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Culturing Diatoms

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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 summarize
29 information and techniques available for long term preservation of diatom cultures. Finally, we also describe
30 the main strategies to make diatom strains amenable to genetic engineering.

31 **1 - Introduction**

32 Diatoms were discovered in the 18th century, but the first cultures were not established until the middle
33 of the next century and cultivation only really developed at the end of the 19th century (Figure 1, Richter
34 1903) pioneered by Miquel (1892). Museums, botanical gardens, zoos and research facilities have served
35 as repositories to preserve collections of fixed specimens, notably type material, for centuries, with such
36 facilities deemed reliable places to safeguard specimens in the state in which they were first deposited,
37 be sure that they are cared for, and be able to distribute them to researchers so that scientific research
38 can be reproduced and expanded (Smith and Ryan 2012). The development of repositories for living
39 material is more recent, Ernst Pringsheim having established the first culture collection catalogue in 1928.
40 Cultures are extremely useful for studies on diatom taxonomy and life cycles, although such work can also be
41 undertaken on natural populations (Mann and Chepurinov 2004). Cultures are, however, absolutely essential
42 for physiological studies, in particular in the domain of photosynthesis, as well as for the biochemical
43 characterization of cells. Cultures are also key for the acquisition of 'omics' data, which is playing a central
44 role for understanding the regulation of cell growth and functions (Armbrust et al. 2004; Bowler et al.
45 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, culture
50 and preservation of diatoms, detailing constraints linked to the specific life cycle of diatoms. We end by
51 reviewing recent advances in the development of techniques for the genetic transformation of cultured
52 diatoms, which are opening new avenues for the production of novel functional knowledge.

53 **2 - Current diversity of diatoms in culture**

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

56 We identified 27 algal collections for which we were able to obtain listings of the diatom cultures they
57 currently maintain (Table 1). More collections contain diatom strains, but they either escaped our search
58 or did not answer our request for information. Some collections harbouring many diatom strains have
59 also disappeared (for example, the KMMCC-Korea Marine Microalgae Culture Center Hur et al. 2015).

60 Some of these collections are registered with the World Data Center for Microorganisms (WDCM, <https://ccinfo.wdcm.org>), while some do not have a website where researchers can order strains. The number
61 of diatom cultures in these collections varies from a few to more than two thousand. Some collections
62 are specialized for marine (e.g., RCC, NCMA) or freshwater (e.g., TCC) ecosystems, while others such
63 as UTEX and BCCM/DCG contain marine, freshwater and terrestrial strains. In total, these collections
64 currently hold more than 6,400 diatom strains (Supplementary Data S1). Depending on the collection, the
65 amount and type of associated metadata varies considerably.

67 In general, strains are taxonomically identified at the species level, although in many cases only identifi-
68 cation at higher taxonomic levels (e.g., class, order, family or genus) is available. The majority of strains
69 belong to the pennate Bacillariophyceae (3,746), followed by the centric classes (Mediophyceae: 1625,
70 Coscinodiscophyceae: 316). More than 300 strains have not been assigned at the class level. Identified
71 strains belong to 216 genera and 828 species. This represents less than 5% of the 18,537 diatoms species
72 listed in Algaebase (Guiry 2012), underlining the need to develop isolation efforts. The best represented
73 genera in culture are *Seminavis*, *Navicula*, *Nitzschia*, *Pseudo-nitzschia*, *Thalassiosira*, *Chaetoceros* and
74 *Skeletonema*, while some of the best represented species are the coastal benthic *Seminavis robusta* and the
75 polar pelagic *Chaetoceros neogracilis* (Figure 3). The prevalence of particular genera and species in culture
76 holdings may reflect the ease with which these species can be isolated, but may also reflect the interest of
77 a particular laboratory or collection in specific genera or species. For example, the genus *Pseudo-nitzschia*,
78 some species of which can produce domoic acid (Bates et al. 2018), has been the focus of numerous
79 research projects, leading to numerous strains being isolated.

80 More effort has been devoted to isolation of marine diatoms (Figure 4) compared with those from freshwater,
81 while terrestrial ecosystems (e.g. soil) are underrepresented. Most isolation has been performed from water
82 column or benthic samples, although microbiomes from animals or plants (e.g., biofilms from turtles or
83 from macroalgae) and ice have received some attention. Most diatom cultures originate from coastal and
84 continental habitats in Europe, the US, Japan, Australia and New Zealand (Figure 5). While polar areas
85 (both Arctic and Southern Oceans) are quite well covered, very few diatom strains have been isolated from
86 oligotrophic oceanic regions, where they are of course less abundant and often harbour symbionts (Foster
87 and O'Mullan 2008), which may hinder cultivation. Although the oldest diatom strains held in a collection
88 were isolated prior to 1960, there has clearly been an increase in the number of strains isolated in the last
89 10 years (Figure 6). A peak was reached around 2016, but this could be simply due to a larger number of
90 cruises from which diatoms were isolated. For example, the Green Edge expedition in the Arctic in 2016
91 led to the isolation of several hundred diatom strains (Gérikas Ribeiro et al. 2020).

92 **3 - Isolation of diatom cultures**

93 **3.1 Sampling**

94 Sampling for diatoms (and more generally for phytoplankton) has to be adapted to the targeted ecosystem.
95 Surface plankton can be collected with a net with a mesh size adapted to the cell size of targeted taxa
96 (e.g. 5 μm , 10 μm , 20 μm , 64 μm). In order to preserve the most fragile cells, it is critical to tow the net
97 at slow speed. Towing time will of course depend on the cell concentration in the water and will be longer
98 when sampling more oligotrophic waters. Samples can also be collected at different depths in the water
99 column with Niskin bottles attached to a cable or mounted on a Rosette sampling device. For samples from
100 benthic sediments or ice, special tools such as box corers or ice corers may be required. For ice, melting
101 at room temperature with the addition of 0.2 μm filtered seawater may be necessary. It is important to
102 record as many parameters as possible when collecting samples, in particular substrate type, ecosystem
103 type, geographical position (longitude and latitude), depth or altitude, temperature, salinity, pH and where
104 possible nutrient concentrations (nitrate, phosphate, silicate, etc.). These parameters will be critical for
105 documenting the origin of the isolated strains, in particular to assess the status of the strain relative to
106 Access and Benefit Sharing regulations (see below), and may also help to define optimal growth conditions.

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

113 The samples can either be processed immediately or enriched by adding a small volume of culture medium
114 (typically 1 to 10% of the volume of the sample) or of filtered natural seawater collected at the same
115 location (to mimic as much as possible in situ conditions). Samples are then incubated at a temperature
116 and photoperiod matching those prevailing in the environment from which the sample was taken. Enriched
117 samples (also called "pre-cultures") should be placed in a culture cabinet and regularly monitored by
118 techniques such as optical microscopy or flow cytometry. Pre-culturing can be used to select for specific
119 characteristics, for example by inoculating a plankton sample in nitrogen deplete medium to pre-select
120 potential diazotrophs.

121 3.2 Isolation strategies

122 Several isolation strategies can be employed on the same sample to maximize the chances of success and
123 the diversity of cultures obtained (for a detailed description of algal isolation techniques, refer to the book
124 by Andersen 2005).

125 **Serial dilution.** Serial dilution, consisting of repeatedly diluting a sample or an enrichment by transferring a
126 small volume of each successive dilution to fresh medium, is one of the most common approaches (Andersen
127 2005). The principle of serial dilution is to dilute the sample to extinction, such that the last dilution that
128 contains any cells is likely to contain just one cell, from which a pure (mono-specific) culture is initiated.
129 Serial dilution is most easily carried out in multi-well plates (typically 24, 48 or 96 wells), but tubes or flasks
130 can also be used. Dilutions are maintained in optimal growth conditions (temperature, light) and culture
131 growth should be observed within a few days, but it is advisable to wait for 1 to 2 weeks before transferring
132 in order to verify the purity of the isolates. Serial dilution is cheap to perform and does not require specific
133 skills or equipment, but is a non-targeted method, meaning there is no guarantee of selecting taxa of
134 interest. It can, however, lead to successful isolation of rare and/or fragile taxa from mixed samples that
135 may be difficult to isolate by other methods. Another significant drawback of this method is that there is
136 no practical way of knowing whether the culture was initiated from a single cell (and hence is clonal) or
137 from two or more cells of the same species. If clonal cultures are required, it is therefore recommended to
138 re-isolate cultures resulting from successful serial dilution using one of the other methods listed below.

139 **Single cell isolation.** Single cell isolation is usually undertaken with a drawn-out Pasteur pipette, which
140 allows cells to be manually selected one at a time from the natural sample or the enrichment under a
141 microscope (ideally an inverted microscope which has more space for manipulating cells). The collected
142 cell is placed in a drop of clean culture medium to be washed. This step will be repeated as many times as
143 necessary until the cell is free from other protists (Figure 7A). Aspiration is controlled by adding either a
144 suction bulb or a tube with a mouthpiece to the end of the pipette. Single cells isolated in this manner are
145 placed in sterile culture medium in a tube or a multi-well plate and maintained in optimal growth conditions
146 in terms of temperature and light. These different steps can be viewed at [www.youtube.com/watch?v=](http://www.youtube.com/watch?v=0hNWVtRGeXI)
147 [0hNWVtRGeXI](http://www.youtube.com/watch?v=0hNWVtRGeXI). Micropipette isolation is very targeted and should result in clonal cultures of species of
148 interest. However, it requires considerable technical expertise and is most effective for reasonably large
149 cells (typically above 10 μm).

150 **Solid medium.** More commonly employed for bacterial culture, the use of solid or semi-solid agar media
151 can also be useful for the isolation of diatom cultures. For both streaking and inclusion techniques, 0.5-2%

152 agarose is added to the culture medium (supplemented with silica for diatoms). For streaking, a drop of
153 a few microlitres (3-25, depending on the richness of the natural sample) is streaked onto the agar plate
154 using the dial method (Figure 7B). Using the ring of the loop, tight striations are very delicately drawn
155 on the first dial, so as not to damage the agar. Then the loop is flamed and left to cool. The box is
156 turned by 90° towards the second dial and a trace is drawn in the same way as the first. This is repeated
157 for the third dial, this time inoculating it with streaks that are not tightly spaced, that do not overlap
158 the previous streaks. This simple technique allows separation of taxa that will appear after a few weeks
159 in the form of spots (corresponding to colonies originating from a single cell) on the agar. To guarantee
160 a clonal culture, it is recommended to transfer the colony to liquid medium and repeat the agar streaking
161 operation at least once. While this streaking method is more specific to isolation from natural samples,
162 the alternative inclusion technique is dedicated to strain purification and is detailed in the last section of
163 this chapter. Once a pure culture has been obtained by either of these methods, it can be cultured on agar
164 plates or transferred to liquid medium. This method is quite easy and economical, but it may take several
165 weeks to establish pure cultures.

166 **Flow cytometry sorting.** An alternative method for cell isolation is the use of a sorting flow cytometer.
167 Flow cytometry can distinguish populations according to pigment autofluorescence (chlorophyll, phycoery-
168 thrin) and light scatter, which is a proxy of size. A flow cytometer with sorting capacity makes it possible
169 to select cells according to their characteristics and to deposit one or several cells in tubes or multi-well
170 plates containing a suitable culture medium. Many diatoms form chains or colonies which hinder flow
171 cytometric analysis and sorting, a constraint that can potentially be offset by vigorous shaking or vortexing
172 of the sample prior to sorting. Due to the presence of the frustule, most diatoms are relatively robust
173 compared to other microalgae and thus survival rates upon passage through a flow cytometer tend to be
174 relatively high. For fragile species, Marie et al. (2017) described a method involving the use of bovine
175 albumin serum (BSA) and antibiotics to mitigate the effect of the stress to which the cells are subjected
176 during flow cytometry (laser, shear stress, bacterial dominance, etc.) and thereby increase success rate of
177 culture isolation. Flow cytometry sorting allows the very rapid preparation of a large number of isolates,
178 but it requires a sorting flow cytometer, which is expensive and in general is difficult to operate. Its success
179 rate can be low, and it is impossible to differentiate diatoms from other phytoplankton based on their flow
180 cytometry characteristics, so, in the case of diatoms, this method is non-targeted.

181 3.3 Culture media for isolation

182 Each of these approaches has its advantages and disadvantages, but it should be emphasized that the
183 isolation medium is a critical element that can select for certain taxa. For marine diatoms, for example,
184 f/2 (Guillard 1975) enriched with silica is often used. Media can be used at different dilutions and can
185 be prepared with seawater originating from different environments. Standard media can also be modified
186 by adding for example soil extract and there is undoubtedly considerable scope for the formulation of new
187 media to broaden the list of diatoms that can be successfully cultured. To limit the growth of bacteria,
188 and thus maximize the chances of successful isolation, the culture medium can be supplemented with a
189 mix of antibiotics (e.g. Penicillin - Streptomycin - Neomycin solution, Sigma-Aldrich P4083). However,
190 there may be cases when microalgal growth depends on bacteria (Riquelm et al. 1988) and antibiotics may
191 be detrimental.

192 3.4 Characterization of isolates

193 Once single diatom cells have been separated by these different approaches, isolations should be regularly
194 monitored either by light microscopy with or without fluorescence or by flow cytometry. Cultures that
195 appear pure should rapidly be transferred to fresh medium. After a period of a few months (beyond which
196 cultures are generally considered to be acclimated), cultures can be taxonomically characterized, which
197 for diatoms typically involves observation by scanning electron microscopy (SEM) and/or sequencing of
198 a short genetic barcode. This information is useful to interpret field metabarcoding surveys and help to
199 provide to external researchers cultures that are reliably identified. Due to the presence of the silica frustule,
200 preparation of diatom cultures for SEM is in general relatively straightforward (filtration onto a membrane
201 filter, drying, sputter coating), but for species that produce organic material that covers the frustule, a
202 cleaning step (typically in boiling nitric acid) may be necessary in order to be able to clearly visualize the
203 frustule (Seaborn and Wolny 2000). For diatoms, barcoding typically involves sequencing of the V4 region
204 of the 18S rRNA gene, which is a short region located roughly between 500 and 1000 base pairs. In
205 some cases the 18S rRNA gene is not variable enough to discriminate between closely related species or
206 subspecies, and it may therefore be necessary to sequence other genes, such as the 28S rRNA gene and/or
207 the ITS region of rRNA operon, or plastidial (e.g. *rbcL*) or mitochondrial (e.g. *cox1*) markers (Evans et al.
208 2007; Rimet et al. 2019). Characterization of cultures allows dereplication of duplicate strains, with several
209 potential strategies that can be used to keep strains of interest. For example, if the same species has been
210 isolated several times from several stations, only one or two strains from each sampling station may be
211 kept.

212 3.5 Information management

213 It is very important to keep all of the information about the isolated strains in a database. Each strain
214 should have a unique and permanent identifier and it is important to add as much metadata as possible
215 related to the sampling site (see above), sampling permits, culture isolation (isolation method, isolator,
216 medium used for isolation, etc.), as well as information pertaining to the taxonomy (ideally including links
217 to reference databases such as AlgaeBase, www.algaebase.org), genotype (links to barcode and genome
218 sequences) and phenotype (images, size, pigment composition, etc.) of the strains. This information
219 is particularly relevant in the context of possible restrictions on the use of strains (and potentially also
220 digital sequence information issuing from strains) related to the Nagoya Protocol of the Convention for
221 Biological Diversity, which sets out guidelines for regulation of access to genetic resources and the fair and
222 equitable sharing of benefits arising from their utilization (so-called "ABS" legislation). Culture collections
223 are ideally placed to facilitate implementation of, and adherence to, such regulations that depend on long-
224 term traceability of information related to strains. For culture collections, it is therefore recommended to
225 avoid using Excel files that are only local to a computer and do not allow easy sharing of information. The
226 best option is a SQL relational database (e.g. MySQL) hosted on a cloud server. Such a database allows
227 all personnel from the culture collection to simultaneously access and update data. It also allows the data
228 to be displayed on a website that external users can access to obtain information about the strains and
229 eventually order them. Although such a database can be developed and maintained in house using tools
230 such as R which allow both the management of the database and the development of websites using R
231 shiny (<https://shiny.posit.co>), another solution is to subcontract to a web development company that will
232 ensure the maintenance of the website, in particular if the website is used to order strains.

233 4 - Culture of diatoms

234 Diatom strains are generally considered to be relatively easy to grow (at least on the short term) using
235 standard microalgal culturing techniques. A wide range of media have been developed for culturing marine
236 microalgae (for an exhaustive list, refer to the book by Andersen 2005). For marine diatoms, one of the first
237 media to be employed was the simple Erd-Schreiber's (ES) medium containing soil extract (Föyn 1934). In
238 the last 50 years, this has been replaced by more sophisticated media such as f/2 (Guillard 1975), K (Keller
239 et al. 1987) and L1 (Guillard and Hargraves 1993). f/2 can be made from its constituent components,
240 but can also be purchased commercially. Low nutrient media such as MET-44 (or f/2 diluted 10 times)
241 can be useful for diatoms that originate from oligotrophic ecosystems. The main specificity for culturing

242 diatoms is that media should contain silica (usually in the form of sodium silicate), which is required by
243 diatoms for production of their frustules. Natural seawater may contain a sufficient quantity of silica to
244 maintain diatom growth without adding a supplement, particularly for species with thin frustules and/or
245 if the water is collected outside periods of diatom blooms which deplete natural silica levels. However,
246 silicate is generally added (at concentrations up to 100 μM) as a precaution when culturing diatoms, and
247 silica supplements are absolutely required when using artificial seawater based medium recipes. For cultures
248 in liquid media, plasticware (polystyrene flasks, tubes or multi well plates) or glass recipients (Erlenmeyer
249 flasks, tubes, etc.) can be used (Figure 8). Some diatom species may die out in certain brands of plastic
250 flasks, whereas they may grow fine in other brands or glass tubes. The reason for this is unclear. The
251 advantage of using pre-sterilized plasticware is to reduce preparation time and the risk of contamination,
252 but it generates a lot of waste and thus has a strong environmental impact. For cultures on solid medium,
253 the recipe is supplemented with 0.5-2% agar and following autoclaving the medium is distributed (before
254 setting) either in Petri dishes or as slants in glass tubes. All cultivation procedures should be conducted
255 under a laminar flow hood to limit bacterial contamination.

256 With respect to temperature, once diatom cultures are established, it is not necessary to exactly reproduce
257 the isolation temperature. Typical routine maintenance temperatures are 4 °C for polar species, 13 °C
258 for temperate species and 20 °C for tropical species. Diatoms contain chlorophylls *a* and *c* which absorb
259 light in the blue and red parts of the light spectrum and the main accessory pigment is fucoxanthin which
260 absorbs light in the 450-540 nm wavelength range. It is generally therefore best to use daylight-quality
261 fluorescent tubes or LED lights which reproduce the natural spectrum, combining several wavelengths (blue,
262 green, red). Although it is possible to grow some diatoms in continuous light, the best results are usually
263 obtained by applying a photoperiod such as 12L:12D. Light intensity is typically set at 80-100 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$
264 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
265 samples. For long-term maintenance of microalgal strains, it is recommended to use dedicated incubators
266 with temperature and light control. Alternatively, walk-in temperature-controlled rooms equipped with
267 shelves and light ramps are very convenient for large collections.

268 For growth in liquid media, transfer to fresh medium should typically be conducted every 2 to 4 weeks,
269 depending on the species and light conditions. Lowering the temperature and/or light level may result
270 in longer periods between transfers and reduce the labour involved. Growth is generally slower on solid
271 medium, and transfer frequency is typically between 2 and 3 months. In most cases, growth of the newly
272 transferred culture can be verified simply by observing the colour, but it may be necessary to use optical
273 microscopy or flow cytometry.

274 It is important to record the growth preferences of each culture (photoperiod, medium, transfer period, etc.)
275 and any relevant information (e.g. change of morphology) in the culture database (see above). In order to
276 limit sources of error and contamination linked to human activity during transfers, it is highly recommended
277 to use a unique code for each strain (see database above) and to identify each culture container with a
278 computerised label (rather than a manual indication which can be misinterpreted or erased) displaying at
279 least the culture code, taxonomy, medium used and date of last transfer. Again, working with a cloud-based
280 database makes this easy to implement.

281 Diatoms have commercial applications in fields such as cosmetics, pharmaceuticals or aquaculture (Sharma
282 et al. 2021) since they produce high value-added molecules (e.g. pigments, lipids, polyunsaturated fatty
283 acids). For such applications, it is often necessary to increase the volume of cultures in order to collect
284 large amounts of biomass. This requires optimising some parameters (medium, light) compared to standard
285 culture conditions. One approach is to gradually increase culture volume starting with a tube (a few
286 millilitres) up to Erlenmeyer flasks (typically several hundreds of millilitres) and then working up to litre
287 scale in dedicated infrastructures such as photobioreactors (Figure 8). Such gradual increase should limit
288 stress. Various parameters can be modified depending on the result to be achieved. For example, increase
289 in photoperiod (up to 24 h continuous light) and light intensity will induce greater photosynthetic activity
290 and change in pigment content, such as antheraxanthin and zeaxanthin, known for their photo-protective
291 and antioxidant activities (Goss et al. 2020). Factors such as pH, temperature, salinity and nutrient
292 concentration are essential for optimal biomass production. When diatoms are used to feed larger organisms
293 such as shellfish or zooplankton it may be necessary to alter medium composition (Liu et al. 2016; Nghiem
294 Xuan et al. 2020). Air bubbling may prevent diatoms from settling or adhering to the walls of containers.
295 Over the last decades, numerous large-capacity microalgae culture systems have been developed for bio-
296 refining and waste treatment (e.g. Matsumoto et al. 2017). While conditions in closed systems are easier
297 to control, this is not the case in open systems (ponds and raceways of up to several hectares) for which
298 external conditions such as seasonality, irradiation, temperature and variable rainfall present additional
299 challenges (Wang and Seibert 2017).

300 **5 - Life cycles**

301 Although many diatoms can be easily isolated from environmental samples (see above) and grow well on
302 commercially available algal culture growth media, long-term maintenance of strains is often challenging
303 because of their peculiar life cycle, resulting in a gradual cell size diminution and final loss of cultures. Unlike
304 most other microalgae, the majority of diatoms have a diplontic life cycle (Figure 9), with a long vegetative

305 phase with diploid mitotically dividing cells, alternated by a comparatively short sexual phase with short-lived
306 haploid gametes (Chepurnov et al. 2004). During mitotic cell division, two slightly unequal daughter cells are
307 formed due to the peculiar architecture of the siliceous cell wall (frustule) and the biosynthesis of new valves
308 inside the confines of the parental cell wall. As a result, the mean cell size of a clonal population gradually
309 decreases during repeated rounds of mitotic divisions, known as the MacDonald-Pfitzer rule (Macdonald
310 1869; Pfitzer 1869). Ultimately, cells become critically small, resulting in malformations and eventually cell
311 death (Mann 2011). While this appears to be the general rule, a minority of diatom species do not decrease
312 in cell size when dividing, while others are able to restore their cell size asexually (Kaczmarska et al. 2022,
313 2013; Mann 2011; Rose and Cox 2013). In most species studied, however, sexual reproduction and the
314 subsequent expansion of a zygote into the auxospore is the most prevalent mechanism to restore large
315 sized cells. Spontaneous and experimentally induced abrupt cell size reduction demonstrates that sexual
316 reproduction in diatoms is dependent on cell size and can take place once cells pass a species-specific size
317 threshold (SST, Chepurnov et al. 2004). Although studied in detail for only a few species, this endogenous
318 cell size sensing mechanism, also referred to as the diatom sex clock, results in an alternation between brief
319 sexual reproduction events and long intervals of vegetative growth, which can span several years (Lewis
320 1984; Mann 2011). While the processes of cell size decrease and auxosporulation are conserved among most
321 diatoms, diverse mating strategies exist, largely coinciding with the main morphological groups of centric,
322 araphid pennate and raphid pennate diatoms (Figure 9). Most centric diatoms are oogamous, producing
323 large egg cells which are fertilized by small, flagellate spermatozoa. In general, they are homothallic (self-
324 fertile), and clonal cells below the SST can, depending on their size, differentiate into egg or sperm cells.
325 However, exceptions to this general pattern exist (e.g. Davidovich et al. 2017). In contrast, species from
326 the evolutionary younger group of pennate diatoms, are often heterothallic, although homothallic species
327 and mixed strategies are also known (Davidovich et al. 2010, 2009; Mann and Poulíčková 2019; Quijano-
328 Scheggia et al. 2009; Vanstechelman et al. 2013), and sexual reproduction therefore requires a partner of
329 the compatible mating type. The phylogenetically older group of araphid pennate diatoms is characterized
330 by non-flagellate motile male gametes and larger immobile female gametes (anisogamy). In contrast,
331 most raphid pennate species typically produce morphologically indistinguishable gametes (isogamy). A
332 key difference with araphid diatoms is that a transfer of function from gametes to the gametangia has
333 evolved, where diploid sexualized cells from opposite mating types interact to form a mating pair in which
334 gametogenesis is subsequently initiated. Pheromone signalling plays a crucial role during diatom sexual
335 reproduction (Frenkel et al. 2014; Sato et al. 2011). The system appears to be most evolved in raphid
336 pennate diatoms and includes both sex inducing pheromones and attraction pheromones influencing the
337 behaviour of gametangial cells, as well as pheromones, so far uncharacterised, involved in gamete attraction

338 (Gillard et al. 2013; Klapper et al. 2021; Moeys et al. 2016).

339 Although cell size is the precondition for sexual reproduction to occur, appropriate environmental conditions
340 should be also present to permit auxosporulation or to trigger it. This is best known for centric diatoms,
341 where spermatogenesis and/or oogenesis can be induced by a shift in environmental conditions such as
342 salinity, temperature, irradiance and its spectral composition, or photoperiod (Amato 2010; Baatz 1941;
343 Godhe et al. 2014; Moore et al. 2017). In pennate diatoms, it appears that growing conditions should
344 be favourable, although the topic has been little explored in a systematic manner. For example, sexual
345 reproduction of the pennate *Pseudo-nitzschia multistriata* was most successful when cultures were in the
346 exponential growth phase (Scalco et al. 2014). In addition, auxosporulation appears to be light dependent,
347 both in terms of irradiance as well as spectral composition (Bilcke et al. 2022; Davidovich 1998; Gillard
348 et al. 2013; Mouget et al. 2009).

349 Understanding the processes involved in cell size reduction and restoration is essential for the long-term
350 maintenance of diatom species in culture collections. To our knowledge, very little is known about possible
351 controls of vegetative cell enlargement and asexual auxosporulation and how widespread it is among di-
352 atoms. Such information would be very interesting as it would allow preservation of the original genotype,
353 in contrast to meiotic recombination during sexual auxosporulation which results in new genotypes. In ad-
354 dition, repeated selfing of homothallic strains may be applied to increase the level of homozygosity, which
355 may be of interest for both fundamental and applied research. In any case, the use of sexual reproduction in
356 the context of maintaining species or strains in a culture collection implies knowledge of the mating strategy
357 SST, and conditions favouring sexual reproduction. In the case of heterothallic species, the availability of
358 multiple strains of compatible genotypes is an obvious additional requirement. Increasingly, the importance
359 of diatom-bacteria interactions is being revealed, including both beneficial as well as inhibitory effects on
360 diatom sexual reproduction (Cirri et al. 2019). However, as this information and resources are available for
361 only few species, many diatom strains may be maintained for variable periods in culture collections, but
362 are eventually bound to be lost.

363 Ideally, strains of large-sized diatoms (well above SST) would be cryopreserved (see below). However, if
364 cryopreservation of large-sized cells is not possible, inducing sexual reproduction in such species is a solution
365 to maintain those species in a collection. It should be noted, however, that this may also promote selection
366 of genotypes adapted to laboratory conditions.

367 One alternative, but as yet little explored, avenue for long-term storage of diatom species in culture
368 collections is exploiting the fact that some species are able to form resting stages (Figure 9), including
369 spores or resting cells. While the former are morphologically distinct from vegetative cells, resting stages

370 have valves that are indistinguishable compared to their vegetative counterparts, but they are characterized
371 by a condensed protoplast (Kaczmarska et al. 2013). In diatoms, resting spore formation is fundamentally
372 different from auxosporulation. Auxosporulation involves swelling of a zygote and the subsequent formation
373 of large initial cells, which then divide into cells with a species-specific morphology. In contrast to many
374 other groups of microalgae where resting spores are formed from zygotes, diatom resting spore develop from
375 mitotically dividing cells, usually as a result of environmental stressors such as e.g. nitrogen deprivation. In
376 some species it has been demonstrated that these spores remain viable for centuries to millennia (Härnström
377 et al. 2011; Kaczmarska et al. 2013; Sanyal et al. 2022), opening the prospect of exploring anoxic, dark
378 conditions to maintain such species in culture.

379 **6 - Cryopreservation**

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

391 Cryopreservation is the storage of viable cells at ultra-low temperatures of liquid nitrogen (-196 °C) and/or
392 its vapour phase (-156 °C). The underlying principle of cryopreservation is that at ultra-low temperatures,
393 vital functions, such as enzymatic activity and cell division, are slowed to the point of cessation, but not
394 death (Benson 2008). A fundamental discovery in implementing successful cryopreservation was made by
395 Polge et al. (1949) working with fowl sperm. They observed that at low temperatures, the sperm could
396 be "protected" by using glycerol. Since then, cryopreservation has been used as a means to store (or
397 "cryobank") biological materials including seeds, viruses, bacteria, and mammalian and plant tissue culture
398 cells as a hedge against loss of valuable research resources. Unicellular algae have been cryopreserved since
399 the early 1960s, although new methods were required due to poor success rates and alteration of algal
400 ultrastructure (Morris 1976; Plattner et al. 1972). Morris (1978) successfully cryopreserving 252 of 284

401 strains of Chlorococcales (green algae) using a two-step cooling protocol . Cryopreservation methodology
402 for algae has continued to evolve (Day et al. 2010).

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

406 **Freezing Methods** Although a range of freezing protocols has been developed for the cryopreservation of
407 algae (Ramon et al. 2002), they fall into two main categories: (1) rapid cooling, and (2) 'two-step' cooling.
408 The rapid cooling technique consists of plunging the algal material, suspended in culture medium with
409 an appropriate cryoprotective agent, rapidly into liquid nitrogen at $-196\text{ }^{\circ}\text{C}$. At such rapid cooling rates
410 however, the internal solution of the material becomes supercooled, increasing the possibility of damaging
411 intracellular structure due to ice formation (Karlsson and Toner 1996; Meryman 1966). Most freezing
412 protocols avoid this by utilizing a two-step cooling process, with controlled or semi-controlled cooling from
413 room temperature (generally at a rate of ca. $-1\text{ }^{\circ}\text{C min}^{-1}$) to a holding temperature of around -30
414 $^{\circ}\text{C}$ before the material is plunged into liquid nitrogen to complete the freezing process. The first step
415 can be performed using simple devices such as Mr Frosty[®] or more sophisticated controlled-rate freezers.
416 Damage is thought to be prevented using this method because the reduced cooling rate allows sufficient
417 time for osmotic equilibrium to be maintained by shrinkage of the cell. Another method, common in higher
418 plants, to minimize intracellular damage upon freezing is the process of vitrification, which is ultra-rapid
419 cooling in the presence of high concentrations of cryoprotective agents,(first described by Rall and Fahy
420 1985). Although not widely used to date, vitrification does appear to have some potential use in the
421 cryopreservation of algal material. A more detailed description of vitrification methods can be found in
422 Steponkus et al. (1992) and Karlsson and Toner (1996). A more recently employed method with algae is
423 encapsulation-dehydration (Hirata et al. 1996). This technique involves the dehydration of encapsulated
424 algal cells by means of sterile air drying followed by immersion and storage in liquid N_2 . The method has the
425 advantage that no toxic cryoprotectants are required, hence upon thawing there can be direct cultivation
426 without the need for repeated washing of the sample.

427 **Cryoprotectants** Cryoprotective agents (CPAs) are added to offer protection from cell damage during
428 freezing and thawing processes. CPAs are low molecular weight compounds that passively traverse the
429 plasma membrane to equilibrate the solute concentration between the cell interior and the extracellular
430 matrix (Day and Brand 2005). Different CPAs may act in different ways such as lowering the temperature
431 at which intracellular water freezes (Franks 1985), minimizing osmotically driven decreases in cell volume
432 during freezing (Canavate and Lubian 1995) or altering membrane properties such as solute permeability

433 (Santarius 1996). As an important mode of action of CPAs is the stabilization of membranes, and con-
434 sidering that membrane damage can be a primary initiator of free radical reactions, it is not surprising to
435 find that several CPAs may also act as free radical scavengers (Benson 1990; Canavate and Lubian 1995).
436 Hubálek (2003) reviewed the use of cryoprotectants in microorganisms. For microalgae, DMSO is the most
437 commonly used CPA.

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

450 **Thawing** Thawing is critical for the reactivation of cultures. Several parameters are critical, notably
451 osmotic and toxic shocks and light stress. In order to limit osmotic shocks and the intracellular and
452 resulting extracellular damages, frozen cultures are preferably thawed rapidly by immersing them for about
453 three 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
454 effect on the cells and to prevent this, it is important to rapidly dilute the thawed aliquot in a sufficient
455 volume of fresh culture medium. It is possible to include a centrifugation step to pellet the cells and
456 remove the CPA before re-culturing, but this step induces additional stress and decreases survival chances.
457 In order to slowly revive the cultures, they are placed in optimal culture conditions (medium, temperature),
458 while light is introduced progressively after a 24 to 48 hours phase of darkness or semi-darkness, thus
459 limiting the light stress. It takes a few weeks for the cultures to recover a growth rate identical to that
460 before cryopreservation. During these few weeks, monitoring is performed by light microscopy and/or flow
461 cytometry.

462 **Overall success of cryopreservation for diatoms** Cryopreservation success for microalgae varies widely
463 between different taxonomic groups. For example, dinoflagellates are very difficult to cryopreserve (Hage-
464 dorn et al. 2015). Diatoms are relatively easy to cryopreserve, although success rates vary significantly

465 among diatom lineages (Stock et al. 2018). In the collections we surveyed (Table 2), pennate diatoms
466 appear easier to cryopreserve than centric diatoms, especially the Coscinodiscophyceae. However, there
467 are large variations between genera (Table S1). For example, while all strains of *Seminavis* tested can
468 be cryopreserved, only 50% of *Thalassiosira* strains are successful. This could be an effect of cell size,
469 as Paredes et al. (2021) reported that in terms of estimated cell volume, approximately two thirds of the
470 small ($< 1000 \mu\text{m}^3$) and intermediate ($1000\text{--}2500 \mu\text{m}^3$) categories of diatoms from the RCC collection for
471 which cryopreservation was attempted were successfully cryopreserved, whereas few of the larger (> 2500
472 μm^3) category survived cryopreservation. Paredes et al. (2021) also reported that in terms of geographic
473 origin, approximately only two thirds of temperate and polar diatom strains for which cryopreservation was
474 attempted were successfully cryopreserved, whereas almost all tropical strains (albeit from a lower total
475 number) grew after thawing.

476 **Long-term conservation.** Although some studies have shown that long-term storage (15 years) maintains
477 the same properties as short-term storage (less than one year) (Nakanishi et al. 2012), other studies have
478 raised some potentially significant issues. Müller et al. (2007) found that genomic alterations occurred
479 after cryopreservation of some microalgae cultures, as repeatable differences in AFLP patterns, although
480 no visible phenotypic changes were observed. They highlighted that although all biological functions cease
481 at cryogenic storage temperatures, prolonged storage can induce some chemical damage, for example due
482 to the formation of free radicals and ionizing radiation, which can damage nucleic acids and ultimately
483 influence genetic stability. With the drastic reduction in sequencing costs, Foo et al. (2023) advocate that
484 whole genome analysis would provide comprehensive information on whether cryopreservation alters the
485 genetic and functional characteristics of a strain of interest.

486 7 - Diatom strains amenable to genetic engineering

487 The study of the molecular basis of diatom life began in the 1990s with the development of DNA-mediated
488 transformation in a few selected species. This important achievement to analyse and modulate the function
489 of diatom genes was first achieved by biolistic transformation in *Cyclotella cryptica* and *Navicula saprophylla*
490 via the pioneering work of Dunahay et al. (1995). Subsequently, this method was adapted to other species
491 such as *Phaeodactylum tricornutum* (Apt et al. 1996; Falciatore et al. 1999), *Thalassiosira weissflogii*
492 (Falciatore et al. 1999), *Cylindrotheca fusiformis* (Poulsen and Kröger 2005) and several others (Table 3).
493 Biolistic transformation is based on the helium-accelerated particle bombardment of diatoms with exogenous
494 DNA coated on gold or tungsten particles, followed by selection of the transformed cells with antibiotics.
495 There are also some reports describing diatom transformation by electroporation, where electric currents

496 are used to increase cell membrane permeability and DNA delivery (Table 3).

497 More recently, bacterial-mediated conjugation transformation was developed for the most studied diatom
498 model species *P. tricornutum* and *T. pseudonana* (Karas et al. 2015). With this approach, DNA is delivered
499 as an episome or artificial chromosome using genetically engineered *Escherichia coli* bacteria that promote
500 DNA transfer via direct contact with diatoms via cell-to-cell pili. Following transformation, the transgenic
501 material is replicated and maintained in the diatom cells due to the presence of a centromeric element,
502 CEN6-ARSH4-HIS3, included in the episome. Bacterial conjugation is becoming the method of choice
503 for diatom synthetic biology because the efficiency of transformation is very high (100-1000 transformants
504 per 10^8 cells with bacterial conjugation vs 1-800 transformants per 10^8 cells with biolistics, Moosburner
505 et al. 2022). It also allows reproducible expression levels of transgenes in different transgenic lines because
506 the episome is not integrated into the diatom genome. Conversely, with the biolistic and electroporation
507 transformation, plastid-containing DNA is integrated into multiple copies and in multiple sites in the
508 genome, meaning independent transgenic lines show very different expression levels (George et al. 2020).
509 Moreover, random integration into the genome can also result in undesirable changes in genomic loci at
510 integration sites or surrounding regions. To overcome these problems and to obtain conclusive functional
511 information, it is always necessary to characterize independent transgenic lines. Episomal genetic elements
512 may, however, be lost in the absence of antibiotic selection. Due to the high cost of the antibiotics
513 commonly used for selection, this represents a disadvantage if a large quantity of transgenic lines is required
514 for functional characterisation of diatom gene products or biotechnological exploitation of transgenic cell
515 extracts. On the other hand, loss of the episome by removal of antibiotics may also be an advantage in
516 achieving transient expression of the transgene, which is a desirable effect in gene functional studies (e.g.,
517 to compare phenotypes related to the presence or absence of a specific gene) or to remove transgenic
518 proteins that may modify the DNA, and thus generate an unintended genomic change (Moosburner et al.
519 2022).

520 Several factors are critical to the success of genetic transformation, which may limit the application of these
521 tools to certain diatom species (Table 3): The ability to maintain the selected species under laboratory
522 conditions is obviously the first important requirement, but many diatom species currently remain refractory
523 to laboratory culture. Moreover, as different transformation methods also cause cell damage and mortality,
524 transformation of diatoms is usually performed with a high quantity of cells (typically around 10^8) as
525 starting material. For diatom species that do not reach a high cell density under laboratory conditions, it
526 is therefore necessary to use large culture volumes and a cell concentration step prior to transformation,
527 which may represent a limitation to routine use of the technology. In addition, genetic transformation relies
528 on the use of an appropriate selection marker to isolate transgenic lines in a population of untransformed

529 cells. Diatoms, like many algae, are resistant to many commercially available antibiotics. However, some
530 antibiotics have been identified that kill diatoms under particular conditions of cell concentration and
531 salinity (to be defined for each species) and these can be used to isolate transgenic lines expressing specific
532 antibiotic resistance genes (Table 3). For successful transformation, it is also preferable to use axenic
533 cultures, as bacterial contamination can alter the growth of diatoms on the selection medium and thus
534 lead to the growth of false transgenic lines. However, many diatoms do not grow properly in the absence
535 of bacteria, which can complicate genetic transformation efforts.

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

544 Co-transformation of two vectors is also documented in several diatom species, whereby a selectable marker
545 gene on a plasmid can be used to co-deliver in the same diatom cell another non-selectable transgene, such
546 as a gene expressing a protein of interest fused to a protein tag or a reporter gene. However, the possibility to
547 use multiple selectable markers largely facilitates genetic manipulations requiring the expression of different
548 transgenes. This can be useful to simultaneously modify multiple genes or for the characterisation of the
549 function of a gene (e.g., gene mutagenesis in a first transformation round, followed by complementation of
550 a wild-type or a mutated version of the gene of interest in a second transformation, see Giovagnetti et al.
551 2022). Multiple selectable antibiotics have so far only been identified for a few species (Table 3). For *P.*
552 *tricornutum*, chemical based selectable markers have also been recently developed (Serif et al. 2018), based
553 on the APT (Adenine Phosphoribosyltransferase, enzyme of the adenine salvage pathway) and UMPS
554 (Uridine-5'-monophosphate synthase, enzyme of the de novo pyrimidine biosynthesis pathways) genes,
555 whose inactivation results in the resistance to the toxic compounds 2-fluoroadenine and 5-fluoroorotic acid,
556 respectively.

557 Independently of the transformation method employed, transgenic lines are visible after several weeks of
558 growth on the selectable media (between 2–5 weeks depending on the species and the method). Growth of
559 the transformed diatom cells on an agar plate greatly facilitates the isolation and characterisation of clonal
560 transgenic lines derived from the same transformation event. For some species such as *P. multistriata*

561 and *Pseudo-nitzschia arenysensis* that are difficult to grow on plates, the selection of transgenic lines
562 following transformation is undertaken in liquid media in the presence of the antibiotic (Sabatino et al.
563 2015). However, this protocol is more time-consuming as it requires the subsequent isolation of individual
564 transgenic cells from a pool of transformed cells in order to perform molecular characterization. In some
565 centric species such as *T. pseudonana* or *Cyclotella cryptica*, which are also difficult to grow on agar plates,
566 the isolation of transgenic lines can be successfully achieved by growing cells inside a low % (0.25%) agar
567 matrix (Turnsek and Dupont 2017) or on the agar surface, but by using high purity agar.

568 Over the last twenty years, the main application of genetic transformation has been the study of diatom
569 gene function. Powered by the availability of -omic information and tools to modulate gene expression,
570 these studies have been instrumental in characterizing specific cellular and metabolic features of diatoms
571 and in elucidating the mechanisms contributing to their ecological success in the environment. The readers
572 could refer to Falciatore and Mock (2022) for a recent overview on the “Molecular Life of Diatoms” and to
573 Moosburner et al. (2022) and the Chapter “Genetic Regulation of Diatom Photosynthesis: Understanding
574 and Exploiting Genetic Diversity” in this book for additional details on the tools currently available for
575 genetic engineering and genome editing in various diatom species.

576 Seminal information to drive genetic engineering in diatoms has also increased the potential of these
577 algae for biotechnological exploitation. Diatoms naturally generate relevant foodstuff substances (e.g.,
578 omega-3 fatty acids like EPA) and produce lipids, especially triacylglycerides (TAGs), for carbon storage
579 (Bozarth et al. 2009). Thus, efforts have been made to establish diatoms as source material in various
580 industrial applications (e.g., production of health foods, biomolecules, feed for aquaculture, jet fuels) and
581 to overcome limitations of biomass production. It has already been shown that the production of high
582 added value molecules such as lipids can be increased by modifying specific diatom genes (Hao et al. 2018;
583 Muto et al. 2015). Diatoms can also be exploited in targeted drug delivery using genetically engineered
584 diatom biosilica (Delalat et al. 2015) or as a platform for the production of heterologous products such
585 as plant-derived monoterpenoids (Fabris et al. 2020) or monoclonal antibodies (Hempel et al. 2017). The
586 application of genetic engineering to a wide range of diatom species will further strengthen the field of
587 diatom biotechnology in the future.

588 **8 - Concluding remarks**

589 Culture collections are critical for diatom research. Less than 5% of described species are currently main-
590 tained in culture, which is a small proportion given that the real extent of diatom biodiversity is estimated
591 to be in the region of 100,000 species. This highlights the necessity to continue and amplify isolation

592 efforts, especially for species from environments that have been relatively undersampled to date, such as
593 plant and animal microbiomes or sediments. It is also important that researchers that isolate diatoms (and
594 other algae) routinely deposit them in public culture collections, and for public culture collections to provide
595 clear information on their strain deposition policies. In most instances, this will require communication
596 with public collections prior to sample collection, and perhaps even a written sample management plan if
597 dedicated funding is sought.

598 As important as it is for scientists to know what level of support they may expect from public culture
599 collections, it is even more critical that culture collections remain funded to be able to maintain existing
600 cultures and expand the range of strains available for research and industry (Becker et al. 2019). There are
601 numerous examples of culture collections containing unique diatom strains that have disappeared (e.g., the
602 Scandinavian Culture Collection of Algae and Protozoa at the University of Copenhagen) or are demonstra-
603 bly threatened (e.g., individual collections in the US that consolidated into what is now the NCMA) due to
604 lack of funding and annual budgets 'in the red'. In some countries (e.g. France), collections are supported
605 on the long term by public institutions such as universities (e.g. Sorbonne University), in particular by the
606 creation of permanent (centrally funded) positions for technical personnel, as well as via the improvement
607 of infrastructures and acquisition of equipment on structural funds. They still, however, remain dependent
608 on obtaining competitive grants to operate on a daily basis. Prevention of the loss of culture collections
609 due to lack of funding will require development of funding models that diversify beyond the limited scope
610 of distribution of cultures. Some examples that have proven effective are providing aligned products, such
611 as harvested cells from larger (20 L) cultures and/or chemical extracts (e.g., DNA, RNA, protein) from
612 strains. Leveraging long term storage infrastructure can open up opportunities to hold "private Collections"
613 for companies, which can yield annually recurring revenues. Similarly, as economic sectors utilizing algae
614 continue to expand, culture collections should consider the possibility of non-exclusive licensing of strains to
615 ensure that they are not disadvantaged in the process of commercialization. While it is unlikely that there
616 will be a single consistent financial model across all culture collections, it is important as a community that
617 we strive for consistency where possible to ensure the broader stability of these important global resources.

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Table 1: List of culture collections containing diatoms. Only collections that contain diatoms and answered our survey are included. WDCM corresponds to collection ID in the World Data Center for Microorganisms (<https://ccinfo.wdcm.org>). Some website URLs have been shortened.

Acronym	WDCM	Name	# of diatom strains	Country	Website	Email
ACOI		Coimbra Collection of Algae	16	Portugal	http://acoi.ci.uc.pt/index.php	algoteca@ci.uc.pt
ACUF		Algal Collection University Frederico II	20	Italy	http://www.acuf.net/index.php?lang=en	gabpinto@unina.it
ALISU		Culture Collection of the Universidade de Lisboa	9	Portugal		aaferreira@fc.ul.pt
ANACC		Australian National Algae Culture Collection	336	Australia	https://www.csiro.au/ANACC	ian.jameson@csiro.au
ARC	1096	Algal Resources Collection	9	USA	https://www.algalresourcescollection.com/	desouzac@uncw.edu
BCCM/DCG	1039	BCCM/DCG Diatoms Collection	536	Belgium	https://bccm.belspo.be/about-us/bccm-dcg	bccm.dcg@ugent.be
BMAK		Aidar & Kutner Microorganisms Collection	73	Brazil	https://bit.ly/3ldfDA6	fsalcorr@usp.br
CCALA	905	Culture Collection of Autotrophic Organisms	4	Czech Republic	https://ccala.butbn.cas.cz/	Josef.Juran@ibot.cas.cz
CCAP	522	Culture Collection of Algae and Protozoa	165	UK	https://www.ccap.ac.uk/	ccap@sams.ac.uk
CICCM	1042	Cawthron Institute Culture Collection of Micro-algae	135	New Zealand	https://cultures.cawthron.org.nz/	Krystyna.Ponikla@cawthron.org.nz
CMR_RBI		Culture collection of the Center for Marine Research, Ruder Boskovic Institute	152	Croatia		ruso@cim.irb.hr
CPCC	605	Canadian Phycological Culture Centre	31	Canada	https://bit.ly/3lgecRA	hdroshton@uwaterloo.ca
DSMZ	274	Leibniz-Institut Deutsche Sammlung von Mikroorganismen und Zellkulturen	50	Germany	https://www.dsmz.de/	Silke.Pradella@dsmz.de
FINMARI CC		FINMARI Culture Collection	30	Finland		heidi.hallfors@syke.fi
MBRU		Russian Marine Biobanks	24	Russia	https://bit.ly/42T57Ws	naizdaicher@mail.ru
MCC	591	National Institute for Environmental Studies Collection	124	Japan	https://mcc.nies.go.jp/	mcc@nies.go.jp
MEDIC		Marine Epibiotic Diatom Collection	126	South Africa	http://ipt.sanbi.org.za/lptsanbi/resource?r=diatom	roksana.majewska@nwu.ac.za
MNHN-ALCP		Collection du Muséum d'Histoire Naturelle, Paris	3	France	https://bit.ly/3o7jec0	sahima.hamlaoui@mnhn.fr
MUC		Mersin University Collection	16	Turkey		elifeker@gmail.com
NBRC	825	Culture Collection Division, National Institute of Technology and Evaluation	14	Japan	https://www.nite.go.jp/nbrc/catalogue/	
NCMA	2	Provasoli-Guillard National Center for Marine Algae and Microbiota	585	USA	https://ncma.bigelow.org/	ncma@bigelow.org
NORCCA	498	Norwegian Culture Collection of Algae	290	Norway	https://norcca.scrol.net/	bente.edvardsen@bio.uio.no
RCC	829	Roscoff Culture Collection	939	France	https://roscoff-culture-collection.org/	rcc@sb-roscoff.fr
SZCZ		Szczecin Diatom Culture Collection	2151	Poland	https://bit.ly/432RI3X	andrzej.witkowski@usz.edu.pl
TCC	1030	Thonon Culture Collection	193	France	https://www6.inrae.fr/cartel-collection.eng/	frederic.rimet@inrae.fr
UTEX	606	Culture Collection of Algae at the University of Texas at Austin	390	USA	https://utex.org/	dnobles@austin.utexas.edu

Table 2: Cryopreservation status by class for cultures from several collections (BCCM, RCC, NCMA, UTEX, DSMZ, MBRU, MCC). Plus sign corresponds to success and Minus sign to no success. Cultures not tested were not included. Refer to Table S1 for data at the genus level.

Class	+	-	% success
Bacillariophyceae	921	304	75
Bacillariophyta others	25	3	89
Coscinodiscophyceae	11	45	20
Mediophyceae	436	263	62

Table 3: 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	Ifuku et al. 2015
		bsr	Blasticidin-S	Yin and Hu 2021

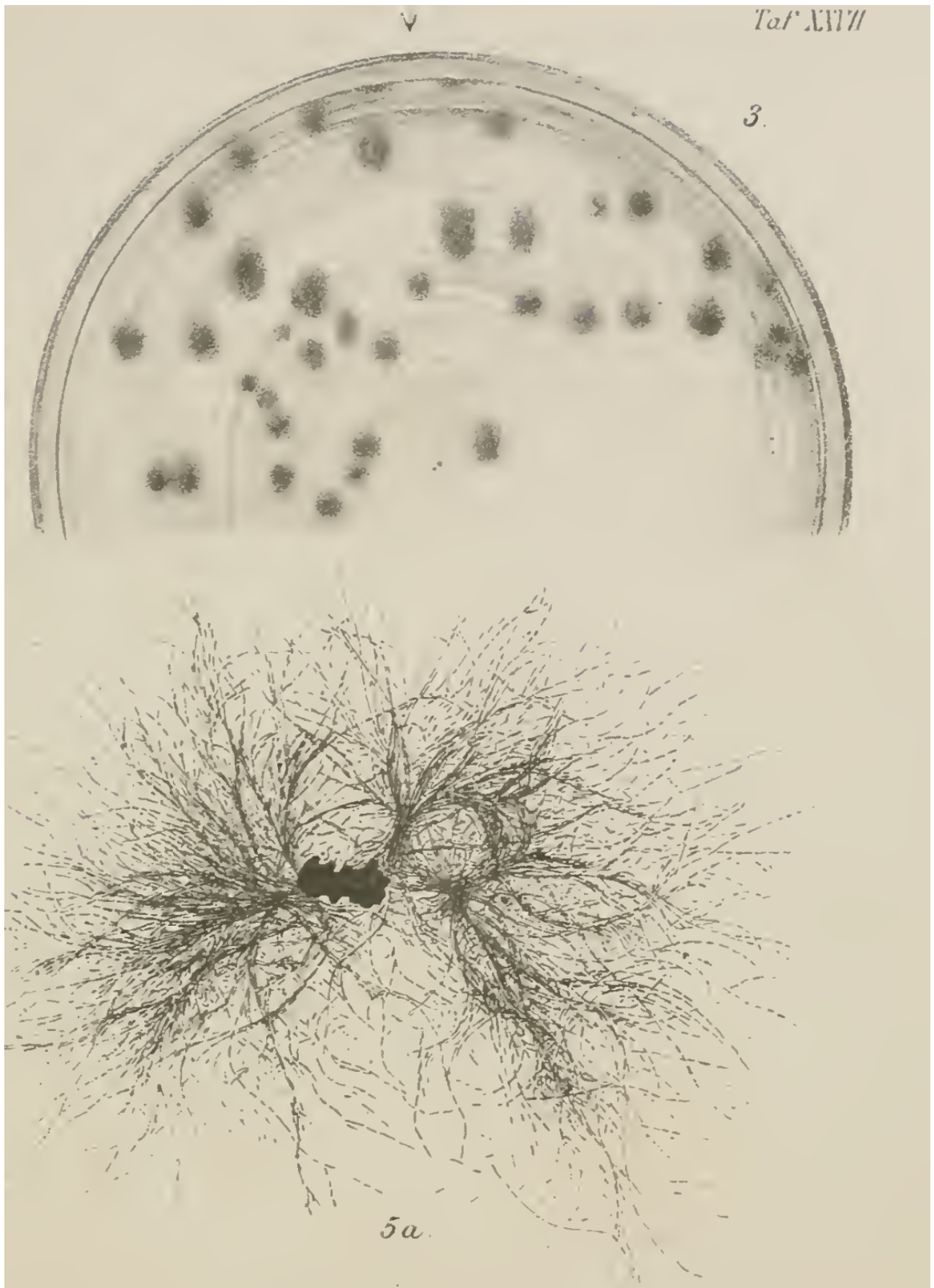


Figure 1: Colonies of *Nitzschia palea* growing on agar (reprinted from Richter 1903).

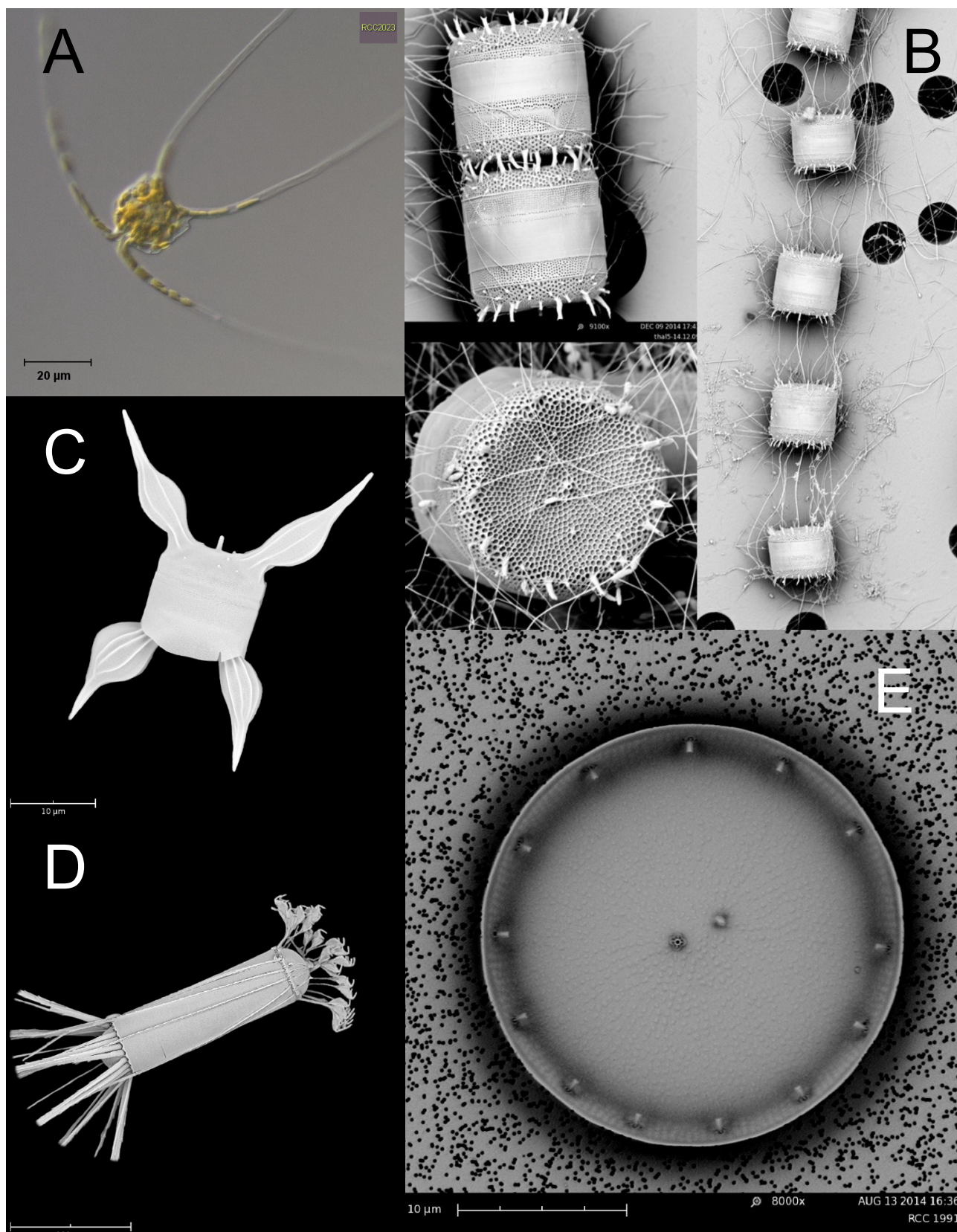


Figure 2: Examples of marine diatoms. A. *Chaetoceros peruvianus* RCC2023. B. *Thalassiosira delicatula* RCC2560. C. *Chaetoceros bulbosus* from a natural sample off New Zealand. D. *Corethron pennatum* from a natural sample off New Zealand. E. *Shionodiscus bioculatus* RCC1991.

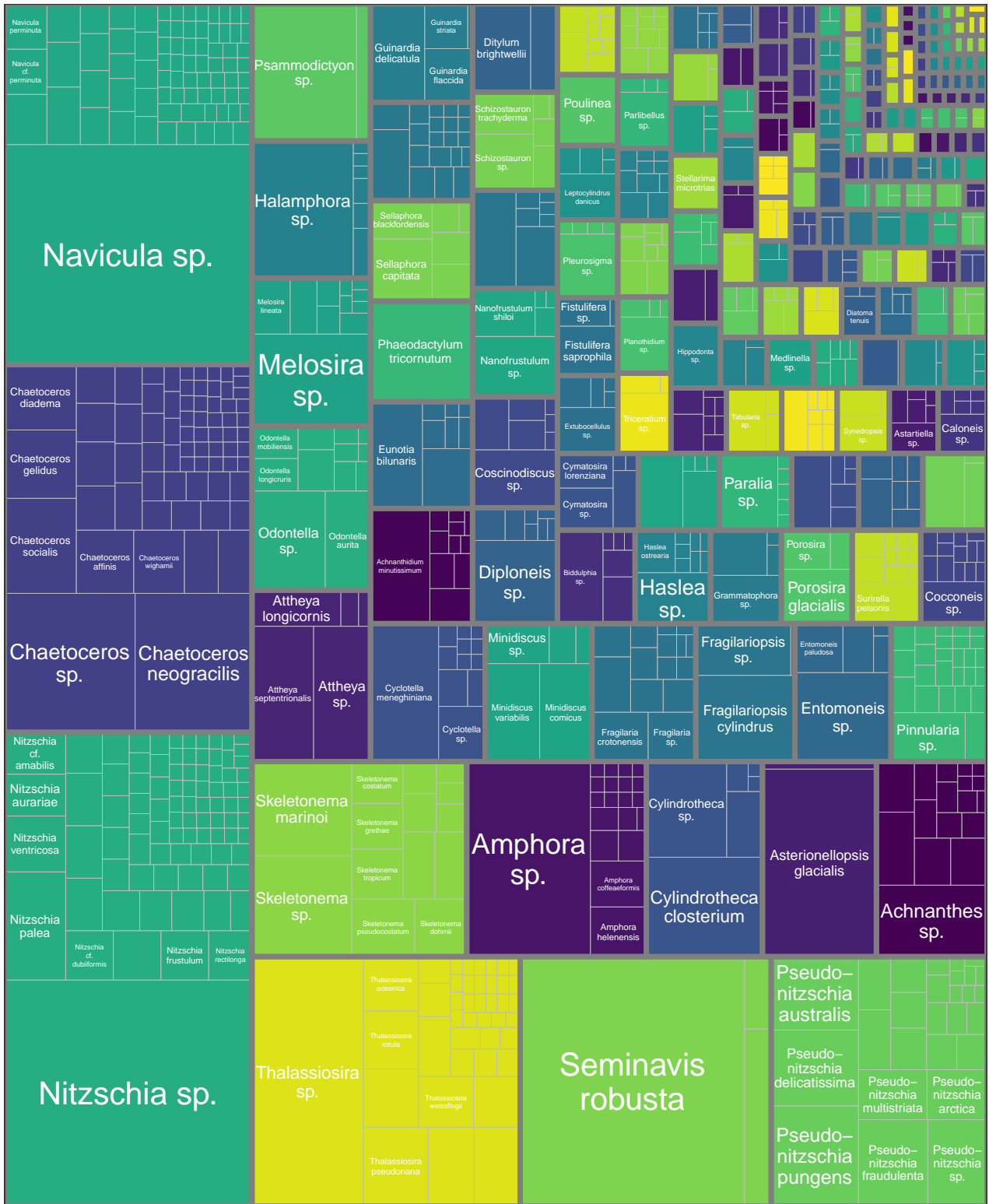


Figure 3: Diatom genera and species available cultures. Rectangle surfaces are proportional to number of strains.

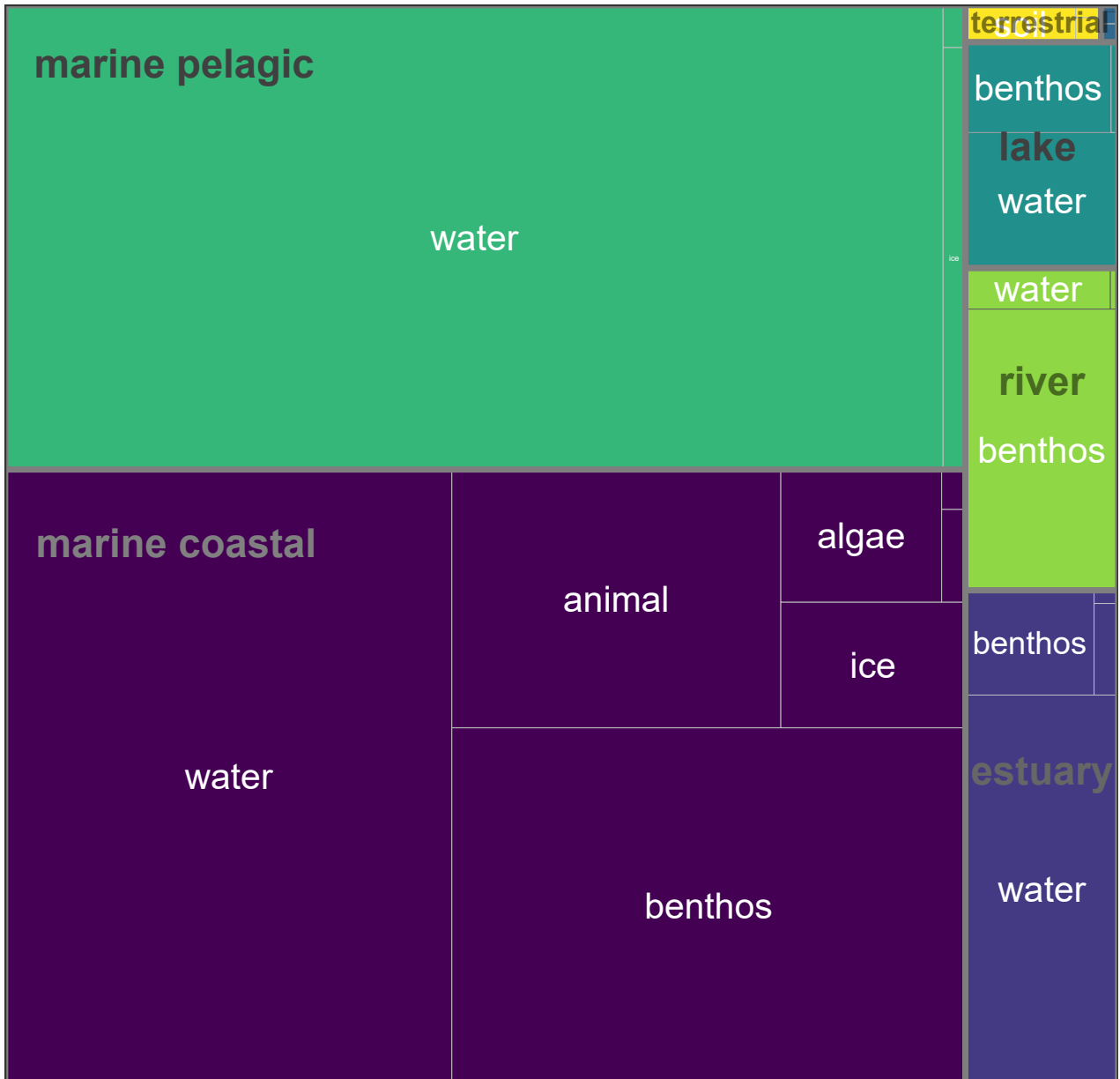


Figure 4: Origin of diatom cultures according to ecosystems and substrates. Rectangle surfaces are proportional to number of strains.

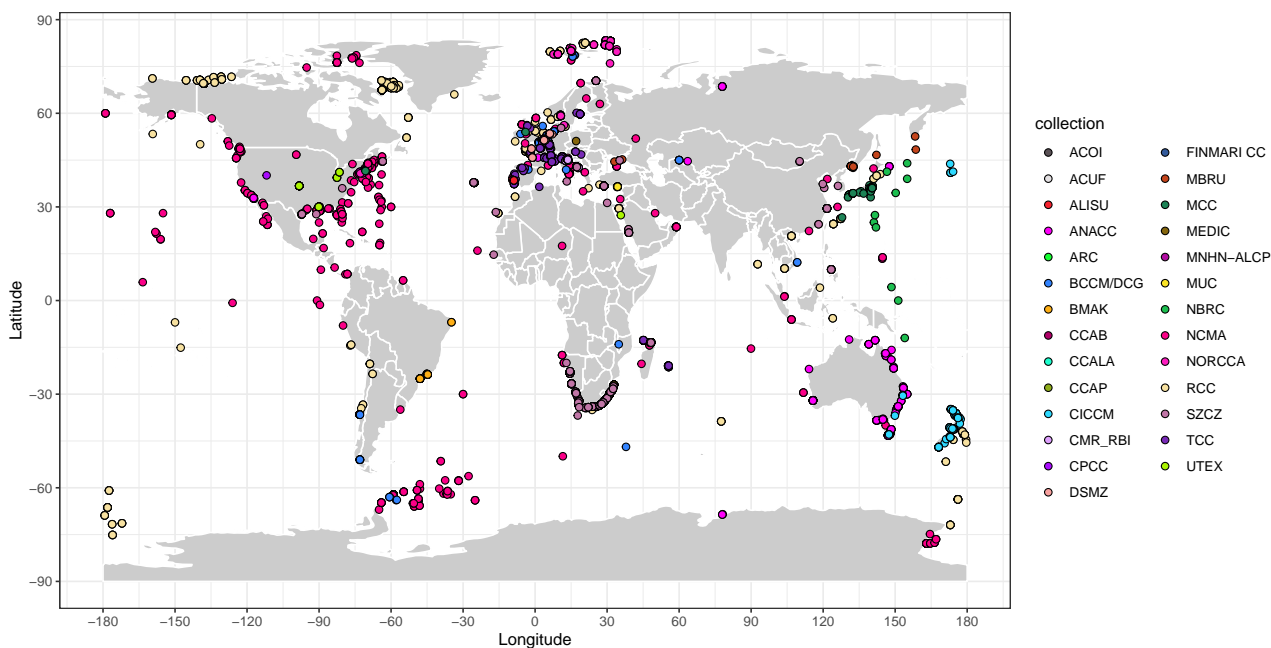


Figure 5: Isolation sites for diatom cultures for the different collections.

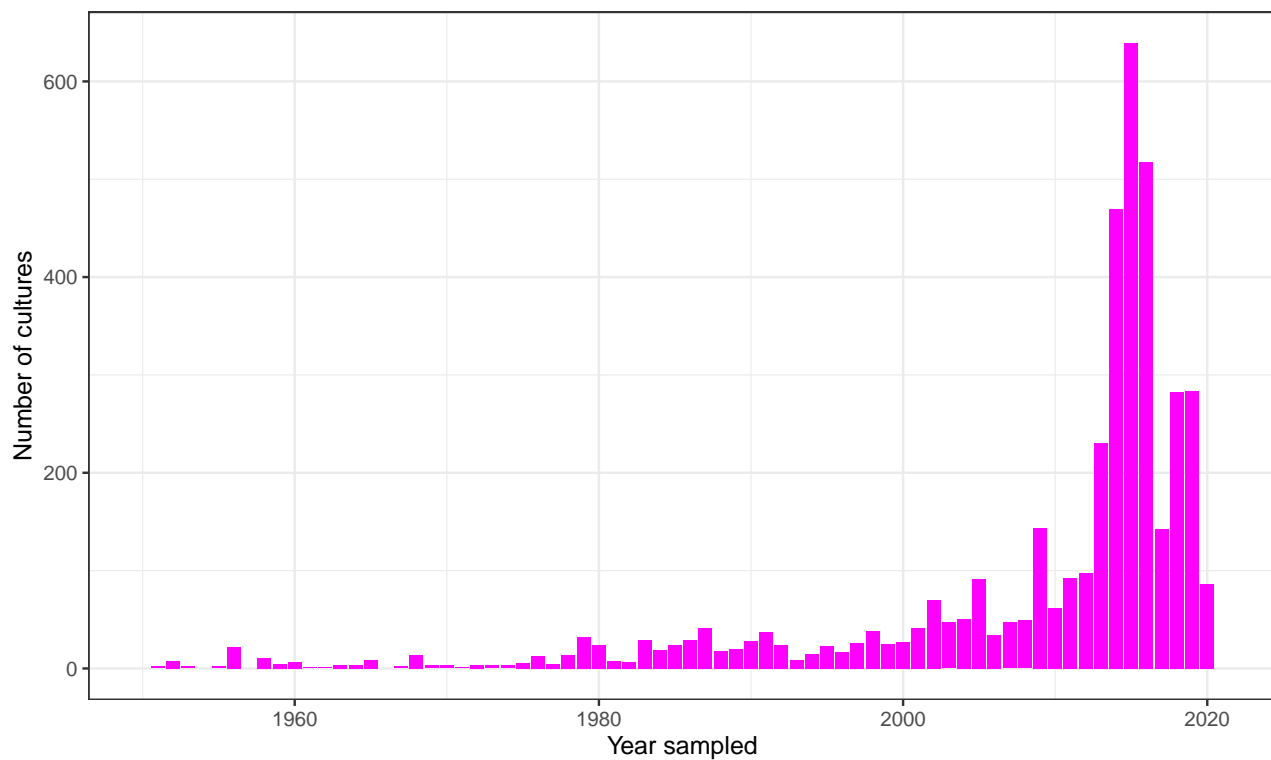


Figure 6: Year of sampling for diatoms listed in Supplementary Data S1. The peak around 2016 could be linked to the large number of sampling expeditions during this period.

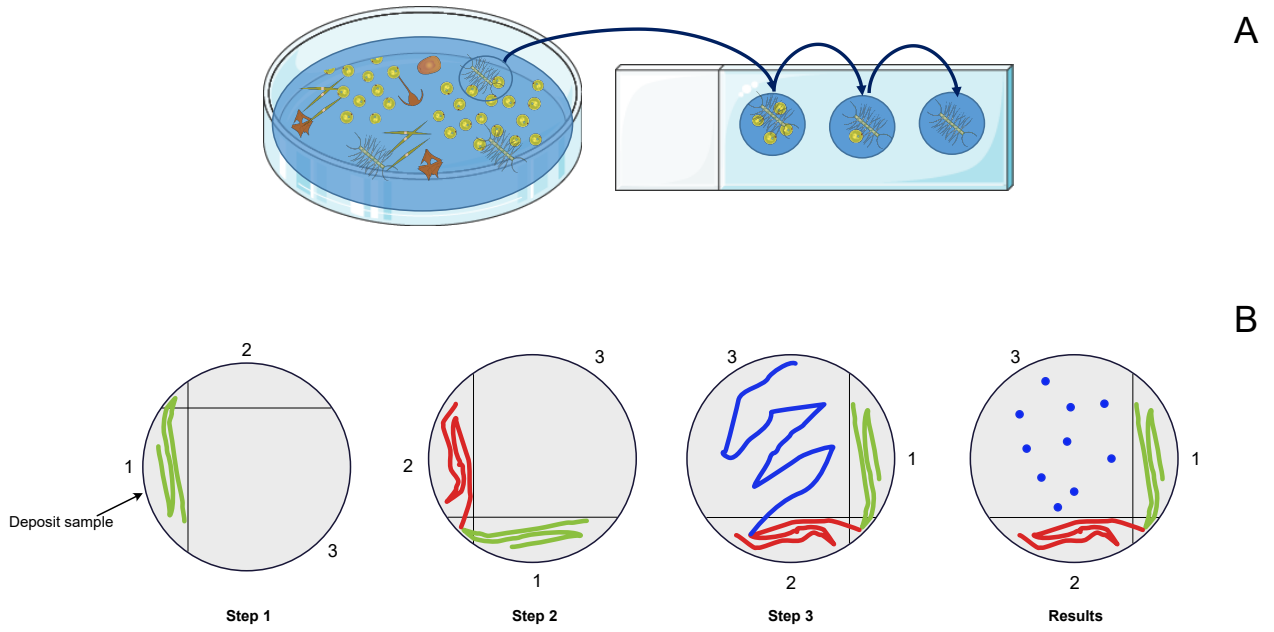


Figure 7: Culture isolation strategies. A. Single cell isolation. B. Quadrant method on agar plates.



Figure 8: Cultures of diatoms at the Roscoff Culture Collection using either small culture flasks, tubes or larger glass containers with a bubbling system.

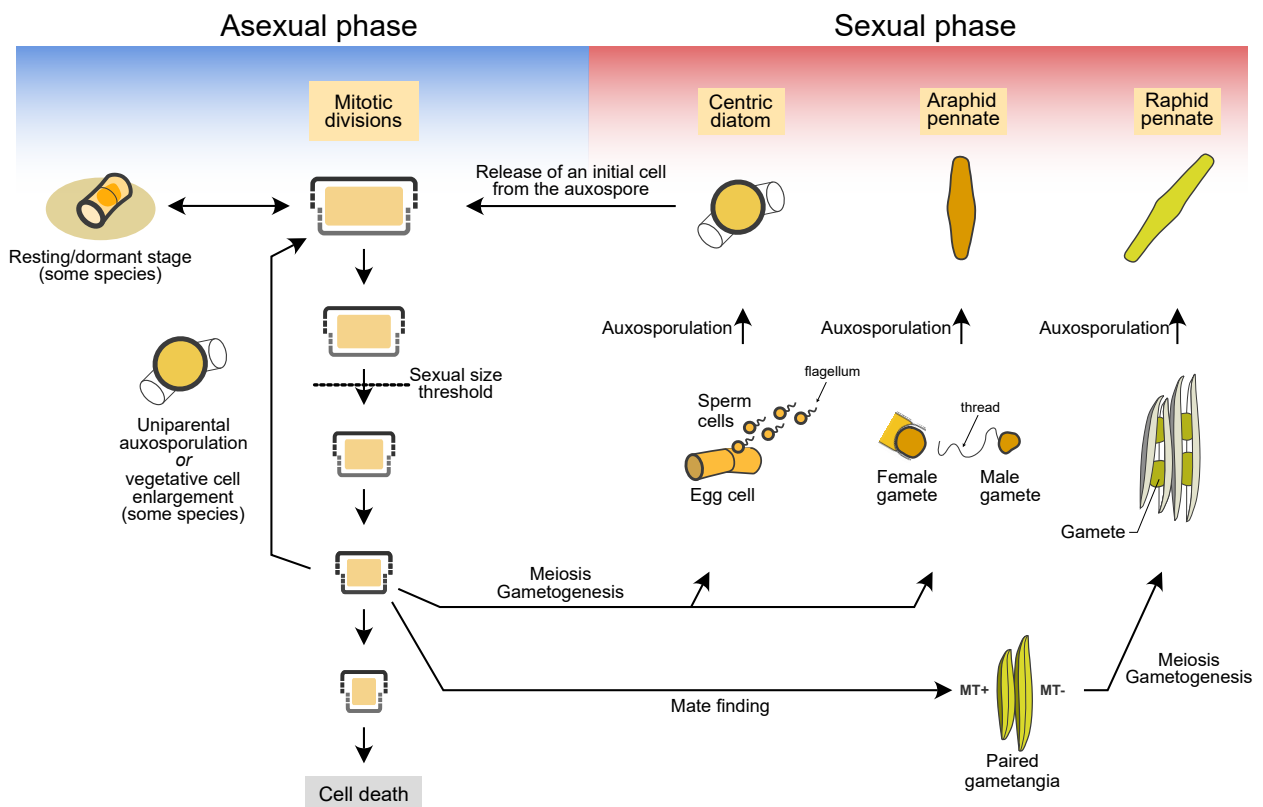


Figure 9: The life cycle of diatoms: a graphical representation of vegetative cell size decrease and the main strategies to restore cell size.

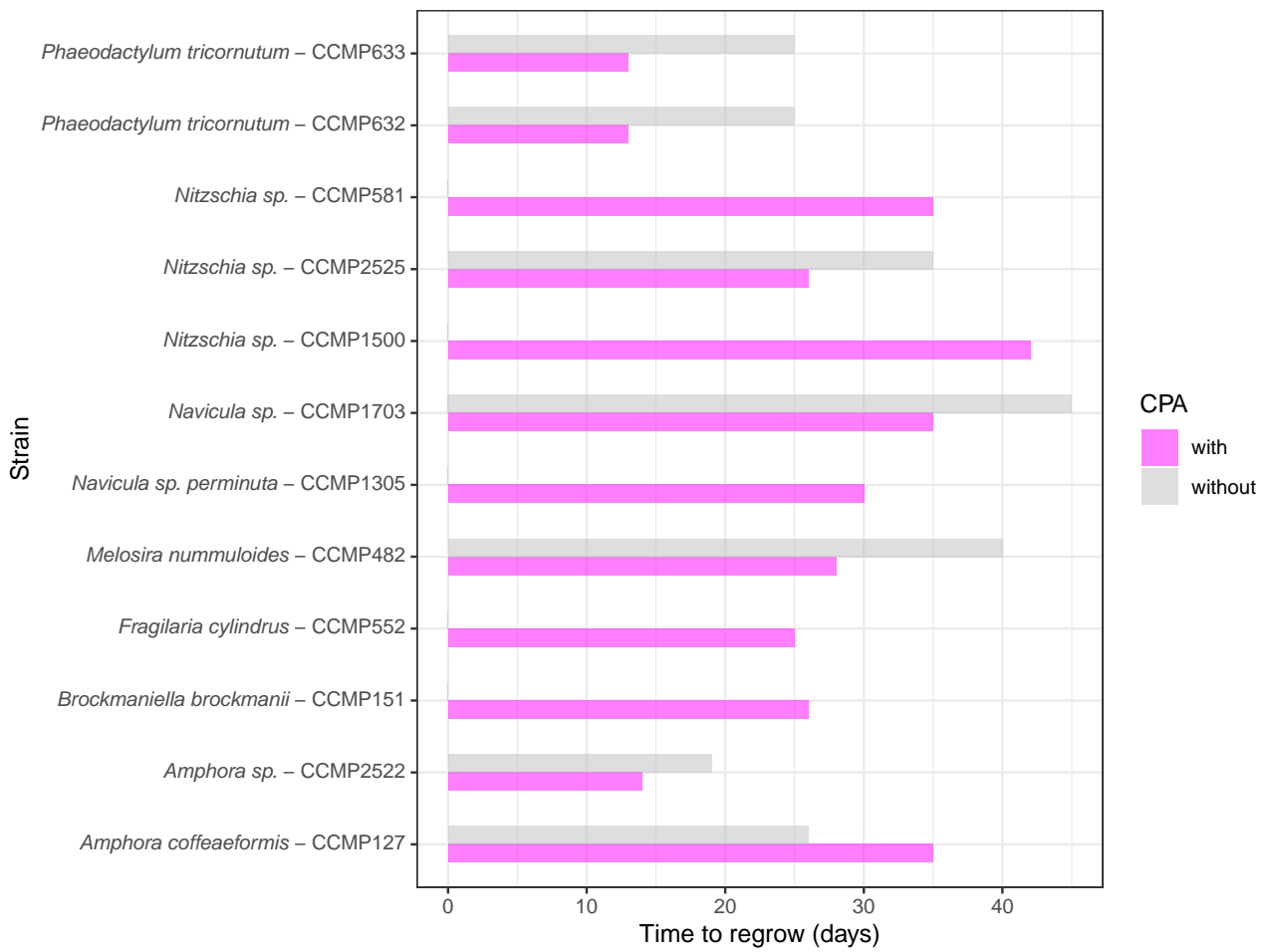


Figure 10: Growback times for diatom strains cryopreserved with and without cryoprotective agents (CPA) added.

933 Supplementary Material

934 **Supplementary Data S1.** List of diatom strains inventoried with associated metadata (tax-
935 onomy, origin, cryopreservation status): <https://doi.org/10.5281/zenodo.8163805>. Taxonomic
936 assignation of genera (i.e. class, order, family) is according to AlgaeBase ([https://www.
937 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. In the genus columns, taxa ending with X, XX, or XXX correspond to strains that could only be identified at the family, order or class level, respectively.

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	32	20	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

Table S1: (continued)

Class	Genus	+	-	% success
Bacillariophyceae	Fragilariales_XX	1	0	100
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

Table S1: (continued)

Class	Genus	+	-	% success
Bacillariophyceae	Pseudo-nitzschia	5	8	38
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

Table S1: (continued)

Class	Genus	+	-	% success
Coscinodiscophyceae	Triceratium	2	2	50
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