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Monster potential meets potential monster: pros and cons of deploying genetically modified microalgae for biofuels production

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Biofuels production from microalgae attracts much attention but remains an unproven technology. We explore routes to enhance production through modifications to a range of generic microalgal physiological characteristics. Our analysis shows that biofuels production may be enhanced *ca* fivefold through genetic modification (GM) of factors affecting growth rate, respiration, photoacclimation, photosynthesis efficiency and the minimum cell quotas for nitrogen and phosphorous (N:C and P:C). However, simulations indicate that the ideal GM microalgae for commercial deployment could, on escape to the environment, become a harmful algal bloom species *par excellence*, with attendant risks to ecosystems and livelihoods. In large measure, this is because an organism able to produce carbohydrate and/or lipid at high rates, providing stock metabolites for biofuels production, will also be able to attain a stoichiometric composition that will be far from optimal as food for the support of zooplankton growth. This composition could suppress or even halt the grazing activity that would otherwise control the microalgal growth in nature. In consequence, we recommend that the genetic manipulation of microalgae, with inherent consequences on a scale comparable to geoengineering, should be considered under strict international regulation.

1. Introduction

The production of liquid transport biofuels from terrestrial crop plants is a proven technology [1] that continues to attract controversy. Much concern is levelled at the comparative societal, ethical and political values of using land and fertilizer for energy rather than feeding populaces. An alternative to the use of photosynthetic higher plants is to use photosynthetic microalgae. The term 'microalgae' typically describes any photosynthetic microbe, either prokaryotic cyanobacteria or eukaryotic protists. While some of these organisms are capable of synthesizing biochemical precursors for biofuels heterotrophically [2], net C-fixation requires a predominately photosynthetic metabolism under conditions of adequate illumination and (usually) inorganic nutrients. It is these photosynthetic microalgae that we consider here. Microalgae have been suggested to be ideal organisms for biofuels production owing to their rapid growth rate, high oil content, suitability for growth on marginal land, and no direct conflict with the growth of food crops [3,4]. However, the path to successful deployment of microalgal biofuels is most challenging [5], with the cost estimates for production currently far exceeding fossil fuel prices [6,7].

Irrespective of the form of biofuels produced from microalgae, the objective is to transfer the normal flow of newly fixed carbon (C), from generating structural biomass, towards the accumulation of energy-dense C storage products (starch, lipid). While nutrient (especially nitrogen, N) limitation prior to crop harvesting is needed to optimize biofuels production, light limitation is a far more likely event at this stage of microalgae crop growth owing to the

self-shading properties of dense, highly pigmented, microalgal suspensions [8]. As with all commercial crops, approaches to overcoming such inherent limitations to production have attracted considerable interest [9–11].

In this work, we consider various issues associated with the advantages and disadvantages of applying genetic modification (GM) to microalgae to enhance biofuels production. Similar arguments to those we present here apply to any manipulation of the phenotypic characteristics of microalgae; we use the term GM to imply any alteration of wild-type characteristics [12] that would not likely occur naturally. Because these organisms are single-celled microbes with minimum generation times of less than a day, they may be considered more readily amenable to GM than are higher plants. However, it is worth noting that in reality the path to GM of these organisms is far from trivial [9,12].

While deployment of GM biofuels-optimized microalgae may appear to offer great potential, there are counterpoints to this promise. While microalgae in nature, as phytoplankton, are important components of the trophic web leading to fisheries, one may question whether microalgae optimized for biofuels production would readily fit benignly into ecology, or whether they may form harmful algal blooms (HABs). The large-scale growth of micro-organisms that can be readily transferred across and between continents (e.g. with migrating wildfowl or in the ballast water of ships [13]) thus warrants careful consideration.

We have conducted our analysis through screening *in silico* GM algal populations, avoiding the attendant environmental and ethical risks of *in vivo* trials. *In silico* models of algal community physiology, though widely used in many oceanographic scenarios [14], and deployed for simulations of microalgal biomass production [8], have hitherto not been applied in earnest to examine algal biofuels production. Here, we use a variant of a well-documented algal physiology model [8,15,16] to investigate options for enhancing microalgal biofuels production through GM routes. We then take the resultant biofuels-optimized GM organism and consider the implications for predator–prey interactions if such an organism escaped into the natural environment. The results indicate that the configuration of a biofuels-optimized organism also describes an organism that, on escape to the natural environment, has the potential to form HABs on a scale greater than naturally occurring species do.

2. Material and methods

2.1. Base algal model

All models represent a compromise between complexity and computational load. The model used here is broadly typical of the more complex examples of mechanistic adaptive microalgal models. The model describing the growth of microalgae was developed from a long line of models [15,17]. This model type has a firm basis in physiology, and has been well-validated in its performance against data for various phytoplankton species, growing under different conditions and various combinations of light, N, P, Fe and/or Si limitation [14–20]. The implementation here included a description of the interactions between light, N and P, with photoacclimation according to the ‘Flynn–Geider’ configuration described in Flynn *et al.* [21].

Algal-C was allocated to nitrogenous components (protein and nucleic acids) and non-nitrogenous structural components, with the balance as surplus-C attributed to components for

potential exploitation as biofuels. The simulated contribution to biofuels material is calculated by reference to the cellular C:N ratio, and the absolute minimum cellular C:N (CN_{min}), using the following equation.

$$Cex_C = \frac{CN_{cell} - CN_{min}}{CN_{cell}} = \frac{CN_{cell} - (CN_{core} + Cstruc_N)}{CN_{cell}}. \quad (2.1)$$

The value of CN_{min} can be determined experimentally from N-replete ammonium-grown microalgae. It comprises two main components: the C:N of the core cellular nitrogenous components (CN_{core}), which is primarily protein and nucleic acids, and the non-nitrogenous structural material (primarily membranes and cell wall), which is described here as a C:N value referenced to the N in the core ($Cstruc_N$). The value of CN_{core} is estimated to have a value of *ca* 3.2 gC (gN)^{−1}, from the C:N value of protein and nucleic acids, and the contribution that these two make to the whole cell [22]. $Cstruc_N$ is given as $CN_{min} - CN_{core}$; the typical value of CN_{min} is around 4 [19,22], yielding a value of this non-nitrogenous $Cstruc_N$ in nutrient-replete cells of 0.8 gC (gN)^{−1}. The biochemical fractionation between different carbohydrates, fatty acids and lipids within the surplus-C (Cex_C) is not described further within the model because there are insufficient data as yet to support such a development. The fractionation does not affect the central conclusions of our analysis (considered further in §4).

2.2. Non-genetic modification and genetic modification configurations

The microalgal model described interactions between light (including photoacclimation), nutrients (N and P) and growth. The base (non-GM) model was configured to represent a typical microalgae with respect to C:N:P:Chl [15,16,19], and produces maximum-simulated areal production rates similar to peak values in nature (*ca* 4 gC m^{−2} d^{−1}) [23]. The default values of constants used for the non-GM configuration, and the ranges explored for GM configurations, are given in table 1. These are all phenotypic features for which there are, likely, many genotypic regulators. For example, altering the photosystem antenna size [25] affects phenotypic features of the initial slope of the photosynthesis–irradiance curve (α^{Chl}) and also, depending on how the cell responds by altering the number of photosynthetic reaction centres, potentially the maximum pigment content ($ChlC_{max}$). An explanation of the features considered is given in the following.

2.2.1. Maximum growth rate (μ_{max})

This sets the maximum possible growth rate under optimal conditions. The maximum growth rate attainable in simulations was less than μ_{max} because growth was simulated in a light–dark cycle (see below). Engineering factors affecting this feature may require a consideration of the source wild-type cell line, cell cycle controls, and limitations on respiratory functions affecting synthesis and cell maintenance [26].

2.2.2. Respiration rates (basal and metabolic respiration)

Basal respiration (BasRes; described here as a proportion of μ_{max}) includes that associated with cell maintenance, while metabolic respiration (ProtRes; described here as C respired for the assimilation of N from intracellular ammonium into protein and nucleic acids) is associated with new net synthesis of structural components. Added to respiration is the cost of reducing nitrate to ammonium before assimilating nitrate-N (equivalent to 1.71 gC per g nitrate-N [27]). Engineering a decrease in respiratory costs may require a consideration of features such as protein turnover rates and functioning of key biochemical pathways.

Table 1. Parameter values for the base non-GM model and ranges explored for the GM counterparts. Values are also given for the physico-chemical culture system; also see text. The nutrient regime equates to that of the classic *f/2* medium [24] containing 882 μM N and 36.2 μM P.

parameter	additional comment	unit	physics and chemistry	microalgae	
				non-GM	GM range tested
surface light	noon value at 0° latitude; see text	$\mu\text{mol photon m}^{-2} \text{s}^{-1}$	2180		
light–dark cycle	see text	fraction	0.5		
optical depth		m	0.1		
dilution		d^{-1}	0.05–0.6		
nutrient-N		gN m^{-3}	12.35		
nutrient-P		gP m^{-3}	1.12		
μ_{max}	maximum growth rate in continuous light	d^{-1}		1.386	0.5–4
ChlC_{max}	maximum pigment content per g of cell-C	gChl (gC)^{-1}		0.06	0.01–0.06
α^{Chl}	initial slope of photosynthesis–irradiance curve	$(\text{gC mol}^{-1} \text{photon}) \times (\text{m}^2 \text{g}^{-1} \text{Chl})$		7	1–14
M	controls photoacclimation rate	dimensionless		2	1–4
NC_0	minimum N quota	gN (gC)^{-1}		0.05	0.01–0.1
PC_0	minimum P quota	mgP (gC)^{-1}		5	1–10
KQN	controls efficiency of cell-N usage	dimensionless		10	0.0625–10
KQP	controls efficiency of cell-P usage	dimensionless		0.01	0.001–1
BasRes	basal respiration as a fraction of μ_{max}	dimensionless		0.05	0.01–0.15
ProtRes	metabolic respiration referenced to N-growth	$\text{gC respired} \times (\text{gN assimilated})^{-1}$		1.5	0.75–3
Cstruc_N	structural C relative to nitrogenous core	gC (gN)^{-1}		0.8	0.5–2
CN_{core}	C : N for the nitrogenous core of the cell (protein, nucleic acids, etc.)	gC (gN)^{-1}		3.2	2–4

2.2.3. ChlC_{max}

This sets the maximum pigment content, described here as chlorophyll per unit of cell-C. In crude terms this limits the ‘greenness’ of the individual cell. With decreasing light, notably owing to self-shading within the cell suspension, photoacclimation within the cell stimulates an increase in photopigment content, to capture more photons for the individual cell. Through natural selection the value of ChlC_{max} is expected to become elevated [8], attaining as much as $0.08 \text{ g Chla (g cell-C)}^{-1}$ [28]. However, such elevated levels cause internal self-shading and critically also self-shading at the population level which decreases efficiency for photosynthesis, and hence decreases overall production. Total productivity is enhanced greatly if the level of photoacclimation (the value of ChlC_{max}) is limited, though this is unlikely to be a stable selective trait [8]. Engineering this feature may require a consideration of altering photosystem antenna size [29] and/or the number of photosynthetic reaction centres.

2.2.4. α^{Chl}

This phenotypic feature affects the overall efficiency of the light–chemistry conversion process, with units of $\text{gC (mol photon)}^{-1} \times (\text{m}^2 \text{g}^{-1} \text{Chl})$. The rate of photosynthesis is thus a function of the available light, the pigment content (ChlC ; §2.2.3) and the value of α^{Chl} (for further information, see [15,17,21,30]). Various factors affect the value of α^{Chl} , including the photochemistry within the Z-scheme, and the level of self-shading within the cell [31]. Internal self-shading is affected by the antenna size (Chl per reaction centre), overall Chl:C (affected by ChlC_{max}) and cell size. The fundamental basis of life on Earth is thought to have been fixed some 2 Ga ago [32], with natural selection then optimizing the packaging of these key biochemical processes. Accordingly, enhancing the efficiency of the basis of photochemistry, a fundamental feature of cell biochemistry, would literally be a real life-changing event.

2.2.5. Photoacclimation rate (M)

This is described by parameter M in Flynn *et al.* [21], and affects the rate at which pigment content is upregulated with photoacclimation when the illumination falls (see above on ChlC_{max}). Here, the most important feature affected by this rate was the increase in Chl:C on entry into the dark phase of the diel light–dark cycle. Engineering this feature would require modifying the acclimation response rate to darkness and/or to C-limitation.

2.2.6. NC_0 and KQN

These parameters, respectively, describe the minimum cellular N:C (the subsistence quota for N [19] and the efficiency of N utilization (specifically the efficiency of the action of biosynthetic pathways associated with N-compounds [16,19]. Lowering NC_0 would also, most likely, involve a decreased need for basal respiration (i.e. a lowering of protein turnover and damage–repair activities, with a decreased need for associated proteins/enzymes and RNA), and a lowering of the DNA content. Evidence from past experimental studies indicates that KQN sets a linear relationship between cellular N:C and growth rate [16,19]. To decrease KQN , to make the relationship between N:C and growth rate curvilinear, it would require a fundamental change in protein and enzyme synthesis and efficiencies of their operation.

2.2.7. PC_0 and KQP

These are the P counterparts to NC_0 and KQN . There is far greater variability in these parameter values than for NC_0 and KQN , and they are recognized as important features in competition between microalgae [33,34]. Because of the mixed structural and energetic/regulatory functions of P (contrasting with the mainly structural functions of N), the value of KQP is much lower than KQN , and the resultant often strongly curvilinear relationship between P:C and growth rate indicates that the cells alter the efficiency of P usage as P becomes limiting [16,19]. To engineer changes in PC_0 and KQP would require decreasing the content of P-containing structural components (DNA, RNA, membranous phospholipids), and enhance the efficiency of use for the remainder.

2.2.8. CN_{core} and Cstruc_N

These parameters, respectively, describe the ratio by mass of the nitrogenous material in the cell (as C:N, comprising proteins, DNA and RNA) and the amount of organic non-nitrogenous structural material relative to the nitrogenous component (as C:N, comprising cell wall, membranes); see text associated with equation (2.1). To engineer changes in CN_{core} would require a decrease in the amount of DNA and RNA (as these contain a lower C:N than does protein [22], if not a complete rebuild of the very nature of the biochemistry of life. Cell walls in microalgae are not as substantial as those in higher plants, so most of the material in Cstruc_N comprises membranes containing phospholipids. Decreasing Cstruc_N is thus likely to decrease PC_0 . Default values used here are: $\text{CN}_{\text{core}} = 3.2$; $\text{Cstruc}_\text{N} = 0.8$ (see §2.1).

2.3. Microalgal simulations

Growth was simulated with illumination conditions for a cloudless mid-summer day at 0° latitude. An astrological function was used describing the sigmoidal daylight variation with a noon maximum instantaneous photon flux density of $2180 \mu\text{mol m}^{-2} \text{s}^{-1}$ and, accounting for reflectance off the water surface with changing sunlight incidence, giving a day average of $675 \mu\text{mol m}^{-2} \text{s}^{-1}$. It was assumed that conditions of temperature, CO_2 and pH were optimal throughout. The macro-nutrient

regime was either that of $f/2$ [24] with inorganic N available at $880 \mu\text{M}$ and phosphate at $36.2 \mu\text{M}$, or at some multiple of those concentrations (e.g. $5 \times f/2$). Simulations to explore optimal configurations of growth and phenotype characteristics were run to steady state in chemostat-style conditions, assuming a homogeneous distribution of cells over an optical depth of 0.1 m, a depth shown previously to be in the optimal range to balance areal and volumetric production rates [8]. Physico-chemical limitations to the supply of nutrients (including CO_2 injection and the maintenance of pH) were assumed to have been overcome.

Areal production is reported for biomass and biofuels (with units of $\text{gC m}^{-2} \text{d}^{-1}$). As simulations were run in chemostat-style, steady-state mode, dilution rates in the plots equate to day-averaged growth rates. The biofuels component represents a portion of biomass, ranging typically from near zero to *ca* 70 per cent of the C-biomass, as according to equation (2.1).

2.4. Predator–prey simulations

For the predator–prey simulations, the base (non-GM) configured microalga or its GM-configured counterpart (table 2) were simulated as being grown together with a zooplankton predator. The zooplankton model [35] has been validated against various datasets, and used previously in the type of simulation deployed here [36]. The zooplankton parameters were set for a microzooplanktonic predator as per details in Mitra [35]; values for the maximum ingestion rate (G_{max}) and the half-saturation constant for ingestion (K_{pred}) are in table 2. The stoichiometric (C:N:P) basis of the trophic interactions described in the predator–prey simulations have a well known, firm basis in the literature [37–39]. In essence, an increasing disparity between the C:N:P of the microalgal prey and its zooplankton predator has a deleterious impact, adversely affecting growth of the predator and nutrient (ammonium and phosphate) regeneration.

Predator–prey simulations were run in a dynamic system describing a mixed layer depth of 10 m, with mixing into and out of the mixed layer at 0.05d^{-1} , and assuming an initial (and sub-mixed layer) nitrate concentration of $10 \mu\text{M}$. Phosphate was supplied at a mole ratio (nutrient N:P) of either 16 or 64, equating to pristine or eutrophically skewed conditions, respectively. Light at the surface was described as for the culture simulations (§2.3), however, as the simulation developed the depth-integrated light field available to the microalgae decreased rapidly from an average day-light value of *ca* $500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ at the start of the simulation to below $50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ at the peak of the bloom.

The predator–prey simulations presented here assume no GM of algal fatty acid composition or of other factors that may adversely affect palatability to the predator [39]. As such, the results from the predator–prey simulations represent best-case scenarios. GM of the fatty acid content (profile) has already been explored [40–42], though the implications of this on palatability to grazers await clarification.

3. Results

Additional results are presented in electronic supplementary material, figures S1–S4.

3.1. Optimization of biofuels production

The key results from our analyses of GM optimization of production are summarized in figure 1; other results are given in the electronic supplementary material, figures S1–S4. For optimizing biofuels production, the most important phenotypic physiological features are maximizing growth rate

Table 2. Parameters for model runs shown in figure 2 and as indicated in figure legends for electronic supplementary material, figures S5–S11. See §2, and table 1 for further information.

parameter	unit	physics and chemistry	microalgae		
			non-GM	GM	zooplankton
surface light (maximum, see text)	$\mu\text{mol photon m}^{-2}\text{s}^{-1}$	2180			
light–dark cycle	fraction; see text	0.5			
mixing depth	m	10			
mixing rate	d^{-1}	0.05			
nutrient-N	mgN m^{-3}	140			
nutrient-P (mole N : P = 16)	mgP m^{-3}	19.38			
nutrient-P (mole N : P = 64)	mgP m^{-3}	4.84			
μ_{max}	d^{-1}		2	4	
ChlC_{max}	gChl (gC)^{-1}		0.06	0.02	
α^{Chl}	$(\text{gC mol}^{-1}\text{ photon}) \times (\text{m}^2\text{ g}^{-1}\text{ Chl})$		7	14	
M	dimensionless		2	1	
NC_0	gN (gC)^{-1}		0.05	0.025	
PC_0	mgP (gC)^{-1}		5	2.5	
KQN	dimensionless		10	10	
KQP	dimensionless		0.01	0.01	
BasRes	dimensionless		0.05	0.05	
ProtRes	$\text{gC respired} \times (\text{gN assimilated})^{-1}$		1.5	1.5	
G_{max}	$\text{gC prey} \times (\text{gC predator})^{-1}$				3
K_{pred}	mgC prey m^{-3}				10

(μ_{max} ; figure 1a), minimizing the maximum photopigment content (ChlC_{max} ; figure 1b) and maximizing the efficiency of the light capture process (α^{Chl} ; figure 1c). There are important, yet typically overlooked, differences between optimizing production of microalgal biomass versus production of biofuels. Biofuels content (excess-C content) relates inversely to the N-limited status of the cells (equation (2.1)), thus for maximum biofuels production (figure 1a) cells need to be grown under N-limiting conditions at their lowest relative growth rate (μ/μ_{max}). Therefore, although microalgae are typically grown commercially in systems operating at low dilution rates (and hence low μ), highest biofuels production will be realized through the use of cells with the highest potential for growth (high μ_{max}). Furthermore, avoiding light limitation is an essential prerequisite in the optimization of biofuels production, thus limiting self-shading (by lowering ChlC_{max}) and maximizing light conversion to C-fixation (raising α^{Chl}) are critically important features. The form of the plots in figure 1a reflects this interplay between nutrient status, pigmentation and thus self-shading, and production. Simulations using high nutrient loads (e.g. $5 \times f/2$) yielded low biofuels production, because the simulated organisms never exhausted the available nutrients (not shown).

Lowering the minimum cellular content of nitrogen (NC_0 , figure 1d) and of phosphorus (PC_0 , electronic supplementary material, figure S1a), and enhancing the efficiency of the use

of cellular N and P (KQP and KQN, electronic supplementary material, figure S1b,c), give relatively minor headline enhancements of biofuels production, although there are additional advantages that would likely affect the financial viability of the whole venture (see §4). There are also several other physiological characteristics of lesser importance. Minimizing respiration rates (see the electronic supplementary material, figure S2a,b) prevents the loss of a proportion of the biofuels-C accumulated during day to support night-time respiration. Decreasing the rate of photoacclimation, the process by which microalgae increase their pigment content in response to light limitation (including at night-time), is also useful (see the electronic supplementary material, figure S2c), as it slows the self-shading event that decreases the accumulation of excess-C. Lowering the C:N ratio of core nitrogenous components and of the amount of C allocated to cell structure also have potential to slightly enhance biofuels production (see the electronic supplementary material, figure S3).

In reality, no GM changes will occur alone. In electronic supplementary material, figure S4, we show the combined effects of changing factors associated with photosynthesis, N or P physiology. Of these, the changed photosynthetic configurations (see the electronic supplementary material, figure S4a) are the most powerful, with scope even when used alone to raise production by *ca* threefold. While productivity gains through enhancing the efficiency of the use of N and P (KQN

Figure 1. Biomass and biofuels areal production. Variation of areal production of biomass (i) and biofuels (ii) against dilution rate (i.e. growth rate, μ) for different physiological characteristics under chemostat-style steady-state conditions. (iii) The percentage of biomass as biofuels; note the different axes directions. (a) Maximum growth rate ($\mu_{\max}$); (b) maximum chlorophyll content ($\text{ChlC}_{\max}$); and (c) overall phenotypic efficiency of the photochemistry (α^{chl} , given here with units of $(\text{gC mol}^{-1} \text{ photon}) (\text{m}^2 \text{ g}^{-1} \text{ Chla})$). (d) Minimum (subsistence) quota for N (NC_0 , N : C). Note that at high dilution rates biofuels production falls as the microalgae become N-sufficient.

and KQP) appear relatively minor, cost effectiveness in fertilizer usage will improve.

3.2. Predator–prey interactions

Having explored the optimal configuration of microalgae for biofuels production, we now consider whether zooplankton

grazing could likely contain the escape of such an organism to nature. Here, the growth conditions are very different, with a large optical depth and low nutrient concentrations. We compared the predator–prey interactions between a zooplankton predating [35] either a naturally configured (non-GM) microalgal prey or a GM biofuels-optimized microalgal prey. For this, we used only a mid-range GM

Figure 2. Predator–prey simulations. Simulated interaction between a microalgal prey (algae) and its zooplanktonic predator (zoo). Simulations were run with the microalgae configured to represent a non-GM (thin line) or a biofuels-optimized GM strain (thick line; table 2). In (a), the mole nutrient ratio is $N:P = 16$ representing pristine water bodies. In (b), $N:P = 64$ representing the skewed nutrient content seen in eutrophic coastal waters. Temporal development of the interaction would depend on initial conditions. Plots show development of the algal and zooplankton biomass, and changes in the algal $N:C$ and $P:C$ ratios. Decreases in algal $N:C$ and $P:C$ indicate changes in nutrient stress (through exhaustion of external nutrient supply).

configuration (table 2; cf. table 1), but even this shows greatly improved biomass and biofuels production capabilities over the non-GM form (see the electronic supplementary material, figure S5).

Our simulations with the non-GM microalgae show the expected importance of elemental stoichiometry ($C:N:P$) in the predator–prey interaction (figure 2), with algal prey of a high C content (low $N:C$ and/or $P:C$) being of poor nutritional value. The adverse impact of food quality on zooplankton becomes particularly apparent under P -limitation, i.e. under nutrient supply conditions with skewed $N:P$ ratios typical of eutrophication (figure 2b). The combination of characteristics in the GM biofuels-optimized microalgae gives a clear enhanced potential for such organisms forming a poorly grazed bloom (figure 2). First, this status is attained through more rapid growth, forming higher population densities than given by the comparative non-GM configuration for a given nutrient load, and thus out-stripping zooplankton predation control. Second, there is the ability of the GM microalgae to become more C -rich, exacerbating the already damaging skewed stoichiometry in nutrient-limited microalgae, and hence disrupting the trophic dynamics which may otherwise restrain net microalgal growth. Of the individual GM characteristics considered, those for μ_{max} , $ChlC_{max}$ and

especially PC_0 appear most important as potentially damaging characteristics in such an organism released to nature (see the electronic supplementary material, figures S6–S11). We also explored other combinations of physical-nutrient descriptions (shallower versus deeper, with different nutrient loads), obtaining broadly similar responses, with the GM biofuels-optimized microalgae always displaying an enhanced scope for forming large poorly grazed blooms (not shown).

4. Discussion

4.1. The advantages of deployment of genetic modification microalgae for biofuels production

Our analysis shows a potential for an increase in biofuels production from microalgae by perhaps fivefold through modifying phenotypic characteristics. The exact gain will depend on many factors, but a gain of fourfold is attainable by deploying the GM versus the non-GM configurations described in table 2, and these are not the extreme GM configurations tested (table 1). The optimal configuration for a biofuels producing microalgae is to have (in approximate order of importance) a high μ_{max} , high α^{chl} , low $ChlC_{max}$

low minimum P : C and N : C contents, low photoacclimation and dark respiration rates, and high efficiency in the use of P and N. Collectively, these features endow the organism with an ability to grow rapidly in low-light conditions, use relatively little nutrients, more rapidly attain higher biomass and biofuels levels than normal, and be capable of attaining more extreme C : N and C : P levels, and hence contain more biofuels potential per unit of biomass. While such guidelines would help focus selection of wild-type algal strains, most likely a real enhancement would require specific attention to GM of these phenotypic facets.

One feature, high maximum growth rates ($\mu_{\max}$), may appear surprising as a preferred characteristic given that continuous culture (chemostat-style) systems are typically run at low dilution rates, thus minimizing consumption of fresh media. The reason for the importance of a high $\mu_{\max}$ is because the production of excess-C-rich metabolites that may act as stock for biofuels is driven primarily as a stress response to an excess supply of fixed-C over supply of nutrients (notably of N). The greater the disparity between the growth rate (μ) and the potential maximum rate ($\mu_{\max}$), the greater the potential for the accumulation of excess-C; this is a function of the well-documented relationship between cellular nutrient quotas and μ [16,19,43]. However, prolonged growth at low dilution rates (forcing low growth rates) selects for a decrease in $\mu_{\max}$ [44], presumably as the metabolism of the organisms downshifts through adaptation, thus minimizing metabolic stress [26]. This likely presents a challenge for the deployment of microalgae selected or genetically modified to achieve high growth rates, as with time the characteristic is likely to be lost (selected against) during growth at enforced low growth rates.

Lowering the minimum cellular content of nitrogen (NC_0 , figure 1*d*) and phosphorus (PC_0 , electronic supplementary material, figure S1*a*), and enhancing the efficiency of the use of cellular N and P (KQP and KQN, electronic supplementary material, figure S1*b,c*) appears to provide only minor enhancements of biofuels production. However, there are important operational and other commercial benefits of such configurations through minimizing nutrient usage [5,45]. This is especially important for P as it is projected that readily available, relatively cheap, sources of phosphate will become increasingly limiting over the coming decades [46]. Additionally, the lower the P-demand by the microalgae the more likely it is that cells become N and not P limited when grown at a given nutrient N : P; this aids development of high C : N [16,19] and hence further enhances the potential for biofuels production.

Some of the features identified in our analysis would be easier to engineer than others and, critically, some will be more stable to mutation, selection and competition pressures. Already the photosystem antennae size has been subjected to GM [25,29]; this has some leverage on decreasing $ChlC_{\max}$ and enhances production (figure 1*b*). While possession of a low $ChlC_{\max}$ is likely not a stable trait [8], minimizing the use of nutrients is likely to be stable as it confers well-documented advantages [47]. Modifications of features influencing fundamental aspects of the generation of photo-reductant, affecting the value of α^{Chl} , are likely to give stable traits though they will be far more challenging (perhaps currently impossible) to achieve.

A feature of the biofuels value of the microalgae that we do not explore in our analysis is the biochemical

differentiation of the excess-C between starch and lipid/fatty acids. The nature of this surplus-C is important from a physiological perspective (as the synthesis of lipid is expensive relative to that of carbohydrate [6]), as well as from a biofuels perspective [5]. While it is quite likely that attempts will continue to be made to genetically modify the biochemistry of this material [9–11], and indeed other facets of the organism (such as features affecting the ease of harvesting, tolerance to temperature and salinity, etc.), the characteristics that we consider represent primary features affecting microalgal growth and costs in terms of nutrient demand and production. The issue of such biochemical modification of lipid and/or carbohydrate does not affect the central message of the research presented here, not least because it does not alter the key features of the stoichiometric-inspired predator–prey interactions that we consider. Indeed, what will exacerbate the deterioration of the predator–prey interaction (increasingly the likelihood of a HAB) is a change in the quality of the fatty acid such that it no longer contains metabolically important polyunsaturated fatty acid (PUFA) [48] and/or it contains a higher proportion of biofuels-desirable short-chain saturated fatty acids [40,41], or even includes exotic fatty acids that are indigestible, unpalatable or toxic.

4.2. Potential environmental risks posed by biofuels-optimized GM microalgae

For microalgae to provide any significant contribution to biofuels production, they will need to be grown over vast areas. It is most unlikely that all of that growth would be under cover, and even then it is unrealistic to expect that leakage or spillage of some proportion of the many thousands of cubic metres of culture that would be harvested per week would never occur. In all reality then, we need to consider the impact of such a leakage to the environment.

The features of a biofuels-optimized microalga, and their likely genetic stability, have important implications for ecology when such an organism enters the natural environment. Previously, we reported that during algal–algal competition a microalga with a lower $ChlC_{\max}$ while being superior as a clonal crop organism, would be at considerable selective disadvantage and would likely be eradicated in the natural environment [8]. However, as we warned in that previous work, this assumes that the control of growth by predators is equally distributed and does not discriminate in favour of the low $ChlC_{\max}$ -configured organism. Predator–prey systems are sensitive to such discriminations, and microalgae that appear outwardly poorly competitive with other microalgae can still grow to form dominant ungrazed blooms through such mechanisms [49]. The configuration of biofuels-optimized microalgae that we identify here is, other than the issue of $ChlC_{\max}$, highly competitive in comparison with the default configuration. If such an organism became the dominant primary producer it would inevitably form dense blooms, for that is what it is designed for, albeit in ponds or other culture systems.

While the ability to grow rapidly under low light is important for competition with other phototrophs, of the factors we explored, it is the extreme C : N and C : P ratios in biofuels-optimized microalgae that create the greatest risk to trophic dynamics. Such extreme ratios, and the ability to continue to grow rapidly with high fatty acid and/or starch content creates a severe nutritional stoichiometric challenge for zooplankton growth [35,38,39,49]. This limits predation upon

the simulated GM organism, especially under P-limitation (figure 2b; electronic supplementary material, S11).

While the potential formation of ungrazed HABs indicated in figure 2 simply reflects an imbalance in stoichiometric ecology, characteristics such as fatty acid [48] and toxin content [49,50] are of vital ecological importance, affecting zooplankton feeding and growth [39,51]. Any approach that alters fatty acid profiles in microalgae, especially to the biofuels-preferred shorter, saturated forms [40] which have little or no nutritional value to zooplankton, would undoubtedly exacerbate the significance of the already highly damaging stoichiometric imbalance (figure 2). Indeed, even when taken in isolation, modifying microalgae to alter their fatty acid content may be expected to adversely affect predation and increase the potential for them forming ungrazed (perhaps ungrazable) blooms.

The implications of changes in palatability and toxin production (as secondary metabolites in nutrient-stressed microalgae), which are likely to co-occur with such fatty acid modifications, are well known [39,49–51]. In consequence, it is most probably that biofuels-optimized microalgae will be less palatable than assumed in the simulations shown here, giving rise to what Mitra & Flynn [39] refer to as negative stoichiometric modulation of predation, a process that effectively shuts down predation very rapidly as C:N rises. The outcomes from such trophic interactions will thus likely be even starker in comparison with the default, wild-type, expectations.

One could endeavour to counter the above problems by developing traits that place biofuels microalgae at a distinct competitive disadvantage against their naturally occurring counterparts on escape to natural waters. However, configuring a crop organism in this way would also make it vulnerable to failure against contaminants in a culture system. The fact is that for microalgae to be a robust commercially successful organism for biofuels production requires that it can outcompete any contaminating microalgae, and also proliferate in the presence of any zooplanktonic (predator) pests. Altering factors such as growth rate or nutrient affinity, so that GM microalgae would only grow well at high nutrient concentrations, would place them at a disadvantage in competition with contaminants in culture systems, and would in any case be selected against even within a clonal crop culture when growing under the nutrient limitation that is required to stimulate biofuels production.

One potential solution to this conundrum is to optimize growth of GM biofuels-optimized microalgae in extreme environments, for example with respect to temperature or pH [52], conditions that would not commonly occur in nature. Whether such growth conditions place an acceptable additional financial and logistic burden on the whole enterprise would need careful consideration, given the massive volumetric scale of biomass production required to provide a significant biofuels production. Such an approach would also itself not be immune from posing risks to the environment.

An alternative approach is not to increase biomass production to yield metabolites for biofuels production, but to modify biochemistry to redirect the synthesis of organics away from growth and towards fatty acids, which the cells then release for direct harvesting from the growth medium [33]. This approach could be viewed as having parallels with events that already occur in nature. The production and release of excess polysaccharide from nutrient-stressed microalgae in nature is not uncommon, and causes well-documented problems associated with foams

and transparent exopolymeric material [53,54]. This released material then promotes the growth of ecosystem disruptive algal blooms through inhibition of grazing, and can also create serious pollution events along coasts [55]. While the GM approach to direct extracellular production of material destined for biofuels carries various attraction (notably with respect to harvesting), it may thus also carry with it causes of environmental concern as well. Immobilizing the microalgae on some fixed substrate could overcome the risk, assuming that the cells could only grow on the substrate and that challenges of adequate illumination (and hence production) can be overcome among the attached microalgae.

Finally, it is worth noting that GM terrestrial crops differ greatly from GM microalgae with respect to the potential for environmental damage. While higher plants can be made sterile to limit their spread, by their very nature GM microalgae must be capable of reproduction. Higher plants undergo typically one generation a year; microalgae reproduce daily. Our understanding of the impacts of GM higher plants upon ecology has developed over a few decades, a period of reproductive cycles that GM microalgae would achieve in a week. It will thus take something of the order of a century of higher plant generations to compare with a fraction of 1 year's growth of microalgal generations. While GM terrestrial plant crops have been deployed without obvious catastrophic impacts on ecology (though certainly not without controversy on this point; [56]), it is not possible to extrapolate an argument that GM microalgae would be similarly benign.

5. Conclusions

There has been much claimed for the potential of algal biofuels to contribute significantly to energy sustainability and security, but detailed analyses indicate that for financial and logistic realization costs per litre of biofuels need to come down significantly before such a dream can be realized [5,6,45]. A major advance may be achieved by attaining a step change in microalgal productivity. Significantly, our previous analysis [8] suggests that areal productivity using 'typical' microalgae is likely to be little better than that seen under optimal conditions in nature [23]. While in culture ponds the volumetric production is much higher, and hence harvesting and dewatering costs are decreased accordingly, the implication is that areal production using wild-type strains is limited by the total light incident to the culture system and by the underlying physiology of the organisms. That physiology has evolved over millions of years from basic metabolic building blocks with origins to the emergence of life on Earth [32]. To go beyond this (natural maximum) productivity of $ca\ 4\ g\ C\ m^{-2}\ d^{-1}$ thus requires a change in the physiology of the organisms. It is most probably that this can only be achieved through radical GM, creating organisms that are literally new to nature.

Our work indicates a clear potential for GM in the commercial development of microalgal biofuels, with scope for raising production by perhaps half an order of magnitude (figure 1; electronic supplementary material, S5). Coupled with more efficient processing technologies, GM microalgae could make microalgal biofuels a viable and cost-effective option. However, our study also suggests a very real risk that the engineered product could come to represent the perfect HAB species (figure 2), with all the attendant risks to the environment, to environmental services and human health

that HABs present [57]. This is not to say that all GM approaches will exhibit the same potential risks to nature. However, and accepting that not all of the GM traits may be stable in nature, given the ease with which GM microalgae could be transferred around the planet the potential risk of GM microalgae to nature should not be underestimated. There already exists ample warning of the damage that can be caused from the inadvertent trans-ocean transfers of 'exotic' natural HAB species [13], with no evidence that naturally occurring zooplankton can contain the problem. Indeed, disruption to biodiversity by invasive alien species is well known and all too common (e.g. for aquatics, [58]). In this capacity, the mass cultivation of any microalga isolated from a source distant to the site of commercial deployment is also a matter of concern.

The spread of GM microalgae of the type of configuration we identify would be effectively impossible to halt. As GM of factors likely affecting palatability of microalgae is already

being conducted in the name of biofuels production [10,41,42], there is a real risk that the genie is already part way out of the bottle. If GM biofuels-optimized microalgae were to destroy fisheries then a main driver for microalgal biofuels research, the argument that such biofuels production would not compete with production of biomass for food [3,4], may prove to be totally misplaced. Accordingly, a strong argument can be made for the regulation of GM of microalgae at an international level, because the potential for damage could have global consequences, echoing recent concerns over geoengineering [59]. Whether, against arguments for sovereign fuel security, regulation could be enforced, is a dilemma that society may soon have to face up to.

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