediately after treatment (t_0) an average of 200 cells per well was counted using an Axiovert inverted microscope and densities (number of cells per mm^2) were calculated. At t_0, total cell densities were determined. After 14 d (t_{14}) separate cell counts were performed of (1) dead cells (shrivelled and colourless cell content or empty frustule) and (2) all cells (dead and alive together). Again, we counted an average of 200 cells per well in both t_{14} cell counts. However, if the number of dead cells was too low for this, we counted the exact number of dead cells present in the entire well. An increase in the total cell density in a well between t_0 and t_{14} validated 'growth' and thus survival of the replicate. A strain was considered 'tolerant' for a treatment if at least two out of three replicates showed positive growth. A species was designated as 'tolerant' for a treatment if at least one of its strains was considered tolerant. The percentage of viable cells (uncorrected %VC) equalled $100 \times [1 - (\text{number of dead cells per mm}^2 \text{ at } t_{14} / \text{total number of cells per mm}^2 \text{ at } t_0)]$. In an attempt to correct for possible effects of differences between strains in 'background mortality' (i.e. % of dead cells in control conditions) on the stress treatment mortality (which is a combination of background mortality and the stress mortality), we calculated for each strain a 'corrected %VC' by adding the average background mortality to the average uncorrected % VC after treatment. Both uncorrected and corrected %VC were further analysed.

Statistical analysis

For each treatment, statistical analyses were performed on two measured variables: (1) the tolerance of a species (presence/absence of growth) and (2) the survival rate, measured as the average percentage of viable cells of a species (%VC). We performed the analyses on both the uncorrected and the corrected %VC. As our data were not normally distributed, we used nonparametric statistical tests. Habitat types were subdivided in two categories (terrestrial and aquatic). Strains of the same species occurring in different habitat types were seen as different ecological entities and treated as separate species. All statistical analyses were performed with Statistica 7.0 (StatSoft, Inc., Tulsa, OK, USA), and the significance level was set at $P = 0.05$.

First, we tested for each of the six stress treatments whether the treatment resulted in a lower average %VC of the different species compared to the control treatment using nonparametric Wilcoxon matched pairs tests (Sokal & Rohlf 2000). Likewise, Wilcoxon matched pairs tests were used to test for differences in %VC between the different stress treatments. Based on environmental samples, it has been noted that the average valve length of populations of the terrestrial diatom species *Pinnularia borealis* Ehrenberg and *Hantzschia amphioxys* decreases with a decreasing soil moisture content (Van de Vijver & Beyens 1997; van Kerckvoorde 2000). Therefore, we additionally calculated the Pearson product-moment correlations between length and %VC for each treatment. This was done for all species together and for the strains of *Pinnularia borealis* and *Hantzschia amphioxys* separately.

Second, we determined for each stress treatment whether the tolerance (presence/absence of growth) of species differed significantly between habitat types. For each treatment, a contingency table with the numbers of tolerant and intolerant species per habitat type was made and analyzed with the nonparametric Pearson's chi-square test (Sokal & Rohlf 2000) to test for effects of habitat type. This test works with observed frequencies and takes into account the asymmetrical setup.

Finally, for each treatment we tested whether the average %VC of species varied between habitat types using the nonparametric Mann–Whitney *U* test (Sokal & Rohlf 2000).

RESULTS

Diatom strains

In total 69 strains of 34 species (25 terrestrial and 9 aquatic) were used, belonging to 13 genera (Table 2; Figs 1–35). The terrestrial samples revealed taxa that are often encountered in terrestrial habitats, such as *Pinnularia borealis*, *Hantzschia amphioxys*, *Nitzschia austriaca* Hustedt, *N. perminutum* (Grunow) M. Peragallo, *N. palea* (Kützing) W. Smith and *Mayamaea atomus* (Kützing) Grunow var. *permitis* (Hustedt) Lange-Bertalot (formerly known as *Navicula atomus*) (Ettl & Gärtner 1995). Especially the first two species are among the most frequently reported terrestrial diatoms (Patrick 1977; Flechtner *et al.* 1998; van Kerckvoorde 2000; Van de Vijver *et al.* 2004). The aquatic strains belonged to aquatic species such as *Amphora pediculus* (Kützing) Grunow and *Eunotia implicata* Nörpel *et al.* Strains of five species were isolated from both habitats. The terrestrial and aquatic isolates from *Navicula radiosa* Kützing (Figs 15–17), *N. libonensis* Schoeman (Figs 12, 13) and *N. veneta* Kützing (Figs 9, 11) were morphologically identical, while those from *Cymbella subaequalis* (Figs 31, 32) and *Rhopalodia gibba* (Figs 28, 29) differed in valve width and in outline and fibula density, respectively (Table 2).

Survival in control conditions

Most of our tested strains had a %VC of more than 90% in the control conditions. However, some strains – for example, three strains of *Hantzschia amphioxys*, six strains of *P. borealis*, *P. obscura* Krasske and *Nitzschia austriaca* – had %VC of less than 70%. These strains were probably in poor health. In the case of *N. austriaca*, *P. obscura*, one strain of *P. borealis* and one strain of *H. amphioxys* morphotypes 1 and 2, heating improved the survival percentage compared to the control survival. The uncorrected and corrected %VC in the control condition was not significantly different between habitat types ($P = 0.78$ for both the uncorrected and the corrected dataset).

Tolerance to heating

Heating was tolerated by the majority of the species (Table 2), but all four temperature treatments had a significantly lower %VC compared to the control conditions ($P < 0.001$) (Fig. 36). There were also differences in %VC between the different heating treatments ($P < 0.05$ for both uncorrected and corrected %VC) (Fig. 36). All strains survived gradual heating to +30°C (Fig. 37) with no significant difference in %VC between habitat types (Fig. 38).

Gradual heating to +40°C was survived by a significantly lower number of aquatic species compared to terrestrial species ($P = 0.013$) (Fig. 37). The aquatic *Amphora pediculus*, strains of *Navicula radiosa* from both habitats and the aquatic strains of *Cymbella subaequalis* and *Rhopalodia gibba* were killed by the gradual heating (Table 2). In contrast, the terrestrial strains of *Cymbella subaequalis* and *Rhopalodia gibba* survived gradual heating to 40°C. The %VC was highly variable among species of the same habitat with extremes between 0% and 98% (Table 2; Fig. 38), while no significant differences were detected between habitats.

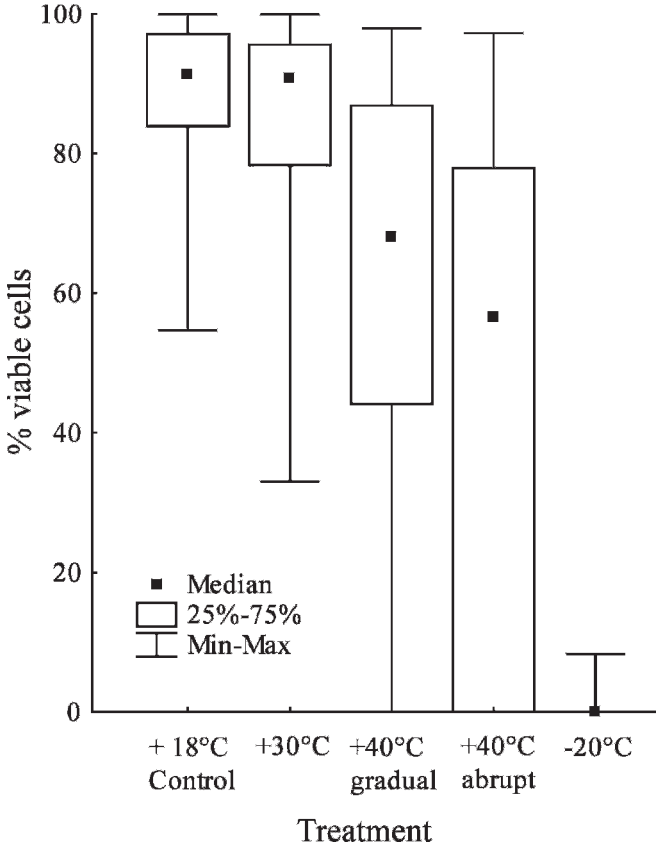


Fig. 36. Box plots of the percentages of viable cells (%VC) of the diatom species for the different temperature treatments without separation in habitat type. $N = 34$ for each treatment.

Abrupt heating to +40°C was more lethal than the gradual heating as three more strains isolated from

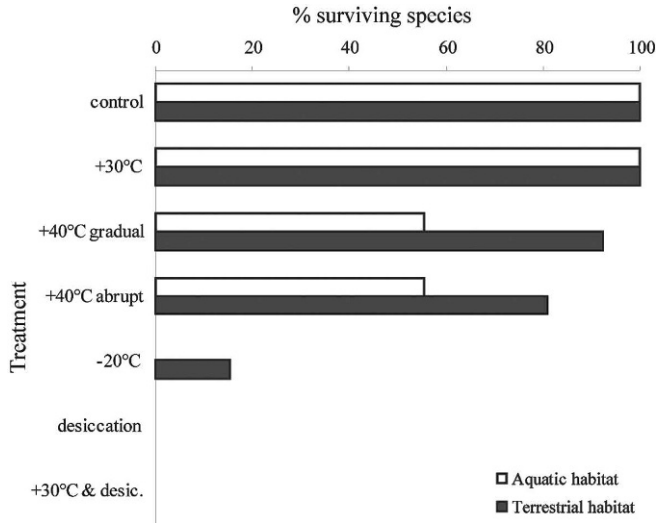


Fig. 37. Percentages of tolerant species (representing growth in at least two out of three replicates of at least one strain) per habitat type for each treatment. White bars represent aquatic taxa, black bars represent terrestrial taxa. For terrestrial taxa $N = 25$; for aquatic taxa $N = 9$.

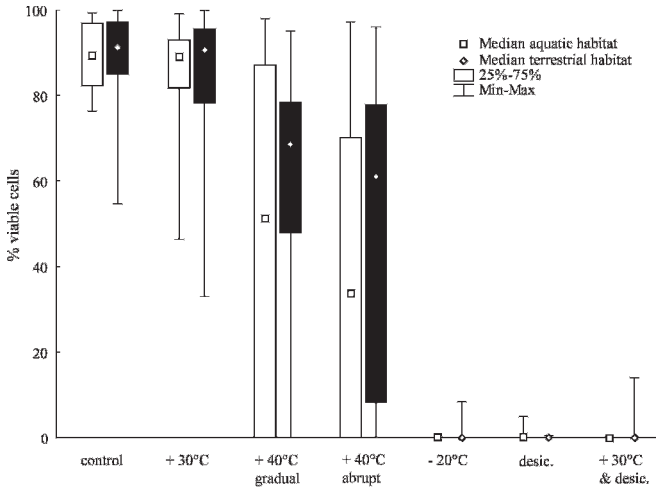


Fig. 38. Box plots of percentages of viable cells (median, box: first and third quartiles, whiskers: minimum and maximum) after the different temperature and desiccation tolerance treatments (control 18°C, +30°C, +40°C gradual, +40°C abrupt, −20°C, desiccation, +30°C and desiccation) for aquatic species (white bars) and terrestrial species (black bars). For terrestrial taxa $N = 25$; for aquatic taxa $N = 9$.

terrestrial habitat were intolerant: *Achnanthidium minutissimum* (Kützing) Czarnecki, *Pinnularia divergentissima* (Grunow) Cleve and *Cymbella subaequalis* (Table 2). Survival frequencies were not dependent on habitat type ($P = 0.142$) (Fig. 37), and, similar to the gradual heating, the %VC was highly variable, and no significant differences were detected between habitat types (Fig. 38). For both habitat types together, a significantly lower %VC was present after abrupt heating compared to gradual heating to +40°C ($P = 0.017$ for both uncorrected and corrected %VC) (Fig. 36).

Tolerance to freezing

Freezing at −20°C was lethal for most of the isolates (Table 2). None of the aquatic strains survived. Only some strains of three terrestrial species tolerated the treatment, being *Pinnularia borealis* (5 out of 10 strains), *Mayamaea atomus* var. *permitis* (both tested strains) and *Hantzschia amphioxys* morphotype 2 (one out of four) and morphotype 4 (one out of two) (Table 2). The %VC fluctuated greatly between replicates, from 2% to 64% (Table 2). No significant differences were observed between habitat types based on survival frequencies ($P = 0.217$) (Fig. 37) or on %VC ($P > 0.05$ for both uncorrected and corrected %VC) (Fig. 38).

Tolerance to desiccation

None of the species consistently tolerated desiccation, with or without preconditioning (Table 2; Fig. 37). Growth was recorded in a single replicate of five strains from three different species, *Pinnularia borealis* (two strains), *Hantzschia amphioxys* morphotype 4 (one strain) and *Navicula radiosa* (two strains). The first two are typical terrestrial species, while resistant strains of the third species were isolated from an aquatic and a terrestrial sample (Table 2). %VC in these replicates varied between 5% and 84% (Table 2).

Influence of valve length

Valve length appeared unimportant in explaining %VC of all species analyzed together (R^2 not higher than 0.08, $P > 0.05$) and the %VC of the strains of *P. borealis* and *H. amphioxys* separately.

DISCUSSION

All temperature and desiccation treatments conducted in this study clearly represented adverse conditions for vegetative diatom cells, as the percentage viable cells (%VC) significantly decreased in all six treatments compared to the control condition. Furthermore, our results indicate a high sensitivity of freshwater diatoms to abrupt heating, freezing and desiccation. Forty percent of the tested species died after abrupt heating to +40°C, only three species survived freezing and not a single species consistently survived desiccation. Our results agree with published data for freshwater planktonic diatoms that report tolerance to high temperatures only up to 30–40°C (Suzuki & Takahashi 1995; Butterwick *et al.* 2005) and no survival when exposed to desiccation (Jaworski & Lund 1970). They are in stark contrast, however, with stress tolerances reported for vegetative cells of terrestrial and aquatic cyanobacteria and green algae, with some representatives being able to survive desiccation periods ranging from 1 d to several weeks (Potts 1999; Sabacka & Elster 2006; Gray *et al.* 2007) and freezing temperatures ranging from −40°C to liquid nitrogen (Tamaru *et al.* 2005; Sabacka & Elster 2006).

While desiccation and freezing both result in the dehydration of cells (Welsh 2000), our results revealed an even lower survival for desiccation compared to freezing. This agrees with observations that desiccation is a more injurious stress than freezing (Oldenhof *et al.* 2006; Sabacka & Elster 2006). Interestingly, some strains of the terrestrial species *H. amphioxys* and *P. borealis* did survive freezing or desiccation in a single replicate. As the percentages of surviving cells were very low, this may have been due to the combination of a low survival probability and a low number of tested cells per well (between 300 and 1000). This means that some cells of these species are capable of surviving freezing or desiccation, possibly by means of differential expression of universal molecular mechanisms such as osmolytes (Welsh 2000), stress proteins (Bierkens *et al.* 1998; Rousch *et al.* 2004), antioxidants or fatty acid composition (Holmstrup *et al.* 2002; Rousch *et al.* 2003; Vicré *et al.* 2004; Dunlap *et al.* 2007) or by their cell cycle phase (Hodgson *et al.* 1992). In addition, two strains of *N. radiosa* showed survival percentages for desiccation of 74% and 84% in a single replicate. Possibly, the cells were clustering during desiccation or were in a different culture growth phase despite the fact that we attempted to keep all cultures in exponential phase. Also, a mechanism involving intercellular communication of stressful conditions and a subsequent response of the individual cells could be hypothesised. While these observations indicate that cells of at least some (terrestrial or habitat-unspecific) diatom species can be in a physiological state in which they can

survive desiccation or freezing, the reasons for this rare survival are not clear and should be further investigated.

In contrast to desiccation to the air, previous experiments with sediments show that most terrestrial species survive in sediment with over 50% moisture content (Evans 1959). Soils show a large vertical gradient in water content in the shallow surface layer (Gao *et al.* 2008), and the observed vertical migration of semi-terrestrial diatoms and cyanobacteria in drying ponds (Evans 1959) could thus be their main mechanism to avoid desiccation. Moreover, in natural conditions, the slower desiccation rate in sediments allows more species to survive (Evans 1959; Hostetter & Hoshaw 1970), possibly due to the longer time interval during which cells could initiate protective mechanisms (Oldenhof *et al.* 2006) or form physiological resting stages (McQuoid & Hobson 1996). In our study, preconditioning by heating the cells to +30°C did not enhance their tolerance to desiccation despite the known positive influence of pretreatment on stress tolerance by the initiation of protection mechanisms in various organisms (Bierkens *et al.* 1998; Bayley *et al.* 2001; Sung *et al.* 2003; Dunlap *et al.* 2007). However, in the heating treatments there was an effect of preconditioning, as several species survived gradual but not abrupt heating to 40°C. Moreover, abrupt heating had in general a more negative influence on %VC than gradual heating. On the other hand, some strains of, for example, *Rhopalodia gibba*, *Nitzschia* and *H. amphioxys* did have a higher survival after the abrupt heating compared to the gradual heating, a fact that could be due do the longer duration of stress during the gradual heating.

The sensitivity of freshwater diatoms to freezing, desiccation and abrupt heating may influence their dispersal capacities and consequently rates of allopatric speciation. Diatom speciation has been generally tied to evolution within ancient water bodies or landscapes (Kociolek & Spaulding 2000; Rossiter & Kawanabe 2000); whereas, terrestrial diatoms are thought to be the least likely to become reproductively isolated and undergo speciation due to higher dispersal abilities (Spaulding *et al.* in press). Nevertheless, our results clearly indicate that vegetative cells of most taxa do not survive harsh adverse conditions, implying that most diatoms – terrestrial species included – are likely to be limited in their dispersal capacities. This is also in agreement with the rare occurrence of living diatoms in the air (Van Overeem 1937; Schlichting 1961, 1964; Brown *et al.* 1964; Roy-Ocotla & Carrera 1993) or on waterfowl (Schlichting 1961). We therefore argue that physically unprotected vegetative diatom cells will not survive long-distance dispersal by wind or birds. This was already suggested by the high differentiation for microsatellite markers between populations of the benthic freshwater diatom *Sellaphora capitata*, even when located only some hundreds of kilometres from each other (Evans *et al.* 2009). A limited dispersal is also in agreement with recent taxonomic revisions, which led to the discovery of a large number of regionally endemic species in isolated areas, for example, in the genera *Diadesmis* Kützing (Van de Vijver *et al.* 2002), *Luticola* D.G. Mann (Van de Vijver & Mataloni 2008) *Muelleria* (Frenguelli) Frenguelli (Spaulding *et al.* 1999) and *Stauroneis* Ehrenberg (Van de Vijver *et al.* 2005). However, the occasional survival of a single replicate from our experiments and the rare occurrence of single, living diatom cells on waterfowl and in air traps indicate that diatom cells can indeed be occasionally dispersed. For instance, 2 yr after the volcanic activity had ceased on Surtsey Island, 33 km of the coast of Iceland, 69 different diatom species were reported, mainly terrestrial species also occurring in Iceland (Behre & Schwabe 1970 in Kristiansen 1996). Therefore, we conclude that, although occasional dispersion events indeed may be successful, vegetative diatom cells will not be massively dispersed by wind and waterfowl.

Besides the high sensitivity of both terrestrial and aquatic species to our treatments, our results do not reveal a marked and consistently higher tolerance of terrestrial diatom species compared to aquatic species. All species, regardless of habitat, were very sensitive to desiccation and, apart from three species, to freezing. The very broad ranges of %VC between species inside the two habitat types for the other treatments – ranging from 0% to 100% – indicate highly species-specific tolerances, which were for a large part independent of habitat. Similar species-specific stress tolerances were already reported in other microalgae (Butterwick *et al.* 2005; Gray *et al.* 2007).

As we were mainly interested in the general survival capacities of vegetative diatom cells and habitat-dependent survival differences among species, we did not explicitly test for intraspecific variation in %VC. Although the experimental design was not standardised enough to draw firm conclusions on this matter, and some strains were obviously in a less healthy physiological state than others, there seem to be some differences in stress tolerance between strains of the same species. For instance, one strain of *H. amphioxys* morph. 4 survived freezing much better than the other strains. The same was true for *P. borealis*, in which cells of half of the strains tolerated freezing. Also in the heating to +40°C treatments, differences in %VC between strains were obvious for several species. Future experiments should focus on determining the extent of intraspecific genetic variation for these traits.

Nevertheless, apart from these inter- and intraspecific differences, the habitat-dependent tolerances for gradual heating to +40°C and freezing do point at a better adaptation of vegetative cells of the terrestrial species to their more extreme habitat. First, a higher number of terrestrial species survived gradual heating to +40°C compared to aquatic species, which is probably an adaptation to the extreme diurnal variations in temperature in the upper soil layer (Gao *et al.* 2008). The positive responses of the terrestrial strains of *Rhopalodia gibba* and *Cymbella subaequalis* to this treatment compared to the aquatic strains underscore this habitat-specific difference. Interestingly, the aquatic and terrestrial strains of both species also differed morphologically. These different ecological entities might represent different species, similar to the ecophysiologically and genetically separated aquatic and terrestrial lineages of green algae (Lewis & Lewis 2005; Gray *et al.* 2007; Zoe *et al.* 2008). In diatoms, it has recently become clear that a high (pseudo)cryptic species diversity exists (Sarno *et al.* 2005; Mann & Evans 2007; Vanormelingen *et al.* 2008a), and closely related species can occupy different niches (Vanelslander *et al.* 2009). Alternatively, it

may concern different locally adapted populations, although it is unclear in that case why there is a correlation with valve morphology. In contrast, *Navicula radiosa* strains from both habitat types responded in exactly the same way to our tolerance experiments and showed no morphological differences. As such, *N. radiosa* may be a truly generalist species occurring in both habitats. This needs further study, however.

A second clue for a habitat-specific adaptation of diatoms – although not statistically underpinned – resulted from the freezing experiment. The nine surviving strains all belonged to the typical terrestrial taxa *Hantzschia amphioxys*, *Pinnularia borealis* and *Mayamaea atomus* var. *permitis* (Ettl & Gärtner 1995). Similarly, the only diatom reported in literature to tolerate freezing is the terrestrial *Stauroneis anceps* Ehrenberg originating from temperate desert crusts (Hostetter & Hoshaw 1970). The higher tolerance to freezing may have arisen as an adaptation to the more extreme variations in temperature in terrestrial habitats (Gao *et al.* 2008).

Interesting to note is the possible influence of climatic conditions on the observed stress tolerance differences between aquatic and terrestrial diatoms. All our strains were isolated from warm temperate, fully humid areas with warm summers (Kottek *et al.* 2006). Various organisms from more extreme climates have a wider tolerance range for temperature and drought compared to their counterparts from milder climates (e.g. Sinclair *et al.* 2003; Xiao *et al.* 2008; Tomanek 2008; Dong & Somero 2009). As such, the relatively small difference in stress tolerance between terrestrial and aquatic diatoms in our study could result from the temperate climate they inhabit and may be larger in more extreme climates.

In conclusion, in the present study we detected a high sensitivity of vegetative cells of benthic freshwater diatoms to desiccation, freezing and abrupt heating. This is in agreement with the high population differentiation observed in a freshwater benthic diatom and may explain the widespread endemism observed in freshwater diatoms. Secondly, vegetative cells of terrestrial diatoms are more tolerant to temperature extremes than their aquatic counterparts, probably as an adaptation to the more extreme terrestrial environment. Additional studies should address the influence of climate on diatom stress tolerance, the molecular mechanisms underlying stress tolerance and the evolutionary divergence of terrestrial and aquatic diatom lineages. Ultimately, linking differential levels of stress tolerance among species to population differentiation and the geographical distribution of species will reveal how stress tolerance influences dispersal and colonization and thus shapes diatom diversity and distribution.

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