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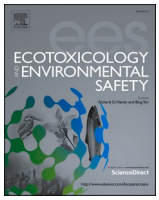

Environmental risk assessment for genetically modified microalgae

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ABSTRACT

Advances in genomic techniques have great potential to accelerate the development of the microalgal sector and to address key challenges related to upscaling. Genetically modified microalgae (GMM) can exhibit enhanced biomass productivity, increased accumulation of valuable biocompounds, or express other beneficial traits compared to their non-modified counterparts. However, the application of GMM and their associated cultivation methods may lead to unintentional release, potentially affecting adjacent ecosystems. Here we apply a problem formulation approach to support the environmental risk assessment of novel GMM relative to their wild-type counterparts. We focus on potential effects on ecosystem services provided by natural microalgae communities. We present plausible pathways to harm through which GMM could exert adverse effects and formulate relevant research questions to evaluate the likelihood of each step within these pathways. Existing knowledge on the hazards associated with invasiveness, gene transfer and toxicity of GMM is compiled and discussed. Together, these elements provide a practical framework for identifying relevant hazards and guiding data generation to support environmental risk assessments of GMM.

1. Introduction

Microalgae constitute a diverse group of photosynthetic microorganisms with fast growth rates and high biomass productivity that play important roles in aquatic ecosystems, where they support primary production, nutrient cycling, and food-web dynamics (Glibert, 2024). They are widely distributed in aquatic habitats, thriving in freshwater, brackish, and marine ecosystems across climatic zones from polar to tropical latitudes, and are also present in terrestrial environments (Metting Jr, 1996). Microalgae include both eukaryotic photoautotrophs (kingdom Chromista, *sensu* (Ruggiero et al., 2015)) and prokaryotic cyanobacteria (kingdom Bacteria, phylum Cyanophyta). In the context of production, however, distinguishing between these two groups is generally not considered necessary, and the term microalgae is commonly applied to both.

Because microalgae produce a wide range of valuable biomolecules, including proteins, fatty acids and pigments (Gallego, 2025), they are used in diverse applications such as pharmaceutical products, food and feed additives, chemical feedstocks, bioremediation of pollutants (including CO_2 fixation), and agricultural biostimulants (Gallego et al., 2025). Such applications require different cultivation systems and technologies that will depend on the trait of interest and cell type (eukaryotic/prokaryotic), each associated with different levels of risk regarding accidental release into the environment. The two main cultivation systems for large-scale microalgae production (closed systems, *i.e.*, photobioreactors and fermenters; and open systems, *i.e.*, ponds) differ in economic feasibility, productivity, risk of biological contamination and potential for environmental spillage. Open cultivation systems depend on environmental conditions and are relatively easy to operate (especially if located in warm regions), but they are more susceptible to contamination by undesirable microorganisms than closed systems, which are usually more productive as they operate under controlled conditions (Gallego et al., 2025; Mata et al., 2010; Bani et al., 2021).

Microalgal production systems are increasingly being explored for large-scale applications, alongside broader use of genomic and molecular tools. This trend is reflected in a recent horizon-scanning study that identified the traits most frequently targeted for modification in genetically modified microalgae (GMM) (Gallego et al., 2026). Given this growing interest, GMM are now regulated under most (if not all) jurisdictions. Regulatory authorities must address their environmental,

Abbreviations: GMM, Genetically modified microalgae; GMO, Genetically modified organism; GM, Genetically modified; ERA, environmental risk assessment; HAB, harmful algal bloom; EDAB, ecosystem-disturbing algal bloom; HGT, Horizontal gene transfer; VGT, Vertical gene transfer.

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economic and human health impacts before entering the market (More et al., 2020). In most jurisdictions, GMM require regulatory approval, which includes an environmental risk assessment (ERA) that addresses the potential consequences of unintentional release (Beacham et al., 2017). Beyond existing approval requirements, several countries are currently revisiting regulatory approaches for GM microorganisms as their applications expand (*e.g* (Rubinstein et al., 2025). In practice, controlled release of GMM from open pond systems has already been assessed (Szyjka et al., 2017) and, in some cases, approved for use (OGTR, 2024). Within the EU, various policy initiatives (including the European Green Deal, the Farm to Fork Strategy, and the Sustainable Blue Economy), promote the development of the microalgal sector (European Commission, 2022). However, the use of GMM continues to raise environmental concerns (Eckerstorfer et al., 2025).

In Europe, the level of containment of GMM is defined by the cultivation system, rather than by the cell type or other intrinsic risks concerning prokaryotes and eukaryotes. Open and closed microalgal production systems are regulated differently, under Directive 2001/18/EC and 2009/41/EC, respectively (European Union, 2001, 2009). Directive 2001/18/EC deals with deliberate release of GMOs into the environment, and GMM cultivated in open systems would require a case-by-case assessment, including also other aspects such as animal and human safety (European Union, 2001). Directive 2009/41/EC on the contained use of GM microorganisms refers to contained microalgal facilities (indoor and outdoor), and establishes four categories (and containment levels) based on the risk to human health and the environment (European Union, 2009). GMM usually fall within categories 1 and 2, with optional or required inactivation of the microalgae in used material and waste, respectively (see Annex III). Methods to inactivate GMM (*e.g.*, composting, sterilization) might be required under this Directive. The Directive mandates a case-by-case risk assessment for all contained uses, including the evaluation of potential consequences of accidental release for human health and the environment.

Different regulatory authorities worldwide provide guidance for assessing the environmental risks of GM microorganisms including microalgae (More et al., 2020; Mullins et al., 2024; USEPA, 2025). As for any other GMO, risk assessment is conducted in a comparative way, *i.e.*, the characteristics of the GMM are compared to the non-transformed wild type to identify potential areas of concern (EFSA, 2011; European Union, 2009). When a GMM strain is intended for commercial use, it must undergo an ERA. This process typically begins with problem formulation (Paoli et al., 2022) which comprises three main steps (Devos et al., 2019). First, the valued components of the environment must be identified, *i.e.*, the protection goals. While these protection goals are generally defined by regulators and policy makers, several conceptual frameworks can support their identification. For example, the Millennium Ecosystem Assessment (MEA) offers a practical way to translate broad policy objectives into operational protection goals through the concept of ecosystem services (Devos et al., 2015; Millennium Ecosystem Assessment, 2001). Microalgae provide supporting (oxygen and biomass, nutrient cycling), regulating (water depuration, climate regulation), provisioning (food, feed, bioactive compounds, genetic resources), and cultural (recreational and aesthetic values) ecosystems services (Naselli-Flores and Padisak, 2023). Second, plausible pathways to harm (PTH) are described to clarify how a stressor may affect the identified protection goals (Devos et al., 2019). Each PTH represents a logical, step-by-step sequence of events that must occur for GMM to cause an adverse environmental effect. In a third step, testable risk hypotheses can be formulated based on individual steps within these PTHs. Relevant existing knowledge is compiled, and, where necessary, new studies are conducted to test these hypotheses.

Here, we aim to provide a unified, systems-based framework for the assessment of GMM strains -both existing and emerging- cultivated under open or closed system conditions. Our conceptual model offers a general and structured approach to support environmental risk assessors by compiling, in a non-exhaustive manner, existing knowledge and methodological approaches relevant to the ERA of GMM across different scenarios. Specifically, we define the protection goal as the proper functioning of aquatic ecosystems that provide essential services, including water quality for irrigation and drinking purposes, fisheries, recreational use, and habitat for plants and animals (European Union, 2000; European Commission, 2001). We then describe several plausible PTHs through which GMM may cause adverse effects, including invasiveness, gene transfer, and toxicity/allergenicity. Building on these pathways, we formulate research questions to support the ERA, compile existing data, and propose appropriate testing approaches. In this study, we assess both prokaryotic cyanobacteria and eukaryotic microalgae together, as both are subject to the same regulatory framework. However, we acknowledge that their distinct biological and ecological characteristics may influence their potential environmental impacts.

2. Plausible pathways to harm (PTH)

The unintentional release of GMM into the environment may pose various risks to surrounding ecosystems. For illustrative purposes, we define our protection goal as the maintenance of properly functioning aquatic ecosystems that provide essential services, including water quality for irrigation and drinking purposes, fisheries, recreational use, and habitats for plants and animals. Based on this protection goal, we identified plausible pathways that could lead to environmental harm (PTH), *i.e.*, the deterioration of ecosystem functioning and associated services. These pathways are summarized in Fig. 1. The figure outlines sixteen sequential steps which are discussed below in the same order as they appear in the figure.

2.1. Environmental release of GMM

The ability of GMM to survive and persist in natural habitats following accidental release depends on several key factors. These include: 1) their ability to thrive under the specific environmental conditions (niche requirements), 2) their capacity to compete with native microorganisms, including microalgae (competitive ability for resources), and 3) their ability to avoid predation (*e.g.*, through morphological traits or the production of secondary metabolites) (Fig. 1, **step 1**).

2.1.1. Environmental requirements

When GMM are released into the environment, certain environmental conditions are necessary for them to establish and grow (Fig. 1, **step 1**). Such required conditions (niche requirements) are usually species-specific and can vary enormously (niche breadth). The species of choice is thus an important factor to be considered not only for commercial purposes (Lane, 2022), but also for minimizing the risk of proliferation outside the cultivation system. Certain species with narrow and/or extreme ecological niches have been modified and improved using gene-technology tools (Varshney et al., 2015).

Extremophiles can thrive in hostile habitats and tolerate extreme pH, temperature, salinity, irradiance, CO_2 or high metal concentrations (Malavasi et al., 2020). For example, *Galdieria sulphuraria,* which produces commercially relevant pigments, is a thermo-acidophilic species that grows at temperatures close to 50°C and pH < 4 (Retta et al., 2024). The cyanobacterium *Thermosynechococcus elongatus*, also used in biotechnology, thrives in hot springs, with optimal growth rates between 55°C and 60°C. Species that perform well under such conditions can be preferred in large-scale production systems because of the low risk of contamination by other microorganisms (Varshney et al., 2015; Lafarga et al., 2021). More importantly, extreme niche conditions act as a biocontainment strategy to prevent the growth of GM microorganisms outside of the authorized system (George et al., 2024; Sebesta et al., 2022).

Knowing the environmental conditions in the surrounding area of the production system is relevant to preventing the undesirable

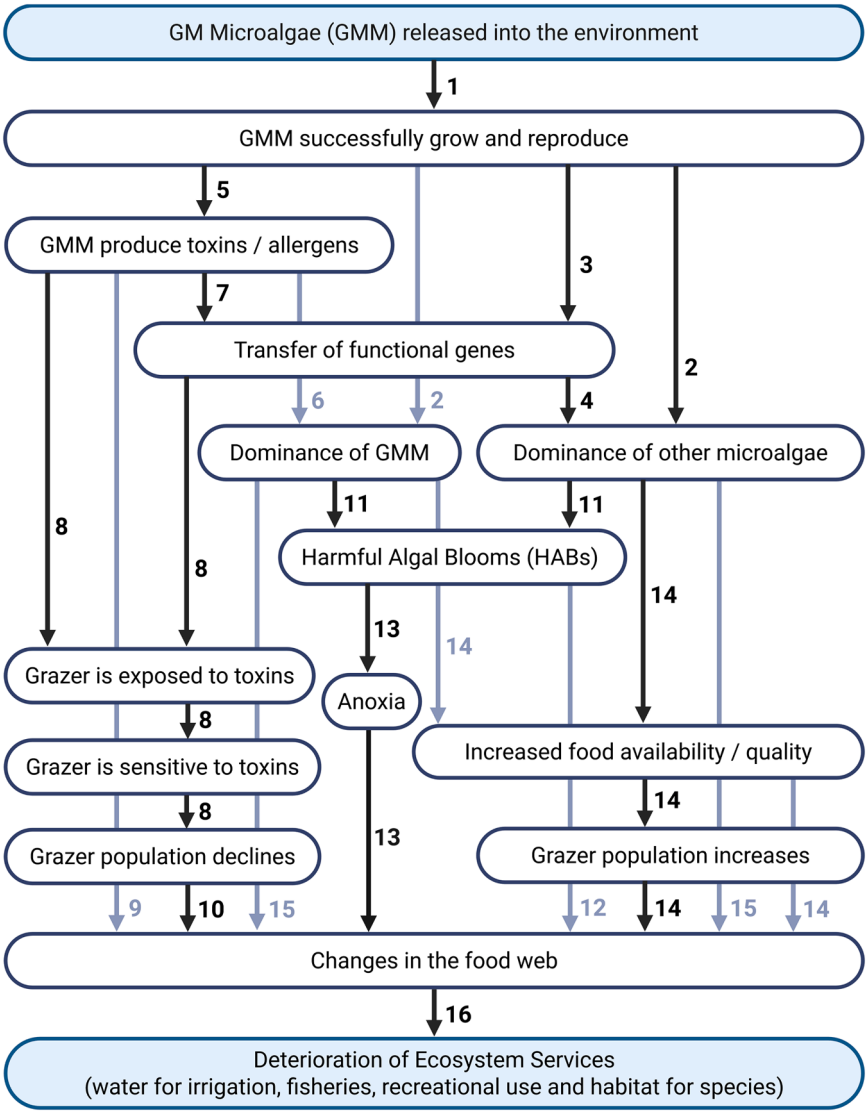

Fig. 1. Plausible pathways to harm in case of an unintentional release of GM microalgae (GMM) into the environment. For each specific step (1–16), testable risk hypothesis can be formulated and subsequently addressed to assess whether harm is likely to occur. Sixteen distinct steps are identified and discussed in detail in the main text. Grey arrows and numbering are included solely to improve figure readability and to avoid overlapping with text boxes.

proliferation of GMM strains in case of a release. For instance, *Limnospira platensis* (commercially known as 'spirulina') shows optimal growth rates under high pH conditions (pH 8.5–10) (Malavasi et al., 2020; Costa et al., 2019; Ogbonda et al., 2007) and would not likely survive in acidic systems.

Since environmental conditions in ecosystems usually fluctuate due to seasonal, meteorological, biological and other factors, it is preferable that GMM do not thrive under such changing conditions. Strains capable of forming cysts or dormant stages, such as *Haematococcus pluvialis* (aplanospores) or *Scenedesmus/Tetradesmus* spp. (autospores) may exhibit enhanced tolerance to environmental fluctuations, thereby improving their survival potential. Stress-tolerant species (*i.e.*, those with a wide niche) such as the industrially relevant *Chlorella vulgaris* are highly adaptable to different environmental conditions and can colonize nearly every aquatic and terrestrial habitat (Paoli et al., 2022; Devos et al., 2019).

To test whether a GMM strain can tolerate natural environmental conditions, one can apply the same environmental tolerance tests used for wild-type strains in microcosms, which ensure high replicability (Thomas et al., 2026). However, such approaches are often limited to short-term assessments and may have a limited capacity for extrapolation to natural, long-term conditions (Table 1).

2.1.2. *Competitive ability for essential resources*

Released GMM must compete with the natural communities for resources in order to persist in the ecosystem (Fig. 1, **step 1**). As environmental conditions are relatively homogeneous in aquatic habitats -if compared to terrestrial habitats-, microalgae primarily compete for a few essential resources, that is, certain nutrients and light. Any newly arrived microalga into a natural ecosystem can go through two different scenarios: it can be outcompeted by the established microalgal community, or it can successfully establish and coexist within the native communities. In the latter case, the GMM may cause changes in the microalgal community, *e.g.*, altering species dominance (Fig. 1, **step 2**).

GMM may experience metabolic burden, reduced competitiveness, and lower stress tolerance compared with their wild-type counterparts (*e.g.,* Ajjawi et al., 2017; Poliner et al., 2018), which can limit their ability to persist under natural environmental conditions. To test whether any newly arriving species in any ecosystem can persist within natural communities, 'direct invasion' microcosm experiments can be conducted (*e.g.,* Gallego et al., 2019; Narwani et al., 2013). This approach relies on quantifying the growth rates of the GMM, its

Table 1
Overview of the research questions addressed in this study and the corresponding potential test approaches, method types, (with supporting references), advantages, and limitations.

PTH Step	Research question	Potential test approach	Method type (references)	Method advantages	Method limitations
1	Do GMM tolerate natural environmental conditions?	Environmental tolerance tests Cultivation and detection methods (flow-cytometry, imaging, microscopic identification)	• Microcosms (Thomas et al., 2026)	• High replicability	• Short-term focus • Limited extrapolation
2,6	Do GMM successfully grow and reproduce, potentially becoming dominant?	Direct invasion experiments	• Microcosms (Gallego et al., 2019; Narwani et al., 2013)	• Informative • Cost-effective	• Constrained to pairwise combitations • Resident community must be at steady state
			• Mesocosms (*in situ*) (Buchberger and Stockenreiter, 2018)	• Realistic (effect of invasion on multispecies community)	• Higher cost • Uncontrolled oscillations of environmental variables
		Measurement of competitive ability traits	• Laboratory experiments (monocultures) (Huisman et al., 1999; Passarge et al., 2006; Burson et al., 2019; Gallego and Narwani, 2022) • Microcosms (Burson et al., 2018; Bestion et al., 2017; Edwards et al., 2011; Armstrong et al., 2023) • Models (Kruk et al., 2021)	• Potential to predict strain dominance in natural communities	• R^* needs to be calculated for each limiting resource
		Morphological proxies for competitive ability (size, shape, functional classifications)	• Existing compendia (Kremer et al., 2014; Olenina, 2006; Reynolds, 2006; Kruk et al., 2010; Reynolds et al., 2002; Margalef, 1978; Reynolds, 1984; Smayda, 2001) (Olenina, 2006) • Imaging and single-cell approaches	• Simple and time-effective	• Limited applicability (functions only as a proxy)
		Physiological proxies for competitive ability (N fixation, mixotrophy,…)	• Laboratory controlled experiments (Litchman and Klausmeier, 2008) and ref. herein)	• Predictive power under resource limitation	• Limited applicability (functions only as a proxy)
		Measurement of nutrient utilization and stoichiometry traits	• Laboratory controlled experiments • Models (Edwards et al., 2012; Hillebrand et al., 2013)	• Informative • Allow comparisons with wild-type strains	• Findings might not be generalizable beyond the experimental growth conditions
		Grazing resistance experiments	• Microcosms (Ger et al., 2018; Thomas et al., 2024) • Mesocosms (Rong et al., 2021; Chislock et al., 2013)	• Very informative (over longer durations)	• Scale limitations • Require robust design to avoid oversimplification
5,7,8	Are grazers and higher-tier organisms exposed to toxins produced by GMM?	Detection of phycotoxins (ELISA, HPLC, chromatographic methods, biosensors)	• Laboratory measurements (United States Environmental Protection Agency, 2024; Meriluoto et al., 2017; Sanseverino et al., 2017) • Biosensors (Anfossi et al., 2013; Zahraee et al., 2023; Cunha et al., 2018) • Multiplex systems (Chorus and Welker, 2021)	• Fast	• Expensive equipment
8	Are grazers and higher-tier organisms sensitive to toxins produced by GMM?	Standard toxicological tests (*e.g.*, genotoxicity, rodent studies)	• Laboratory controlled tests (one or more grazers, one or more toxins) (Chorus and Welker, 2021; More et al., 2022; Louzao et al., 2022; Castrec et al., 2018)	• Replicability	• High costs
11	Do GMM form harmful algal blooms (HABs)?	Forecasting methods	• Remote sensing (Caballero et al., 2025) • Data-driven models (Sagova-Mareckova et al., 2021)	• Accurate on recurrent blooms	• Rely on monitoring data
9,10,12,13,14,15	Do GMM or other microalgae cause changes in the food web?	Top-down cascade effects	• Whole-lake manipulations (Sasaki et al., 2025) and ref. herein) • Observational studies (Di Pane et al., 2022)	• Most realistic scenarios	• High complexity limits results interpretation • Stricter regulatory approval due to higher risk
			• Mesocosms, enclosures, pond experiments (Sasaki et al., 2025) and ref. herein) • Meta-analyses	• Detection of indirect effects • Synthesis of multiple studies	• High operational costs • Very limited data available for GMM; potential inability to detect novel interactions
16	Has the provision of ecosystem services deteriorated?	Monitoring	• Water quality indicators (chlorophyll-*a*, biotic indices, pH, dissolved oxygen,…) (Wang, 2024; Boyer et al., 2009)		• Only to be conducted post-release

wild-type counterpart (and each species of the natural community at risk of invasion), growing alone and in combination with the invader (Grainger et al., 2019; Clark, et-al, 2026). Such an approach can be informative and cost-effective, but it is inherently constrained to pairwise combinations. In addition, the resident strain must be at equilibrium at the time of invasion (which can be achieved in chemostats-like microcosms), and the system must be limited to a certain number of species, despite the fact that natural communities often consist of dozens of taxa (Orr et al., 2025) (Table 1).

A trait that quantifies the competitive ability of a strain under a single limiting resource is the 'minimum resource requirement' (Tilman's R^*), *i.e.*, the lowest concentration of any limiting resource (R) at which a microalgal species can still thrive (Tilman, 1981, 1982). R^* can be calculated in monocultures as a function of the half-saturation constant for growth (K_s) and the maximum specific growth (μ_{max}) (Tilman, 1982). If a GM strain shows a higher R^* than its wild-type strain, it will be less competitive than its counterpart (for that limiting resource). R^* reliably predicts competitive outcomes in laboratory experiments with two species (Miller et al., 2005; Wilson et al., 2007; Huisman et al., 1999; Passarge et al., 2006; Burson et al., 2019), and its applicability extends to natural communities, where it can be used to predict phytoplankton species dominance (Gallego and Narwani, 2022; Chase and Leibold, 2009) (Table 1). For instance, *Ceratium furcoides* blooms (causing red tides) were successfully predicted by comparing its R^* to those of the co-occurring native species with similar morphological traits (Kruk et al., 2021). Thus, the potential dominance of a GM strain may be predicted in the same way as invasive microalgae.

The competitive ability of a species can also be estimated using morphological and physiological proxies (Litchman and Klausmeier, 2008) (Table 1). Cell size is the most relevant proxy because it impacts growth, metabolism and access to resources (small cells have a higher surface/volume ratio than large cells, affecting the resource uptake ratio and ultimately growth rate) (Litchman and Klausmeier, 2008; Finkel et al., 2009). Efforts to produce compendia on microalgal species biovolume have been done for $>$ 1200 freshwater (Kremer et al., 2014) and $>$ 690 marine species (Olenina, 2006), by using standardized equations for biovolume calculation (Hillebrand et al., 1999; Sun and Liu, 2003).

Under resource deficiency, other microalgal traits can be advantageous, such as nitrogen fixation and mixotrophy (*i.e.*, alternation of autotrophic and heterotrophic modes of nutrition), and hence confer a competitive advantage to the strain. Some cyanobacteria like *Nostoc* spp. and *Aphanizomenon* spp. have the ability to fix atmospheric nitrogen, and most dinoflagellates and chrysomonads (usually mixotrophs/phagotrophs) can utilize organic carbon as a resource (Reynolds, 2006). High photosynthesis rates (common in some diatoms and cyanobacteria), and the presence of gas vesicles that allow buoyancy to maximize light utilization (common in cyanobacteria), may provide a competitive advantage when light is limiting (Reynolds, 2006). This is particularly relevant when GMM are modified to reduce the antenna size to increase biomass productivity, as the strain might outcompete the local species under high irradiance of a specific wavelength (Kumar et al., 2021), but will be likely outcompeted under low light.

Phytoplankton nutrient utilization traits (maximum nutrient uptake rate, half-saturation constant for nutrient uptake, growth rates) (Edwards et al., 2012) and stoichiometry-related traits (available and internal N:P ratios) (Hillebrand et al., 2013) may characterize GMM and natural competitors. Important parameters, such as temperature, are linked to these traits (Weber de Melo et al., 2025; Heinrichs et al., 2025). Taxonomic-based phylogeny might be promising to easily infer traits, when more 'omics' data are available (Bruggeman, 2011). In addition, functional classifications (aggregations of species with similar traits) can contribute to the characterization of GMM and their wild-types, by capturing commonalities in morphology, colonization capacity or stress tolerance (Reynolds, 2006; Kruk et al., 2010; Reynolds et al., 2002; Margalef, 1978; Reynolds, 1984; Smayda, 2001), but their applicability might be limited (Table 1).

Characterization of the competitive ability traits for any GMM compared to its wild-type counterpart can be done under controlled conditions (in laboratory and microcosms experiments that simulate environmental conditions), or with models (see reviews in Miller et al., 2005; Litchman and Klausmeier, 2008). Microcosms with multispecies competition and relevant environmental factors are especially informative (Burson et al., 2018; Bestion et al., 2017; Edwards et al., 2011; Armstrong et al., 2023). Mesocosm *in situ* experiments with natural phytoplankton communities are the most realistic experimental scenarios to predict the success or failure of the invasion, but upscaling lab and microcosms experiments adds complexity to the test, as more trophic levels and uncontrolled oscillations of environmental variables occur (Vijayaraj et al., 2022) (Table 1). For instance, to understand the effect of a potential invading species on the natural community, an invader can be added to an established resident community, as shown in (Buchberger and Stockenreiter, 2018). Such experiments also demonstrate that invaders failing to establish may alter the resident microalgae community composition (Buchberger and Stockenreiter, 2018; Weithoff et al., 2017).

2.1.3. Grazing avoidance strategies

The success of a GMM in an established microalgal community is also driven by grazing resistance (Fig. 1, **step 1**). When grazing pressure is overcome, the microalgae (either the GMM or other species) may increase in abundance and become dominant (Fig. 1, **step 2**). By 'grazers', we refer to organisms that feed on phytoplankton, mainly by suspension and filter feeding, *e.g.*, zooplankton and bivalves. Grazing resistance depends on different microalgal traits *e.g* (Litchman and Klausmeier, 2008), including morphological traits (large cell size, colony formation, presence of spines, unusual shapes), physiological traits (low nutritional value, toxin production, production of exopolymer substances) and behavioral traits (motility) (Pancic and Kiorboe, 2018).

Microalgae can achieve larger sizes by increasing their cell size, but also by forming colonies. For instance, *Desmodesmus* spp. and *Scenedesmus* spp. can form 8-cell colonies to avoid ingestion by grazers, and colony formation can be induced by the presence of *Daphnia* (or their released chemicals) (Hessen and Van Donk, 1993; Lampert et al., 1994). Other structures, such as spines and silica walls reduce the edibility of certain microalgae (Pancic and Kiorboe, 2018; Hessen and Van Donk, 1993). Elongated shapes are rarely ingested by copepods and cladocerans (as opposed to spherical cells), due to their size-limited feeding structures (Lürling, 2020).

Some microalgae cannot be digested after ingestion or possess low nutritional value, leading grazers to preferentially exploit more nutritious alternative food sources. This is the case for some cyanobacteria that lack essential fatty acids and sterols required for zooplankton reproduction (Ger et al., 2016). Other microalgae produce toxins that can have detrimental effects on grazers (see Section 2.3 on toxicity), while a third group can secrete mucilage sheaths, or increase the thickness of their cell walls, thereby becoming highly resistant to digestion following ingestion (Reynolds, 2007). Additionally, some species can actively 'swim' by using flagella, to escape from grazers and to take up nutrients. This is the case for certain dinoflagellates and raphidophytes (Pancic and Kiorboe, 2018).

Grazing experiments with GMM could follow designs similar to those used in microcosms (Ger et al., 2018) or mesocosms (Rong et al., 2021) experiments with wild-type strains, which can be very informative over longer durations, but are inherently constrained by limitations related to experimental scale (Table 1).

Trade-offs between grazer resistance and microalgae competitive ability traits are common (see reviews in Litchman and Klausmeier, 2008; Pancic and Kiorboe, 2018). Resistance to grazing usually increases with microalgal cell size, but then their surface/volume ratio (and consequently, the nutrient uptake rate) decreases (Litchman and Klausmeier, 2008; Pancic and Kiorboe, 2018). To account for such

trade-offs, simple food web models (Ehrlich et al., 2020) and more complex models integrating physical, chemical and biological variables have been used to predict how grazing shapes microalgal traits and communities across seasonal to evolutionary timescales (Branco et al., 2020; de Senerpont Domis et al., 2013; Sommer et al., 1986, 2012). Existing models on phyto- and zooplankton could be extended to GMM and incorporate traits previously obtained in the laboratory (growth rate, nutrient uptake rates, etc.) to predict their occurrence and dominance in natural ecosystems.

2.2. Transfer of functional genes

If GMM successfully establish and reproduce in the environment, the transfer of one or more functional genes to the natural community gene pool could occur at a frequency that might vary with the density of the GMM in the ecosystem (Fig. 1**, step 3**). If the recipient(s) of such gene transfer gains a competitive advantage, it may disrupt the food web, particularly if it becomes dominant (Fig. 1**, step 4**).

The risk of gene transfer depends on the microalgal cell type involved. Cyanobacteria can transfer genes horizontally (HGT) via transformation, transduction and conjugation of genetic material. These processes have been extensively studied (Lorenz and Wackernagel, 1994; Dreiseikelmann, 1994). Some cyanobacteria can actively take up DNA from the environment and thus exhibit a natural competence for transformation (Schirmacher et al., 2020). This natural competence requires the expression of a secretion system pilus and the competence *com* gene locus (Bhaya et al., 2025; Dubnau, 1999). To date, at least 73 cyanobacteria species have been identified with such gene loci (Schirmacher et al., 2020). The expression of these genes is known to be controlled by a circadian clock (Taton et al., 2020). DNA ingestion occurs after fragmentation into single strands (Schirmacher et al., 2020). Then, most of the DNA is used as a nutrient source. However, in cases of chromosomal strand breaks and presence of homologous sequences in the free DNA, a transient complex of donor and recipient DNA can form in the cytoplasm. This allows the integration of single-stranded DNA segments (from the donor) by recombination processes (Lorenz and Wackernagel, 1994). In the case of GMM, the DNA containing the genetic modification(s) could be released upon cell death and taken up by native competent bacteria, though the likelihood of integration into their genome is small.

Another HGT process involving plasmids is conjugation. In (cyano) bacteria, physical contact occurs between donor and recipient cells, followed by the transfer of single-stranded DNA (Dreiseikelmann, 1994). This process requires the presence of a conjugative plasmid that carries the necessary genes for DNA transfer, including mobilisation genes, a *cis*-acting origin of transfer, and genes that facilitate the formation of a stable conjugation pair (Zechner et al., 2017; Llosa et al., 2002; Cabezón et al., 2015). Such conjugative and mobilizable plasmids exist within some native cyanobacteria populations (Muro-Pastor et al., 1994; Herrero and Flores, 2008). In the case of GMM, for such a transfer to occur, the transferred genes must be present on a conjugative plasmid within the cell.

Further, transducing phages can contribute to HGT in cyanobacteria. Such phages can transfer specific bacterial DNA segments adjacent to their integration site (special transduction) or occasionally pack bacterial DNA into infectious phage particles (general transduction), thereby infecting hosts from multiple bacterial families (Lang et al., 2012; Verheust et al., 2010). Some cyanophages (Caudoviricetes) are known to have a broad host range and/or the potential for general transduction at low frequencies (Sullivan et al., 2003; Weigele et al., 2007; Laurenceau et al., 2021; Clokie et al., 2003). Unless such cyanophages are used for cloning in GMM, gene transfer via transduction can be ruled out.

Unlike cyanobacteria, eukaryotic microalgae generally cannot actively transfer DNA horizontally (León and Fernández, 2007; Shi et al., 2021). The only evidence of natural DNA uptake refers to ancient events involving endosymbiont integration (Maruyama et al., 2011), and gene transfer between microalgal hosts and infecting viruses (Maruyama et al., 2011; Krueger-Hadfield, 2024; Monier et al., 2009; von Dassow and Montresor, 2010).

Eukaryotic microalgae can only transfer genes vertically by inhabiting genetic material through sexual reproduction (Krueger-Hadfield, 2024) or receive genes unidirectionally through transduction by viruses (Monier et al., 2009). Sexual reproduction is also a rare event in most microalgae and would only allow the transfer of genes within the same species or very closely related species (Krueger-Hadfield, 2024). Only some chlorophytes, dinoflagellates and coccolithophores have life cycles with sexual reproduction (von Dassow and Montresor, 2010; Dvořák et al., 2015). *Chlamydomonas reinhardtii* can reproduce sexually (Sekimoto, 2017) and hybridization has been observed (Nakada et al., 2014). Data on microalgae life cycles are still limited (von Dassow and Montresor, 2010), thereby introducing uncertainty into ERAs. For example, the sexual stage of the industrially relevant diatom *Phaeodactylum tricornutum* has never been observed (Chepurnov et al., 2004; Mao et al., 2020) (but indirect proof was documented in (Li et al., 2012)), and sexual reproduction in *G. sulphuraria* has been only recently observed (Hirooka et al., 2022). Maintaining the (potentially sexual) strain in an asexual state during biomass production would minimize the risk of hybridization and vertical gene transfer. Nutrient shifts, light interruption or changing salinities can cause stress and trigger sexual reproduction (Godhe et al., 2014; Figueroa et al., 2006; Armbrust et al., 1990). For instance, some diatoms produce gametes when exposed to ammonium during stationary phase (Moore et al., 2017).

Overall, the frequency of gene transfer events involving GMM is either negligible, as in the case of eukaryotic microalgae, or low, as observed for cyanobacteria, provided that neither conjugative plasmids with a broad host range and mobilization ability nor broadly transducing prophages are present in cyanobacteria hosts. The transfer of functional genes can be verified through established genotypic, transcriptomic, proteomic or phenotypic analyses, *e.g* (EFSA, 2011; Doron et al., 2016; EFSA, 2021; Pandey and Azad, 2022).

2.3. Toxicity/allergenicity of microalgal compounds to grazers and higher-tier organisms

Certain microalgae naturally produce toxins and other metabolites that can accumulate in the cell or be released into the surrounding water (Long et al., 2021). Phycotoxins are typically secondary metabolites associated with toxic effects, whereas allergenicity is generally linked to (non-toxic) proteins. In the following sections, we focus exclusively on toxic compounds produced by microalgae, as allergenicity represents a comparative hazard pathway.

2.3.1. Exposure to toxins

Some phycotoxins have been exploited as biocides, biofertilizers, chelators and supplements (Haque et al., 2017; Jha and Zi-Rong, 2004; Haraguchi et al., 2024; Li et al., 2001), and even cultivated at large scale (Wang et al., 2015). If the strain producing the phycotoxin is genetically modified, or if gene(s) encoding phycotoxins are transferred into another microalgal strain, this could pose a risk to organisms across multiple trophic levels (Fig. 1**, steps 5, 7**). First, local phytoplankton species could be outcompeted by the GMM because of the competitive advantage conferred by chemical inhibition (allelopathy) (Fig. 1**, step 6**). Second, the gene(s) responsible for toxicity could also be transferred into other members of the microalgal community, causing changes in the community structure (Fig. 1**, step 4**). Third, grazer populations (mainly zooplankton and bivalves) would be exposed to phycotoxins when consuming GMM or any microalgae that have received the functional gene (Fig. 1**, steps 7, 8**). Fourth, aquatic organisms could be exposed to phycotoxins that are released into the water (Fig. 1**, step 9**).

Any phycotoxin produced by GMM could follow these exposure routes: direct ingestion of the toxin (or through grazers that contain the

toxin), or direct exposure (through skin and external tissues, or inhalation). Moreover, exposure can also induce selective mechanisms of tolerance. Some cladocerans, for example, have adapted to periodic exposure and consumption of toxic cyanobacteria (Hairston et al., 2001; Lemaire et al., 2012) and copepods can also consume toxic dinoflagellates without harm (Turner, 2014). Other grazers simply avoid eating the toxic microalgae. For instance, bivalves have developed strategies such as shell closure, clearance rate (the ability to remove toxic microalgal cells from the water suspension), or production of biodeposits to limit contact between the bivalve tissues and the toxic microalgae (Hegaret et al., 2007; Sauvey et al., 2021). Toxin exposure will depend on the type of toxin produced, but more importantly, on the grazing rates and selection strategy, which are usually species-specific (see reviews in Turner, 2014; Turner and Tester, 1997). Freshwater cladocerans, considered generalist grazers with high filtration rates, are more exposed to phycotoxins than copepods (Peters and Downing, 1984) and can even reduce the populations of toxic microalgae by grazing. In contrast, calanoid copepods tend to ingest fewer toxic microalgae when alternative, more palatable microalgae are available (Ger et al., 2018; Turner and Tester, 1989).

Since toxin production in non-GM strains varies enormously in space and time, with some environmental factors triggering toxin production and/or release (Huisman et al., 2018; Paerl and Huisman, 2009; Brandenburg et al., 2019; Agathokleous et al., 2022; Van de Waal et al., 2009; Rinta-Kanto et al., 2009; Bates et al., 2018), we expect GMM to show a similar pattern. One of the first studies on ERA of GMM (Henley et al., 2013) suggested that GMM producing a known toxin would pose a lower environmental risk than those producing a novel toxin, since the latter could provide a competitive advantage to the GMM (and a higher grazing resistance). To our knowledge, there is no evidence (based on experience with GM plants) that novel toxins appear after genetic modification, but pre-existing toxins could increase in concentration, as observed for steroidal glycoalkaloids in potato (Ladics et al., 2015). If GMM are known to produce a toxin (because their wild-type counterparts do), then toxin levels must remain below hazardous levels.

If a toxic gene is deliberately introduced, cultivation should be restricted to closed systems to reduce risk for animals, humans, plants or the environment. To date, only a single GMM strain has been engineered to heterologously express phycotoxins for applied purposes such as drug lead development (Videau et al., 2016). However, several other studies have engineered microalgae to investigate physiological processes or gene function and expression investigations, *e.g.,* Stern et al., (2025).

To detect phycotoxins a number of methods are established, including ELISA, HPLC and chromatographic methods (LC-MS) (USEPA, 2024; Meriluoto et al., 2017; Sanseverino et al., 2017), biosensors with gold nanoparticles (Anfossi et al., 2013) and aptamer-based biosensors (see reviews in Zahraee et al., 2023; Cunha et al., 2018). Biosensors can be used in water samples (drinking water, freshwater, seawater), shellfish and even fish (Zahraee et al., 2023). Multiplex systems allow quantification of multiple phycotoxins in a single system (see Chorus and Welker, 2021 and references herein). Although these methods are generally accurate and rapid, they often require specialized and expensive equipment (Table 1).

2.3.2. Sensitivity to toxins

Phycotoxins produced by GMM may cause sublethal effects and increase the mortality of grazers (Fig. 1**, step 8**), and other organisms, potentially altering the entire food web due to cascading effects (Fig. 1**, steps 9, 10**) (ToxTool, 2024; Lassus et al., 2016; Jos and Cameán, 2020). Here, we describe the hazard posed by phycotoxins acquired through the exposure routes presented in Section 2.3.1.

Nearly 200 microalgal species have been listed to have harmful effects on other organisms (including vertebrates) or human health, mostly through direct consumption (Lassus et al., 2016). If any of these harmful strains are genetically modified, we expect them to exert a similar impact on higher trophic levels as their non-modified wild-type counterparts. Toxin-producing microalgae ingested by zooplankton grazers can cause various deleterious effects, such as low egg production, hatching and survival, as well as lethargy (Turner, 2014). For instance, toxic dinoflagellates may inhibit the development of copepod nauplii (Turner, 2014; Bagoien et al., 1996). Oyster, clam, cockle and mussel populations can decrease due to physiological stress, alterations in the immune defense (haemocytes), tissue degeneration and even necrosis after ingestion of phycotoxins (see reviews in Lassudrie et al., 2020; Neves et al., 2021). Some microalgae can also be ichthyotoxic and cause massive fish kills in both marine and freshwater habitats (Lassus et al., 2016; Hallegraeff et al., 2023). Such fish-killing harmful mechanisms are caused by mechanical irritation or clogging of gills, or by compounds with cytotoxic and lytic activity (Rasmussen et al., 2016). Microalgae causing ichthyotoxic events include the genera *Margalefidinium, Chattonella, Prymnesium, Chrysochromulina, Karenia, Pfiesteria, Heterocapsa, Heterosigma,* amongst others *(*Lassus et al., 2016*)*. Marine genus *Gambierdiscus* produces toxins that bioaccumulate in the fish flesh of tropical reef fish, and in the viscera of herbivorous fish, respectively (Lassus et al., 2016). In freshwater habitats, microcystin-LR causes hepatic necrosis in rainbow trout after ingestion of *Microcystis aeruginosa* blooms (Tencalla et al., 1994), and induces high oxidative stress in the liver, kidney and gills of tilapia fish (Jos et al., 2005). *Prymnesium parvum*, a microalga with global distribution in freshwater and brackish habitats (Lassus et al., 2016), produces toxins that induce gill irritation and respiratory failure in commercially relevant fish (Svendsen et al., 2018).

Phycotoxins can accumulate in the food chain and eventually cause intoxication in vertebrates and humans by ingestion of the toxin vectors. Bivalve mollusks are the most common vectors of toxins, but higher-order consumers, such as carnivorous gastropods and crustaceans, can also accumulate phycotoxins and cause food poisoning (Shumway, 1995). Different types of poisoning categories have been described in humans, based on their main mechanism of action (Nicolas et al., 2017; Krasner et al., 2025). *Alexandrium, Pyrodinium* and *Gymnodinium* produce saxitoxins, which cause muscle paralysis (Nicolas et al., 2017). Brevetoxins from *K. brevis* cause gastrointestinal and neurological symptoms (Watkins et al., 2008). Okadaic acid and other lipid-soluble toxins promote tumor formation (Valdiglesias et al., 2013). Gambierol, produced by *Gambierdiscus toxicus,* causes gastrointestinal and neurological symptoms, including hallucinations, that can persist for years (Nicolas et al., 2017; Cuypers et al., 2008). Domoic acid in humans, seabirds and marine mammals can result in brain damage (Krasner et al., 2025).

Drinking water contaminated with phycotoxins may also cause toxicity in animals. Direct ingestion of cyanotoxins (cyanobacteria toxins) has caused poisoning and death of wild and domestic mammals worldwide, (see reviews in Briand et al., 2003; Stewart et al., 2008). Human poisoning by cyanotoxins after consuming contaminated drinking water has also been reported (see review in Hitzfeld et al., 2000), causing severe liver damage and tumor-promoting, pulmonary and neurological effects (Carmichael, 2001). The most fatal intoxication in humans with cyanotoxins caused the death of more than 60 patients (Pouria et al., 1998; Jochimsen et al., 1998). Sensitivity to non-toxic microalgae has also been reported in humans after direct contact through the skin (Hofbauer, 2021), and by inhalation (Hofbauer, 2021).

Toxicity of phycotoxins produced by GMM could follow standard toxicological approaches based on genotoxicity and rodent studies (More et al., 2022; Louzao et al., 2022). Currently, alternative *in silico* methods and *in vitro* bioassays using cell cultures, usually combined with integrated testing strategies, are useful to characterize cyanobacterial blooms. The World Health Organization (WHO) provides an overview for cyanotoxicity testing because it is the most common microalgal toxicity in freshwaters, and also includes tests based on standardized guidelines issued by ISO and OECD (Chorus and Welker, 2021). Toxicological studies can be carried out on single toxin and organism groups but can also detect hazardous effects of different types of toxins (Louzao

et al., 2022; Castrec et al., 2018) (Table 1).

In case it is deemed necessary, laboratory and microcosm experiments on grazers exposed to toxic microalgae are useful tools to evaluate responses to phycotoxin exposure and sensitivity. Designs should account for the co-evolutionary dynamics between toxic microalgae and their grazers, as in Lemaire et al. (2012). Experimental studies limited to a single algal strain and set of conditions may underestimate the variability of toxin production across strains and environments, and the potential for grazers to co-evolve to avoid or resist phycotoxins (Turner, 2014) (Table 1).

2.4. Harmful algal blooms (HABs)

Introducing GMM into an aquatic ecosystem may alter the community structure, potentially leading to dominance by GMM or by another species (Fig. 1, **step 2**), and in some cases, triggering harmful algal blooms (HABs) (Fig. 1, **step 11**). HABs are extensive proliferations of microalgae in open-ocean, coastal, and freshwater ecosystems. Ultimately, HABs may disrupt the entire aquatic food web with negative impacts on food security, tourism, local economies, and human health (Fig. 1, **step 12**) (Gobler, 2020). We can classify HABs into two groups: 1) toxic HABs that produce phycotoxins with direct effects on plankton, grazers, animals and humans (Sections 2.3.1 and 2.3.2), and 2) non-toxic HABs, which are indirectly harmful by causing turbidity and oxygen depletion (Fig. 1, **step 13**), leading to food web alterations, biodiversity loss and deterioration of water quality (Lassus et al., 2016). This second type of HAB is often called EDAB (ecosystem-disrupting algal bloom), since ecosystem functions can be severely altered or degraded.

Nutrient levels, stratification, irradiance, pH and water temperature may trigger the development and persistence of an algal bloom (Wells et al., 2015) or exacerbate the frequency of HABs (Paerl and Huisman, 2009; Gobler, 2020). Microalgae competitive ability traits and grazing avoidance traits of the blooming species (Section 2.1.2 and 2.1.3) may inform us about the invasiveness of the species. Examples of invasive (and toxic) wild-type species with superior traits include marine dinoflagellate *Prorocentrum minimum* and the diatoms *Odontella sinensis, Paralia sulcata* and *Pseudo-nitzschia multistriata*. In freshwater habitats, we can distinguish between the toxin-producing cyanobacteria (*Microcystis aeruginosa, Cylindrospermopsis raciborskii, Aphanizomenon flos-aquae, Planktothrix agardhii),* the haptophyte *Prymnesium parvum,* and the non-toxic *Gonyostomum semen* and diatoms *(Didymosphenia geminata, Thalassiosira incerta, Skeletonema subsalsum)*. The identity of the bloom-forming taxa shapes the severity of the ecological impact, with cyanobacteria driving the most severe disruptions (Amorim and Moura, 2021).

Nutrient-phytoplankton-grazer interactions may exacerbate or prolong the duration of the blooms (Sunda et al., 2006). Because high-density algal blooms are poor food sources for herbivore grazers, grazer mortality increases and grazing pressure declines. This allows the bloom to proliferate, depleting nutrients and light availability. Moreover, shading by EDAB species causes the loss of benthic phototrophs (*e.g.*, freshwater macrophytes, seagrasses), releasing nutrients that are rapidly taken up by EDAB species and further fueling their growth (Sunda et al., 2006). More importantly, the loss of grazers and benthic phototrophs will likely alter the entire ecosystem trophic web, leading to elevated mortality of aquatic fauna that rely on small grazers as prey and benthic phototrophs as habitat. Examples of EDABs causing hypoxia and massive fish kills in marine and freshwater environments can be found in Lassus et al., (2016), Hallegraeff et al., (2023) and Rasmussen et al., (2016).

If a GMM bloom develops, management strategies commonly applied to naturally occurring HABs (artificial mixing, addition of flocculants, biomanipulation, etc.) can be implemented to minimize the negative effects on the ecosystem (see review in Harris et al., 2024). Forecasting methods (remote sensing and data-driven models) relying on monitoring data can be relatively accurate for recurrent blooms (Caballero et al., 2025; Sagova-Mareckova et al., 2021) (Table 1).

2.5. Changes in the food web and deterioration of ecosystem services

When GMM or other microalgae proliferate due to the introduction of the GMM in the ecosystem, effects at the ecosystem level may arise. Elevated microalgal biomass increases food availability for grazers, potentially impacting higher trophic levels (Fig. 1, **step 14**). Depending on the temporal dynamics of grazer populations, predator pressure and the nutritional quality of the available food, ecosystems may shift toward clear-water states or, alternatively, toward microalgae-dominated conditions characterized by hypoxia and inefficient energy transfer. Degradation of water quality and associated ecosystem services (including nutrient cycling, climate regulation, food and genetic resources, recreational and aesthetic value) (Naselli-Flores and Padisak, 2023) may also occur through changes in the identity of the dominant phytoplankton species (Fig. 1, **step 15**).

It is well understood that changes to one component of an aquatic ecosystem can cause trophic cascades up and down the food web, with negative consequences for aquatic ecosystem services (Fig. 1, **step 16**) (Carpenter and Kitchell, 1996). Shifts in phytoplankton may induce major changes in ecosystems, impacting the food-web structure and biogeochemical cycles *e.g.,* Di Pane et al., (2022). In lakes, relatively stable states dominated by microalgae, with high turbidity and loss of macrophytes, may persist for years after the initial disturbance that shifted the system from a clear-water state (Scheffer et al., 1993; Ibelings et al., 2007).

Chlorophyll *a* measurements (common proxy for total phytoplankton biomass), phytoplankton community composition, biotic indices and other water-quality variables (dissolved oxygen, pH, etc.) are effective for detecting ecosystem-service deterioration in aquatic environments (Wang, 2024; Boyer et al., 2009). Top-down cascade effects can also be tested in laboratory experiments, as in Ger et al. (2018).

3. Implementation of PTH for GMM

Several measures can be implemented to reduce the risk of GMM escaping and causing damage to the environment. The need to implement these measures, however, depends on the potential risk of the specific GMM strain and its trait(s) and must be adapted case by case. Ideally, the potential to cause adverse effects is already considered during the development of GMM, as in the Safe(r) Innovation Approach by the Organization for Economic Co-operation and Development (OECD) (Devos et al., 2019).

In general, closed cultivation systems should be prioritized over open systems because of the lower risk of microalga escape (Jha and Zi-Rong, 2004). Since open systems are considered as production systems with deliberate release (subject to Directive 2001/18/EC), enclosure in glass- or greenhouses should be used as a measure of physical containment to minimize the risk of escape (Annex II, section D.1) (European Union, 2001). If closed cultivation systems are used, it is preferred to place them indoors, as the material of the photobioreactor can deteriorate faster due to direct exposure to sun and meteorological events. Facilities should furthermore comply with standard biosafety measures. For instance, sterilization of instruments or devices must be done after use, in accordance with protocols developed for microorganisms of risk category 1 or 2 (which include GMM).

Some measures can be applied to minimize the risk of GMM becoming invasive if accidentally released into natural ecosystems. First, external additives could be used to allow the growth of GMM in the production system, but without such additives they will likely not survive in a natural environment. This includes the use of elements that are essential for growth (chemical auxotrophy), such as vitamins B_1, B_7 and B_{12} (see review in Tang et al., 2010). Auxotrophy can also be induced genetically (synthetic auxotrophy) (Clark et al., 2018; Slattery et al.,

2020; Hirota et al., 2017; Motomura et al., 2018; McCarthy et al., 2017; Wang et al., 2016). There are different ways to generate synthetic auxotrophs: First, the knock-out of essential genes to create metabolic defects has been successful in cyanobacteria, with strains dependent on very high CO_2 levels (Clark et al., 2018), and in eukaryotes, with adenine-dependent microalgae (Slattery et al., 2020). Second, metabolic pathways can be engineered to enable microalgae to utilize alternative substrates that are scarce under natural conditions (*i.e.*, phosphite instead of phosphate, or ammonium as the only nitrogen source) (Hirota et al., 2017; Motomura et al., 2018). This strategy has been developed in cyanobacteria, green microalgae and diatoms (Motomura et al., 2018; McCarthy et al., 2017; Wang et al., 2016). Third, through the insertion of lethal genes that only activate when the concentration of a signal molecule changes (as a consequence of escape) (Sebesta et al., 2022). Lethal genes include toxin-antitoxin (TA) systems and phage lysis proteins (Sebesta et al., 2022). Controllable kill switches with TA gene pairs have long been effective in inducing cell death in bacteria (Molin et al., 1993; Wright et al., 2015), and more recently, in eukaryotic microalgae of commercial interest, such as *Chlorella vulgaris* and *Messastrum gracile* (Ng et al., 2016; Gan and Cha, 2024). In cyanobacteria, TAs have been characterized for *Synechocystis* sp. PCC 6803 (Čelešnik et al., 2016; Kopfmann and Hess, 2013), *Aphanizomenon flos-aquae* and *Microcystis aeruginosa* (Songailiene et al., 2020; Klemenčič et al., 2021).

A comprehensive characterization of GMM, in comparison with the non-transformed wild type, helps to determine their potential to successfully colonize natural habitats in the event of escape, including all the traits described in Section 2.1. We recommend gathering data on the strain environmental niche requirements (pH, temperature, alkalinity, etc.), nutrition- and stoichiometry-related traits (maximum nutrient uptake rate, half-saturation constant, growth rate, internal nutrient ratios), competitive ability traits (R^* and proxies such as cell and/or colony size, pigment composition, photosynthetic-efficiency rate, etc.), and grazing resistance traits (morphological, physiological and behavioral traits that reduce edibility). Whilst some traits are relatively easy to measure but might have a low predictive value, *e.g.*, size, others might require careful experimental design but can be more informative *e.g.*, R^* in Gallego and Narwani (2022). 'Direct invasion' microcosm experiments with pairwise combinations of strains can predict the stable coexistence (or competitive exclusion) of the GMM in a natural community and inform whether the GMM are more competitive than its wild type. At the microalgal community level, colonization experiments with multiple strains can be conducted in micro- and mesocosms, either by constructing artificial communities as in Lamprecht et al. (2022), or by using natural communities as in Buchberger and Stockenreiter (2018). Microcosms allow precise manipulation and are more cost-effective and easier to replicate, at the expense of limitations in long-term dynamics.

At a large scale, only a limited number of field trials and authorizations for GMM have been reported (Szyjka et al., 2017; OGTR, 2024), and these included specific containment measures and monitoring. The cultivation facility of marine GMM in a location surrounded by freshwater bodies with bunds to prevent runoff is considered a reasonable level of containment (OGTR, 2024). Also, glasshouses and polythene tunnels have been proposed as physical containment barriers (Beacham et al., 2017), and dispersal trap ponds enable monitoring of the dispersal of GMM cultivated in open ponds (Szyjka et al., 2017).

One concern is that the novel genetic element may end up in other naturally occurring species or strains *via* gene transfer. To our knowledge, eukaryotic strains pose a negligible HGT risk compared to prokaryotic cyanobacteria, but sexual hybridization (VGT) might still occur -albeit rarely- among eukaryotes. To prevent this, the characterization of GMM should also include the environmental conditions (nutrients, light levels, etc.) triggering sexual reproduction. The risk of HGT in cyanobacteria can be minimized with strategies originally designed for bacteria, such as the use of entangled genes (the recombinant gene is entangled with a kill-switch gene, and a rescue gene located elsewhere in the genome prevents cell death) (Chemla et al., 2025). Genome recoding (changing a codon from one amino acid to another) also reduces HGT for DNA transfer to and from the environment (Chemla et al., 2025; Zürcher et al., 2022), but it can result in loss of gene function.

The use of potentially harmful strains (HABs, EDABs) should be considered only when absolutely necessary and strictly confined to closed production systems. If GMM are known to produce toxins, it needs to be analyzed whether toxin levels have increased in response to the genetic modification and whether the increased levels pose a risk to higher-trophic organisms. Exposure experiments can combine laboratory, microcosm and semi-natural mesocosm experiments, as each provides relevant information on short- and long-term dynamics. For example, controlled microcosm studies with simple artificial communities (two microalgae and two grazers) can inform us about the mechanisms and traits underlying microalgae-grazing interactions (Thomas et al., 2024). Limnocorral experiments may allow for different grazer genotypes and co-evolution of grazing resistance against toxic microalgae (Chislock et al., 2013). Toxicity tests to detect the harmful effects of phycotoxins on their grazers or on higher levels of the trophic web include analytical and bioassay methods such as genotoxicity and rodent studies (Anfossi et al., 2013; Chorus and Welker, 2021; More et al., 2022; Louzao et al., 2022).

Early detection of GMM-HABs would rely on periodic monitoring (nutrient levels and oxygen levels, plankton samples). Once identified, management strategies can be implemented (physical treatments, chemical approaches and biological treatments) (Harris et al., 2024).

To detect changes in the food web, complex experimental approaches commonly used in aquatic ecology can be conducted (see review in Sasaki et al., 2025), but a trade-off might arise between realistic conditions and the complexity (and safety) of the experiment. Mesocosms in facilities with physical containment systems would be safer than *in situ* mesocosms, but the latter are more realistic and informative.

4. Summary

In this conceptual study, we describe several measures that could be implemented to reduce the likelihood of GMM escaping from production systems, establishing themselves in natural ecosystems, or causing adverse environmental impacts, in accordance with the protection goal used for illustration. Furthermore, we identify key aspects that could be considered under the 'safe by design' principle when developing GMM for use in biotechnological production systems. Problem formulation and pathways to harm (PTH) facilitate the identification of testable risk hypotheses and appropriate tests to be conducted when assessing the environmental risks of GMM. This framework guides both protocol development and hazard identification and supports the ERA process by focusing on data generation that reduces uncertainty. Combined with the methods and techniques outlined above, as well as the selection of safe recipient strains (*i.e.,* application of safe-by-design principles), it is possible to generate interpretable and reproducible data that supports ERA of GMM. Table 1 provides an overview of the methodologies available to address questions related to the potential environmental risks of GMM. However, there is a need for further method development and harmonization, as well as agreement on effect thresholds that would indicate risk, to strengthen the ERA.

CRediT authorship contribution statement

Irene Gallego: Conceptualization, Investigation, Visualization, Writing - original draft, Writing - review & editing. **André Friedrich:** Writing – review & editing. **Johan Robbens:** Writing – review & editing. **Jörg Romeis:** Conceptualization, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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