University of the Highlands and Islands
Oilthigh na Gàidhealtachd agus nan Eilean

UHI Research Database pdf download summary

Microzooplanktonic grazers - A potentially devastating threat to the commercial success of microalgal mass culture

Day, John; Gong, Yingchun; Hu, Qiang

Published in:
Algal Research

Publication date:
2017

Publisher rights:
© 2017 The Authors. Published by Elsevier Ltd.

The Document Version you have downloaded here is:
Peer reviewed version

The final published version is available direct from the publisher website at:
10.1016/j.algal.2017.08.024

Link to author version on UHI Research Database

Citation for published version (APA):
Day, J., Gong, Y., & Hu, Q. (2017). Microzooplanktonic grazers - A potentially devastating threat to the commercial success of microalgal mass culture. *Algal Research*, *27*, 356-365.
https://doi.org/10.1016/j.algal.2017.08.024

General rights
Copyright and moral rights for the publications made accessible in the UHI Research Database are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights:

1) Users may download and print one copy of any publication from the UHI Research Database for the purpose of private study or research.
2) You may not further distribute the material or use it for any profit-making activity or commercial gain
3) You may freely distribute the URL identifying the publication in the UHI Research Database

Take down policy
If you believe that this document breaches copyright please contact us at RO@uhi.ac.uk providing details; we will remove access to the work immediately and investigate your claim.

Download date: 27. Nov. 2022

1 Microzooplanktonic grazers – a potentially devastating threat to the commercial
2 success of microalgal mass culture

3

4 John G. Day[a*], Yingchun Gong [bc], Qiang Hu [bcd*]

5

6 Culture Collection of Algae and Protozoa, Scottish Association for Marine Science, Scottish Marine
7 Institute, Oban, PA37 1QA, Scotland, UK [a]; Center for Microalgal Biotechnology and Biofuels, Institute of
8 Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China [b]; Key Laboratory for Algal Biology,
9 Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China [c]; SDIC Microalgae
10 Biotechnology Center, China Electronics Engineering Design Institute, Beijing 100142, China [d]

11 [*] Corresponding authors: John G. Day (John.Day@sams.ac.uk); Qiang Hu (huqiang@ihb.ac.cn)

12

13 ABSTRACT

14 Eukaryotic microalgae and prokaryotic cyanobacteria are globally the most important primary producers,
15 forming the base of food web in aquatic ecosystems. As such, they are eaten by a huge diversity of
16 protistan taxa (e.g., amoeba, flagellates and ciliates), as well as zooplanktonic and larger metazoan grazers.
17 As in terrestrial agriculture, grazing has the potential to devastate the microalgal "crop" and this has
18 obvious implications to the commercial success of the developing microalgal industry. Whilst in
19 conventional agriculture thousands of years of exploitation of a relatively small number of crop plants, has
20 resulted in tools, knowledge and strategies that can manage this issue, in the case of microalgal mass
21 culture this is relatively undeveloped. This review explores our current understanding of the issue and
22 where further research is needed, focusing on the diversity of grazers and how microalgae under various
23 environmental regimes and culture conditions avoid being annihilated. In addition, the implications of
24 algal mass culture, where the objective is to maintain a virtual monoculture, are discussed in the context of
25 how infection could be prevented/ minimised and if infection occurs, how this may be managed to prevent

excessive losses in productivity or quality of the algal crop. The ultimate objective would be the development of robust methodologies for the early detection of “infection” of microalgal mass-cultures. This would allow the timely implementation of best management practices to prevent/reduce, damage caused by grazing. In reality, whilst there will be areas of commonality, as in terrestrial agricultural crops, methods will be need to be specifically tailored for each algal taxon, cultivation system and location.

Keywords:

Algal biofuel

Amoeba

Biological constraints

Ciliates

Grazers

Rotifers

1. Introduction

Microalgae are sunlight-driven cell factories that photosynthetically convert carbon dioxide, water and mineral nutrients to potential products such as: biofuels, human foods, animal feeds and high value compounds. The advantage of using microalgae rather than other higher plants have been well documented and include factors such as: many taxa have very high photosynthetic efficiencies and biomass productivities and can grow in conditions that are not favourable for terrestrial biomass production [1]. Thus, microalgae can provide an alternative to current unsustainable over-exploitation of natural resources, with possibility of providing a solution to the environmental dilemma of food versus energy production on high quality arable land. Whilst man has used microalgae, in particular *Spirulina/Arthrospira*, as a food for hundreds if not thousands of years [2, 3], the origins of the current development of the microalgal biotech

50 sector may be traced to the 1940's, where attempts to grow microalgae were focussed on finding
51 alternative sources of chemicals for use in munition manufacturing during the Second World War, by
52 examining the production of lipids by various micro-algae [4, 5]. Later, during the oil-crisis of the 1970's,
53 when the price of crude oil was high, microalgae were "revisited" for their potential in biofuels based on
54 their ability to accumulate oil, usually in the form of triacylglycerol's [6]. Over the past ten years there has
55 been an upsurge in interest in the commercial potential of microalgae stimulated by factors including:
56 concerns associated with anthropogenic climate change, energy supply and security and increased interest
57 in higher value metabolites for use in the food, pharma and wellness sectors. A variety of strategies have
58 been proposed including coproduction of biofuels with high-value products [7]. To date, technologies have
59 been developed for the production of a range of high and intermediate-value products at a commercial
60 scale, such as health foods, aquaculture feeds and niche-market "healthy" oils and industrial oils, as well as
61 specific high-value products. The latter are mostly lipid-based nutraceuticals or cosmeceuticals such as
62 carotenoids and omega-3 polyunsaturated fatty acids [8]. Some of the algae producing products, such as
63 beta-carotene, are cultivated profitably at large scale in artificial, saline lagoons, and raceway pounds [9].
64 Other microalgae cultivated under less harsh environmental conditions, such as *Haematococcus pluvialis*
65 for astaxanthin production, are more susceptible to being outcompeted by other microalgae and are most
66 commonly grown in more enclosed photobioreactor systems [10, 11].

67 Key to the commercial success is the cost of production at the commercial scale. However, there is
68 relatively limited public domain knowledge about costs of microalgal cultivation and processing at the
69 commercial scale. It has been suggested that model-based simulations, combined with pilot-plant
70 production data, can fill this gap and a recent study by Ruiz et al. [12] performed a techno-economic
71 evaluation of the whole process chain including cultivation, biorefinery and market exploitation for a 100
72 hectares facility in six locations. Their projections indicated a current cost per unit of dry biomass of 3.4
73 Euro kg^{-1} for microalgae cultivation in Spain (excluding biorefining products), with an expected reduction
74 to 0.5 Euro kg^{-1} in the next ten years. At the current production costs a range of high-value products (e.g.,
75 polyunsaturated fatty acids and pigments) would be currently profitable, but products aimed at the food
76 and bulk chemical commodities markets require further production cost reductions to become
77 economically viable. Efficient and sustainable microalgal cultivation is only likely to be commercially

profitable if conducted in either photobioreactors, or open pond systems sited on land, within which growth conditions can be controlled and optimised. For most if not all envisaged products, a large number of factors require optimisation including choice of microalga(e), nutrients, pH etc., in addition to the engineering aspects of microalgal production and downstream processing. However, a key factor commonly overlooked is the issue of “crop” loss due to grazing. The implications of a reduction in productivity and quality of harvested biomass, due to removal of the microalgae by grazers, at best reduces profitability and where a catastrophic culture “crash” results from grazing pressure this could be an industry threatening issue.

2. Grazers

Algae are crucial to the “health” of the planet, contributing approximately 50% of the total global photosynthetic activity [13] and forming the basis of the food chain for over 70% of the world's biomass [14]. In almost all natural aquatic environments top down control, i.e., grazing by ciliates, amoeba, rotifers and other zooplankton form a key aspect of the food web and as such have significant influence on ecosystems and are critical to the effective functioning of the microbial loop [15]. Although there is much variability from site to site worldwide, on average meiofauna, i.e., organisms with a body size of <1mm, alone graze at a rate of 0.01 h^{-1}, or 1% of the standing stock of both heterotrophs and autotrophs per hour [16]. There is a considerable body of literature on microzooplanktonic grazers, which includes reviews on freshwater and marine ecosystems [17, 18], mesocosm studies [19] and the roles of grazers in manmade environments such as wastewater treatment plants [20]. Clearly, their capacity to ingest microalgae has major implications to the development of algal mass culture systems, with respect to productivity, sustainability and commercial viability. The key factors that require consideration are the impact that grazers have on algal productivity and, in mixed populations, competition between different algal taxa. This largely depends on the mode of grazing and the selectivity of the grazer(s). It is known that an individual grazer taxon may exhibit preference for certain food items and selection has been shown to be influenced by prey size [21, 22], motility [23], as well as the chemical characteristics of the food particle/alga [24]. Furthermore, some species are able to discriminate against inert particles [25]. However, the morphological variety of the both grazers and their potential food (Fig. 1) is such that in reality most potentially commercially exploitable algae are at risk of being eaten by grazers.

106 Industry threatening grazers range from macroscopic insects to microflagellates, barely larger than the
107 alga(e) they ingest. Aquatic insect larvae have been reported to graze in *Spirulina* ponds [26, 27] and are
108 effectively unavoidable in open freshwater pond systems. Larger zooplankton such as the Brine shrimps
109 *Artemia* and *Paraartemia* may be a significant problem in marine, or even hypersaline pond systems where
110 the salt levels drop below 15% (w/v) NaCl [6]. In freshwater, brackish and marine media-based
111 production systems rotifers and cladocerans may be the major grazing zooplankton having the potential to
112 reduce algal concentrations and production to low levels within just a few days or weeks [28, 29]. For
113 example, rotifers and cladocerans at high densities ($>10^5$ L^{-1}) have been reported to reduce algal cell
114 density by 90% within 2 days [30] and Cauchie et al. [31] measured a 99% reduction in algal chlorophyll-a
115 due to *Daphnia* grazing over several days in an open pond system. Debatably protozoa, because of their
116 size, diversity and speed of reproduction, pose the largest threat to commercial exploitation of algae. The
117 authors have observed a 90% reduction in the cyanobacterium *Oscillatoria* within 5 to 6 days, with a
118 corresponding 100-fold increase in the grazing ciliate *Nassula* [32]. In a recent study by the authors, scale-
119 up of *Chlorella* production in an open pond system was severely constrained by repeated contamination
120 and grazing by one species of chrysophyte, which was identified as *Poterioochromonas malhamensis* [33].
121 Additionally, we have observed on cultivation of *Scenedesmus* that the microalga usually grew well,
122 however, culture quality and productivity deteriorated when it was invaded by vampyrellids [34]. This
123 effect is not restricted to freshwater taxa, grazing ciliates have been observed to clarify dense outdoor mass
124 cultures of *Dunaliella salina* within 2 days [35]. Furthermore, in *Dunaliella* ponds when the salinity drops
125 below 20% (w/v) NaCl, amoeba and ciliates can rapidly decimate the algal culture [36]. In the context of
126 ensuring consistency of productivity, grazing is a widespread problem and there is a growing literature on
127 the topic (Table 1). It is worth noting that many of the microzooplanktonic grazers listed are capable of
128 forming resistant resting stages, cysts spores etc. that may remain viable for many years. These may
129 remain dormant in sediments or biofilms within production facilities, or be spread by wind currents [37]
130 and as such form a major threat to open pond production systems in particular. Defences of algae against
131 predation and the prevention of infection of production facilities are discussed in the following sections.

132 3. Natural defences

133 3.1 Morphological adaptation

134 Planktonic microalgae, especially nanoplanktonic species with dimensions of 2 - 30 µm are highly
135 susceptible to zooplankton grazing [69, 70]. However, algae with larger cell sizes, 20 - 30 µm or more in
136 longest dimension, are generally less susceptible to being ingested by microzooplanktonic grazers, simply
137 because of their size [69]. This morphological "solution" of becoming too large to be consumed conflicts
138 with the selection pressure of resource acquisition, which generally favours algae with smaller cell sizes
139 and short doubling times [71]. The capacity for rapid resource acquisition and growth is a major factor in
140 the selection of biotechnologically exploitable algal strains [8] and to some extent explains the focus on
141 organisms with small cell size including *Chlorella* and *Nannochloropsis.*

142 Many algal taxa have relatively plastic phenotypes, with different cell sizes and morphological
143 features being observed at different points in their life cycle, or under different environmental conditions.
144 The prokaryotic cyanophyte *Microcystis aeruginosa*, a common bloom-forming organism, on cultivation
145 in the lab invariably grows as a unicellular suspension. In nature and under experimental conditions,
146 including when a culture of the flagellate grazer *Ochromonas* sp. was placed in dialysis tubing in a culture
147 of the cyanobacterium, *M. aeruginosa* colony formation was induced [72]. This behaviour has also been
148 reported by Yang et al. [73] as a response to direct grazing pressure on *M. aeruginosa* by *Ochromonas* sp.
149 In both cases the colonies formed exceeded the upper size limit for ingestion by the flagellates, thus
150 protecting the algae from predation. Grazing protection has also been observed in filamentous
151 cyanobacteria, for example *Aphanizomenon* has been observed to form flake-like assemblages in the
152 presence of the grazer *Daphnia*, but grew as single filaments in their absence [74]. Similar responses have
153 been reported for eukaryotic microalgae, for example the green alga *Chlamydomonas reinhardtii* usually
154 occurs as single, bi-flagellated cells in culture. However, as for other members of the Volvocales, under
155 appropriate environmental conditions, *C. reinhardtii* can also form palmelloid life stages. In a study on
156 exponentially growing unicellular *C. reinhardtii*, cultured in the presence of a grazer, the rotifer
157 *Brachionus calyciflorus,* the alga formed mucoid, palmelloid colonies within 25 hours [41]. A similar
158 response, also attributed to info-chemicals released by grazers, has been observed in *Scenedesmus*
159 *subspicatus* and *S. acutus* where colonial forms are less vulnerable to grazing, but they have higher sinking
160 rates and, thus, may be more likely to suffer from light limitation than single-celled forms [63]. A further
161 example of grazer-induced mucilaginous colony production is by the bloom-forming prymnesiophyte

162 *Phaeocystis globosa* which may be induced to form colonies by the heterotrophic dinoflagellates *Noctiluca*
163 *scintilans* and *Gyrodinium dominans* [75]. The poor nutritional quality of the colonies can result in low or
164 inefficient feeding by copepods [76]. To date, there has been a limited amount of work on the specificity
165 of microzooplanktonic grazer-induced phenotypic plasticity, although the study by Luo et al. [77] indicates
166 that there is a specific interaction, at least at the genus level, although it was observed that different strains
167 of the same species demonstrated different levels of response. In field samples of *Micractinium pusillum*,
168 the alga forms characteristic cuboidal or tetrahedral colonies with long and strong bristles, much more
169 frequently than in cultured samples, which on long-term routine maintenance in culture collections lose
170 their bristles/ spines [78]. Medium from cultures of *B. calyciflorus* was demonstrated to induce long
171 bristles (50—100 μm) in *M. pusillum*; however, medium from *Daphnia* cultures failed to induce bristle
172 formation [77]. On testing a related chlorophyte, *Chlorella vulgaris*, no effect was observed on challenging
173 cultures with samples of either grazer. Further work is needed to gain greater understanding of the nature
174 and function of info chemicals and their capacity to induce protective phenotypic change in algae, but the
175 implications to their use to protect algal crops has obvious potential.

176

177 3.2 Chemical antipredator defence

178 It has been known for many years that microalgae are capable of producing bioactive metabolites,
179 with one of the first reports being on the production of an antibacterial fatty acid, Chlorellin, isolated from
180 cultures of *C. vulgaris* [79]. The bioactive metabolites produced by algae encompass a wide range of
181 chemical entities, with activities against viruses, bacteria, fungi, protozoa, as well as potential use in the
182 treatment of cancers and other health problems [80, 81, 82]. It may be speculated that algae produce these
183 chemicals to give them an ecological advantage and it is known that in natural ecosystems substances can
184 be released by cyanobacteria into the waterbody that influence feeding rates of zooplankton, as
185 demonstrated by Ostrofsky et al. [83] who reported inhibition of feeding rates by compounds released by
186 *Anabaena flos-aquae* under both lab and field conditions. This phenomenon appears to be relatively widely
187 spread across both cyanobacterial and microalgal taxa and it would appear logical to assume that toxin
188 production is specifically related to grazing inhibition. However, published evidence suggests otherwise,
189 for example the systematic study undertaken by Wang et al. [84]. This study investigated the effects on

190 the rotifer *B. plicatilis* of 10 strains of *Alexandrium* including eight toxic (PSP-toxin producers) and three
191 non-toxic isolates, their media and material from lysed cells. The feeding experiments demonstrated that
192 the rotifers readily ingested cells of a non-toxin containing isolate of *A. tamarense*, as well as *A.*
193 *lusitanicum*, and *A. minutum*, both of which contain PSP-toxin, but grazed relatively less, or not at all, on
194 the other seven *Alexandrium* strains tested. Ingestion of PSP toxin containing cells from these two known
195 toxic species had no impact on rotifer mortality; however, even though no PSP toxins were found in
196 *Alexandrium* sp1 and *Alexandrium* sp2, these resulted in a collapse of the rotifer populations, as did the
197 other five toxin containing isolates tested. It thus appears likely that toxic mechanism(s) other than PSP
198 were responsible for causing the lethal effects on the rotifers.

199 To date, in most cases, there is little published on either the structure or functional mode of algal-
200 produced antipredator chemicals. However, a significant body of work has been undertaken on the model
201 grazer, the dinoflagellate *Oxyrrhis marina,* which on ingestion and subsequent lysis of the alga *Emiliania*
202 *huxleyi*, converts dimethylsulphoniopropionate (DMSP) from the alga, to dimethyl sulphide (DMS) via the
203 enzyme DMSP lyase [85]. In a subsequent study [86] it was demonstrated that this reaction deters
204 protozoan herbivores, presumably through the production of highly concentrated acrylate, and that *O.*
205 *marina* differed in its ability to ingest and survive on algae with high-activity DMSP lyase, but
206 preferentially select strains with low enzyme activity when offered prey mixtures. This response was not
207 restricted to a single grazer, as addition of DMSP has also been reported to reduce grazing on *E. huxleyi* by
208 the dinoflagellates *Amphidinium longum* and *Gymnodinium* sp., as well as the ciliate *Coxliella* sp. [87].

209 An alternative protective strategy involves the release of chemicals that do not prevent, or reduce, grazing
210 on the alga, but reduce fecundity of the grazer. Diatoms are major primary producers at the base of the
211 marine food web and their main predators include the herbivorous copepods. Secondary metabolites,
212 including defensive oxylipins, released by these algae immediately after grazing-induced cell damage, are
213 targeted not against the predators themselves, but rather at interfering with their reproductive success [88].
214 This strategy is obviously slower acting than directly killing the grazer, or deterring it from ingestion of
215 algal cells, but has the potential to facilitate managed coexistence of the algae and grazers.

216 A significant amount of additional research is required to identify the range of anti-grazer compounds
217 that are produced by algae, how their production can be controlled, or stimulated, and elucidation of their

218 mode(s) of action. The possibility of harness the potential of the natural "chemical warfare" between the
219 algae and their grazers has commercial implications and could be a crucial component in the management
220 of future algal production facilities. Clearly, this is a future area of research that warrants further
221 investigation.

222

223 **4. Microalgal mass culture**

224 In the context of commercial exploitation the scale of production in algal biotechnology can vary
225 hugely with 10's or 100's of litres being appropriate in the context of the needs for small-scale aquaculture
226 applications, but for other products considerably larger volumes/ foot-prints are needed. For example, the
227 βcarotene production facility in Hutt Lagoon Australia is ~740 hectares and future algal biofuel facilities,
228 in order to be economically viable, will need to be significantly larger again. Irrespective of the
229 volume/size of production plant, or whether the algae are being cultivated in closed photobioreactors, or
230 open pond-based systems, infection by microzooplanktonic grazers poses a threat that could potentially
231 devastate the algal crop, or at best reduce productivity and hence commercial viability.

232

233 4.1 Detection

234 By the time an algal culture is being heavily grazed the effects may be obvious by eye as a gross
235 change in colour, with a reduction in the green colouration as the algal cell density reduces and in extreme
236 cases an increase in brown colouration as the grazers "bloom" and the algae die and lyse. Whilst changes
237 in colouration, optical density, or turbidity could all form the basis of an automated monitoring system,
238 these are unlikely to be sufficiently sensitive to detect the early stages of infection. Therefore, by the time
239 the grazers have been detected it may be too late to apply any strategies that may be available to control or
240 manage the infection. It is, therefore, important to be able to have an "early warning system" one can
241 potentially prevent grazing becoming a problem.

242 Microscopy is the most obvious approach to detection and enumeration. Sample size may vary, but is
243 restricted in the case of Haemocytometers to <1.0 µl, or if a larger chamber such as a Sedgwick Rafter cell

244 [89] is employed to 1.0 ml. Additionally, the size and configuration of the Haemocytometer may preclude
245 its use in detecting or enumerating large grazers including rotifers and copepods. An alternative to examine
246 larger volumes (50-100 ml) is to fix samples and then employ sedimentation chambers, where the samples
247 can be observed using an inverted microscope [90]. This approach has the advantage that much lower
248 densities of grazers may be detectable directly from the sample. However, the samples need a settlement
249 phase, generally 12 - 20 h, so there is a significant delay in being able to detect, or enumerate, any grazers
250 present. Furthermore, from the authors experience it is problematic when algal cell densities are high (> 1
251 x 10^6 cells ml^{-1}) as the algae may "bury" the grazers and although it works well for larger zooplankton,
252 many protozoans will lyse on being treated with some fixatives. However, in many cases employing
253 Lugol's (2%, final fixative concentration) is effective and if cells are too dark a few drops of sodium
254 thiosulfate can be added to bleach the sample.

255 More recently cytometric approaches have been trialled using a FlowCAM [91, 92] that can readily
256 detect a wide range of grazers including: amoeba, flagellates, ciliates and zooplankton. This method has a
257 better detection capability than microscopy, with reported detection limits of <1 individual ml^{-1} for *B.*
258 *calyciflorus* in an algal culture (*Chlorella* sp.) with a cell density of 10^7 cells ml^{-1} [92] and <10 cells ml^{-1}
259 for both ''large'' and ''small'' ciliate grazers, *Euplotes vannus* (~80 x 45 µm) and an unidentified
260 holotrichous ciliate (~18 x 8 µm) respectively, in *N. oculata* cultures with a cell density of 10^7 cells ml^{-1}
261 [91]. Furthermore, small ciliates could be detected in cultures with algal densities of up to 1.4 x 10^8 cells
262 ml^{-1} (>0.5 g l^{-1} dry wt.) [91]. This approach has a number of further advantages in so far as it has the
263 potential to be fully automated and, although samples are still relatively small, flow rates of between 0.1 –
264 1.0 ml min^{-1} are readily achievable. However, there are limitations to the system, as it was not technically
265 possible under the configurations employed, to use the system to directly examine cultures with >2.0 x 10^8
266 *N. oculata* cells ml^{-1}, as this was beyond the computational/ image capturing capacity of the FlowCAM
267 [91]. Furthermore, at cell densities >3.0 x 10^8 *N. oculata* cells ml^{-1}, using the flow-cells available, the
268 system invariably became blocked [91].

269 An alternative strategy that could be employed is to use a targeted molecular approach whereby
270 known individual grazers, or groups of grazers could be identified employing multiple polymerase chain
271 reaction (PCR)-based tools. On using allele-specific probes and monitoring contaminants using

272 Quantitative PRC (QPCR) "weedy" invader algae may be detected at levels as low as one in 10^8 cells in a
273 culture [93]. Furthermore, this study demonstrated that the QPCR method developed was 10^4 times more
274 sensitive than flow cytometry in the detection of *Tetraselmis striata* cells serially diluted in
275 *Nannochloropsis salina* culture [93]. The approach has also been employed to detect the parasitic ciliate
276 *Cryptocaryon irritans*, which causes "white spot disease" in marine fishes, from the natural environment at
277 cell densities of <1 cells ml^{-1} [94]. Furthermore Carney et al. [95] have demonstrated that microbiome
278 analysis by Second Generation Sequencing has the capacity to identify both eukaryotes and bacteria agents
279 associated culture crashes in raceway mass cultures of *Nannochloropsis salina.* In addition to known
280 grazers, such as *Brachionus* and the gastrotrich *Chaetonotus,* additional organisms with the potential to
281 ingest *N. salina* including the suctorian ciliate *Acineta,* the potentially parasitic vampyrellid, *Vampyrella*
282 and the amoeba *Nolandella* were identified [95]. The rapid developments in the use of high-throughput
283 community sequencing and the availability and increasing sophistication of bioinformatic tools suggest to
284 the authors that ultimately molecular approaches will be the most probable way forward for the early
285 detection of grazers in algal crops.

286 Irrespective of the detection method that may be employed, unless the option for on-line continuous
287 monitoring is available, the key to early detection will be the instigation of a suitable sampling regime.
288 This will need to be tailored to the requirements of the individual production system, but it is suggested
289 that a minimum of daily sampling at a number of points in each production unit (pond, bioreactor, etc.) is
290 needed. Furthermore, sampling of sediments and/or any biofilms, if present, should be included, as these
291 may be potential sources of inocula that could result in infection. In addition, effective grazer DNA/RNA
292 sample preparation is critical, particularly in high-density microalgal cultures where the cell density of
293 microalgae may be much greater than that of the invader grazers at early stages of infection. This makes it
294 difficult to get enough grazer DNA/RNA for an effective gene-level analysis, as the grazer DNA/RNA
295 materials may be lost or masked by the overwhelming amount of microalgal DNA/RNA isolated from an
296 infested sample. A method for selectively separating or enriching grazer DNA/RNA from an algae-
297 dominant background needs to be developed.

298

299 4.2 Prevention of infection

300 There is a limited number of publications specifically suggesting how to reduce, or prevent, infection
301 of algal crops by grazers. The most obvious approaches involve engineering orientated solutions and/or
302 having housekeeping procedures in place that reduce the risk of initial infection. For closed
303 photobioreactors these are more readily achievable. An appropriately high specification photobioreactor,
304 ideally with the possibility of sterilizing both the unit and medium, with all manipulations undertaken
305 aseptically will theoretically not become infected. However, such ideals are not practicable on scale-up,
306 due to their cost implications, unless the product is of high commercial value. In reality, cleaning and
307 sanitisation rather than sterilization is achievable and these procedures must be tailored to the unit with the
308 objective of minimising the possibility or introduction of cysts/eggs of microzooplanktonic grazers. At the
309 planning stage engineering options that minimise connections between separate production units to avoid
310 cross-contamination should be included. Furthermore, on planning and construction it should be ensured
311 that there are no areas where detritus can remain between production batches, as they might retain live or
312 resting stages of the grazers. In open systems the challenge is even greater and in reality little can be done
313 to avoid infection beyond approaches like using netting to prevent the loss of algae associated insect larvae
314 grazing, which has been successfully implemented in in *Spirulina* ponds [6]. In addition, employing
315 physical barriers, such as the use of green houses, or "Poly-tunnels" may reduce the risk of wind-borne
316 infection by cysts and eggs of grazers [32]. In reality, management protocols and the possible use of
317 treatments or other approaches to manage infection and subsequent grazing will provide the only
318 practicable solutions in production scale facilities.

319

320 4.3 Treatment

321 A wide variety of approaches, with varying degrees of success, have been trialled to control grazers of
322 microalgae and cyanobacteria (Table 2). Direct engineering solutions to prevent or reduce algal grazing are
323 fraught with difficulties and physical separation is not always possible, as in many cases both microalgae
324 and their grazers are similar in size (preventing size-fractionation or centrifugation approaches). These
325 techniques are in addition relatively expensive and time consuming, so some tested engineering
326 approaches will not be practicable above lab-scale. However, cavitation induced by ultrasonication has

327 significant potential and has been demonstrated to be effective in inactivating large zooplankton, such as
328 *Ceriodaphnia dubia*, *Brachionus plicatilis* and *B. calyciflorus*, with little effect on microalgae [96].

329 Chemical treatment, analogous to the use of pesticides in agriculture, has been considered, but these
330 present issues associated with efficacy, cost and environmental damage. The use of many broad-spectrum
331 metabolic inhibitors, such as Cycloheximide, is inappropriate as in most cases both the algal crop and the
332 potential grazers are eukaryotes. There are a few targeted anti-grazers pesticides, for example the
333 protozoan specific inhibitor Cytochalasin [97], or quinine sulphate, which has been demonstrated to
334 rapidly destroy ciliates in an outdoor pond when dosed at 10 mg l^{-1}, whilst not damaging the alga *D. salina*
335 [35]. Additionally, the anti-protozoan drug Metronidazole has been successfully used in treating
336 *Scenedesmus* sp. cultures invaded by the zooflagellate *Amphelidium* sp. [59]. However such chemicals,
337 where they are available, would be prohibitively expensive to use at production scale, in addition to any
338 collateral environmental damage that may result from their application. It is worth noting that various
339 approaches have been used successfully in small-scale cultures, for example reasonable success was
340 obtained with formalin, methylene blue and malachite green at low concentrations in *Chlorella* and
341 *Dunaliella* cultures respectively [42. 98]. Other strategies that have been trialled for their suitability for use
342 in open ponds include: the use of copper to selectively inhibit *B. calyciflorus* growth in a mass culture of
343 *Chlorella kessleri* [99]; hyperchlorite in *Nannochloropsis* cultures to control protozoa and disadvantage
344 invasive strains [100]; treatment with the use of ammonia to treat rotifers and cladocerans in open ponds;
345 and pH adjustments (decrease to pH 3 for 1–2 hours, followed by adjustment to pH 7.5) have been used to
346 target rotifers [101, 102] and heterotrophic flagellates [66]. Glyphosate and ozone have been used to
347 maintain *Nannochloropsis* cultures [99, 103]. Whilst the use of conventional pesticides including:
348 Dipterex, Parathion, and dichlorodiphenyltrichloroethane (DDT) have been historically demonstrated to
349 control zooplankton in laboratory-scale *Chlorella* sp. cultures, these have not been used in large-scale
350 culture [44] and today would no longer be considered environmentally acceptable.

351 The above treatments are not likely to be applicable at scale and alternative more readily scalable and
352 cost-effective approaches are required. Options that involve temporarily changing environmental
353 conditions such as osmotic potential, pH, or temperature, all have significant engineering and cost
354 implications. However, where systems have been installed to increase CO_2 levels to boost algal

355 productivity these could in parallel be employed to control the grazer population. Increasing pond night-
356 time CO_2 concentration by gas injection of pure CO_2, has been demonstrated to result in the rapid control
357 of a zooplankton bloom in a 1.5 m^3 microalgal cultures [104]. Furthermore, the authors [33] have recently
358 investigated the effect of culture pH (i.e., 6.0, 6.5, 7.0 and 7.5), maintained by supply of compressed air
359 bubbles containing various concentrations of CO_2, on *Chlorella sorokiniana* culture stability in the
360 presence of a grazing threat by the flagellate *P. malhamensis* and several other protozoa. When CO_2 levels
361 were low, culture crashes due to grazing were observed, and it was speculated that increased CO_2 partial
362 pressure in the culture medium enhanced diffusion of CO_2 into the cytoplasm of *P. malhamensis*, reducing
363 the intracellular pH leading to cell death [33]. This approach has been successfully trialled in the field in a
364 100 l raceway pond where increasing the CO_2 supply to a *C. sorokiniana* culture reduced the pH from 6.8
365 to 6.0 leading to a significant reduction in *P. malhamensis* numbers and elimination of other grazers within
366 8 h of initiating the treatment [33].

367 An alternative approach that is included here for completeness is to employ top-down biological
368 control, such as the introduction of higher trophic predators. This could include larger copepods or
369 predatory rotifers. This approach has been employed by Mitchell and Richmond [105] to use rotifers to
370 remove microalgal contaminants from *Spirulina*; however, for many potential algal crops using this
371 approach to target grazers is likely to be problematic as they will invariably consume both microalgae and
372 their grazers. The alternative bottom up biological control approach of introducing targeted grazer
373 pathogens is equally problematic. Furthermore, the authors are unaware of any known, cultivatable, target-
374 specific protozoan or other microzooplanktonic pathogens, or viruses, which could be introduced to
375 instigate biological control.

376 **5. Further approaches to crop protection**

377 It is certain that there is no "one size fits all" solution(s) to the prevention and control of grazers in
378 microalgal mass culture. Furthermore, there is a great deal of work needed before effective strategies are
379 available and the likelihood is that bespoke solutions will be required for each alga and production plant.
380 Prevention of infection is invariably more likely to be effective than treatment, or the management of
381 contaminant grazers, but there are constraints with respect of the economic viability of many potential
382 products if high cost, sterilisable, bio-secure bioreactors have to be employed. A number of important gaps

383 in our current knowledge and opportunities have been identified above and these are synthesised in the
384 three paragraphs below.

385 Firstly, "know your enemy", although there is a considerable body of work on different grazer taxa,
386 there is still, to some extent, a lack of coordinated research that interconnects all aspects of this topic in the
387 context of algal biotechnology. Montagnes et al. [122] started this process but were specifically discussing
388 natural populations focussing on the needs for research to understand: searching, contact, capture,
389 processing, ingestion, digestion at community, population, and individual levels, and the need to develop
390 linkages in research focussing on food selection, food webs and modelling. Understanding the interactions
391 between the organisms involved and the capacity to manage mixed populations to the extent that grazing
392 of the crop is not at a level that results in either catastrophic crop loss, or a reduction in economic viability,
393 will be crucial to sustainability in the microalgal industry.

394 Secondly, breeding strain selection and the potential of modern molecular methods. Understanding
395 susceptibility to predation at the molecular and metabolomic levels will potentially allow the manipulation
396 of cultures or use of mutants that are less susceptible to grazing. For example, it has been hypothesized
397 that the SwmB protein defends *Synechococcus* from ingestion by *O. marina* by interfering with attachment
398 of dinoflagellate prey capture organelles or cell surface receptors [123]. Work has also been undertaken to
399 explore grazing resistance of *Synechococcus* by a heterolobosean amoeba. Challenging a library of mutants
400 of *S. elongatus* by an amoeba led to the identification of a grazer-resistant knockout mutant of the wzm
401 ABC O-antigen transporter gene, SynPCC7942_1126 and three other genes involved in O-antigen
402 synthesis and transport conferred resistance to being grazed [124]. Better understanding of how specific
403 protective traits, such as spine formation, may be induced at the molecular level could lead to the
404 development of GM algae that are commercially exploitable. Similarly, the capacity to grow in extremes of
405 osmotic potential could become the basis of a platform of products and processes, as the molecular
406 mechanisms become understood and transformation systems are developed. All of the options involving
407 the future industrial-scale cultivation and exploitation of transgenic algae raise issues with respect to
408 ethics, public acceptability, safety and stability that are beyond the scope of this review. However an
409 alternative, although less targeted, approach involving strain selection, potentially in conjunction with
410 conventional mutagenesis may also provide a platform to develop grazer resistant algal isolates.

411 Finally, integrated solutions that incorporate pest identification, tracking and management, as
412 developed by Sapphire Energy Inc. to address the challenge of *Scenedesmus dimorphus* infection by
413 *Amoeboaphelidium protococcarum* [125], will be required for each facility where a significant threat of
414 grazers occurs. In reality, this will involve all algal production facilities even those extremophiles where
415 the threat may be readily managed via osmotic potential or pH. The implementation of solutions that can
416 prevent crashes and offers the capacity to manage contamination in open ponds is crucial to the future
417 economic viability of algal biotechnology.

418

419 **Authors contributions**

420 The conception and design of the review were carried out by all authors. Data acquisition was primarily
421 performed by JGD and YG. JGD performed the initial drafting of the article, this and subsequent versions
422 were reviewed and revised by HQ and YG. YG prepared Fig 1. All authors contributed to the final
423 approval of the article.

424

425 **Acknowledgements**

426 JGD acknowledges National Capability funding for the Culture Collection of Algae and Protozoa (CCAP)
427 from the UK Natural Environment Research Council (NERC). This work was partially funded by the State
428 Development & Investment Corporation and China Electronics Engineering Design Institute, China (No.
429 Y34115-1-Z01-0007).

430

431 **References**

432 [1] A.S. Carlsson, J.B. van Beilen, R. Moller, D. Clayton, EPOBIO: Realizing the economic potential of
433 sustainable resources - Bioproducts from non-food crops, in: D. Bowles (Ed.), Micro and macro algae:
434 Utility for industrial applications. CPL Press, Newbury, UK, 2007, pp. 1-82.

435 [2] P. Dangeard, Sur une algae blue alimentaire pour I'homme *Arthrospira platensis*. Actes Soc Linn
436 Barcaux Extr Proces-verbeaux 91 (1940) 39-41.
437 [3] W.V. Farrar, Tecuitl: a glimpse of Aztec food technology. Nature 221 (1966) 341-342.
438 [4] J.S. Burlew, Algal culture from laboratory to pilot plant. Carnegie Inst. Wash. Publ. 600, 1953, pp 357.
439 [5] R. Harder, H.V. Witsch, Ütber Massenkultur von Diatomeen. Ber. deut. bot. Ges, 60 (1942) 146 - 152.
440 [6] M.A. Borowitzka, Culturing Microalgae in outdoor ponds, in: Andersen, R.A. (Ed.) Algal Culturing
441 Techniques. Academic Press, New York, M.A., 2005, pp 205-217.
442 [7] P.J. leB Williams, L.M.L Laurens, Microalgae as biodiesel & biomass feedstocks: Review & analysis
443 of the biochemistry, energetics & economics. Energy Environ. Sci. 3 (2010) 554-590.
444 [8] S.P. Slocombe, Q.Y. Zhang, M. Ross M, A. Anderson, N.J. Thomas, A. Lapresa, C.N. Campbell, K.D.
445 Black, M.S. Stanley, J.G. Day, Unlocking nature's treasure-chest: screening for oleaginous algae.
446 Scientific Reports 5 (2015) 9844
447 [9] M.A. Borowitzka, High-value products from microalgae—their development and commercialisation. J.
448 Appl. Phycol. 25 (2013) 743–756.
449 [10] M. Olaizola, (Commercial production of astaxanthin from *Haematococcus pluvialis* using 25,000-liter
450 outdoor photobioreactors. J. Appl. Phycol. 12 (2000) 499-506.
451 [11] J. Li, D. Zhu, J. Niu, S. Shen, G. Wang, An economic assessment of astaxanthin production by large
452 scale cultivation of *Haematococcus pluvialis*. Biotechnol. Adv. 29 (2011) 568-574.
453 [12] J. Ruiz, G. Olivieri, J. de Vree, R. Bosma, P. Willems, J.H. Reith, M.H.M. Eppink, D.M.M.
454 Kleinegris, R.H. Wijffels, M.J. Barbosa, Towards industrial products from microalgae. Energy Environ.
455 Sci. 9 (2016) 3036-3043
456 [13] W. Wiessner, E. Schnepf, R.C. Starr, Algae, environment and human affairs. Biopress Ltd., Bristol,
457 UK, 1995.
458 [14] R.A. Andersen, Algae, in J. Hunter-Cevera, A. Belt (Eds) Maintaining cultures for biotechnology and
459 industry. Academic Press, Inc., London, 1996, pp. 29-64.

460 [15] E.B. Sherr, B.F. Sherr, Significance of predation by protists in aquatic microbial food webs. Antonie
461 Van Leeuwenhoek Int. J. Gen. Mol. Microbiol. 81 (2002) 293-308.

462 [16] P. Montagna, Rates of metazoan meiofaunal microbivory: A review. Vie et Milieu 45 (1995) 1-9.

463 [17

464 [17] R.W. Pierce, J.T. Turner, Ecology of planktonic ciliates in marine food webs. Rev. Aquat Sci 6 (1992)
465 139-181.

466 [18] D. Vaque, J.M. Gasol, C. Marrase, Grazing rates on bacteria – The significance of methodology and
467 ecological factors. Mar. Ecol. Prog. Ser. 109 (1994) 263-274.

468 [19] U. Sommer, K.H. Peter, S.Genitsaris, M. Moustaka-Gouni, M Do marine phytoplankton follow
469 Bergmann's rule sensu lato? Biol Rev. 92 (2017) 1011-1026.

470 [20] B. Ayo, E. Santamaria, A. Latatu, I. Artolozaga, I Azua, J Iriberri, Grazing rates of diverse
471 morphotypes of bacterivorous ciliates feeding on four allochthonous bacteria. Lett. Appl. Microbiol. 33
472 (2001) 455-460.

473 [21] A. Andersson, U. Larsson, A. Hagstrom, Size-selection grazing by a microflagellate on pelagic
474 bacteria. Mar. Ed. Prog. Ser. 33 (1986) 51-57.

475 [22] S.S. Epstein, M.P. Shiaris, Size selective grazing of coastal bacterioplankton by natural assemblages
476 of pigmented flagellates, colorless flagellates and ciliates. Microb. Ecol. 23 (1992) 211-225.

477 [23] J.M. Gonzalez, E.B. Sherr, B.F. Sherr, Differential feeding by marine flagellates on growing versus
478 starving, and on motile versus nonmotile, bacterial prey. Mar. Ecol. Prog. Ser. 102 (1993) 257-267.

479 [24] P.G. Verity, Feeding in planktonic protozoans: Evidence for non-random acquisition of prey. J.
480 ProtozooI. 38 (1991) 69-76.

481 [25] H.L.J. Jones, B.S.C. Leadbeater, J.C. Green, Mixotrophy in marine species of *Chrysochromulina*
482 (Prymnesiophyceae): ingestion and digestion of a small green flagellate. J. Mar. Biol. Assoc. UK 73
483 (1993) 283-296.

484 [26] L.V. Venkatamaran, T.C.S. Kanya, Insect contamination (*Ephydra californica*) in the mass outdoor
485 cultures of blue green, *Spirulina platensis*. Proc. Indian Acad. Sci. 90 (1981) 665-677.

486 [27] A. Belay, Mass culture of *Spirulina* outdoors: The Earthrise Farms experience, in: Vonshak, A. (Ed.)
487 *Spirulina platensis* (*Arthrospira*): Physiology, cell biology and Biochemistry. Taylor & Francis, London,
488 A., 1997, pp. 131-158.

489 [28] T. van Harmelen, H. Oonk, Microalgae biofixation processes: applications and potential contributions
490 to greenhouse gas mitigation options TNO Built Environmental and Geosciences. Apeldoorn. Prepared for
491 the International Network on Biofixation of CO2 and greenhouse gas abatement with Microalgae.
492 Operated under the International Energy gency Greenhouse Gas R&D Programme, The Netherlands
493 (Order No. 36562), 2006.

494 [29] J.R. Benemann, Open Ponds and Closed Photobioreactors – Comparative Economics 5th Annual
495 World Congress on Industrial Biotechnology and Bioprocessing, Chicago, April 30, 2008.

496 [30] W.J. Oswald, Algal production – problems, achievements and potential, in: G. Shelef, C.J. Soeder
497 (Eds.), Algae Biomass. Elsevier North/Holland/Biomedical Press, Amsterdam, Netherland, 1980, pp. 1–8.

498 [31] H.M. Cauchie, L. Hoffmann, M.F. Jaspar-Versali, M. Salvia, J.P. Thomé, *Daphnia magna* Straus
499 living in an aerated sewage lagoon as a source of chitin: ecological aspects. J. Zoology 125 (1995) 67–78.

500 [32] J.G. Day, Grazers – the overlooked threat to sustained production of future algal biofuels. Biofuels 4
501 (2013) 459-461.

502 [33] M.Y. Ma, D.N. Yuan, Y. He, M. Park M, Y.C. Gong, Q. Hu, Effective control of *Poterioochromonas*
503 *malhamensis* in massive culture of *Chlorella sorokiniana* GT-1 by maintaining CO_2-mediated low culture
504 pH. Algal Res. (Accepted)

505 [34] Y.C. Gong, D.J. Patterson, Y. Li, Z. Hu, M. Sommerfeld, Y. Chen, Q. Hu, *Vernalophrys algivore*
506 gen. nov., sp. nov. (Rhizaria: Cercozoa: Vampyrellida), a new algal predator isolated from outdoor
507 mass culture of *Scenedesmus dimorphus*. Appl. Environ. Microbiol. 81 (2015) 3900-3913.

508 [35] I. Moreno-Garrido, J.P. Canavate, Assessing chemical compounds for controlling predator ciliates in
509 outdoor mass cultures of the green algae *Dunaliella salina*. Aq. Eng. 24 (2001) 8-14.

510 [36] F. Post, L. Borowitzka, M. Borowitzka, B. Mackay, T. Moulton, The protozoa of a Western
511 Australian hypersaline lagoon. Hydrobiologia 105 (1983) 95-113.

512 [37] S. Rodriguezzaragoza, Ecology of free-living amebas. Crit. Rev. Microbiol. 20 (1994) 225–241.

513 [38] P.L. Starkweather, Trophic relationships between the rotifer *Brachionus calyciflorus* and the blue-
514 green alga *Anabaena flos-aqua* Verh. Int. Ver. Limnol. 21 (1981) 1507-1514.

515 [39] W.E. Scott, F.M. Chutter, Introduction: infections and predators. Univ. Orange Free State Publ. Ser. C
516 3 (1981) 103-109.

517 [40] C. Méndez, E. Uribe, Control de *Branchionus* sp. y *Amoeba* sp. en cultivos de *Arthrospira* sp. Latin
518 American J. Aquatic Res. 40 (2012) 553-561.

519 [41] M. Lurling, W. Beekman Palmelloids formation in *Chlamydomonas reinhardtii*: defence against
520 rotifer predators? Ann. Limnol. - Int. J. Lim. 42 (2006) 65-72.

521 [42] S. Rothbard, Control of *Euplotes* sp by formalin in growth tanks of *Chlorella* sp used as growth
522 medium for the rotifer *Brachionus plicatilis* which serves as feed for hatchlings. Bamidgeh 27 (1975) 100-
523 109.

524 [43] X. Zhang, X., M.M. Watanabe, Grazing and growth of the mixotrophic chrysomonad
525 *Poterioochromonas malhamensis* (Chrysophyceae) feeding on algae. J. Phycol. 37 (2001) 738-743.

526 [44] Loosanoff, V., J. Hanks, and A. Ganaros, Control of certain forms of zooplankton in mass algal
527 cultures. Science 125 (1957) 1092-1093.

528 [45] J. Flores-Burgos, S.S.S. Sarma, S. Nandini, Effect of Single Species or Mixed Algal (*Chlorella*
529 *vulgaris* and *Scenedesmus acutus*) Diets on the Life Table Demography of *Brachionus calyciflorus* and
530 *Brachionus patulus* (Rotifera: Brachionidae). CLEAN - Soil Air Water 33 (2010) 614-621.

531 [46] F. Bader, H. Tsuchiya, A. Fredrickson, Grazing of ciliates on blue–green algae: Effects of ciliate
532 encystment and related phenomena. Biotechnol. Bioeng. 18 (1976) 311-332.

533 [47] L. Xinyao, S. Miao, L. Yonghong, G. Yin, Z. Zhongkai, W. Donghui, W. Weizhong, A. Chencai,
534 Feeding characteristics of an amoeba (Lobosea: *Naegleria*) grazing upon cyanobacteria: food selection,
535 ingestion and digestion progress. Microbial Ecol. 51 (2006) 315-325.

536 [48] B.B. Fischer, S. Roffler, R.I. Eggen, Multiple stressor effects of predation by rotifers and herbicide
537 pollution on different *Chlamydomonas* strains and potential impacts on population dynamics. Environ.
538 Toxicol. Chem. 31 (2012) 2822–28.

539 [49] H.A Vanderploeg, G.-A. Paffenhöfer, Modes of algal capture by the freshwater copepod *Diaptomus*
540 *sicilis* and their relation to food-size selection. Limnol. Oceanogr. 30 (1985) 871–885.

541 [50] X. Liu, M. Shi, Y. Liao, Y. Gao, Z. Zhang, D.H. Wen, W.Z. Wu, C.C. An, Feeding Characteristics of
542 an Amoeba (Lobosea : *Naegleria*) Grazing Upon Cyanobacteria: Food Selection, Ingestion and Digestion
543 Progress. Microbial Ecol. 51 (2006) 315-325.

544 [51] S. Savas, Z. Guclu, Filtration and ingestion rates of the rotifer *Brachionus plicatilis* fed five species of
545 microalgae at different cell densities. Microbiology 9 (2006) 216-222.

546 [52] M. Yúfera, E. Pascual, Effects of algal food concentration on feeding and ingestion rates of
547 *Brachionus plicatilis* in mass culture. Hydrobiologia 122 (1985) 181-187.

548 [53] Å. Brabrand, B.A. Faafeng, T.Källqvist, J.P. Nilssen, Biological control of undesirable cyanobacteria
549 in culturally eutrophic lakes. Oecologia, 60 (1983) 1-5.

550 [54] J. Raymont, M. Adams, Studies on the mass culture of *Phaeodactylum*. Limnol. Oceanogr. 3 (1958)
551 119-136.

552 [55] A.D. Ansell, J.E.G. Raymont, K.F. Lander, E. Crowley, P. Shackley P Studies on the mass culture of
553 *Phaeodactylum.* II. The growth of *Phaeodactylum* and other species in outdoor tanks. Limnol. Oceanogr. 8
554 (1963) 184-206.

555 [56] M. Ucko, E. Cohen, H. Gordin, S. Arad, Relationship between the unicellular red alga *Porphyridium*
556 sp. and its predator, the dinoflagellate *Gymnodinium* sp. Appl. Environ. Microbiol. 55 (1989) 2990-2994.

557 [57] C.H. Rosetta, G.B. McManus, Feeding by ciliates on two harmful algal bloom species, *Prymnesium*
558 *parvum* and *Prorocentrum minimum*. Harmful Algae 2 (2003) 109-126.

559 [58] R. Goulder, Grazing by the ciliated protozoon *Loxodes magnus* on the alga *Scenedesmus* in a
560 eutrophic pond. Oikos 23 (1972) 109-115.

561 [59] P. Heussler, S. Castillo, F.M. Merino, Parasite problems in the outdoor cultivation of *Scenedesmus*.
562 Arch. Hydrobiol. Beih. 11 (1978) 223–227.

563 [60] M. Lurling, W. Beekman, Grazer induced defenses in *Scenedesmus* (Chlorococcales; Chlorophyceae):
564 coenobium and spine formation. Phycologia 38 (1999) 368—376.

565 [61] S. Mayeli, S. Nandini, S. Sarma, The efficacy of *Scenedesmus* morphology as a defense mechanism
566 against grazing by selected species of rotifers and cladocerans. Aquatic Ecol. 38 (2005) 515-524.

567 [62] D.O. Hessen, E. Van Donk, Morphological changes in *Scenedesmus* induced by substances released
568 from *Daphnia*. Arch. Hydrobol. 127 (1993) 129-140.

569 [63] A. Dillon, J.D. Parry, Amoebic grazing of freshwater *Synechococcus* strains rich in phycocyanin.
570 FEMS Microbiol. Ecol. 69 (2009) 106-112.

571 [64] K.M. Chen, J. Chang, Influence of light intensity on the ingestion rate of a marine ciliate,
572 *Lohmanniella* sp. J. Plankton Res. 21 (1999) 1791-1798.

573 [65] S. Sevgi, G. Zekiye, Filtration and ingestion rates of the rotifer, *Brachionus plicatilis* fed five species
574 of microalgae at different cell densities. Isr. J. Aquac. Bamidgeh 58 (2006) 39-45.

575 [66] E. Touloupakis, B. Cicchi, A.M.S. Benavides, G. Torzillo, Effect of high pH on growth of
576 *Synechocystis* sp. PCC 6803 cultures and their contamination by golden algae (*Poterioochromonas* sp.).
577 Appl. Microbiol. Biotechnol. 100 (2016) 1333-1341.

578 [67] A.T. Ma, E.F. Daniels, N. Gulizia, B. Brahamsha, Isolation of diverse amoebal grazers of freshwater
579 cyanobacteria for the development of model systems to study predator–prey interactions, Algal Res. 13
580 (2016) 85-93.

581 [68] Z. Chen, X. Lei, B. Zhang, L. Yang, H. Zhang, J. Zhang, Y. Li, W. Zheng, Y. Tian, J. Liu, First report
582 of *Pseudobodo* sp, a new pathogen for a potential energy-producing algae: *Chlorella vulgaris* cultures.
583 PloS one 9 (2014) e89571.

584 [69] K.G. Porter, Selective grazing and differential digestion of algae by zooplankton, Nature 244 (1973)
585 179–180.

586 [70] R.W. Sterner, The role of grazers in phytoplankton succession, in U. Sommer [ed.], Plankton ecology:
587 Succession in plankton communities. Springer, 1989, pp 107-l70.

588 [71] J.T. Lehman, Algal biomass unaltered by food web changes in Lake Michigan. Nature 332 (1988)
589 537-538.

590 [72] U. Burkert, P. Hyenstrand, S. Drakare, P. Blomqvist, Effects of the mixotrophic flagellate
591 *Ochromonas* sp. on colony formation in *Microcystis aeruginosa*. Aquatic Ecol. 35 (2001) 9–17.

592 [73] Z. Yang, F.X. Kong, X.L. Shi, H. Cao Morphological response of *Microcystis aeruginosa* to grazing
593 by different sorts of zooplankton. Hydrobiologia 563 (2006) 225–230.

594 [74] M. Lynch, *Aphanizomenon* blooms: Alternate control and cultivation by *Daphnia pulex*, in W.C.
595 Kerfoot (Ed.), Evolution and ecology of zooplankton communities. University Press of New England,
596 Hanover, 1980, pp 299-304.

597 [75] H.H. Jakobsen, K.W. Tang, Effects of protozoan grazing on colony formation in *Phaeocystis globose*
598 (Prymnesiophyceae) and the potential costs and benefits. Aquat. Microb. Ecol. 27 (2002) 261–273.

599 [76] V. Schoemann , S. Becquevort, J. Stefels, V. Rousseau, C. Lancelot, *Phaeocystis* blooms in the global
600 ocean and their controlling mechanisms: a review. J. Sea Res. 53 (2005) 43-66.

601 [77] W. Luo, S. Pflugmacher, T. Proschold, N. Walz, L. Krienitz, Genotype versus Phenotype variability
602 in *Chlorella* and *Micractinium* (Chlorophyta, Tebouxiophyceae). Protist 157 (2006) 315—333.

603 [78] J.G. Day, R.A. Fleck RA, Cryo-injury and the implications this has to the conservation of biological
604 resources. Microalgae Biotechnol. 1 (2015) 1–11.

605 [79] R. Pratt, T.C. Daniels, J.J. Eiler, J.B. Gunnison, W.D. Kumler, J.F. Oneto, H.H. Strain, Chlorellin, an
606 antibacterial substance from *Chlorella*. Science 99 (1944) 351–352.

607 [80] K.W. Glombitza, Antibiotics in algae, in H.A. Hoppe, T. Levring, Y. Tanaka (Eds) Marine Algae in
608 pharmaceutical science. Walter de Gruyter, Berlin, 1979, pp 303-342.

609 [81] S. Singh, B.N. Kate, U.C. Banerjee, Bioactive compounds from cyanobacteria and microalgae: An
610 overview. Crit. Rev. Biotechnol. 25 (2005) 73-95.

611 [82] H. Abida, S. Ruchaud, L. Rios, A. Humeau, I. Probert, C. De Vargas, S. Bach S, C. Bowler,
612 Bioprospecting marine plankton. Mar. Drugs. 11 (2013) 4594-4611.

613 [83] M.L. Ostrofsky, F.G. Jacobs, J. Rowan, Evidence for the production of extracellular herbivore
614 deterrents by *Anabaena flos-aquae*. Freshwater Biol. 13 (1983) 501- 506.

615 [84] L. Wang, T. Yana, R. Yua, M. Zhou, Experimental study on the impact of dinoflagellate *Alexandrium*
616 species on populations of the rotifer *Brachionus plicatilis*. Harmful Algae 4 (2005) 371–382.

617 [85] G.V. Wolfe, M. Steinke, G.O. Kirst, Grazing-activated chemical defence in a unicellular marine alga.
618 Nature 387 (1997) 894-897.

619 [86] G.V. Wolfe, M.S. Steinke, Grazing-activated production of dimethyl sulfide (DMS) by two clones of
620 *Emiliania huxleyi*. Limnol. Oceanogr. 41 (1996) 1151-1160.

621 [87] S. Strom, G. Wolfe, A. Slajer, S. Lambert, J. Clough, Chemical defense in the microplankton II:
622 Inhibition of protist feeding by b-dimethylsulfoniopropionate (DMSP). Limnol. Oceanogr. 48 (2003) 230–
623 237.

624 [88] G. Pohnert, Diatom/copepod interactions in plankton: The indirect chemical defense of unicellular
625 algae. Chembiochem, 6 (2005), 946-959.

626 [89] J.G. Jones, A guide to methods for estimating microbial numbers and biomass in fresh water.
627 Freshwater Biological Association Scientific Publication No. 39, Ambleside, IK, 1979.

628 [90] H. Utermöhl, Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. Mitteilungen
629 Internationale Vereinigung Theoretische und Angewandte Limnologie 9 (1958) 1-38.

630 [91] J.G. Day, N.J. Thomas, U.E.M. Achilles-Day, R.J. Leakey, Early detection of protozoan grazers in
631 algal biofuel cultures. Bioresource Technol. 114 (2012) 715–719.

632 [92] Y. Wang, M. Castillo-Keller, E. Eustance, M. Sommerfeld, Early detection and quantification of
633 zooplankton grazers in algal cultures by FlowCAM. Algal Res. 21 (2017) 98–102.

634 [93] S.P. Fulbright, M.K. Dean, G. Wardle, P.J. Lammers, S. Chisholm, Molecular diagnostics for
635 monitoring contaminants in algal cultivation Algal Res. 4 (2014) 41–51.

636 [94] A. Taniguchi, H. Onishi, M. Eguch, Quantitative PCR assay for the detection of the parasitic ciliate
637 *Cryptocaryon irritans*. Fisheries Science 77 (2011) 607–613.

638 [95] L.T. Carney, J.S. Wilkenfeld, P.D. Lane, O.D. Solberg, Z.B. Fuqua, N.G. Cornelius, S. Gillespie, K.P.
639 Williams, T.M. Samocha, T.W. Lane, Pond crash forensics: Presumptive identification of pond crash
640 agents by next generation sequencing in replicate raceway mass cultures of *Nannochloropsis salina.* Algal
641 Res. 17 (2016) 341-347.

642 [96] E.R. Holm, D.M. Stamper, R.A. Brizzolara, L. Barnes, N. Deamer, J.M. Burkholder, Sonication of
643 bacteria, phytoplankton and zooplankton: Application to treatment of ballast water. Mar. Poll. Bull. 56
644 (2008) 1201–1208.

645 [97] R.J.G. Leakey, S.A. Wilks, A.W.A. Murray, Can Cytochalasin B be used as an inhibitor of feeding in
646 grazing experiments on ciliates? Eur. J. Protistol. 30 (1994) 309–315.

647 [98] N. De Pauw, J. Morales, G. Persoone, Mass culture of microalgae in aquaculture systems: Progress
648 and constraints. Hydrobiologia116/117 (1979) 121–134.

649 [99] V. Pradeep, S.W. Van Ginkel, S. Park, T. Igou, C. Yi, H. Fu, R. Johnston, T. Snell, Y. Chen, Use of
650 copper to selectively inhibit *Brachionus calyciflorus* (Predator) growth in *Chlorella kessleri* (Prey) mass
651 cultures for algae biodiesel production, Int. J. Mol. Sci. 16 (2015) 20674-20684.

652 [100] J. Weissman, G. Radaelli, D. Rice inventors. Systems and methods for maintaining the dominance
653 and increasing the biomass production of *Nannochloropsis* in an algae cultivation system. US Patent No.
654 20100196995. 2010.

655 [101] O. Zmora, A. Richmond, Microalgae for aquaculture: Microalgae production for aquaculture, in: A.
656 Richmond (Ed.) Handbook of Microalgal Culture. Blackwell Publishing Ltd, Oxford, UK, 2007, pp 365–
657 379.

658 [102] E.P. Lincoln, T.W. Hall, B. Koopman, Zooplankton control in mass algal cultures. Aquaculture. 32
659 (1983) 331–337.

660 [103] B. Vick, inventor. Glyphosate applications in aquaculture. US Patent No.2009004296. 2010.

661 [104] J. Shapiro, Blue-Green Algae: Why they become dominant. Science 179 (1973) 382-384.

662 [105] S.A. Mitchell, A. Richmond, Use of rotifers for the maintenance of monoalgal cultures of *Spirulina*.
663 Biotechnol. Bioeng. 30 (1987) 164-168.

664 [106] V.L. Loosanoff, J.E. Hanks, A.E. Ganaros, Control of certain forms of zooplankton in mass algal
665 cultures. Science 125 (1957) 1092–1093.

666 [107] V. Montemezzani, I.C. Duggan, I.D. Hogg, R.J. Craggs, A review of potential methods for
667 zooplankton control in wastewater treatment high rate algal ponds and algal production raceways, Algal
668 Res. 11 (2015) 211-226.

669 [108] M.D. Ferrando, E. Sancho, E. Andreu-Moliner, Chronic toxicity of Fenitrothion to an algae
670 (*Nannochloris oculata*), a rotifer *(Brachionus calyciflorus*), and the cladoceran (*Daphnia magna*),
671 Ecotoxicology & Environmental Safety, 35 (1996) 112-120.

672 [109] M. Arauzo, M. Valladolid, Short-term harmful effects of unionised ammonia on natural populations
673 of *Moina micrura*, and *Brachionus rubens*, in a deep waste treatment pond, Water Res. 37 (2003) 25-47.

674 [110] X.W. Yin, C.J. NiuEffect of pH on survival, reproduction, egg viability and growth rate of five
675 closely related rotifer species. Aquatic Ecol. 42 (2008), 607-616.

676 [111] Y. Huang, L. Li, J. Liu, W. Lin, Botanical pesticides as potential rotifer-control agents in microalgal
677 mass culture. Algal Res. 4 (2014) 62-69.

678 [112] S.W. Van Ginkel, T. Igou, Z. Hu, A. Narode, S. Cheruvu, S. Doi, R. Johnston, T. Snell, Y. Chen,
679 Taking advantage of rotifer sensitivity to rotenone to prevent pond crashes for algal-biofuel production.
680 Algal Res. 10 (2015) 100-103.

681 [113] C. Xu, K. Wu, S.W. Van Ginkel, T. Igou, H.J. Lee, A. Bhargava, R. Johnston, T. Snell, Y. Chen,
682 The use of the schizonticidal agent quinine sulfate to prevent pond crashes for algal-biofuel production.
683 Int. J. mol. Sci. 16 (2015) 27450-27456.

684 [114] V. Montemezzani, I.C. Duggan, I.D. Hogg, R.J. Craggs, Screening of potential zooplankton control
685 technologies for wastewater treatment High Rate Algal Ponds. Algal Res. 22 (2017) 1-13.

686 [115] B. Klimek, E. Fiałkowska, J. Fyda, W. Kocerbasoroka, A. Pajdakstós, Ł. Sobczyk, The toxicity of
687 aluminium salts to *Lecane inermis* rotifers: are chemical and biological methods used to overcome
688 activated sludge bulking mutually exclusive? Arch. Environ. Protect. 39 (2013) 127-138.

689 [116] R.A. Moreiram, A. da Silva Mansano, O. Rocha, The toxicity of carbofuran to the freshwater rotifer,
690 *Philodina roseola*. Ecotoxicology 24 (2015) 604–615.
691 [117] L.X. Ke, Y.L. Xi, C.W. Zha , L.L. Dong, Effects of three organophosphorus pesticides on population
692 growth and sexual reproduction of rotifer *Brachionus calyciflorus* Pallas, Acta Ecologica Sinica 29 (2009)
693 182-185.

694 [118] H. Geng, P. Xie, Experimental studies on the effects of toxic *Microcystis aeruginosa* PCC7820 on
695 the survival and reproduction of two freshwater rotifers *Brachionus calyciflorus* and *Brachionus rubens*,
696 Ecotoxicology (2008) 709-715.

697 [119] W.M. Hikal, A.Z. Al-Herrawy, E.S. El-Daly, S.E. Elowa, Assessment of pesticide toxicity using the
698 freshwater amoeba *Rosculus ithacus in vitro*. Pyrex Journal of Research in Environmental Studies 2
699 (2015) 8-12.

700 [120] H. Xu, W. Song, A.Warren, An investigation of the tolerance to ammonia of the marine ciliate
701 *Euplotes vannus* (Protozoa, Ciliophora). Hydrobiologia 519 (2004) 189–195.

702 [121] L. Peng, C.Q. Lan, Z. Zhang, C. Sarch, M. Laporte, Control of protozoa contamination and lipid
703 accumulation in *Neochloris oleoabundans* culture: Effects of pH and dissolved inorganic carbon, Biores.
704 Technol. 197 (2015) 143-151.

705 [122] D.J.S. Montagnes, A.B. Barbosa, J. Boenigk, K. Davidson, K. Jürgens, M. Macek, J.D. Parry, E.C.
706 Roberts, K. Zimek, Selective feeding behaviour of key free-living protists: avenues for continued study.
707 Aquat. Microb. Ecol. 53 (2008) 83–98.

708 [123] S.L. Strom, B. Brahamsha, K.A. Fredrickson, J.K. Apple, A.G. Rodríguez, A giant cell surface
709 protein in *Synechococcus* WH8102 inhibits feeding by a dinoflagellate predator. Environ. Microbiol. 14
710 (2012) 807–816.

711 [124] R. Simkovsky, E.F. Daniels, K. Tang, S.C. Huynh, S.S. Golden, B. Brahamsha, Impairment of O-
712 antigen production confers resistance to grazing in a model amoeba–cyanobacterium predator – prey
713 system. PNAS 109 (2012) 16678-16683.

714 [125] R.C. McBride, S. Lopez,C. Meenach, M. Burnett, P.A. Lee, F. Nohilly, C. Behnke, Contamination
715 Management in Low Cost Open Algae Ponds for Biofuels Production. Ind. Biotechnol. 10 (2014) 221-227.

716

717 **Table l.** Exemplar reports on microzooplanktonic grazers of potentially biotechnologically exploitable algae

Target algae	Predator		Culture system	Volume/ size/ location	Reference
Anabaena flos-aquae	Rotifer	*Brachionus calyciflorus*	Laboratory culture	125 mL flasks	[38]
Anacystis nidulans	Ciliate	*Colpoda ateinii*	Open raceways for algal mass cultivation	N/A	[39]
Arthrospira sp.	Rotifers & Amoebae	*Branchionus* sp. & *Amoeba* sp.	Ponds	1000 m^2	[40]
Chlamydomonas reinhardtii	Rotifer	*B. calyciflorus*	Laboratory culture	100 mL flask	[41]
Chlorella	Ciliate	*Euplotes* sp.	Culture tank	1500 L	[42]
Chlorella	Flagellate	*Poterioochromonas*	Laboratory culture	2 L flask	[43]

Chlorella	Colpoda	Copepodans	Open ponds for algal mass cultivation	6000 L	[44]
Chlorella vulgaris	Rotifers	*B. calyciflorus* & *B. patulus*	Laboratory culture	2 L flask	[45]
Cyanobacterium	Ciliate	*Colpoda ateinii*	Laboratory culture	100 mL glass vessels	[46]
Cyanobacterium	Amoeba	*Naegleria*	Laboratory culture	500 mL flask	[40, 47]
Cyanobacterium	Rotifer	*B. calyciflorus*	Laboratory culture	250 mL flask	[38, 40]
Chlamydomonas reinhardtii	Rotifers	*B. calyciflorus*	Laboratory culture	Six-well culture plate	[48]
Chlamydomonas	Copepod	*Diaptomus sicilis*	Laboratory culture	500 mL flask	[49]
Anacystis nidulans	Ciliate	*Colpoda ateinii*	Laboratory culture	100 mL glass vessels	[46]
Cylindrospermum	Amoebae	*Naegleria* sp.	Natural systems	500 mL flask	[50]
Dunaliella salina	Amoebae	*Naegleria* sp. &	Open ponds	Hutt Lagoon,	[36]

		Cladotricha sp.		Western Australia	
D. salina	Ciliates	unidentified ciliates	Open ponds	400 L and 2000 L	[35]
Microcystis viridis	Flagellate	*Poterioochromonas malhamensis*	Laboratory culture	2 L flask	[43]
Nannochloropsis oculata	Rotifer	*B. plicatilis*	Laboratory culture	250 mL flask	[51, 52]
Oscillatoria agardhii	Ciliate	*Nassula ornata*	Laboratory culture	500 mL flask	[53]
Phaeodactylum	Flagellate	*Monas* sp.	Open tanks for algal mass cultivation	1200 L and 2400 L	[54, 55]
Porphyridium	Flagellate	*Gymnodinium* sp.	Open raceways for algal mass cultivation	N/A	[56]
Prorocentrum minimum	Ciliates	Tintinnids & non-loricate ciliates	Laboratory culture	six-well culture plates	[57]
Scenedesmus	Ciliate	*Loxodes magnus*	Natural systems	1 Ha pond	[58]

Scenedesmus	Flagellate	*Amphelidium* sp.	Laboratory culture & open ponds for algal mass cultivation	N/A	[59]
Scenedesmus	Rotifer	*B. calyciflorus*	Laboratory culture	300 mL flask	[60, 61]
Scenedesmus	Cladocerans	*Ceriodaphnia dubia*	Laboratory culture	100 mL flask	[61]
Scenedesmus	Cladocerans	*Daphnia*	Laboratory culture	100 mL flask	[61, 62]
Scenedesmus subspicatus	Cladocerans	*Daphnia magna*		100 mL flask	[62]
Scenedesmus acutus	Rotifers	*B. calyciflorus* & *B. patulus*	Laboratory culture	2 L flask	[45]
Scenedesmus sp.	Ciliate	*Loxodes magnus*	Natural systems	1 Ha pond	[58]
Scenedesmus dimorphus	Amoeba	*Vernalophrys* sp.	Open raceways for algal mass cultivation	600 L	[34]

Synechococcus	Amoeba	Unspecified amoebae	Natural systems	Esthwaite, UK, Lake District	[63]
Various	Ciliate	*Lohmanniella* sp.	Laboratory culture	30 mL	[64]
Various	Rotifer	*Brachionus plicatilis*	Laboratory culture	500 mL	[65]
Synechocystis sp. PCC 6803	Flagellate	*Poterioochromonas* sp.	Laboratory culture & various algae culture systems	800 mL	[66]
Synechococcus	Amoeba	*Vrihiamoeba italica*	Laboratory culture	24-well cell culture plates	[67]
Chlorella vulgaris	Flagellate	*Pseudobodo* sp.	Laboratory culture	20 mL flask	[68]

718

719 N/A Information not available

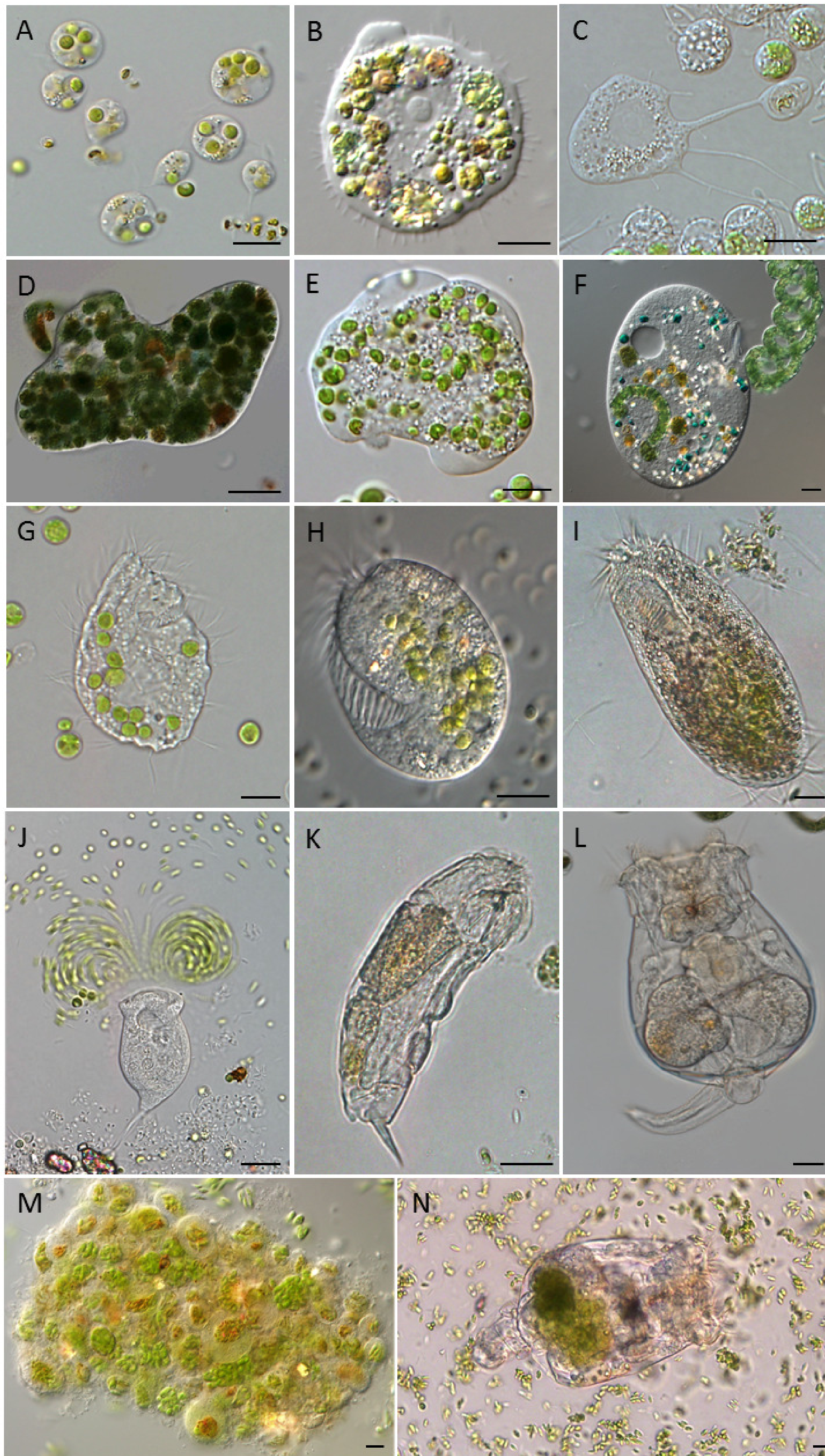
720 **Table 2.**Approaches that have been trialled in lab-, pilot - and large-scale algal cultivation systems to
721 control microzooplanktonic grazers

Grazer	Species	Control measures	Microalga	Effectiveness	Cultivation system	Reference
Cladocera	*Moina tenuicornis*	High-concentration CO_2	not provided		Outdoor mass culture	[107]
Cladocera	*Daphnia magna*	Fenitrothion	*Nannochloris oculata*	24h-LC_{50}: $6.7*10^{-5}$ mg L^{-1}	toxicity testing	[108]
Cladocera	*Moina micrura*	Unionised ammonia	*Oocystis pusilla*	Mortality rates:63%	waste treatment pond	[109]
Copepoda	*Diaphanosoma brachyurum*	Ammonium hydroxide	microalgae	24h LD100:<20 mg L^{-1}	high-rate algal pond	[102]
Copepoda						[106]
Rotifer	Rotifer	Filtration	--		Outdoor ponds	[6]
Rotifer	*Brachionus* spp.	pH adjusting	*Chlorella pyrenoidosa*		Laboratory	[110]
Rotifer	*B. plicatilis*	Toosendanin, Celangulin, Azadirachtin	*Chlorella* sp., *Nannochloropsis* sp.		Laboratory	[111]
Rotifer	*B. calyciflorus*	Copper Ion	*C. kessleri*		Laboratory	[99]
Rotifer	*B. calyciflorus*	Rotenone	*C. kessleri*		Laboratory	[112]
Rotifer	*B. calyciflorus*	Quinine Sulfate	*C. kessleri*		Laboratory	[113]

Rotifer	*Brachionus* sp.	Urea and Ammonium Bicarbonate	*Arthrospira* sp.		Laboratory & outdoor mass culture	[40]
Rotifer	*Brachionus* spp.	High-concentration CO_2	Not provided		Outdoor mass culture	[114]
Rotifer	*B. plicatilis*	Toosendanin, celangulin, Azadirachtin	*Chlorella, Nannochloropsis* sp.	24 h-LC_{50}: $2.132*10^{-3}$mg L^{-1}; 0.175 mg L^{-1}; 18.386 mg L^{-1}	Lab-based toxicity tests	[111]
Rotifer	*Lecane inermis*	Al-salts	_	24 h LD_{100}: 0.012 mg $Al^{3+}dm^{-3}$.	Lab-based toxicity tests	[115]
Rotifer	*Philodina roseola*	Carbofuran	_	48h EC50: 13.36 mg L^{-1}	Lab-based toxicity tests	[116]
Rotifer	*B. calyciflorus*	Chlorpyrifos	*Scenedesmus obliquus*	10.0 mg L^{-1}	Lab-based toxicity tests	[117]
Rotifer	*B. calyciflorus*	Copper	*C. kessleri*	1.5 ppm	Open pond	[99]
Rotifer	*B. calyciflorus*	Rotenone	*C. kessleri*	24 h-LC_{50}: 0.074 M	Lab-based toxicity tests	[112]
Rotifer	*B. calyciflorus* & *B. rubens*		*Microcystis aeruginosa*	sensitive	Lab-based toxicity tests	[118]
Amoebae	*Amoeba* sp.	Urea (U) and ammonium bicarbonate (AB)	*Arthrospira* sp.	60 mg L^{-1} (U) or 100 mg L^{-1} (AB)	outdoor mass cultures	[40]
Amoebae	*Rosculus ithacus*	Chemical mixture *		0.0020 to 0.0064 mg L^{-1}	Lab-based toxicity tests	[119]

Amoebae	*Amoeba*	Urea and Ammonium Bicarbonate	*Arthrospira* sp.		Laboratory & Outdoor mass culture	[40]
Amoebae	Unknown Amoeba	Salinity shift	*Dunaliella* sp.		Outdoor mass culture	[36]
Ciliate	unknown ciliates	Quinine Sulfate	*D. salina*		Laboratory & outdoor mass culture	[35]
Ciliate	Unknown ciliates	Salinity shift	*Dunaliella* sp.		Outdoor mass culture	[36]
Ciliate	*Euplotes vannus*	NH_4Cl		2-h LC50: 7870 mg total ammonia-N L^{-1}	Conventional open system	[120]
Ciliate	Predator ciliates	Quinine sulphate	*D.salina*	24-h LC_{100}:12–14 mg L^{-1}	Outdoor algal mass cultures	[35]
Flagellate	*Poterioochromonas* sp.	High pH	*Synechocystis* sp.		Laboratory & outdoor mass culture	[66]
Flagellate	Unidentified	pH 7.5–9.5 & bicarbonate 0–160 mM	*N. oleoabundans*	inhibited protozoan growth	Laboratory	[121]

* include Methomyl, dimethoate, malathion, dicuran, cypermethrin, carbendazim, fenitrothion and butachlor



725

726 Fig. 1 Exemplar microzooplanktonic grazers: A. *Poterioochromonas malhamensis* grazing on *Chlorella*; B.
727 *Nuclearia* grazing on *Spirulina*; C. *Nuclearia* grazing on *Scenedesmus*; D. Heterolobosean amoebae
728 grazing on *Spirulina*; E. *Saccamoeba* grazing on *Nannochloropsis*; F. *Frontonia* grazing on *Spirulina*; G.
729 *Colpoda* grazing on *Chlorella*; H. *Euplotes* grazing on *Nannochloropsis*; I. *Sterkiella histriomuscorum*
730 grazing on *Scenedesmus*; J. *Vorticella convallaria* grazing on *Scenedesmus*; K. *Lecane inermis* in
731 *Chlorella* culture; L. *Brachinous plicatilis* in *Spirulina* culture; M. Vampyrellid grazing on *Scenedesmus*;
732 N. *Brachinous calyciflorus* grazing on *Scenedesmus*. Scale bar=10μm. Except Fig. 1M was cited from
733 Gong et al. (2015, Fig. 6E), all the others images were unpublished data from Yingchun Gong.