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Culturing Diatoms

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2 Daniel Vaultot^{1,*}, Gust Bilcke^{2,3}, Peter Chaerle⁴, Angela Falciatore⁵, Priscillia Gourvil⁶, Michael W. Lomas⁷,
3 Ian Probert⁶, Wim Vyverman⁴

4

5 ¹ Sorbonne Université, CNRS, UMR7144, Station Biologique, Place Georges Teissier 29680, Roscoff, France

6 ² VIB Center for Plant Systems Biology, Technologiepark 71, Ghent, B-9052, Belgium

7 ³ Department of Plant Biotechnology and Bioinformatics, Ghent University, Technologiepark 71, Ghent,
8 B-9052, Belgium

9 ⁴ BCCM/DCG, Lab. Protistology & Aquatic Ecology, Krijgslaan 281 – S8, 9000 Gent, Belgium

10 ⁵ Laboratoire de Biologie du chloroplaste et perception de la lumière chez les micro-algues, UMR7141,
11 CNRS, Sorbonne Université, Institut de Biologie Physico-Chimique, Paris, 75005, France

12 ⁶ Roscoff Culture Collection, Sorbonne Université, CNRS, FR2424, Station Biologique, Place Georges
13 Teissier 29680, Roscoff, France

14 ⁷ National Center for Marine Algae and Microbiota, Bigelow Laboratory for Ocean Sciences, East Boothbay,
15 Maine, USA

16

17 * Corresponding author: vaultot@gmail.com. Other authors are listed in alphabetical order.

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23 **Abstract**

24 In this chapter, we provide information on cultures of diatoms, starting with a brief introduction of the
25 history of diatom culture collections. We present a synopsis of diatom strains currently available in culture
26 collections and their representation in terms of habitat diversity, geographical distribution, phylogeny and
27 taxonomic diversity. We outline the major techniques for isolating and cultivating diatoms and discuss
28 the problems and possible strategies linked to diatom life cycles and habitat requirements. We provide an
29 overview of available techniques for genetic transformation of diatoms and strategies for forward genetics.
30 Finally, we summarize information and techniques available for long term preservation of diatom cultures,
31 focusing on cryopreservation.

32 **1 - Introduction**

33 Diatoms were discovered in the 18th century, but the first cultures were not established until the middle
34 of the next century and cultivation only really developed from the early 1900s (Figure 1, Richter 1903).
35 Museums, botanical gardens, zoos and research facilities have served as repositories to preserve collections
36 of fixed specimens, notably type material, for centuries, with such facilities deemed reliable places to
37 safeguard specimens in the state in which they were first deposited, be sure that they are cared for, and be
38 able to distribute them to researchers so that scientific research can be reproduced and expanded (Smith
39 and Ryan 2012). The development of repositories for living material is more recent, Ernst Pringsheim
40 having established the first culture collection catalogue in 1928. Cultures are extremely useful for studies
41 on diatom taxonomy and life cycles, although such work can also be undertaken on natural populations
42 (Mann and Chepurnov 2004). They are, however, absolutely essential for physiological studies, in particular
43 in the domain of photosynthesis, as well as for the biochemical characterization of cells. Cultures are also
44 key for the acquisition of 'omics' data, that has played a central role for understanding the regulation of
45 cell growth and functions (Armbrust et al. 2004; Bowler et al. 2008).

46 In recent decades, the development of large public microalgal culture collections has increased the quantity
47 and diversity of cultures at the disposal of diatom researchers, in particular from environments such as
48 the polar regions for which few strains were previously available. In this chapter, we inventory culture
49 collections that host diatom strains and provide information on the major approaches to isolation and
50 culture of diatoms, detailing constraints linked to the specific life cycle of diatoms. We conclude by
51 reviewing relatively new approaches, genetic transformation and cryopreservation.

52 **2 - Current diversity of diatoms in culture**

53 Diatoms are extremely diverse in terms of shapes and structures (Figure 2), as well as habitats and physi-
54 ology. Only a small fraction of this diversity is currently represented in cultures.

55 We identified 37 algal collections containing diatom cultures and obtained culture listings for 27 of them
56 (Table 1). The number of diatom cultures in these collections varies from a few to more than 2000.
57 Some collections are specialized for marine (e.g., RCC, NCMA) or freshwater ecosystems (e.g., TCC) while
58 others such as UTEX and BCCM/DCG contain marine, freshwater and terrestrial strains. In total, these
59 collections currently hold more than 6,400 diatom strains (Supplementary Data S1). Depending on the
60 collection, the amount and type of associated metadata varies considerably.

61 In general, strains are taxonomically identified to the species level, although in many cases only identifi-
62 cation at higher taxonomic levels (e.g., class, order, family or genus) is available. The majority of strains
63 belong to the pennate Bacillariophyceae (3746), followed by the centric classes (Mediophyceae: 1625,
64 Coscinodiscophyceae: 316). 316 strains have not been assigned at the class level. Identified strains belong
65 to 216 genera and 828 species. The best represented genera in culture are *Seminavis*, *Navicula*, *Nitzschia*,
66 *Pseudo-nitzschia*, *Thalassiosira*, *Chaetoceros* and *Skeletonema*, while some of the best represented species
67 are *Seminavis robusta* and *Chaetoceros neogracilis* (Figure 3). The prevalence of particular genera and
68 species in culture holdings may reflect the ease with which these species can be isolated, but may also
69 reflect the interest of a particular laboratory or a collection in specific genera or species. For example, the
70 genus *Pseudo-nitzschia*, some species of which can produce domoic acid (Bates et al. 2018), is an organism
71 of high research value and has been the focus of numerous projects, leading to more strains being isolated.
72 More effort has been devoted to isolation of marine diatoms (Figure 4) compared to those from freshwater,
73 while other ecosystems (e.g. soil) are particularly underrepresented. Most isolation has been performed
74 from water, plankton or ice, although microbiomes (e.g., biofilms from turtles or from macroalgae) have
75 received some attention. Most diatom cultures originate from coastal and continental habitats in Europe,
76 the US, Japan, Australia and New Zealand (Figure 5). While polar areas (both Arctic and Southern Oceans)
77 are quite well covered, very few diatom strains have been isolated from oligotrophic oceanic regions, where
78 they are of course less abundant and often harbour symbionts (Foster and O'Mullan 2008), which may
79 hinder cultivation. Although the oldest diatom strains held in a collection were isolated prior to 1960, there
80 has clearly been an increase in the number of strains isolated in the last 10 years (Figure 6).

81 **3 - Isolation of diatom cultures**

82 Sampling for diatoms (and more generally for phytoplankton) has to be adapted to the targeted ecosystem.
83 Surface plankton can be collected with a net with a mesh size (e.g. 5 μm , 10 μm , 20 μm , 64 μm) adapted
84 to the cell size of targeted taxa. In order to preserve the most fragile cells, it is critical to tow the net at
85 slow speed. Towing time will of course depend on the cell concentration in the water and will be longer
86 when sampling more oligotrophic waters. Samples can also be collected at different depths in the water
87 column with Niskin bottles attached to a cable or mounted on a Rosette sampling device. For samples from
88 benthic sediments or ice, special tools such as box corers or ice corers may be required. For ice, melting
89 at room temperature with the addition of 0.2 μm filtered seawater may be necessary. It is important to
90 record as many parameters as possible when collecting samples, in particular substrate type, ecosystem
91 type, geographical position (longitude and latitude), depth or altitude, temperature, salinity, pH and where

92 possible nutrient concentrations (nitrate, phosphate, silicate, etc.). These parameters will be critical for
93 documenting the origin of the isolated strains and may also help to define optimal growth conditions.

94 After collection, samples are often passed through a 100 to 200 μm mesh to remove large particles and
95 unwanted zooplanktonic organisms. If necessary, samples can be concentrated either by gravity filtration
96 onto 0.8 or 3 μm filters and then resuspension in filtered seawater, or by tangential flow filtration (TFF),
97 which is a reverse filtration process that allows gradual removal of water from the sample through a
98 membrane filter and thus concentration of the sample (typically from 2L down to 25ml). TFF uses a
99 peristaltic pump, but is nevertheless amenable to the preservation of delicate cells (Vaulot 2017).

100 The samples can either be processed immediately or enriched by adding a small volume of culture medium
101 (typically 1 to 10% of the volume of the sample) and incubated at a temperature and photoperiod matching
102 those prevailing in the environment from which the sample was taken. Enriched samples (also called "pre-
103 cultures") should be placed in a culture cabinet and regularly monitored by techniques such as optical
104 microscopy or flow cytometry.

105 Several isolation strategies can be employed on the same sample to maximize the chances of success and
106 the diversity of cultures obtained (Andersen 2005).

107 **Serial dilution.** Serial dilution, consisting of repeatedly diluting a sample or an enrichment by transferring
108 a small volume of each successive dilution to fresh medium, is one of the most common approaches. The
109 principle of serial dilution is to dilute the sample to extinction, such that the last dilution that contains any
110 cells is likely to contain just one cell, from which a pure (mono-specific) culture is initiated. Serial dilution
111 is most easily carried out in multi-well plates (typically 24, 48 or 96 wells), but tubes or flasks can also
112 be used. Dilutions are maintained in optimal growth conditions (temperature, light) and culture growth
113 should be observed within a few days, but it is advisable to wait for 1 to 2 weeks before transferring in
114 order to verify the purity of the isolates.

115 **Single cell isolation.** Single cell isolation is usually undertaken with a tapered Pasteur pipette, which
116 allows cells to be manually selected one at a time from the natural sample or the enrichment under a
117 microscope (ideally an inverted microscope which has more space for manipulating cells). The collected
118 cell is placed in a drop of clean culture medium to be washed. This step will be repeated as many
119 times as necessary until the cell is free from other protists (Figure 7). Aspiration is controlled by adding
120 either a suction bulb or a tube with a mouthpiece to the end of the pipette. Single cells isolated in this
121 manner are placed in sterile culture medium in a tube or a multi-well plate and maintained in optimal
122 growth conditions in terms of temperature and light. These different steps can be viewed on video at
123 www.youtube.com/watch?v=0hNwVtRGeXI.

124 **Solid medium.** More commonly employed for bacterial culture, the use of solid or semi-solid agar media
125 can also be useful for the isolation of diatom cultures. For both streaking and inclusion techniques, 0.5-2%
126 agarose is added to the culture medium (supplemented with silica for diatoms). For streaking, a drop of
127 a few microlitres (3-25, depending on the richness of the natural sample) is streaked onto the agar plate
128 using the dial method. This simple technique allows separation of taxa that will appear after a few weeks
129 in the form of spots (corresponding to colonies originating from a single cell) on the agar. To guarantee
130 a clonal culture, it is recommended to transfer the colony to liquid medium and repeat the agar streaking
131 operation at least once. While the streaking method is more specific to isolation from natural samples,
132 the inclusion technique is dedicated to strain purification and is detailed in the "Diatom transformation
133 and genetic engineering" section of this chapter. Once a pure culture has been obtained by either of these
134 methods, it can be cultured on agar plates or transferred to liquid medium.

135 **Flow cytometry sorting.** An alternative method for cell isolation is the use of a sorting flow cytometer.
136 Flow cytometry can distinguish populations according to pigment autofluorescence (chlorophyll, phycoery-
137 thrin) and light scatter, which is a proxy of size. A flow cytometer with sorting capacity makes it possible
138 to select cells according to their characteristics and to deposit one or several cells in tubes or multi-well
139 plates containing a suitable culture medium. Many diatoms form chains or colonies which hinder flow
140 cytometric analysis and sorting, a constraint that can potentially be offset by vigorous shaking or vortexing
141 of the sample prior to sorting. Due to the presence of the frustule, most diatoms are relatively robust
142 compared to other microalgae and thus survival rates upon passage through a flow cytometer tend to be
143 relatively high. For fragile species, Marie et al. (2017) described a method involving the use of bovine
144 albumin serum (BSA) and antibiotics to mitigate the effect of the stress to which the cells are subjected
145 during flow cytometry (laser, shear stress, bacterial dominance, etc.) and thereby increase success rate of
146 culture isolation.

147 Each of these approaches has its advantages and disadvantages. Serial dilution is cheap to perform and
148 does not require specific skills or equipment, but is a non-targeted method, meaning there is no guarantee
149 of selecting taxa of interest. It can, however, lead to successful isolation of rare and/or fragile taxa from
150 mixed samples that may be difficult to isolate by other methods. A significant drawback of this method
151 is that there is no practical way of knowing whether a mono-specific culture was initiated from a single
152 cell (and hence is clonal) or from two or more cells of the same species. If clonal cultures are required,
153 it is therefore recommended to re-isolate cultures resulting from successful serial dilution using one of the
154 other methods presented here. Micropipette isolation is very targeted and should result in clonal cultures of
155 species of interest. However, it requires considerable technical expertise and is most effective for reasonably
156 large cells (typically above 10 μm). Flow cytometry sorting allows very rapid preparation of a large number

157 of isolates, but requires a very expensive sorting flow cytometer, which in general is difficult to operate. Its
158 success rate can be low, and it is impossible to differentiate diatoms from other phytoplankton based on
159 their flow cytometry characteristics, so, in the case of diatoms, this method is non-targeted.

160 In all of these approaches, the isolation medium is a critical element that can select for certain taxa. For
161 marine diatoms, for example, f/2 (Guillard 1975) enriched with silica is often used. Media can be used at
162 different dilutions and can be prepared with seawater originating from different environments. Standard
163 media can also be modified by adding for example soil extract and there is undoubtedly considerable scope
164 for the formulation of new media to broaden the list of diatoms that can be successfully cultured. To limit
165 the growth of bacteria, and thus maximize the chances of successful isolation, the culture medium can be
166 supplemented with a mix of antibiotics (e.g. Penicillin - Streptomycin - Neomycin solution, Sigma-Aldrich
167 P4083). However, there may be cases when microalgal growth depends on bacteria (Riquelm et al. 1988)
168 and antibiotics may be detrimental.

169 Once single diatom cells have been separated by these different approaches, isolations should be regularly
170 monitored either by light microscopy with or without fluorescence or by flow cytometry. Cultures that
171 appear pure should rapidly be transferred to fresh medium. After a period of a few months (beyond which
172 cultures are generally considered to be stable), cultures can be characterized, which for diatoms typically
173 involves observation by scanning electron microscopy (SEM) and/or sequencing of a short genetic barcode.
174 Due to the presence of the silica frustule, preparation of diatom cultures for SEM is in general relatively
175 straightforward (filtration onto a membrane filter, drying, sputter coating), but for species that produce
176 organic material that covers the frustule, a cleaning step (typically in boiling nitric acid) may be necessary
177 in order to be able to clearly visualize the frustule. For diatoms, barcoding typically involves sequencing of
178 the V4 region of the 18S rRNA gene, which is a short region located roughly between 500 and 1000 base
179 pairs. In some cases the 18S rRNA gene is not variable enough to discriminate between closely related
180 species or subspecies, and it may therefore be necessary to sequence other genes, such as the 28S rRNA
181 gene and/or the ITS region of rRNA operon, or plastidial (e.g. *rbcL*) or mitochondrial (e.g. *cox1*) markers
182 (Evans et al. 2007; Rimet et al. 2019). Characterization of cultures allows dereplication of duplicate strains,
183 with several potential strategies can be used to keep strains of interest. For example, if the same species
184 has been isolated several times from several stations, one or two strains from each sampling station can
185 be kept. When isolated strains are entered into a culture collection database, it is important to add as
186 much metadata as possible, in particular related to the sampling site, but also related to the isolation
187 (enrichment, isolation method, isolator, medium used for isolation, etc.).

188 4 - Culture of diatoms

189 Once diatom strains are established, they are generally considered to be relatively easy to grow (at least
190 on the short term) using standard microalgal culturing techniques. A wide range of media have been
191 developed for culturing marine microalgae (for an exhaustive list refer to the book by Andersen 2005). For
192 marine diatoms, commonly used media used are f/2 (Guillard 1975), L1 (Guillard and Hargraves 1993)
193 and K (Keller et al. 1987). The main specificity for culturing diatoms is that media should contain silica
194 (usually in the form of sodium silicate), which is required by diatoms for production of their frustules.
195 Natural seawater may contain a sufficient quantity of silica to maintain diatom growth without adding
196 a supplement, particularly for species with thin frustules and/or if the water is collected outside periods
197 of diatom blooms (when silica levels are depleted). However, when culturing diatoms silicate is generally
198 added (at concentrations up to 100 μM) as a precaution, and silica supplements are absolutely required
199 when using artificial seawater based medium recipes. For cultures in liquid media, plastic ware (polystyrene
200 flasks, tubes or multi well plates) or glass recipients (Erlenmeyer flasks, tubes, etc.) can be used (Figure 8).
201 The advantage of using pre-sterilized plastic ware is to reduce the risk of contamination, but it generates
202 a lot of waste and thus has a strong environmental impact. For cultures on solid medium, the recipe is
203 supplemented with 1 to 3% agar and following autoclaving the medium is distributed (before setting) either
204 in Petri dishes or as slants in glass tubes. All cultivation procedures should be conducted under a laminar
205 flow hood to limit bacterial contamination.

206 With respect to temperature, once diatom cultures are established, it is not necessary to exactly reproduce
207 the isolation temperature. Typical routine maintenance temperatures are 4 °C for polar species, 13 °C
208 for temperate species and 20 °C for tropical species. Diatoms contain chlorophylls *a* and *c* which absorb
209 light in the blue and red parts of the light spectrum and the main accessory pigment is fucoxanthin which
210 absorbs light in the 450-540 nm wavelength range. It is generally therefore best to use daylight quality
211 fluorescent tubes or LED lights which reproduce the natural spectrum, combining several wavelengths (blue,
212 green, red). Although it is possible to grow some diatoms in continuous light, the best results are usually
213 obtained by applying a photoperiod such as 12L:12D. Light intensity is typically set at 80-100 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$
214 for strains established from surface samples and around 30 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for strains established from deeper
215 samples. For long-term maintenance of microalgal strains, it is recommended to use dedicated incubators
216 with temperature and light control. Alternatively, walk-in temperature-controlled rooms equipped with
217 shelves and light ramps are very convenient for large collections.

218 For growth in liquid media, transfer to fresh medium should typically be conducted every 2 to 4 weeks,
219 depending on the species and light conditions. Lowering the temperature and/or light level may result

220 in longer periods between transfers and reduce the labour involved. Growth is generally slower on solid
221 medium, and transfer frequency is typically between 2 and 3 months. In most cases, growth of the newly
222 transferred culture can be verified simply by colour, but it may be necessary to use optical microscopy or
223 flow cytometry.

224 **5 - Life cycles**

225 Although many diatoms can be easily isolated from environmental samples (see above Andersen 2005)
226 and grow well on commercially available algal culture growth media, long-term maintenance of strains
227 is often challenging because of their peculiar life cycle, resulting in a gradual cell size diminution and
228 final loss of cultures. Unlike most other microalgae, diatoms have a diplontic life cycle (Figure 9), with
229 a long vegetative phase with diploid mitotically dividing cells, alternated by a comparatively short sexual
230 phase with short-lived haploid gametes (Chepurnov et al. 2004). During mitotic cell division, two slightly
231 unequal daughter cells are formed due to the peculiar architecture of the siliceous cell wall (frustule)
232 and the biosynthesis of new valves inside the confines of the parental cell wall. As a result, the mean
233 cell size of a clonal population gradually decreases during repeated rounds of mitotic divisions, known
234 as the MacDonald-Pfitzer rule (Macdonald 1869; Pfitzer 1869). Ultimately, cells become critically small,
235 resulting in malformations and eventually cell death (Mann 2011). While this appears to be the general
236 rule, a minority of diatom species do not decrease in cell size when dividing, while others are able to restore
237 their cell size asexually (Kaczmarska et al. 2022, 2013; Mann 2011; Rose and Cox 2013). In most species
238 studied, however, sexual reproduction and the subsequent expansion of a zygote into the auxospore is the
239 most prevalent mechanism to restore large sized cells. Spontaneous and experimentally induced abrupt cell
240 size reduction demonstrates that sexual reproduction in diatoms is dependent on cell size and can take
241 place once cells pass a species-specific size threshold (SST, Chepurnov et al. 2004). Although studied in
242 detail for only a few species, this endogenous cell size sensing mechanism, also referred to as the diatom
243 sex clock, results in an alternation between brief sexual reproduction events and long intervals of vegetative
244 growth, which can span several years (Lewis 1984; Mann 2011). While the processes of cell size decrease
245 and auxosporulation are conserved among most diatoms, diverse mating strategies exist, largely coinciding
246 with the main morphological groups of centric, araphid pennate and raphid pennate diatoms (Figure 9).
247 Most centric diatoms are oogamous, producing large egg cells which are fertilized by small, flagellate
248 spermatozoa. In general, they are homothallic (self-fertile), and clonal cells below the SST can, depending
249 on their size, differentiate into egg or sperm cells. However, exceptions to this general pattern exist (e.g.
250 Davidovich et al. 2017). In contrast, species from the evolutionary younger group of pennate diatoms,

251 are often heterothallic, although homothallic species and mixed strategies are also known (Davidovich et
252 al. 2010, 2009; Mann and Pouličková 2019; Quijano-Scheggia et al. 2009; Vanstechelma et al. 2013),
253 and sexual reproduction therefore requires a partner of the compatible mating type. The phylogenetically
254 older group of araphid pennate diatoms is characterized by non-flagellate motile male gametes and larger
255 immobile female gametes (anisogamy). In contrast, most raphid pennate species produce morphologically
256 indistinguishable gametes (isogamy). A key difference with araphid diatoms is that a transfer of function
257 from gametes to the gametangia has evolved, where diploid sexualized cells from opposite mating types
258 interact to form a mating pair in which gametogenesis is subsequently initiated. Pheromone signalling plays
259 a crucial role during diatom sexual reproduction (Frenkel et al. 2014; Sato et al. 2011). The system appears
260 to be most evolved in raphid pennate diatoms and includes both sex inducing pheromones and attraction
261 pheromones influencing the behaviour of gametangial cells, as well as pheromones, so far uncharacterised,
262 involved in gamete attraction (Gillard et al. 2013; Klapper et al. 2021; Moeys et al. 2016).

263 Although cell size is the precondition for sexual reproduction to occur, appropriate environmental condi-
264 tions should be also present to permit auxosporulation or to trigger it. This is best known for centric
265 diatoms, where spermatogenesis and/or oogenesis can be induced by a shift in environmental conditions
266 such as salinity, temperature, irradiance and its spectral composition, or photoperiod (Amato 2010; Baatz
267 1941; Godhe et al. 2014; Moore et al. 2017). In pennate diatoms, it appears that growing conditions
268 should be favourable. For example, sexual reproduction of the pennate *Pseudo-nitzschia multistriata* was
269 most successful when cultures were in the exponential growth phase (Scalco et al. 2014). In addition,
270 auxosporulation appears to be light dependent, both in terms of irradiance as well as spectral composition
271 (Bilcke et al. 2022; Davidovich 1998; Gillard et al. 2013; Mouget et al. 2009).

272 Understanding the process of sexual reproduction and the conditions under which it takes place is essential
273 for the long-term maintenance of diatom species in culture collections. This implies knowing the mating
274 strategy, SST, and conditions favouring sexual reproduction. In the case of heterothallic species, the avail-
275 ability of multiple strains of compatible genotypes is an obvious additional requirement. Increasingly, the
276 importance of diatom-bacteria interactions is being revealed, including both beneficial as well as inhibitory
277 effects on diatom sexual reproduction (Cirri et al. 2019). However, as this information and resources
278 are available for only few species, many diatom strains may be maintained for variable periods in culture
279 collections, but are eventually bound to be lost.

280 Ideally, strains of large-sized (well above SST) can be cryopreserved, especially in the case of strains that are
281 used as model species or for whole genome sequencing. Increasingly, studies are demonstrating significant
282 genotype-related differences in physiological traits of diatom strains of the same species as well as strong

283 differences in gene expression patterns (Anderson and Rynearson 2020; Pinseel et al. 2022), implying that
284 the loss of genome-sequenced strains can have important implications for studies using other strains. If
285 cryopreservation of large-sized cells is not possible (often small-sized cells are more easily cryopreserved,
286 Chepurnova et al. unpublished) inducing sexual reproduction in such species is a solution to maintain those
287 species in a collection. It should be noted, however, that apart from losing the original genotype(s), this
288 may also promote selection of genotypes adapted to laboratory conditions.

289 One alternative, but as yet little explored, avenue for long-term storage of diatom species in culture
290 collections is exploiting the fact that some species are able to form resting stages (Figure 9), including
291 spores or resting cells. While the former are morphologically distinct from vegetative cells, resting stages
292 have valves that are indistinguishable compared to their vegetative counterparts, but they are characterized
293 by a condensed protoplast (Kaczmarska et al. 2013). In some species it has been demonstrated that these
294 spores remain viable for centuries to millennia (Härnström et al. 2011; Kaczmarska et al. 2013; Sanyal
295 et al. 2022), opening the prospect of exploring anoxic, dark conditions to maintain such species in culture.

296 **6 - Diatom transformation and genetic engineering**

297 The study of the molecular basis of diatom life began in the 1990s with the development of DNA-mediated
298 transformation in a few selected species. This important achievement to analyse and modulate the function
299 of diatom genes was first achieved by biolistic transformation in *Cyclotella cryptica* and *Navicula saprophylla*
300 via the pioneering work of Dunahay et al. (1995). Subsequently, this method was adapted to other species
301 such as *Phaeodactylum tricornutum* (Apt et al. 1996; Falciatore et al. 1999), *Thalassiosira weissflogii*
302 (Falciatore et al. 1999), *Cylindrotheca fusiformis* (Poulsen and Kröger 2005) and several others (Table 2).
303 Biolistic transformation is based on the helium-accelerated particle bombardment of diatoms with exogenous
304 DNA coated on gold or tungsten particles, followed by selection of the transformed cells with antibiotics.
305 There are also some reports describing diatom transformation by electroporation, where electric currents
306 are used to increase cell membrane permeability and DNA delivery (Table 2). Although theoretically faster
307 and less expensive than biolistic transformation, this method is less popular, largely due to the limited
308 reproducibility of the protocol in different laboratories.

309 More recently, bacterial-mediated conjugation transformation was developed for the most studied diatom
310 model species *P. tricornutum* and *T. pseudonana* (Karas et al. 2015). With this approach, DNA is delivered
311 as an episome or artificial chromosome using genetically engineered *Escherichia coli* bacteria that promote
312 DNA transfer via direct contact with diatoms via cell-to-cell pili. Following transformation, the transgenic
313 material is replicated and maintained in the diatom cells due to the presence of a centromeric element,

314 CEN6-ARSH4-HIS3, included in the episome. Bacterial conjugation is becoming the method of choice
315 for diatom synthetic biology because the efficiency of transformation is very high (100-1000 transformants
316 per 10^8 cells with bacterial conjugation vs 1-800 transformants per 10^8 cells with biolistics, Moosburner
317 et al. 2022). It also allows reproducible expression levels of transgenes in different transgenic lines because
318 the episome is not integrated into the diatom genome. Conversely, with the biolistic and electroporation
319 transformation, plasmid-containing DNA is integrated into multiple copies and in multiple sites in the
320 genome, meaning independent transgenic lines show very different expression levels (George et al. 2020).
321 Moreover, random integration into the genome can also result in undesirable changes in genomic loci at
322 integration sites or surrounding regions. To overcome these problems and to obtain conclusive functional
323 information, it is always necessary to characterize independent transgenic lines. Episomal genetic elements
324 may, however, be lost in the absence of antibiotic selection. Due to the high cost of the antibiotics
325 commonly used for selection, this represents a disadvantage if a large quantity of transgenic lines is required
326 for functional characterisation of diatom gene products or biotechnological exploitation of transgenic cell
327 extracts. On the other hand, loss of the episome by removal of antibiotics may also be an advantage in
328 achieving transient expression of the transgene, which is a desirable effect in gene functional studies (e.g.,
329 to compare phenotypes related to the presence or absence of a specific gene) or to remove transgenic
330 proteins that may modify the DNA, and thus generate an unintended genomic change (Moosburner et al.
331 2022).

332 Several factors are critical to the success of genetic transformation, which may limit the application of these
333 tools to certain diatom species (Table 2): The ability to maintain the selected species under laboratory
334 conditions is obviously the first important requirement, but many diatom species currently remain refractory
335 to laboratory culture. Moreover, as different transformation methods also cause cell damage and mortality,
336 transformation of diatoms is usually performed with a high quantity of cells (typically around 10^8) as
337 starting material. For diatom species that do not reach a high cell density under laboratory conditions, it
338 is therefore necessary to use large culture volumes and a cell concentration step prior to transformation,
339 which may represent a limitation to routine use of the technology. In addition, genetic transformation relies
340 on the use of an appropriate selection marker to isolate transgenic lines in a population of untransformed
341 cells. Diatoms, like many algae, are resistant to many commercially available antibiotics. However, some
342 antibiotics have been identified that kill diatoms under particular conditions of cell concentration and
343 salinity (to be defined for each species) and these can be used to isolate transgenic lines expressing specific
344 antibiotic resistance genes (Table 2). For successful transformation, it is also preferable to use axenic
345 cultures, as bacterial contamination can alter the growth of diatoms on the selection medium and thus
346 lead to the growth of false transgenic lines. However, many diatoms do not grow properly in the absence

347 of bacteria, which can complicate genetic transformation efforts.

348 Transformation also relies on the identification of appropriate regulatory regions (promoter and terminator)
349 for controlled expression of endogenous (i.e. from the same species to be transformed) or exogenous (from
350 other diatom species or other organisms) genes in the diatoms. For most transformed diatoms, transgene
351 expression has been successfully achieved by using endogenous regulatory regions. For only few species,
352 transformation has been achieved using regulatory regions from other diatoms or viral promoters (see
353 Falciatore et al. 2020). The identification of endogenous regulatory regions does not represent a major
354 challenge if genomic and transcriptomic information are available, but such information is currently only
355 available for a limited number of diatom species (Mock et al. 2022).

356 Co-transformation of two vectors is also documented in several diatom species, whereby a selectable marker
357 gene on a plasmid can be used to co-deliver in the same diatom cell another non-selectable transgene, such
358 as a gene expressing a protein of interest fused to a protein tag or a reporter gene. However, the possibility to
359 use multiple selectable markers largely facilitates genetic manipulations requiring the expression of different
360 transgenes. This can be useful to simultaneously modify multiple genes or for the characterisation of the
361 function of a gene (e.g., gene mutagenesis in a first transformation round, followed by complementation of
362 a wild-type or a mutated version of the gene of interest in a second transformation, see Giovagnetti et al.
363 2022). Multiple selectable antibiotics have so far only been identified for a few species (Table 2). For *P.*
364 *tricornutum*, chemical based selectable markers have also been recently developed (Serif et al. 2018), based
365 on the APT (Adenine Phosphoribosyltransferase, enzyme of the adenine salvage pathway) and UMPS
366 (Uridine-5'-monophosphate synthase, enzyme of the de novo pyrimidine biosynthesis pathways) genes,
367 whose inactivation results in the resistance to the toxic compounds 2-fluoroadenine and 5-fluoroorotic acid,
368 respectively.

369 Independently of the transformation method employed, transgenic lines are visible after several weeks of
370 growth on the selectable media (between 2-5 weeks depending on the species and the method). Growth of
371 the transformed diatom cells on an agar plate greatly facilitates the isolation and characterisation of clonal
372 transgenic lines derived from the same transformation event. For some species such as *P. multistriata*
373 and *Pseudo-nitzschia arenysensis* that are difficult to grow on plates, the selection of transgenic lines
374 following transformation is undertaken in liquid media in the presence of the antibiotic (Sabatino et al.
375 2015). However, this protocol is more time-consuming as it requires the subsequent isolation of individual
376 transgenic cells from a pool of transformed cells in order to perform molecular characterization. In some
377 centric species such as *T. pseudonana* or *Cyclotella cryptica*, which are also difficult to grow on agar plates,
378 the isolation of transgenic lines can be successfully achieved by growing cells inside a low % (0.25%) agar

379 matrix (Turnsek and Dupont 2017) or on the agar surface, but by using high purity agar.

380 Over the last twenty years, the main application of genetic transformation has been the study of diatom
381 gene function. Powered by the availability of -omic information, these studies have been instrumental
382 in characterizing specific cellular and metabolic features of diatoms and in elucidating the mechanisms
383 contributing to their ecological success in the environment (see Falciatore and Mock 2022, for a recent
384 overview on the “Molecular Life of Diatoms”). Transgenic lines have been extensively used to characterize
385 the cellular localization of diatom proteins, by fusing proteins with various reporter genes, or to monitor their
386 expression or achieve protein purification with specific tags. The development of new cloning technologies
387 for the assembly of multiple DNA fragments based on Gateway vectors (Siaut et al. 2007) and, more recently,
388 Golden Gate technology (Faktorová et al. 2020; Hopes et al. 2016) have provided important complementary
389 resources for the characterisation of diatom gene products in transgenic lines. Genetic transformation has
390 also been critical to modulate the expression of diatom genes, an essential step to understand their function
391 by performing comparative phenotypic analyses with untransformed wild-type strains. Gene over-expression
392 is currently achieved in diatoms by using a variety of strong or inducible promoters (reviewed in Falciatore
393 et al. 2020), while gene silencing can be achieved by generating transgenic lines expressing antisense
394 or inverted-repeated fragments against the gene of interest (De Riso et al. 2009). The development
395 of different gene editing tools to delete or modify diatom gene products represents an additional major
396 achievement of recent years. Nucleotide modifications were first realized in *P. tricornutum* with a vector
397 expressing site-specific nucleases, such as the Meganucleases (MNs) or the transcriptional Activator-Like
398 Effectors Nucleases (TALENs) (Daboussi et al. 2014). Currently, the Clustered Regularly Interspaced Short
399 Palindromic Repeats (CRISPR/Cas9) technology has been adapted as the method of choice for its ease
400 and molecular efficiency, documented in different species such as *P. tricornutum* (Nymark et al. 2016) and
401 *T. pseudonana* (Hopes et al. 2016). Stable gene modifications have also been obtained by delivering the
402 CRISPR/Cas9 system on an episome (Sharma et al. 2018) or with a RNA ribonucleoprotein complex (Serif
403 et al. 2018). These methods have the advantage that the nuclease modifying the genomes can be only
404 transiently expressed in diatom cells, reducing the risk of off-target mutagenesis and random modifications.
405 Readers can refer to Moosburner et al. (2022) for additional details on the genetic engineering and genome
406 editing tools currently available for emerging diatom model species.

407 Seminal information to drive genetic engineering in diatoms has also increased the potential of these
408 algae for biotechnological exploitation. Diatoms naturally generate relevant foodstuff substances (e.g.,
409 omega-3 fatty acids like EPA) and produce lipids, especially triacylglycerides (TAGs), for carbon storage
410 (Bozarth et al. 2009). Thus, efforts have been made to establish diatoms as source material in various
411 industrial applications (e.g., production of health foods, biomolecules, feed for aquaculture, jet fuels) and

412 to overcome limitations of biomass production. It has already been shown that the production of high
413 added value molecules such as lipids can be increased by modifying specific diatom genes (Hao et al. 2018;
414 Muto et al. 2015). Diatoms can also be exploited in targeted drug delivery using genetically engineered
415 diatom biosilica (Delalat et al. 2015) or as a platform for the production of heterologous products such
416 as plant-derived monoterpenoids (Fabris et al. 2020) or monoclonal antibodies (Hempel et al. 2017). The
417 application of genetic engineering to a wide range of diatom species will further strengthen the field of
418 diatom biotechnology in the future.

419 **7 - Cryopreservation**

420 Most algal strains are maintained in a perpetual active culture state and must therefore be periodically
421 transferred to fresh culture medium. Despite being the most commonly employed method of maintenance,
422 perpetual active culture results in a host of potentially significant problems, perhaps the most important
423 being that of genetic drift. As old methods are improved and new methods developed, enigmatic scientific
424 results issuing from studies using cultures can be reevaluated, but this presupposes that the biological
425 material does not change in phenology or genome content over time. Researchers have known for decades
426 that transferred cell lines have their own selective pressures, and that spontaneous mutation rates are
427 higher for microbes in culture than for the same microbe in the natural environment (Lakeman et al. 2009;
428 Phillips et al. 1994). Cryopreservation is an alternative to perpetual culture that ensures genotypic (and
429 consequently physiological) stability over time, but not all microalgal strains can be/have been successfully
430 cryopreserved.

431 Cryopreservation is the storage of viable cells at ultra-low temperatures of liquid nitrogen (LN2, -196 °C)
432 and/or its vapour phase (-156 °C). The underlying principle of cryopreservation is that at ultra-low tem-
433 peratures, vital functions, such as enzymatic activity and cell division, are slowed to the point of cessation,
434 but not death (Benson 2008). A fundamental discovery in implementing successful cryopreservation was
435 made by Polge et al. (1949) working with fowl sperm. They observed that at low temperatures, the sperm
436 could be "protected" by using glycerol. Since then, cryopreservation has been used as a means to store
437 (or "cryobank") biological materials including seeds, viruses, bacteria, and mammalian and plant tissue
438 culture cells as a hedge against loss of valuable research resources. Unicellular algae have been cryopre-
439 served since the early 1960s, although new methods were required due to poor success rates and alteration
440 of algal ultrastructure (Morris 1976; Plattner et al. 1972). Morris and colleagues published an extensive
441 experiment in 1978 successfully cryopreserving 252 of 284 strains of Chlorococcales (green algae) using a
442 two-step cooling protocol (Morris 1978). Cryopreservation methodology for algae has and must continue

443 to evolve (Day et al. 2010).

444 The primary challenge in cryopreservation is to prevent formation of ice crystals that can lead to damage
445 to intracellular material. Achieving this goal has been approached through methodological aspects, namely
446 rate and/or type of freezing process and use of cryoprotectants.

447 **Freezing Methods** Although a range of freezing protocols have been developed for the cryopreservation of
448 algae (Ramon et al. 2002), they fall into two main categories: (1) rapid cooling, and (2) 'two-step' cooling.
449 The rapid cooling technique consists of plunging the algal material, suspended in culture medium with
450 an appropriate cryoprotective agent, rapidly into liquid nitrogen at $-196\text{ }^{\circ}\text{C}$. At such rapid cooling rates
451 however, the internal solution of the material becomes supercooled, increasing the possibility of damaging
452 intracellular structure due to ice formation (Karlsson and Toner 1996; Meryman 1966). Most freezing
453 protocols avoid this by utilizing a two-step cooling process, with controlled or semi-controlled cooling from
454 room temperature (generally at a rate of ca. $-1\text{ }^{\circ}\text{C min}^{-1}$) to a holding temperature of around -30
455 $^{\circ}\text{C}$ before the material is plunged into liquid nitrogen to complete the freezing process. The first step
456 can be performed using simple devices such as Mr Frosty[®] or more sophisticated controlled-rate freezers.
457 Damage is thought to be prevented using this method because the reduced cooling rate allows sufficient
458 time for osmotic equilibrium to be maintained by shrinkage of the cell. Another method, common in higher
459 plants, to minimize intracellular damage upon freezing is the process of vitrification, which is ultra-rapid
460 cooling in the presence of high concentrations of cryoprotective agents, (first described by Rall and Fahy
461 1985). Although not widely used to date, vitrification does appear to have some potential use in the
462 cryopreservation of algal material. A more detailed description of vitrification methods can be found in
463 Steponkus et al. (1992) and Karlsson and Toner (1996). A more recently employed method with algae is
464 encapsulation-dehydration (Hirata et al. 1996). This technique involves the dehydration of encapsulated
465 algal cells by means of sterile air drying followed by immersion and storage in liquid N_2 . The method has the
466 advantage that no toxic cryoprotectants are required, hence upon thawing there can be direct cultivation
467 without the need for repeated washing of the sample.

468 **Cryoprotectants** Cryoprotective agents (CPAs) are added to offer protection from cell damage during
469 freezing and thawing processes. CPAs are low molecular weight compounds that passively traverse the
470 plasma membrane to equilibrate the solute concentration between the cell interior and the extracellular
471 matrix (Day and Brand 2005). Different CPAs may act in different ways such as lowering the temperature
472 at which intracellular water freezes (Franks 1985), minimizing osmotically driven decreases in cell volume
473 during freezing (Canavate and Lubian 1995) or altering membrane properties such as solute permeability
474 (Santarius 1996). As an important mode of action of CPAs is the stabilization of membranes, and con-

475 sidering that membrane damage can be a primary initiator of free radical reactions, it is not surprising to
476 find that several CPAs may also act as free radical scavengers (Benson 1990; Canavate and Lubian 1995).
477 Hubálek (2003) reviewed the use of cryoprotectants in microorganisms. For microalgae, DMSO was far
478 and away the most commonly used CPA. While cryopreservation will conceptually arrest genetic drift (the
479 veracity of this statement is limited by the relatively short time that researchers have been successfully
480 cryopreserving microalgae), a valid question remains whether there is any genetic selection by the cryop-
481 reservation event itself. Müller et al. (2005) showed that for *Chlorella vulgaris* AFLP patterns were identical
482 pre- and post-cryopreservation, suggesting no genetic selection during the event.

483 Cryopreservation of marine algae without CPAs has been reported in a very few instances, and the results
484 for some species are not consistent between studies. Freezing-induced membrane damage results primarily
485 from severe dehydration. Phospholipids are the major component of plant membranes, and their degree
486 of fatty acid unsaturation and length naturally influence both flexibility and permeability of membranes
487 (Ramon et al. 2002). In addition, strains that are desiccation resistant may be predisposed to successful
488 cryopreservation. It has been suggested that extracellular freezing and desiccation ultimately stress cells
489 similarly, namely that both lead to an increase in the osmotic stress on the cell through the deprivation
490 of free water. A number of diatom strains held by NCMA have been cryopreserved with and without the
491 addition of CPA (Figure 10). While some were not successfully cryopreserved without CPA (i.e., CCMP581,
492 CCMP1500, CCMP151, CCMP552), many were successfully cryopreserved in both treatments. A primary
493 observation, however, was a delayed growback time (time to reach pre-cryopreservation cell abundance) in
494 the samples cryopreserved without CPA.

495 **Thawing.** Thawing is critical for the reactivation of cultures. Several parameters are critical, notably
496 osmotic and toxic shocks and light stress. In order to limit osmotic shocks and the intracellular and resulting
497 extracellular damages, frozen cultures are preferably thawed rapidly by immersing them for about three
498 minutes in a water bath at 27°C (e.g. at RCC) or 37°C (e.g. at DCG). The CPA can have a toxic effect
499 on the cells and to prevent this, it is important to rapidly dilute the thawed aliquot in a sufficient volume
500 of fresh culture medium. It is possible to include a centrifugation step to pellet the cells and remove
501 the CPA before re-culturing, but this step induces additional stress and decreases survival chances. In
502 order to slowly revive the cultures, they are placed in optimal culture conditions (medium, temperature),
503 while light is introduced progressively after a 24 to 48 hours phase of darkness or semi-darkness, thus
504 limiting the light stress. It takes a few weeks for the cultures to recover a growth rate identical to that
505 before cryopreservation. During these few weeks, monitoring is performed by light microscopy and/or flow
506 cytometry.

507 **Overall success of cryopreservation for diatoms.** Cryopreservation success for microalgae varies widely
508 between different taxonomic groups. For example, dinoflagellates are very difficult to cryopreserve (Hage-
509 dorn et al. 2015). Diatoms are relatively easy to cryopreserve, although success rates vary significantly
510 among diatom lineages (Stock et al. 2018). In the collections we surveyed (Table 3), pennate diatoms
511 appear easier to cryopreserve than centric diatoms, especially the Coscinodiscophyceae. However, there
512 are large variations between genera (Table S1). For example, while all strains of *Seminavis* tested can
513 be cryopreserved, only 50% of *Thalassiosira* strains are successful. This could be an effect of cell size,
514 as Paredes et al. (2021) reported that in terms of estimated cell volume, approximately two thirds of the
515 small ($< 1000 \mu\text{m}^3$) and intermediate ($1000\text{--}2500 \mu\text{m}^3$) categories of diatoms from the RCC collection for
516 which cryopreservation was attempted were successfully cryopreserved, whereas few of the larger (> 2500
517 μm^3) category survived cryopreservation. Paredes et al. (2021) also reported that in terms of geographic
518 origin, approximately only two thirds of temperate and polar diatom strains for which cryopreservation was
519 attempted were successfully cryopreserved, whereas almost all tropical strains (albeit from a lower total
520 number) grew after thawing. Cell size therefore appears to be an important factor in cryopreservation suc-
521 cess, with cold-adapted (polar) strains apparently not showing an increased resistance to cryopreservation.

522 **8 - Conclusion**

523 Culture collections are critical for diatom research. However, of the ca. 8,400 described diatom species
524 (Guiry 2012), only around 10% are currently maintained in culture, which is a small proportion given that
525 the real extent of diatom biodiversity is estimated to be in the region of 100,000 species. This highlights the
526 necessity to continue and amplify isolation efforts, especially for species from environments that have been
527 relatively undersampled to date, such as plant and animal microbiomes or sediments. It is also important
528 that researchers that isolate diatoms (and other algae) deposit them in public collections and it is of course
529 even more critical that culture collections remain funded to be able to maintain existing cultures and expand
530 the range of strains available for research and industry (Becker et al. 2019).

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Table 1: List of culture collections containing diatoms.

Acronym	Name	# of strains	Country	Web site
ACOI	Coimbra Collection of Algae	16	Portugal	http://acoi.ci.uc.pt/index.php
ACUF	Algal Collection University Frederico II	20	Italy	http://www.acuf.net/index.php?lang=en
ALGOBANK	Algobank-Caen		France	https://borea.mnhn.fr/en/algobank-caen
ALISU	Culture Collection of the Universidade de Lisboa	9	Portugal	
ANACC	Australian National Algae Culture Collection	336	Australia	https://www.csiro.au/ANACC
ARC	Algal Resources Collection	9	USA	https://www.algalresourcescollection.com/
BCCM/DCG	BCCM/DCG Diatoms Collection	536	Belgium	https://bccm.belspo.be/about-us/bccm-dcg
BEA	Banco Español de Algas		Spain	https://marinebiotechnology.org/en/
BMAK	Aidar & Kutner Microorganisms Collection	73	Brazil	https://www.io.usp.br/index.php/infraestrutura-io-2/banco-de-microorganismos.html
CCAC	Central Collection of Algal Cultures		Germany	https://www.uni-due.de/biology/ccac/
CCALA	Culture Collection of Autotrophic Organisms	4	Czech Republic	https://ccala.butbn.cas.cz/
CCAP	Culture Collection of Algae and Protozoa	165	UK	https://www.ccap.ac.uk/
CCBA	Culture Collection of Baltic Algae		Poland	https://ccba.ug.edu.pl/pages/en/home.php
CCCM	Canadian Center for the Culture of Microorganisms		Canada	https://cccm.botany.ubc.ca/
CICCM	Cawthron Institute Culture Collection of Micro-algae	135	New Zealand	https://cultures.cawthron.org.nz/
CMR.RBI	Culture collection of the Center for Marine Research, Ruder Boskovic Institute	152	Croatia	
CPCC	Canadian Phycological Culture Centre	31	Canada	https://uwaterloo.ca/canadian-phycological-culture-centre/
DSMZ	Leibniz-Institut Deutsche Sammlung von Mikroorganismen und Zellkulturen	50	Germany	https://www.dsmz.de/
FACHB	Freshwater Algae Culture Collection at the Institute of Hydrobiology		China	http://algae.ihb.ac.cn/English/
FINMARI CC	FINMARI Culture Collection/SYKE Marine Research Centre and Tvorminne Zoological Station	30	Finland	
IRTA	Institute of Agriculture and Food Research and Technology of Catalonia Collection		Spain	
MBA	MBA Culture Collection		UK	https://www.mba.ac.uk/facilities/culture-collection
MBRU	Russian Marine Biobanks	24	Russia	http://marbank.dvo.ru/index.php/en/marine-genetic-resources
MCC	Microbial Culture Collection - National Institute for Environmental Studies Collection	124	Japan	https://mcc.nies.go.jp/
MEDIC	Marine Epibiotic Diatom Collection	126	South Africa	http://ipt.sanbi.org.za/iptsanbi/resource?r=diatom
MNHN-ALCP	Collection du Muséum d'Histoire Naturelle, Paris	3	France	https://www.mnhn.fr/en/collections/collection-groups/biological-resources-living-and-cryopreserved-cells/microalgae-and-cyanobacteria
MUC	Mersin University Collection	16	Turkey	
NBRC	Culture Collection Division, Biological Resource Center, National Institute of Technology and Evaluation	14	Japan	https://www.nite.go.jp/nbrc/catalogue/catalogueAdvSearch
NCMA	Provasoli-Guillard National Center for Marine Algae and Microbiota	585	USA	https://ncma.bigelow.org/
NORCCA	Norwegian Culture Collection of Algae	290	Norway	https://norcca.scrol.net/
RCC	Roscoff Culture Collection	939	France	https://roscoff-culture-collection.org/
SAG	Sammlung von Algenkulturen der Universität Göttingen		Germany	https://www.uni-goettingen.de/en/culture+collection+of+algae+%28sag%29/184982.html
SMS	Singapore Marine Strains		Singapore	
SZCZ	Szczecin Diatom Culture Collection	2151	Poland	http://geocentrum.usz.edu.pl/en/szczecin-diatom-culture-collection-szcz/
SZN	Stazione Zoologica di Napoli		Italy	
TCC	Thonon Culture Collection	193	France	https://www6.inrae.fr/carrtel-collection_eng/
UTEX	Culture Collection of Algae at the University of Texas at Austin	390	USA	https://utex.org/

Table 2: Summary of published transformable diatom species and of the methods used for genetic transformation. Abbreviations: sh-ble: Streptoalloteichus hindustanus Zeocin/bleomycin resistance gene; nat1: nourseothricin acetyltransferase; nptII; neomycin phosphotransferase II; sat, streptothricin acetyl transferase; bsr, blasticidin-S deaminase.

Species	Method	Marker gene	Antibiotic	References
<i>Cyclotella cryptica</i>	Biolistic	nptII	G418	Dunahay et al. 1995
<i>Navicula saprophila</i>	Biolistic	nptII	G418	Dunahay et al. 1995
<i>Phaeodactylum tricornutum</i>	Biolistic	sh-ble	Phleomycin/Zeocin	Apt et al. 1996
	Electroporation	nat1	Nourseothricin	Falciatore et al. 1999
	Bacterial conjugation	nptII	Neomycin	Zaslavskaja et al. 2001
		sat	Streptothricin	Buck et al. 2018
		bsr	Blasticidin-S	Niu et al. 2012
			Karas et al. 2015	
<i>Thalassiosira weissflogii</i>	Biolistic	sh-ble	Phleomycin/Zeocin	Falciatore et al. 1999
<i>Cylindrotheca fusiformis</i>	Biolistic	sh-ble	Zeocin	Fischer et al. 1999
				Poulsen and Krger 2005
<i>Thalassiosira pseudonana</i>	Biolistic	nat1	Nourseothricin	Poulsen and Krger 2005
	Electroporation	sh-ble	Phleomycin/Zeocin	Bugge and Robertson 2019
	Bacterial conjugation			Karas et al. 2015
<i>Amphora coffeaeformis</i>	Biolistic	nat1	Phleomycin/Zeocin	Buhmann et al. 2014
		sh-ble	Nourseothricin	
<i>Fistulifera solaris</i>	Biolistic	nptII	G418	Muto et al. 2015
<i>Pseudo-nitzschia multistrata</i>	Biolistic	sh-ble	Phleomycin/Zeocin	Sabatino et al. 2015
<i>Pseudo-nitzschia arenysensis</i>	Biolistic	sh-ble	Phleomycin/Zeocin	Sabatino et al. 2015
<i>Fragilariopsis cylindrus</i>	Biolistic	sh-ble	Zeocin	Faktorov et al. 2020
<i>Skeletonema marinoi</i>	Electroporation	sh-ble	Zeocin	Johansson et al. 2019
<i>Chaetoceros muelleri</i>	Biolistic	nat1	Nourseothricin	Miyagawa-Yamaguchi et al. 2011
		Electroporation	sh-ble	Zeocin
			bsr	Blasticidin-S

Table 3: Cryopreservation status by class for cultures from several collections (BCCM, RCC, NCMA, UTEX, DSMZ, MBRU, MCC). Plus sign corresponds to success and Minus no success. Cultures that were not tested were not included.

Class	+	-	% success
Bacillariophyceae	905	294	75
Bacillariophyta_X	25	3	89
Coscinodiscophyceae	11	45	20
Mediophyceae	436	263	62

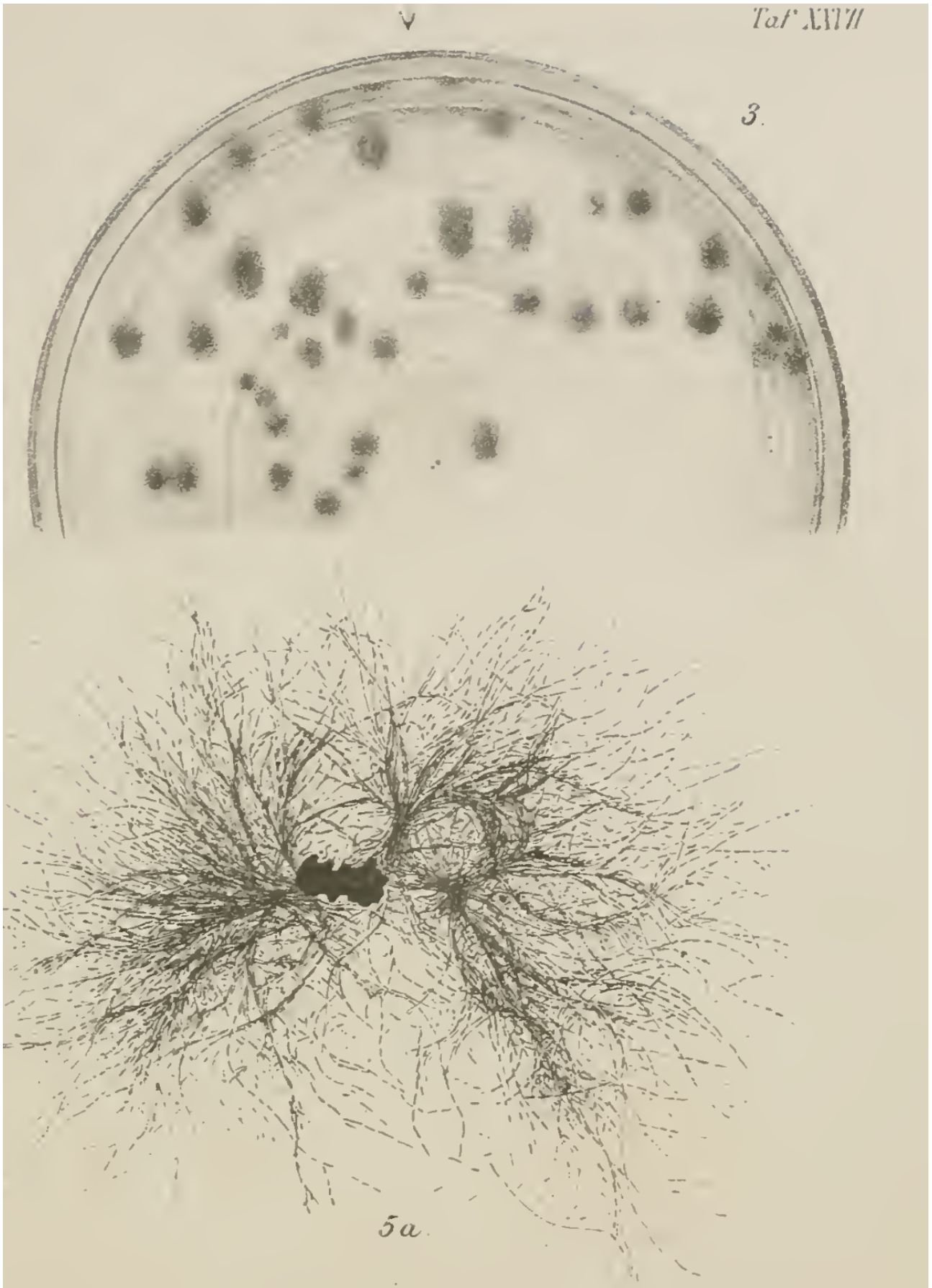


Figure 1: Colonies of *Nitzschia palea* growing on agar (reprinted from Richter 1903).

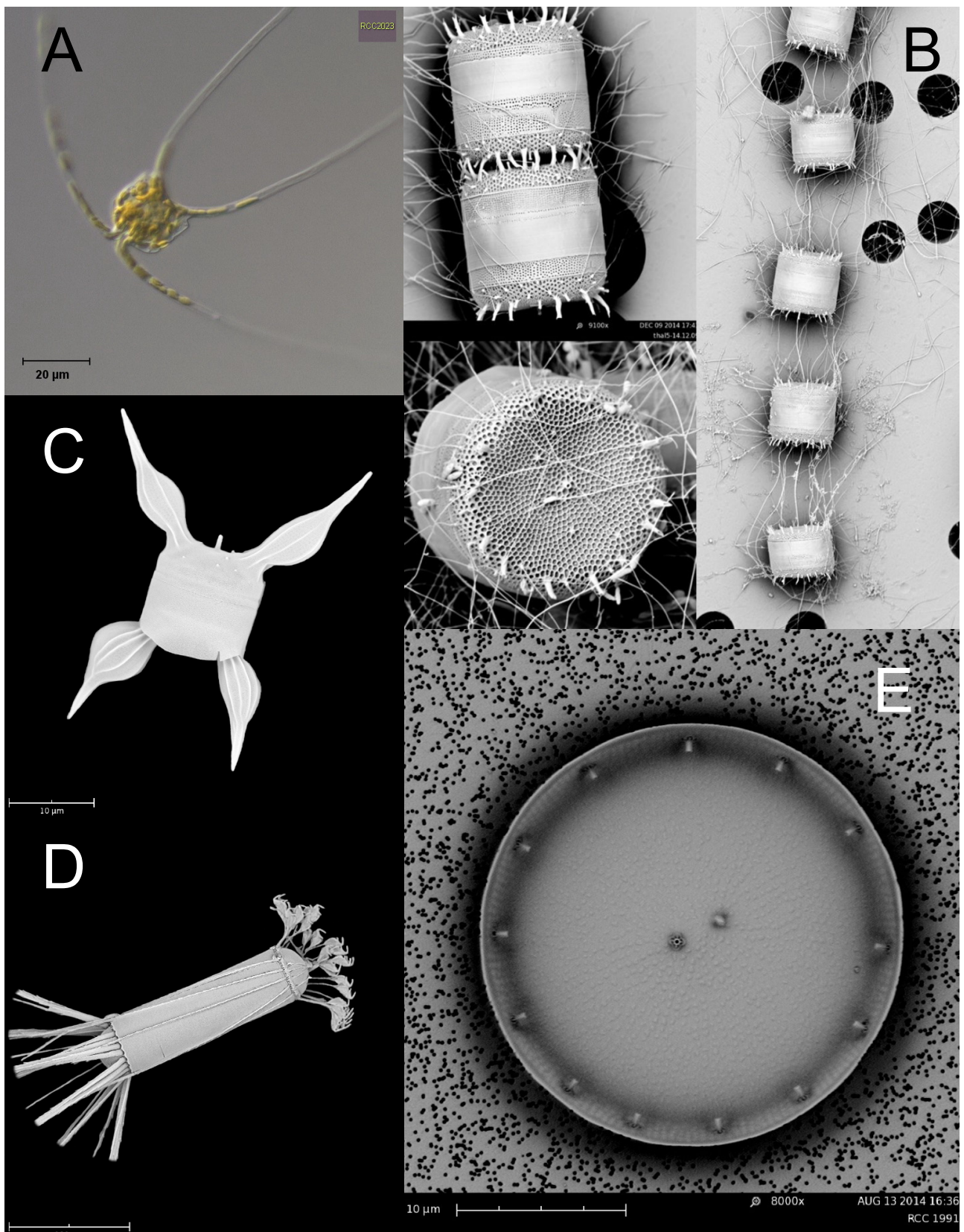


Figure 2: Examples of marine diatoms. A. *Chaetoceros peruvianus* RCC2023. B. *Thalassiosira delicatula* RCC2560. C. *Chaetoceros bulbosus* from a natural sample off New Zealand. D. *Corethron pennatum* from a natural sample off New Zealand. E. *Shionodiscus bioculatus* RCC1991.

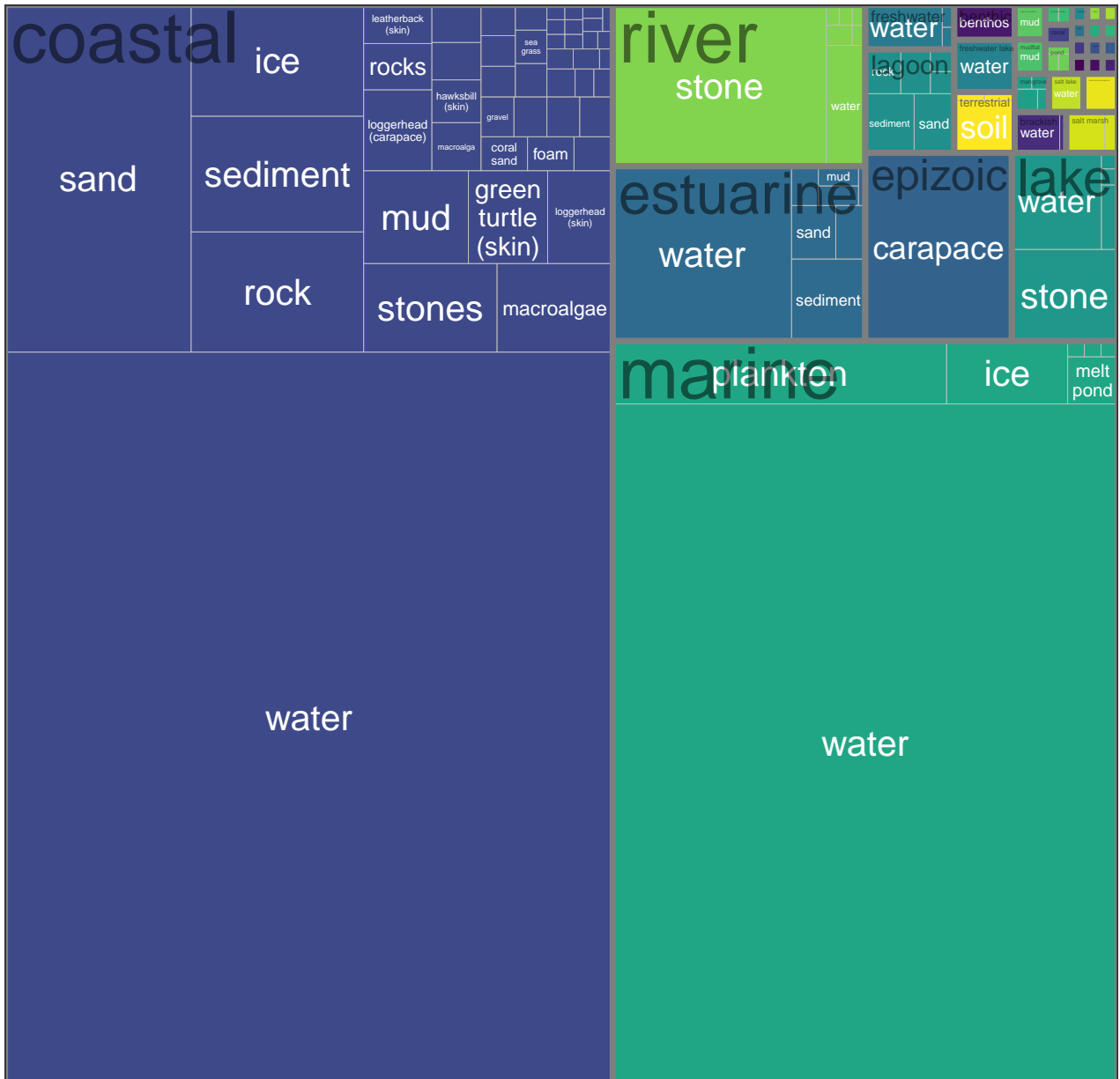


Figure 4: Origin of diatom cultures according to ecosystems and substrates. Rectangle surfaces are proportional to number of strains.

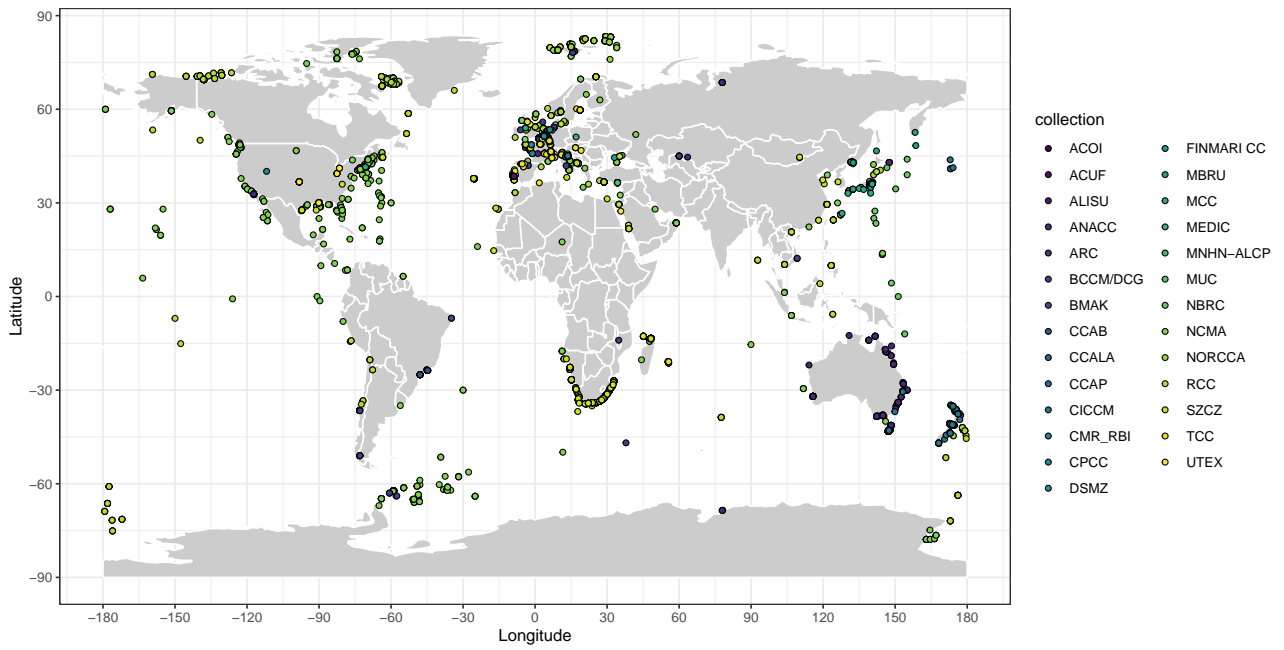


Figure 5: Isolation sites for diatom cultures for the different collections.

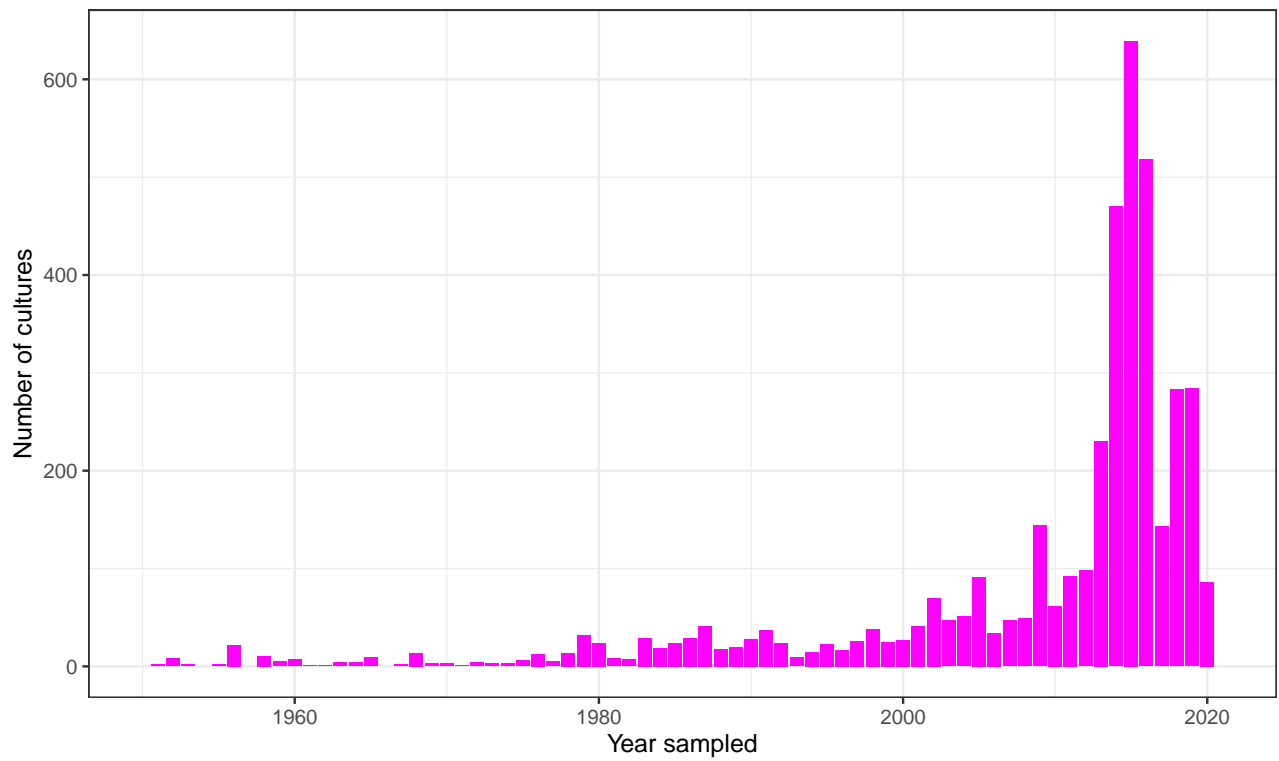


Figure 6: Year of sampling for diatom in collections.

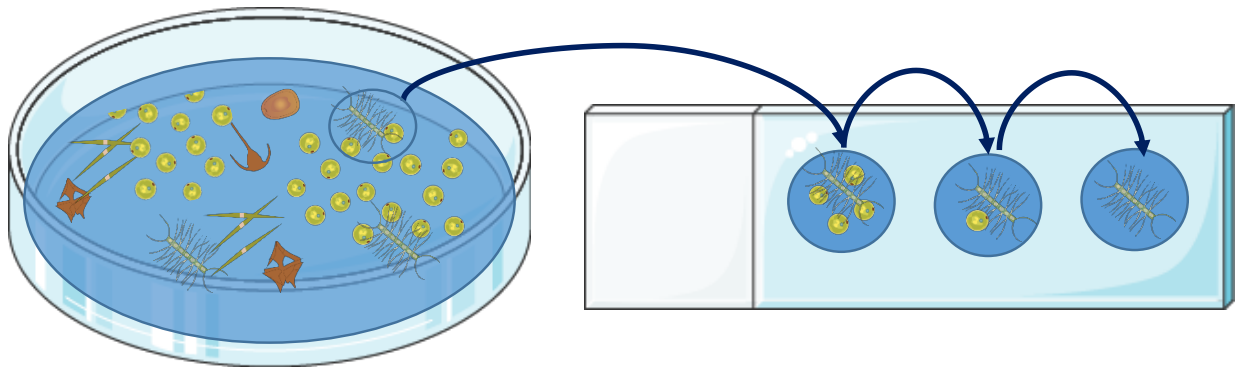


Figure 7: Single cell isolation strategy.



Figure 8: Cultures of diatoms at the Roscoff Culture Collection using either culture flasks or tubes.

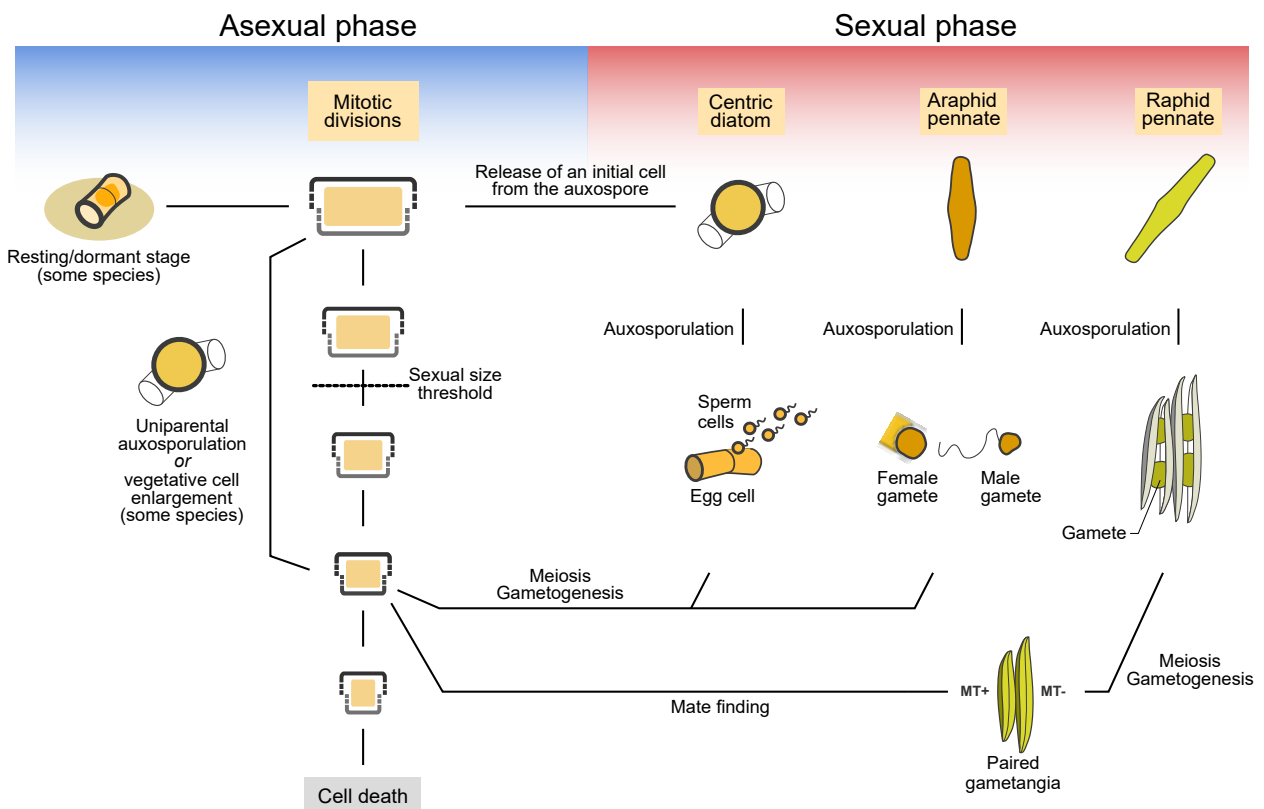


Figure 9: The life cycle of diatoms: a graphical representation of vegetative cell size decrease and the main strategies to restore cell size.

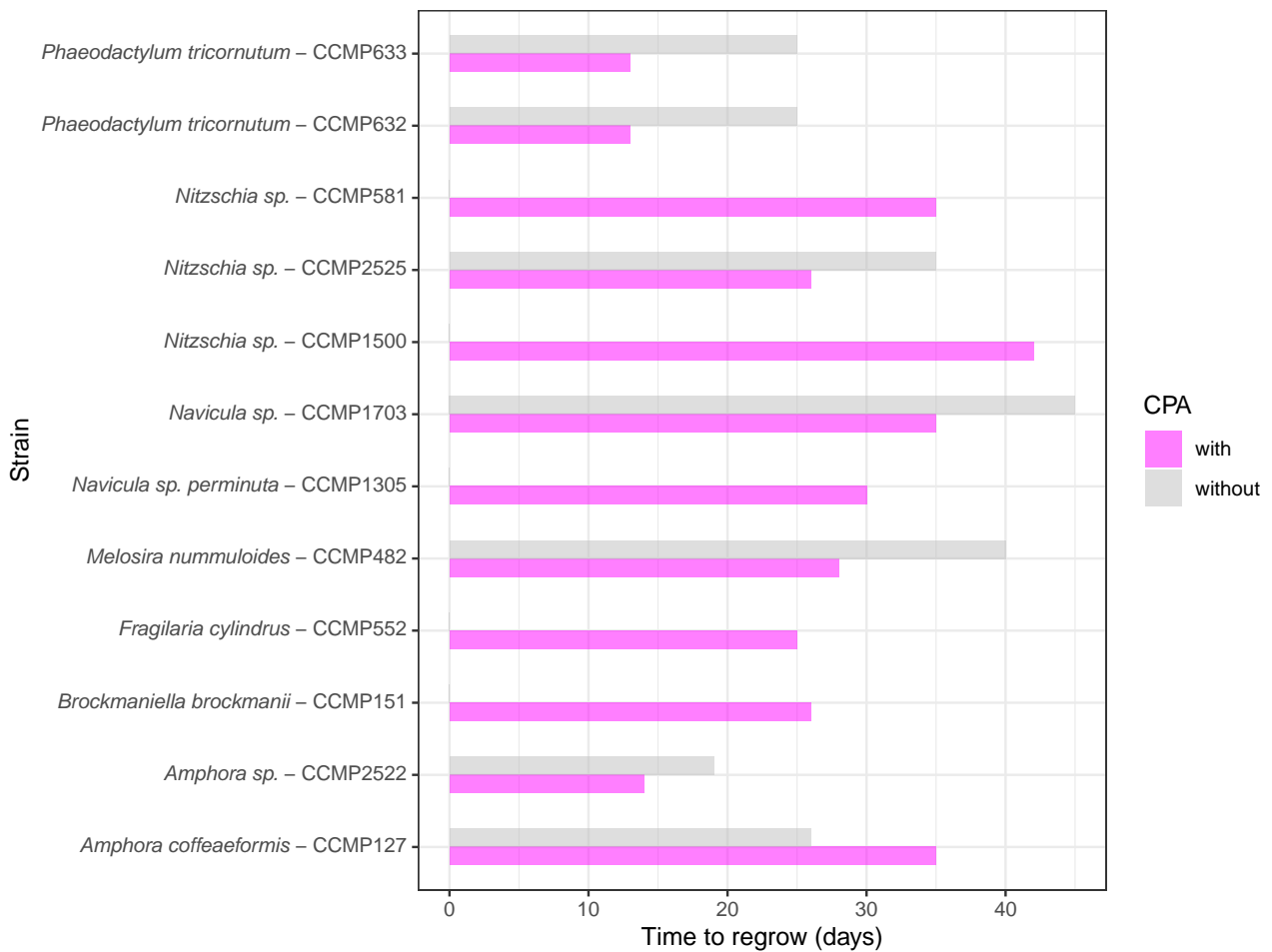


Figure 10: Growback times for diatom strains cryopreserved with and without cryoprotective agents (CPA) added.

835 **Supplementary Material**

836 **Supplementary Data S1.** List of diatom strains inventoried with associated metadata (tax-
837 onomy, origin, cryopreservation status): <https://doi.org/10.5281/zenodo.7641545>. Taxonomic
838 assignation of genera (i.e. class, order, family) is according to AlgaeBase ([https://www.
839 algaebase.org](https://www.algaebase.org)).

Table S1: Cryopreservation status by class and genus for cultures from several collections (BCCM, RCC, NCMA, UTEX, DSMZ, MBRU, MCC). Plus sign corresponds to success and Minus no success. Cultures not tested were not included.

Class	Genus	+	-	% success
Bacillariophyceae	Achnanthes	43	1	98
Bacillariophyceae	Achnanthidium	26	4	87
Bacillariophyceae	Amphiprora	2	0	100
Bacillariophyceae	Amphora	33	12	73
Bacillariophyceae	Anomoeoneis	0	7	0
Bacillariophyceae	Astartiella	1	0	100
Bacillariophyceae	Asterionella	1	0	100
Bacillariophyceae	Asterionellopsis	16	10	62
Bacillariophyceae	Bacillaria	1	0	100
Bacillariophyceae	Bacillariophyceae_XXX	43	4	91
Bacillariophyceae	Biremis	2	0	100
Bacillariophyceae	Caloneis	1	4	20
Bacillariophyceae	Campylodiscus	0	1	0
Bacillariophyceae	Cocconeis	1	1	50
Bacillariophyceae	Craspedostauros	2	0	100
Bacillariophyceae	Craticula	9	11	45
Bacillariophyceae	Cyclophora	0	1	0
Bacillariophyceae	Cylindrotheca	49	5	91
Bacillariophyceae	Cymbella	2	4	33
Bacillariophyceae	Decussata	0	4	0
Bacillariophyceae	Delphineis	1	0	100
Bacillariophyceae	Diadesmis	1	2	33
Bacillariophyceae	Diatoma	2	0	100
Bacillariophyceae	Encyonema	0	2	0
Bacillariophyceae	Entomoneis	11	10	52
Bacillariophyceae	Eolimna	2	0	100
Bacillariophyceae	Eucocconeis	1	2	33
Bacillariophyceae	Eunotia	0	9	0
Bacillariophyceae	Fallacia	2	1	67
Bacillariophyceae	Fistulifera	1	0	100
Bacillariophyceae	Fragilaria	10	3	77
Bacillariophyceae	Fragilariaceae_X	21	0	100
Bacillariophyceae	Fragilariales_XX	1	0	100

Table S1: (continued)

Class	Genus	+	-	% success
Bacillariophyceae	Fragilariforma	1	1	50
Bacillariophyceae	Fragilariopsis	24	2	92
Bacillariophyceae	Gedaniella	2	0	100
Bacillariophyceae	Gomphonema	13	10	57
Bacillariophyceae	Gomphonemopsis	0	1	0
Bacillariophyceae	Grammatophora	9	0	100
Bacillariophyceae	Grammonema	0	1	0
Bacillariophyceae	Gyrosigma	0	2	0
Bacillariophyceae	Halamphora	2	0	100
Bacillariophyceae	Hantzschia	2	9	18
Bacillariophyceae	Haslea	1	0	100
Bacillariophyceae	Humidophila	8	0	100
Bacillariophyceae	Hyalosira	1	0	100
Bacillariophyceae	Licmophora	0	2	0
Bacillariophyceae	Lucanicum	0	1	0
Bacillariophyceae	Luticola	0	1	0
Bacillariophyceae	Mayamaea	3	0	100
Bacillariophyceae	Nanofrustulum	1	0	100
Bacillariophyceae	Navicula	70	35	67
Bacillariophyceae	Naviculales_XX	19	0	100
Bacillariophyceae	Navicymbula	3	0	100
Bacillariophyceae	Neidium	3	8	27
Bacillariophyceae	Neofragilaria	0	1	0
Bacillariophyceae	Nitzschia	119	37	76
Bacillariophyceae	Opephora	1	0	100
Bacillariophyceae	Orizaformis	1	0	100
Bacillariophyceae	Pauliella	1	0	100
Bacillariophyceae	Pinnularia	6	16	27
Bacillariophyceae	Placoneis	2	1	67
Bacillariophyceae	Plagiogramma	1	1	50
Bacillariophyceae	Planothidium	1	1	50
Bacillariophyceae	Pleurosigma	0	1	0
Bacillariophyceae	Psammoneis	0	1	0
Bacillariophyceae	Psammothidium	0	1	0
Bacillariophyceae	Pseudo-nitzschia	5	8	38

Table S1: (continued)

Class	Genus	+	-	% success
Bacillariophyceae	Pseudostaurosira	1	0	100
Bacillariophyceae	Rhopalodia	0	1	0
Bacillariophyceae	Sellaphora	3	2	60
Bacillariophyceae	Seminavis	269	0	100
Bacillariophyceae	Stauroneis	8	9	47
Bacillariophyceae	Staurosira	10	0	100
Bacillariophyceae	Staurosirella	3	1	75
Bacillariophyceae	Stenopterobia	0	5	0
Bacillariophyceae	Striatella	0	1	0
Bacillariophyceae	Surirella	4	21	16
Bacillariophyceae	Synedra	10	3	77
Bacillariophyceae	Synedropsis	9	0	100
Bacillariophyceae	Tabellaria	0	1	0
Bacillariophyceae	Tabularia	0	3	0
Bacillariophyceae	Talaroneis	1	0	100
Bacillariophyceae	Thalassionema	2	3	40
Bacillariophyceae	Tryblionella	1	3	25
Bacillariophyceae	Ulnaria	0	2	0
Bacillariophyta_X	Astrosyne	0	1	0
Bacillariophyta_X	Bacillariophyta_XXXX	8	1	89
Bacillariophyta_X	Phaeodactylum	17	1	94
Coscinodiscophyceae	Amphipenteras	0	1	0
Coscinodiscophyceae	Aulacoseira	0	4	0
Coscinodiscophyceae	Corethron	0	2	0
Coscinodiscophyceae	Coscinodiscus	0	6	0
Coscinodiscophyceae	Guinardia	0	6	0
Coscinodiscophyceae	Hyalodiscus	2	0	100
Coscinodiscophyceae	Melosira	7	1	88
Coscinodiscophyceae	Orthoseira	0	3	0
Coscinodiscophyceae	Paralia	0	3	0
Coscinodiscophyceae	Podosira	0	1	0
Coscinodiscophyceae	Rhizosolenia	0	1	0
Coscinodiscophyceae	Stellarima	0	11	0
Coscinodiscophyceae	Stephanopyxis	0	4	0
Coscinodiscophyceae	Triceratium	2	2	50

Table S1: (continued)

Class	Genus	+	-	% success
Mediophyceae	Amphipentas	0	1	0
Mediophyceae	Arcocellulus	1	0	100
Mediophyceae	Attheya	68	2	97
Mediophyceae	Bacterosira	0	1	0
Mediophyceae	Bellerochea	0	1	0
Mediophyceae	Biddulphia	0	4	0
Mediophyceae	Brockmanniella	1	0	100
Mediophyceae	Cerataulus	0	1	0
Mediophyceae	Chaetoceros	85	55	61
Mediophyceae	Conticribra	3	0	100
Mediophyceae	Cyclostephanos	0	1	0
Mediophyceae	Cyclotella	11	18	38
Mediophyceae	Cymatosira	1	1	50
Mediophyceae	Cymatosiraceae_X	23	0	100
Mediophyceae	Detonula	1	2	33
Mediophyceae	Ditylum	0	13	0
Mediophyceae	Eucampia	0	2	0
Mediophyceae	Eunotogramma	0	1	0
Mediophyceae	Extubocellulus	4	0	100
Mediophyceae	Helicotheca	0	4	0
Mediophyceae	Leptocylindrus	0	1	0
Mediophyceae	Leyanella	1	0	100
Mediophyceae	Lithodesmium	0	3	0
Mediophyceae	Mastodiscus	0	1	0
Mediophyceae	Minidiscus	52	2	96
Mediophyceae	Minutocellus	15	1	94
Mediophyceae	Odontella	4	18	18
Mediophyceae	Papiliocellulus	2	0	100
Mediophyceae	Pleurosira	0	2	0
Mediophyceae	Porosira	3	15	17
Mediophyceae	Proboscia	0	1	0
Mediophyceae	Skeletonema	83	31	73
Mediophyceae	Thalassiosira	78	81	49