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Effects of light variation in algal cultures: a systematic map of temporal scales

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Abstract

Algal aquaculture is a rapidly growing field, with a proliferation of studies exploring algal growth. The expansion of the field not only presents opportunities for synthesis, but also creates challenges in identifying where the strengths and knowledge gaps exist. One tool for formally quantifying the state of knowledge is a systematic map, already useful in many fields, but underutilised in algal research. We used a systematic map to describe variable light regimes in algal cultures. Light variation is ubiquitous in algal cultures and spans a range of temporal scales (microseconds to months), but it is unclear which scales have been explored. We characterised 1393 experiments according to the temporal scale of light variation that was manipulated. Intensely studied light variation frequencies were either very short (< seconds) or long (diel cycles); the prominent gap was frequencies between these extremes (seconds to hours), especially for experiments that lasted for long durations (> months). Experiments that lasted for days were most common, while few studies lasted for months or more. Most studies were conducted in small culture vessels, used instantaneous changes in light regimes, and few studies reported initial stocking density metrics consistently. Our map highlights that the field has accumulated a rich knowledge base that is ripe for synthesis in some areas, particularly very short or relatively long frequency light variation. The map indicates that the key priorities are explorations of intermediate frequencies and our understanding of their effects is limited. Similarly, our understanding of evolutionary responses to variable light regimes of all scales is lagging.

Keywords Systematic mapping · Variable light · Temporal light manipulation · Microalgae · Macroalgae · Algal aquaculture

Introduction

The culturing of algae has expanded rapidly, with global production tripling between 2000 and 2015 (Paul and Borowitzka 2019). Modern algal aquaculture is therefore a relatively new scientific field (Critchley et al. 2020) and our knowledge is likely to be incomplete (Charrier et al. 2017; Kim et al. 2017). However, the proliferation of algal aquaculture research brings its own challenges: In such a rapidly growing field, it can be difficult to identify where there are genuine knowledge gaps versus what is well understood (Critchley et al. 2020). Even factors that have been explored comprehensively in one dimension can be less well resolved in another. Perhaps the best example of this is the role of light in algal aquaculture. Although the effects of mean light intensity on aquaculture are increasingly well characterised (Falkowski and LaRoche 1991; MacIntyre et al. 2002; Juneja et al. 2013; Singh and Singh 2015), our understanding of variability in light intensity remains unclear.

Light is an essential resource for all algae (Litchman and Klausmeier 2008); it can also be the most variable parameter in algal culturing, changing with external inputs (e.g. seasons and clouds), but also with internal factors such as culture density, depth, and mixing rate (Magnusson et al. 2015). That light can be highly variable, and that this variation can be important, has long been acknowledged (Kok 1953; Phillips and Myers, 1954) . However, a comprehensive synthesis of the effects of variable light regimes for algae

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remains lacking, but see Ferris and Christian (1991) and Schulze et al. (2017). Meanwhile, a need for understanding the effects of light variation has become more urgent. For example, Béchet et al. (2013) note that models tend to focus on mean light intensities under constant conditions, rather than more-realistic light regimes that vary. Variable light regimes could alter the productivity and yield of algal cultures (Schulze et al. 2017), but the extent to which they do remains unclear.

Before we can understand how light variation affects algal growth, an essential first step is to delineate the distribution of our knowledge in this area. For our purposes, we will define light variation as any consecutive transitions from one light level (intensity) to another, though a search of the literature indicates that light variation is more complicated than this simple definition implies. Light variation can be applied over a continuum of temporal scales, from nanoseconds to months (Fig. 1). Light manipulations of milliseconds or less are known as high-frequency fluctuations, and manipulations of hours or more are known as low-frequency fluctuations (Fig. 1) (Grobbelaar 1989). These frequencies can have different effects relative to constant light regimes, and to each other (Gómez and Wiencke 1997; Nicklisch and Woitke 1999; Graham et al. 2017; Li et al. 2017; Schulze et al. 2017).

Further compounding the problem is that other relevant factors also vary: the duration of the experiment; the type of culture vessel; and the pattern and predictability of the light manipulation. Each of these factors is likely to influence the response of algae to dynamic light regimes (Havelková-Doušová et al. 2004; Grobbelaar 2006; Magnusson et al. 2015). For example, variable light regimes could reduce growth in the very short term but not in the longer term (Grouneva et al. 2016). Similarly, the culture volume and shape can interact with light, creating differences in the light quality and quantity with depth. In small or shallow cultures, light attenuation will be lessened relative to deeper cultures, particularly at high densities; such that different culture volumes will have different effects on productivity (Moheimani and Borowitzka 2007). The combination of factors that could alter the effects of light variation is extensive but should be soluble if appropriately organised — systematic maps have the potential to organise and describe the state of our knowledge, including its strengths and weaknesses, in this rapidly growing field.

Systematic mapping — an underutilised tool in algal research

Systematic maps group studies into recognisable categories and provide a rigorous and repeatable framework for identifying knowledge gaps, as well as the well-studied areas of the field where inferences should be robust (James et al. 2016). They outline the current state of knowledge relating to a particular topic of interest (James et al. 2016) and provide much-needed overviews of a research field. Systematic maps differ from systematic reviews, as they do not include a meta-analysis; they do not extract the results of studies, nor statistically analyse those results. Instead, they quantify what has been studied rather than what has been found (James et al. 2016). The product of the process is a visual graphic — the map — which displays where the evidence has and has not been accumulated (McKinnon et al. 2016). Using this map, we can determine where research should be targeted (i.e. at the knowledge gaps) and where systematic meta-analyses can be conducted (i.e. the knowledge clusters) and synthesis is possible. While systematic maps are increasingly common in social science (The Campbell Collaboration 2020), medical research (Idrissi et al. 2019), and conservation biology (McKinnon et al. 2016), to our knowledge, they are yet to be applied broadly in aquaculture.

Objectives

The objective of this systematic map was to determine where research efforts have focused in regard to variable light regimes in algal culturing.

The primary questions were:

- At what temporal scales has light has been manipulated in algal culturing?
- What were the durations of these manipulations?

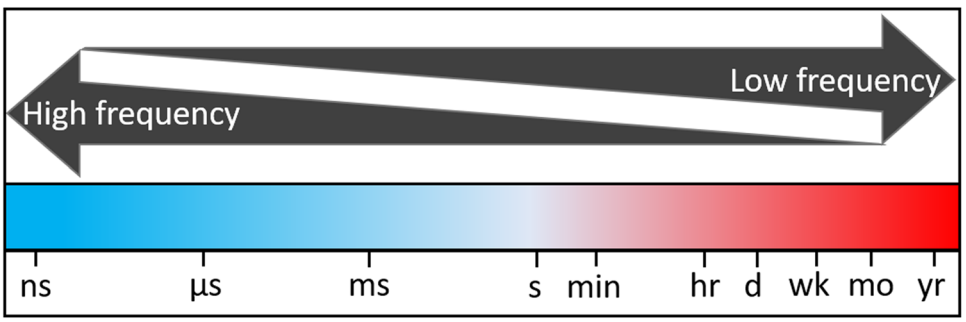

Fig. 1 Temporal continuum of light variation. Light can be manipulated at any point along this continuum from nanoseconds (ns) to years (yr). Frequency increases as the fluctuations become faster and decreases as the fluctuations become slower

The secondary objectives asked:

- What was the waveform of the light manipulation (square, sinusoidal, etc.; see 'Methods')?
- What were the durations of experiments (in terms of algal generations)?
- What culture volumes were used?
- What initial stocking densities were used?

Methods

The systematic mapping process was conducted following the guidelines of the 'Preferred Reporting Items for Systematic Reviews and Meta-Analyses' (PRISMA) statement (Moher et al. 2009) and the Systematic Mapping Methodology (James et al. 2016).

Review team

The review team consisted of one primary reviewer and two stakeholders. The stakeholders assisted in shaping the scope and aims of the review; contributed to the inclusion and exclusion criteria; aided in the decision to include or exclude any records that the primary reviewer was uncertain of; and commented on the most appropriate data visualisation.

Scoping, search strategy, and identification

We searched the literature from May to August 2020. First, to identify an appropriate level of records to screen, we scoped the peer-reviewed publication database, Web of Science, using combinations of search terms (Online resource 1 Table 1). After testing 29 different combinations of search terms, we selected the term '(light OR irradiance) AND fluctuat* AND (m$croalga* OR alga* OR phytoplankton)', as it provided an appropriate balance of sensitivity and specificity, which resulted in 2410 references to be screened (Online resource 1 Fig. 1). Four compilations were identified through the Web of Science database search, providing an additional 91 records to be screened. In addition to these compilations, 50 records were found within papers identified through the database search. Furthermore, we checked for reviews of light manipulations in the journals, Algal Research, Journal of Applied Phycology, New Phytologist, and Applied Microbiology and Biotechnology using the search terms 'Review + light', resulting in an additional 6756 records identified (Online resource 1 Fig. 1).

We also conducted a second search in Web of Science, using the search term '((light OR irradiance) AND fluctuat* AND (m$croalga* OR alga* OR phytoplankton OR seaweed))' in October 2020 to ensure that we had not omitted any seaweed references by not including 'seaweed' in the initial search. Of the 1284 references identified in this search, 18 were included in the database. Once duplicates were removed, the above searches resulted in 10,088 records to be screened at the title and abstract level (Online resource 1 Fig. 1).

Inclusion, exclusion, and screening

Inclusion and exclusion criteria were established prior to the search but were also adapted during the screening process, as it became clear what sort of studies existed (Table 1). To be incorporated in the final database, all inclusion criteria had to be met.

Records were first screened at the title level in Web of Science. If the records appeared to match the inclusion criteria, they were selected using the marked list function in Web of Science (Sevinc 2004). The marked list was then exported and uploaded into the Rayyan QCRI Systematic Reviews web app (Ouzzani et al. 2016), where records were screened at the title and abstract level. We then sorted records into three groups: include, exclude, and maybe. Records in the 'include' and 'maybe' categories were subjected to a full-text assessment and either included or excluded in the database according to the criteria. A list of the full-text articles assessed for eligibility can be found in Online resource 2. Screening and full-text eligibility assessments dramatically reduced the number of records, resulting in 212 records included in the database (Online resource 1 Fig. 1).

Table 1 Inclusion and exclusion criteria for the studies included in the database

Inclusion	Exclusion
Manipulate light through time (i.e. consecutive transitions from one light environment to another)	Studies comparing different treatments of light intensity
Experimental studies	Observational or theoretical studies
The light manipulated must be Photosynthetically Active Radiation (PAR)	Studies manipulating solely ultra violet (UV) radiation
Studies exploring consecutive transitions from one light environment to another	Studies including a diel cycle, but not exploring consecutive transitions from one light environment to another
Light pattern must be definable	Studies exploring solely Pulse-Amplitude Modulation (PAM)

Coding data

A total of 212 records were coded into Microsoft Excel (version 1808) recording information into the following four categories: (1) reference; (2) species information; (3) culture information; and (4) light experiment. Further details on each category are discussed below. A line was coded for each experiment, generally resulting in multiple lines per record, as some studies conducted multiple light manipulations, while others conducted the same light manipulations on different species. In total, 1393 light by species experiment combinations were recorded (Online resource 3).

Reference

The author and year of the publication were coded into Microsoft Excel, as well as the citation being downloaded into EndNote (version X9.3.3).

Species information

The species name, algal type (microalgae or macroalgae), environment (freshwater, marine), and doubling time (time in days required to double) were recorded. The accepted species name was checked in AlgaeBase (Guiry and Guiry 2010) or World Register of Marine Species (Board 2020). For the purposes of this literature map, 'microalgae' includes autotrophic microorganisms (i.e. cyanobacteria and *Arthrospira*). We used doubling time as a rough indication of generation time, by dividing the duration of the experiment by the doubling time of the algal species. Where possible, we used the doubling time specific to the experiment; however, when this was not available, we used a published doubling time. If multiple doubling times occurred within a paper, the average was taken. Similarly, if the doubling time for the specific species was not recorded, then the average doubling time at the genus level was taken. For macroalgae, doubling times were extrapolated from published specific growth rates (SGRs) using the following equation:

$$\frac{1}{SGR} \times 100 \quad (1)$$

If multiple SGRs were published within a paper, the average was taken.

Doubling times are specific to growth conditions. In reality, doubling times may vary several folds relative to our estimates. However, our purpose was to contextualise the length of experiments relative to a reasonable estimate of generation time, so as to determine whether an experiment ran for a sufficiently long time, such that evolutionary responses might be recorded. Additionally, if cultures doubled or more during the experimental period, then the culture would likely be harvested (i.e. diluted) during that time, thus influencing the dynamic light regime.

Culture information

The culture location (indoor vs. outdoor), vessel size (volume), and initial stocking density were recorded. A range of culture volumes were used in the studies in our database and we were interested in how these varied with the other experimental approaches. We grouped culture volumes into six categories: less than 10 mL, 10–99 mL, 100–999 mL, 1–9 L, 10–99 L, and equal to or greater than 100 L. The largest experimental vessel volume was 1400 L, and the smallest was 0.12 mL.

The initial stocking density of cultures was reported across a range of metrics or not stated at all. We grouped these into six categories: not stated, other, grammes per millilitre, cells per millilitre, optical density, and grammes chlorophyll per millilitre. The category 'other' includes measurements of initial stocking density that can only be compared within a study, such as 'X plants per tub' or 'X volume of culture'.

Light experiments

The light–dark cycle, time, and pattern of the manipulations were recorded, as well as the duration of the experiment. Using the doubling times for each species and the duration of the experiments, we determined the number of generations that each experiment lasted. Additionally, we noted whether the study disentangled mean light intensity from light variation.

Light–dark manipulations could be absolute (light versus dark) or relative (low versus high). Manipulation frequencies were classified as lasting for nanoseconds, microseconds, milliseconds, seconds, minutes, hours, diel cycle, days, weeks, months, or years. The time of the manipulation was recorded as the time of the light and dark segments of the period, not the total period. For example, if a study manipulated light in the following pattern: 30 min light, 30 min dark, the manipulation time was recorded as minutes, not hours. An exception to this was diel cycle. We defined diel cycle as one light and one dark segment totalling a period of 24 h. For a light manipulation to be formally classified as diel cycle, the diel cycle needed to vary among treatments within a study. For example, a study needed to compare different light–dark cycles such as 12:12 to 14:10 h. Diel cycle was not formally included as a manipulation when it was superimposed over another light treatment. For example, a study may have one treatment where the light is turned on and off every 30 min for 12 h and then place the culture in darkness for 12 h. A second treatment in the study may turn

the light on and off every 15 min for 12 h and then place the culture in darkness for 12 h. The manipulation time would be classified as minutes, not diel cycle, as diel cycle was superimposed on the light treatment. Thirty-three percent of experiments included in the database had a diel cycle superimposed on the light treatment.

Light can be manipulated according to different patterns in terms of how abrupt the change from dark to light is, how the light changes once the light is on, and how abruptly it turns off. We defined these patterns as square, sinusoidal, or sawtooth waveforms (Fig. 2). A square light manipulation involves an instantaneous change from one light environment to the next (Fig. 2a). A sinusoidal light manipulation follows a sine wave, so that the change in light intensity is gradual (Fig. 2b). It should be noted that some studies manipulated light according to a sinusoidal waveform with oscillations within the wave (Fig. 2c); these were coded as sinusoidal. A sawtooth light manipulation occurs when light gradually, then rapidly increases to a point where it rapidly, then gradually decreases (Fig. 2d). Square waveforms are considered static light regimes, and sinusoidal and sawtooth waveforms are considered dynamic. The waveform can influence how algae respond to changes in light (Havelková-Doušová et al. 2004). An abrupt change is likely to trigger a different response relative to a gradual change, although this may depend on the temporal scale of the manipulation (Nedbal et al. 1996).

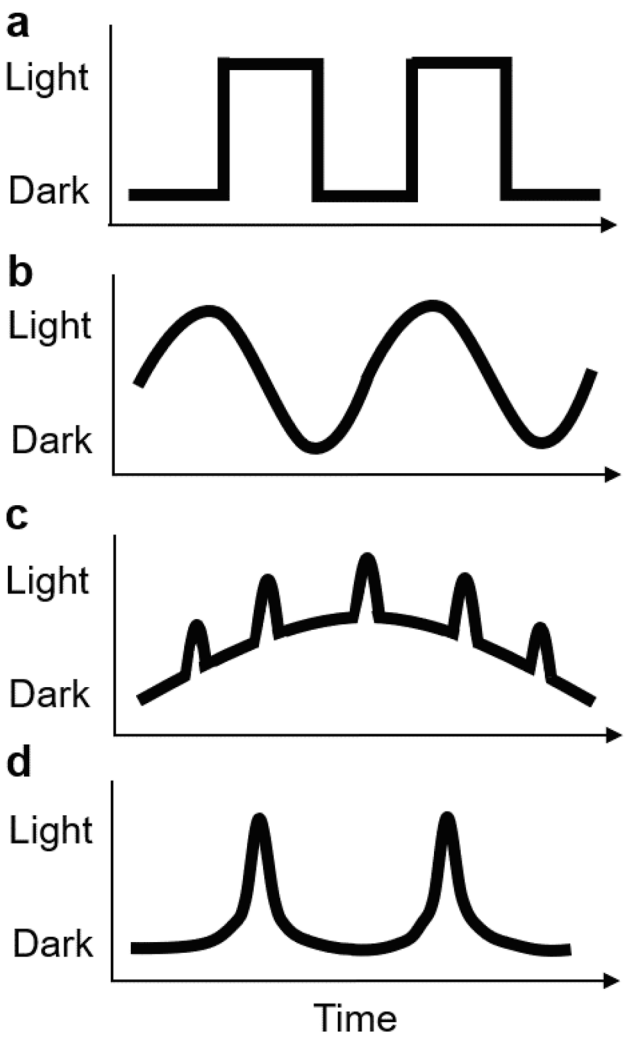

Fig. 2 Schematic of the pattern of the light manipulation. **a** Square waveform, where light instantaneously changes from dark to light. **b** Sinusoidal waveform, where light gradually changes from light to dark. **c** Sinusoidal with oscillations, where light changes in a sinusoidal waveform with peaks of light over time. **d** Sawtooth waveform, where light gradually then rapidly increases reaching a peak where it rapidly then gradually decreases

Seaweed and microalgae show phenotypically plastic responses to dynamic light fields, and their responses differ across timescales and among species (MacIntyre et al. 2000). Photoregulation occurs within seconds and can include changes to the photosynthetic electron transport rate (linear and cyclic) (Grouneva et al. 2016), changes to Rubisco activity (MacIntyre et al. 2000), and non-photochemical quenching (NPQ) involving the Xanthophyll cycle and state-transitions (Dimier et al. 2009). Photoacclimation occurs within hours to days and involves the synthesis or breakdown of molecules, allowing photosynthetic pigments and proteins to vary (Kono and Terashima 2014). At longer time scales (> days), photoadaptation can occur as a result of evolution (Raven and Geider 2003). Photosynthetic efficiencies and yields of seaweed and microalgae are thus likely to be characterised by the species ability to respond to dynamic light (i.e. photoregulation, photoacclimation, or photoadaptation) and the frequencies and patterns of dynamic light. Consequently, we explored the full range of timescales from nanoseconds to years.

The duration of the experiment was categorised into three groups: less than a day (< 24 h), days to weeks, and equal to or greater than a month (≥ 28 days). Algae have short generation times allowing them to evolve and adapt rapidly to their environments (Grobbelaar 2006). If an experiment only lasts for a day or two, it will not capture the evolutionary potential for the algal to respond to their conditions. If we are looking to make inferences that will inform industry, the duration of our studies should reflect those used by industry.

Confounded data occur when the effects of an experimental treatment cannot be separated from other factors that may cause differences among treatments (Quinn and Keough 2002). For example, if there are two experimental treatments, i.e. constant light versus fluctuating light, but the total photon dose for each treatment differs, the experimenter will not know if the difference in the response is due to the difference in total photons, or due to the variable light. Additionally, when studies manipulate multiple factors such as temperature and nutrients, the direct effects of variable light regimes cannot be disentangled. While these studies address

important questions, for the purpose of this systematic map (i.e. exploring variable light regimes), they were considered confounded.

Data analysis

Coded data were interrogated and analysed in r studio (version 1.3.1093) (R Core Team 2018) using the 'tidyverse' set of packages (Wickham 2017). Structural matrices depicting the frequency and distribution of experiments for the temporal scales manipulated were created using heat maps in the package 'ggplot2' (McKinnon et al. 2016; Wickham and Chang 2016).

Results

Species, habitat, and location

The studies included 135 individual algal species, as well as 29 studies on algal communities. The majority of studies (85%) were conducted on microalgae and one study examined both microalgae and macroalgae (a community study). Studies on marine species were more common than freshwater species (118 vs. 88 studies, respectively), and six studies examined both marine and freshwater species. Most studies (88%) were conducted indoors, although seven studies compared indoor and outdoor experiments. The majority of studies did not disentangle mean light intensity from light variation, with only 18% of studies providing equal photon doses across treatments. The majority of studies manipulated light intensity (91%), 15 studies explored changes in light quality with depth, and four studies directly manipulated light colour.

Temporal scale and duration of experiment

Light was manipulated across temporal scales ranging from nanoseconds to years. Studies that manipulated diel cycles were the most common; studies that manipulated light at the scale of nanoseconds and years were least common. Of the studies that manipulated diel cycles, nine examined seasonal changes in diel cycles for at least a year (Karsten et al. 1990; Wiencke 1990a, b; Tom Dieck 1991; Lüning and Kadel 1993; Weykam and Wiencke 1996; Weykam et al. 1997; Lüder et al. 2001; 2002). There were likely many more studies that observe seasonal changes in diel cycles; however, due to our inclusion criteria, studies needed to be experimental, not observational; thus, many studies observing seasonal changes in diel cycles were excluded from our database. The above nine studies, experimentally mimicked natural day lengths, thus were included in the database.

As most studies conducted multiple experiments, the following exploration is on the number of experiments, rather than the number of studies. Experiments manipulating light at the scale of milliseconds and diel cycles were the most common, followed by experiments manipulating light at the scale of seconds and minutes (Fig. 3). Years and nanoseconds were the least commonly manipulated temporal scales (Fig. 3).

In addition to the temporal scale manipulated, we looked at the duration of the experiment. Generally, most experiments ran for days to weeks, and experiments lasting more than a month were rare (10%) (Fig. 3).

The temporal scale of the manipulation tended to be positively correlated with the duration of the experiment (Fig. 3). Typically, experiments that manipulated light variation at higher frequencies (i.e. microseconds) lasted for less than a day, and experiments that manipulated light on longer timeframes (i.e. > weeks) lasted for months or more (Fig. 3). Experiments that lasted for an intermediate timeframe (days–weeks) were generally well-represented across all temporal scales manipulated (Fig. 3).

To further explore the temporal scale manipulated and the duration of the experiment, we grouped the data broadly into macroalgae and microalgae (Fig. 3). For microalgae, the most commonly manipulated temporal scales were milliseconds (25%) and diel cycles (18%), and for macroalgae, these were diel cycles (41%) and weeks (19%) (Fig. 3).

For microalgae, the majority of experiments lasted for days to weeks (75%); for macroalgae, the majority of experiments lasted for multiple months (63%). Experiments lasting for months or more were least common for microalgae (Fig. 3); for macroalgae, experiments lasting less than a day were least common (Fig. 3).

For macroalgae, the duration of the experiment tended to be positively correlated with the temporal scale that was manipulated — experiments lasting for months or more, generally manipulated light at temporal scales greater than hours (Fig. 3). For microalgae, this trend was less apparent, with fewer experiments manipulating light over long temporal scales, nor lasting for long durations (Fig. 3).

Patterns of light manipulations

The majority of experiments manipulated light according to a square waveform (73%), with the least number of experiments manipulating light according to a sawtooth waveform (5%). The waveform tended to depend on the temporal scale of the manipulation — sinusoidal waveforms were more common for seconds to diel cycles, and sawtooth waveforms were used for very short (< 1 s) temporal scales (Fig. 4). Square waveforms were used across all temporal scales but were most common for milliseconds and least common for years and nanoseconds (Fig. 4). For microalgae,

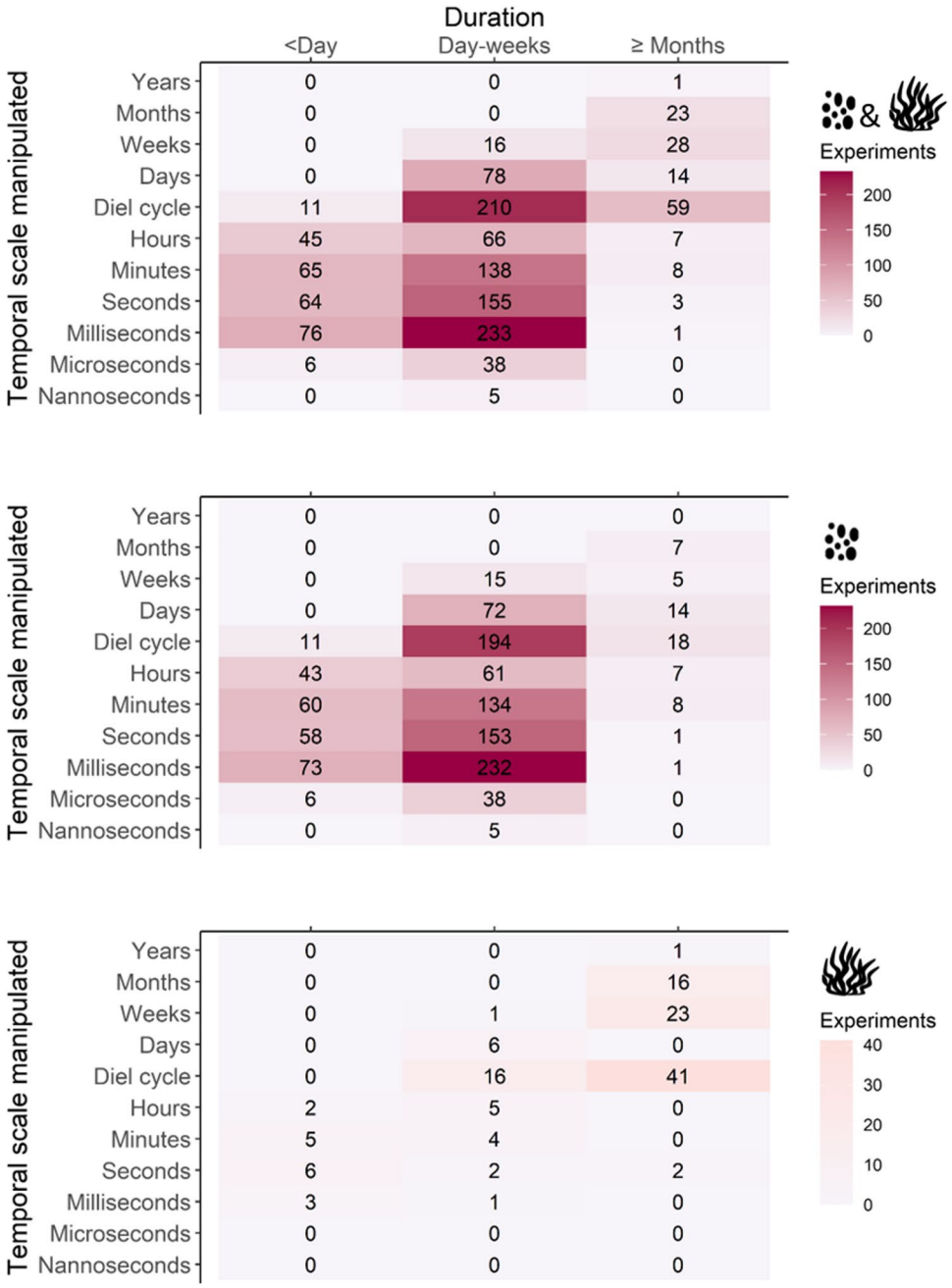

Fig. 3 Structural matrix of the number of experiments according to the temporal scale of the light manipulation and the duration of the experiment for all experiments in the database (top panel), experiments on microalgae (middle panel), and experiments on macroalgae (bottom panel). The colour gradient and numbers within each cell represent the number of experiments conducted. A value of zero indicates no experiments were reported for the temporal scale-duration combination

square waveforms that fluctuated at the scale of milliseconds were most common. For macroalgae, the majority of experiments had a square waveform that followed a diel cycle. No experiments followed a sawtooth waveform for macroalgae (Fig. 4). Twenty-six studies examined the difference between sinusoidal and square waveforms, and three studies examined the difference between square and sawtooth waveforms.

Generation times

For all algae, the majority of experiments lasted for less than 10 generations (Fig. 5). Generally, experiments that lasted for more than 25 generations were conducted on macroalgae (Fig. 5).

Culture volume

Culture sizes differed between the microalgae and macroalgae (Fig. 6). Although culture vessels ranging in volume from 100 mL to 9 L were common for all algae, microalgae were generally cultured in vessels within and below this range and macroalgae tended to be cultured in vessels within and above this range (Fig. 6).

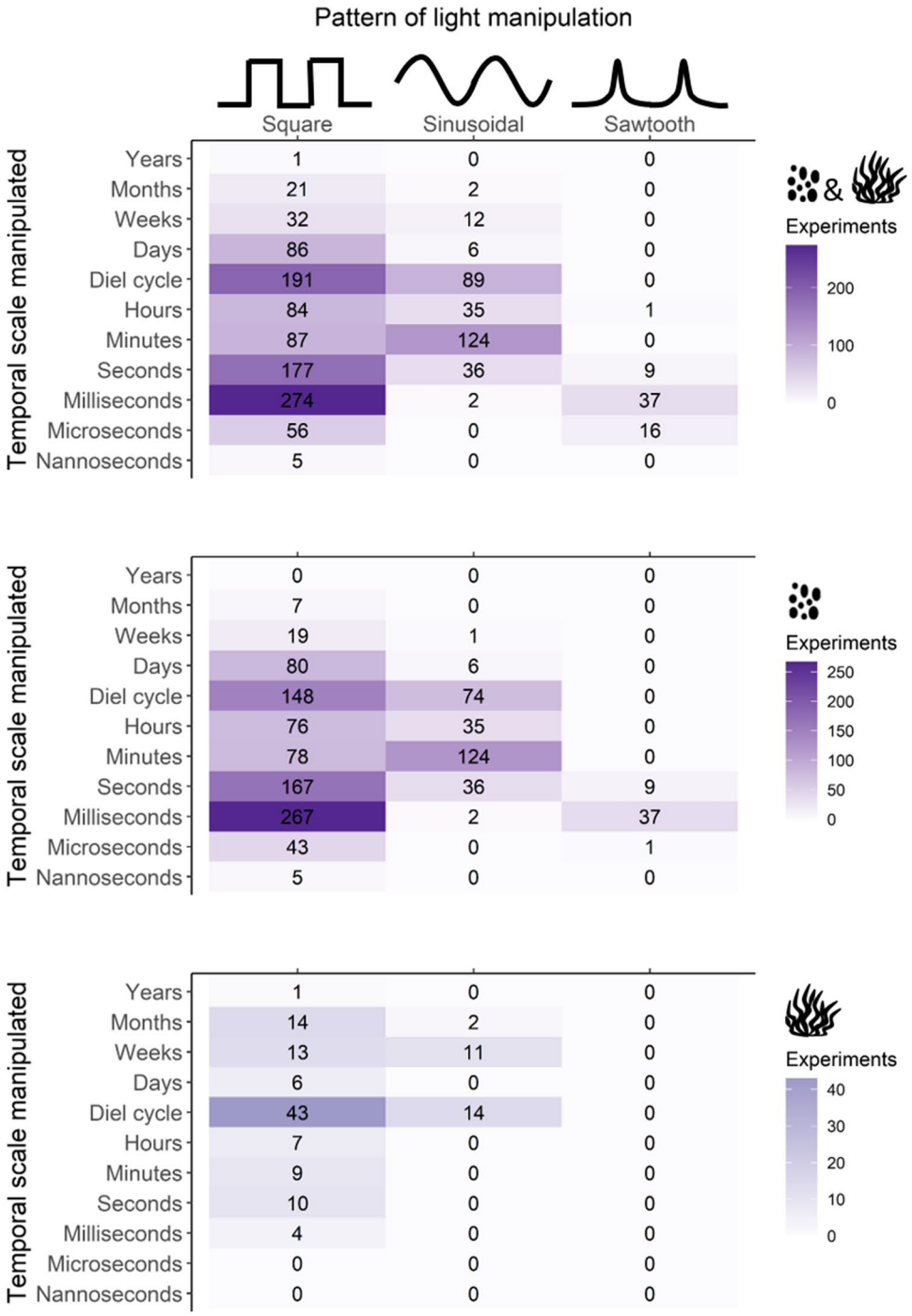

Fig. 4 Structural matrix of the pattern of light manipulations for each temporal scale manipulated for all experiments in the database (top panel), experiments on microalgae (middle panel), and experiments on macroalgae (bottom panel). The colour gradient and numbers within each cell represent the number of experiments conducted. A value of zero indicates no experiments were reported for the temporal scale-pattern of light combination. Pictograms at the top of each column provide visualisation of the pattern of the changing light

In general, experiments on microalgae occurred over days to weeks in intermediately sized culture vessels, unlike macroalgae, where experiments occurred in larger culture vessels for longer timeframes (Fig. 6).

Stocking density

The initial stocking density and how it was measured varied between microalgae and macroalgae (Fig. 7). For microalgae and macroalgae, 47% of experiments did not list the initial stocking density. Other studies mentioned stocking density, but in ways incomparable across studies, such as 'plants per tub' or a vague mention of culture volume rather than concentration. Initial stocking densities for microalgae were reported as grammes per millilitre, cells per millilitre, optical density, and chlorophyll per millilitre. For macroalgae, initial stocking density was

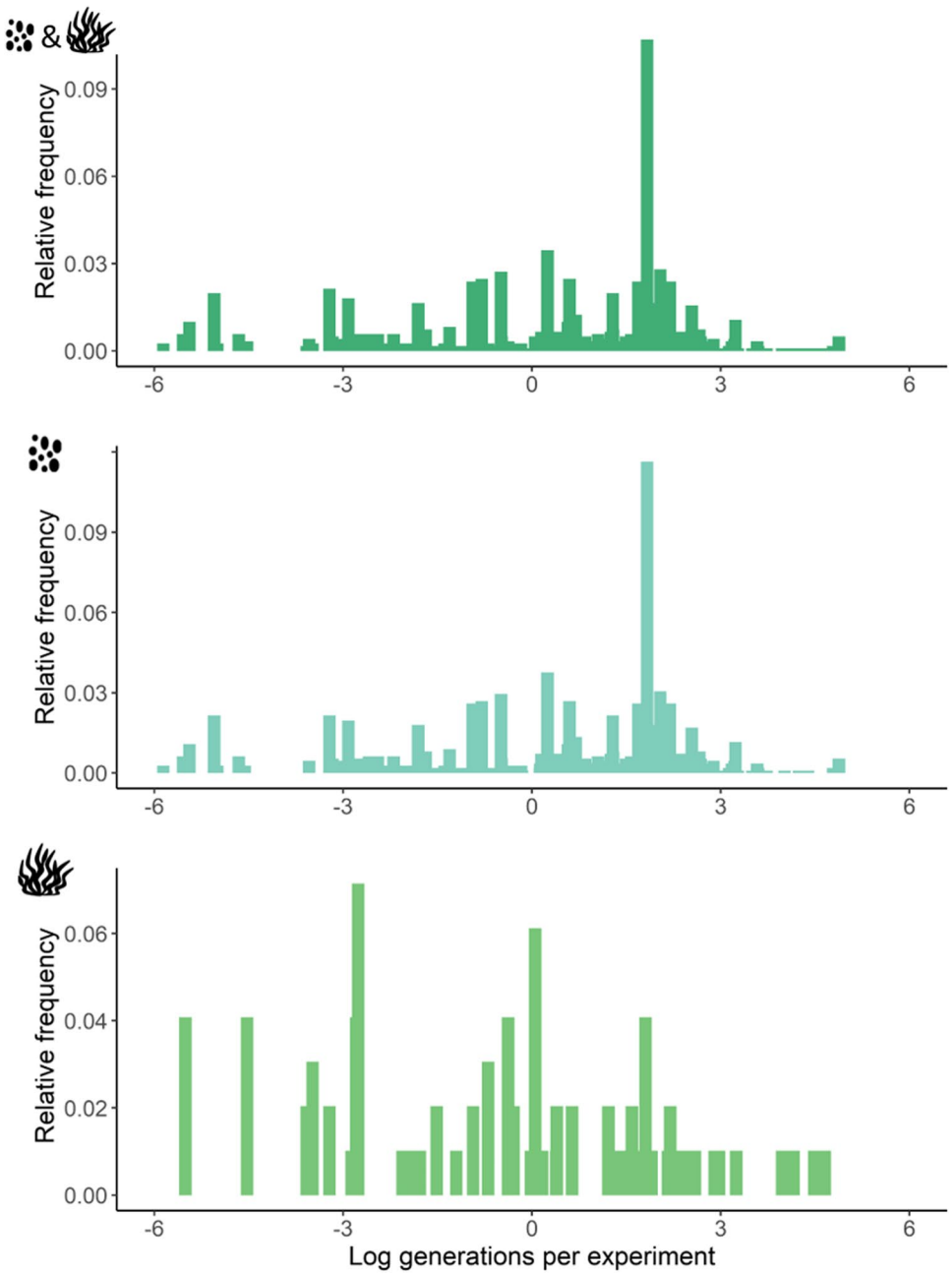

Fig. 5 Relative frequency of natural log generations per experiment for all algae (top panel), microalgae (middle panel), and macroalgae (bottom panel). Relative frequency is calculated within each algal group. Number of generations per experiment was calculated by dividing the duration of the experiment by published doubling times. Note, for macroalgae, specific growth rates were used to extrapolate doubling times (see 'Methods')

recorded as grammes per millilitre or an incomparable measure, such as frond tips per vessel (Fig. 7).

Discussion

Our systematic map identified important knowledge gaps in the field, as well as areas that have been comprehensively examined. Intensely studied temporal scales of light variation include diel cycles and milliseconds, especially for experiments lasting days to weeks. Manipulations of higher frequency variation in experiments that occur for longer periods of time are underrepresented.

Temporal scale and duration of experiment

The prominent gap identified by our map — common to all algae — occurred for intermediate light-manipulation frequencies of seconds, minutes, and hours, in experiments that lasted for months or more. While there are other areas that have received little attention (e.g. temporal scales of nanoseconds for durations of days), we believe that these gaps are of lower priority, as they are probably less typical

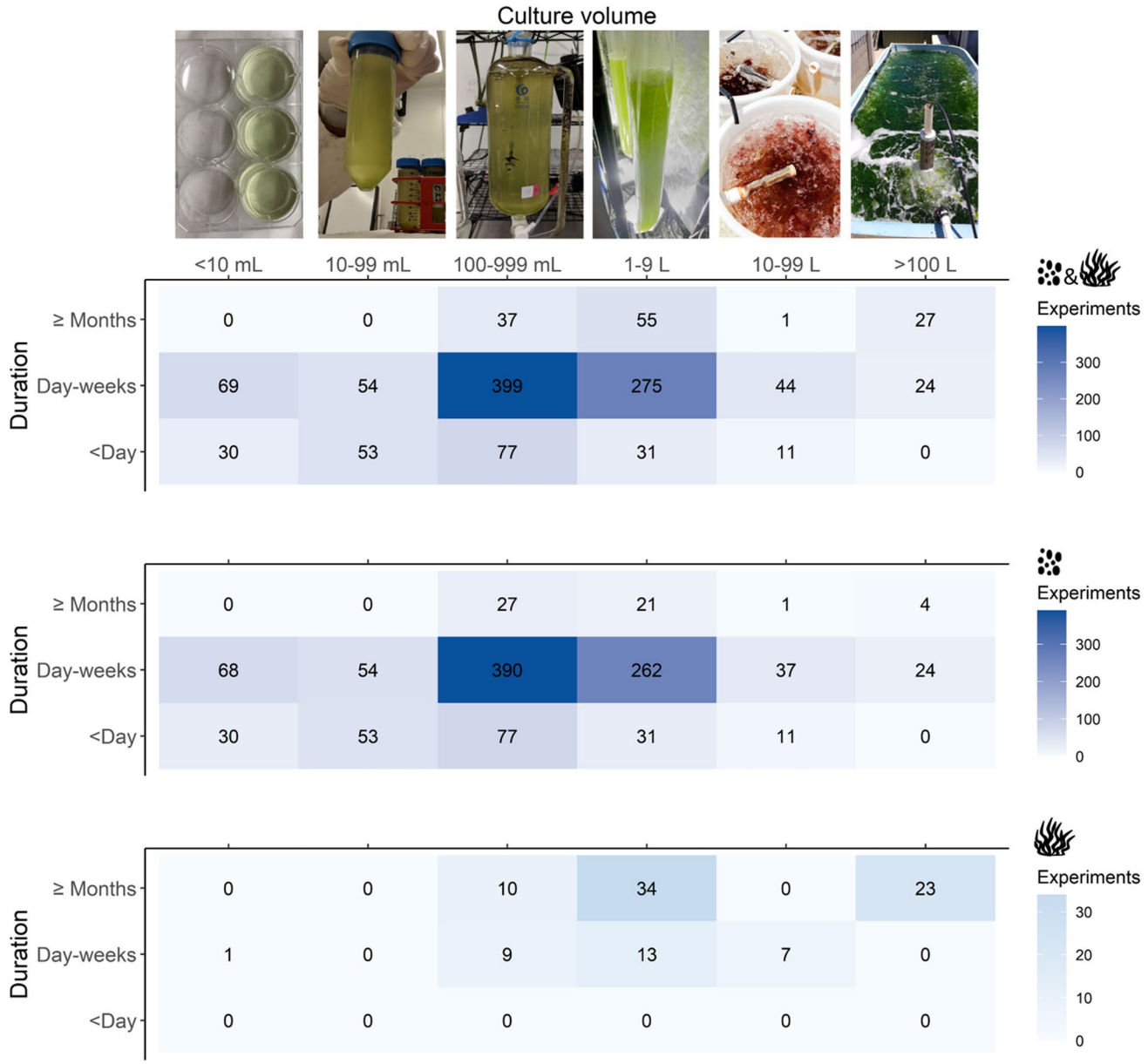

Fig. 6 Variation in culture volumes for the culturing of algae. Photos across the top show the diversity of culture vessel volumes varying from well plates (10 mL) to large-scale culture tanks (2500 L). Top (all algae), middle (microalgae), and bottom (macroalgae) panels display structural matrices of culture volumes and duration of the experiment. The colour gradient and numbers within each cell represent the number of experiments conducted. A value of zero indicates no experiments were reported for the culture volume-duration combination. Photo credit: M. Parascandalo and A. Wegner

of culture conditions. For microalgae, it appears that we have a thorough understanding of the immediate growth consequences of variable light regimes, especially those that range between milliseconds and diel cycles. However, our understanding of the long-term (> weeks) consequences of such fluctuations is limited to only 35 experiments across 12 species and three community studies. Meanwhile, studies on macroalgae tended to run for longer durations but have neglected intermediate-frequency (sub-diel durations of seconds, minutes, and hours) fluctuations in light. Yet it is these intermediate-frequency variations that are likely to dominate industry-scale dense algal cultures, as cells or thalli circulate from shaded regions to irradiated regions of the culture vessels. Our systematic map identified an important disconnect between what temporal scales have been studied, and what is likely to occur in cultures at scale.

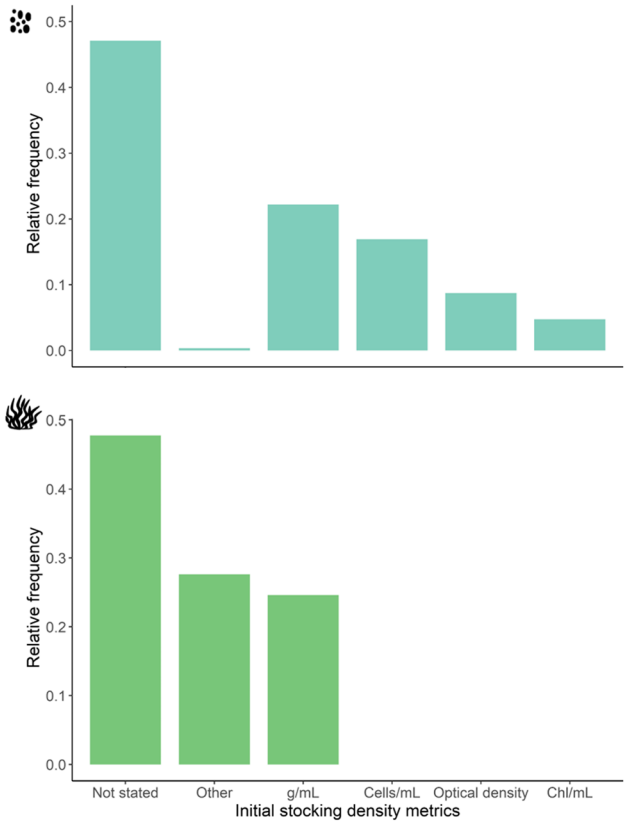

Fig. 7 Metrics used to report initial stocking densities for microalgae (top panel) and macroalgae (bottom panel). 'Other' includes measurements that a not comparable across studies, such as plants per tub, or volume of culture without reference to concentration

The dearth of experimental studies on how light variation affects yields in the longer term means that our understanding of evolution in response to the novel and variable light regimes associated with aquaculture is incomplete. Algal aquaculture creates a strong (and potentially novel) selection regime (Marshall et al. 2018); cultures are partially harvested on a regular basis, leaving a portion of the culture to regrow and be harvested again — a cycle that is repeated many times, lasting for months or years. Increasingly, it seems that algae — both micro and macro — evolve in response to such selection regimes (Monro and Poore 2009; Malerba et al. 2018), and this evolutionary response affects yield (Lawton et al. 2017; Marshall et al. 2018). The majority of experiments in our database lasted for 10 or fewer generations and were not selection experiments sensu stricto. Therefore, the degree to which variable light conditions specifically affect the yields and evolutionary trajectories of these cultures remains unexplored. Future studies could examine the evolutionary consequences of variable light regimes, to determine whether they result in desirable (or undesirable) outcomes.

Patterns of light manipulation

The pattern of light manipulation is likely to influence how algae respond to variable light regimes, yet few studies have explored dynamic patterns (i.e. sinusoidal and sawtooth waveforms). Instead, most studies manipulate light regimes with a square waveform; algae experience instantaneous transitions in light environments. Square waveforms are easy to generate experimentally (e.g. by switching an LED on and off), but they are unlikely to occur outside of an experimental setting (Brindley et al. 2011). Furthermore, responses to square waveforms may not predict responses to more graduated changes, as some studies show different responses between static and dynamic light regimes (Gao et al. 2005; Brindley et al. 2011; Kulk et al. 2011; Orefice et al. 2016). The increased use of dynamic light regimes that mimic natural light variation would help bridge the gap between experimental approaches and conditions in production settings. Advances in technology are making such approaches more accessible; inexpensive and programmable CPU's such as Arduino (Arduino 2015) and Raspberry Pi (Pi 2015) and small LED's are now more manageable and affordable than traditional analogue and fluorescent light technology, allowing dynamic light regimes to be more easily created in the future.

Culture volume

Unsurprisingly, we found that experimental culture volumes were smaller than those used at industry scales — particularly for microalgae. Conducting experiments at scale is challenging, with cost and space requirements often unattainable to researchers. Small culture volumes are far more practical and affordable and are an essential step to understanding how variable light impacts photosynthesis. However, two prominent issues arise in response to the disparity between experimental and industry culture volumes. First, the light regime will differ with the culture volume (Magnusson et al. 2015); algae experience higher-frequency fluctuations in smaller culture vessels (Moberg et al. 2012) compared to larger cultures, where algae experience lower-frequency fluctuations (Demory et al. 2018). Algae respond to changes in light at different rates (MacIntyre et al. 2000); thus, the efficiency of photoacclimation will be governed by the frequency of the light fluctuation (Raven and Geider 2003) which is in turn influenced by the culture volume. Second, population sizes will vary with culture volumes. From an evolutionary perspective, these populations will fundamentally differ, as genetic drift and selection are influenced by population size (Gillespie 2004). The effects of genetic drift — the random changes in gene frequency — are stronger in smaller populations (Falconer and Mackay 1996); while selection — the non-random differential survival of

phenotypically different individuals (Kingsolver and Pfennig 2007) — is less efficacious in smaller populations (Falconer and Mackay 1996). Taken together, this means that adaptive responses, such as photoadaptation, to culture regimes will be slower in smaller populations. Consequently, the evolutionary trajectories of algae will differ between small and large populations, and studies using small culture volumes may misrepresent what occurs at an industry scale. To alleviate any potential mismatch resulting from differential population sizes, future studies should, where possible, use large culture vessels and large population sizes when exploring the consequences of variable light regimes.

Initial stocking density

Our systematic map also revealed an inconsistency in the units reporting initial stocking density. The majority of studies did not report the initial stocking density or reported it as an incomparable metric, such as thalli per vessel, preventing comparisons across studies and with industry. The initial stocking density influences many aspects of the culture, including the internal light regime (Magnusson et al. 2015) and the evolutionary trajectories of the population (Lawton et al. 2017). Without consistent measures of the initial stocking densities, assessments of algal responses to variable light regimes will be less comparable. To allow direct comparisons among experiments, future studies should seek to report initial stocking densities in a common, standardised, and repeatable metric, such as wet weight per volume. Stocking density should also be considered in conjunction with the culture depth, as together they influence the optical density and thus light intensities and patterns of a culture.

Other considerations

Though many studies manipulated variable light regimes, their primary aim was not always to explore the differences between constant and variable light regimes. Consequently, few studies kept the total photon dose (i.e. the total light reaching the culture surface) consistent across treatments, while other studies explored multiple factors, including temperature and nutrients. In these studies, direct inferences about how variable light impacts algal growth cannot be made, as it is unclear whether it is the total light dose or variable light driving the differences in algal growth. While this factor is not essential in studies exploring photosynthetic efficiency, it is essential if we wish to determine the consequences of variable light relative to constant light for industry-relevant variables such as yield. An important next step is to separate total photon dose from variable light regimes, to determine if the effects of variable light regimes are independent of the effects of mean light.

Scaling up predictions from small cultures to large, industrial-level cultures is a major challenge in algal aquaculture (Borowitzka and Vonshak 2017). Often, predictions of yields from laboratory experiments exceed those observed in industry (Grobbelaar 2012). At larger scales, many factors — temperature, light, mixing rates, culture depth, culture density, oxygen exchange rates, nutrient assimilation, etc. — covary (Borowitzka and Vonshak 2017), whereas many of these factors are controlled, and (co)variation is minimised in the laboratory. Although controlling these factors is essential for elucidating the contribution of each factor, particularly in photosynthesis research, predictions for yield at larger scales necessitate explorations at comparable scales. The dearth of larger-scale experiments does not invalidate work at smaller scales, as this work is vital for our understanding of algal photophysiology. However, if we wish to provide effective guidance to industry, we need to conduct experiments that replicate the conditions that occur at larger scales where possible. Our literature map reveals that such studies remain rare. We hope that by identifying this knowledge gap, it will encourage studies that tackle the formidable challenge of working at larger scales.

Conclusion

Our systematic map has highlighted the prominent knowledge gaps and clusters for variable light regimes in algae aquaculture. Static light manipulations of milliseconds and diel cycles for short durations (< weeks) conducted in low-density cultures have provided invaluable insights into photosynthesis research. However, to accurately inform industry, we recommend that future studies seek to emulate culture conditions that are more common in production settings. Similarly, our understanding of evolutionary effects of light regimes is lagging. Overall, our primary recommendations are that future studies focus on experiments that:

- Vary light according to intermediate-frequency temporal manipulations (seconds, minutes, and hours) and last for multiple months using continuous or semi-continuous cultures where the biomass per volume (stocking density) remains relatively constant through regular harvesting; and
- Use dynamic light regimes (sinusoidal and sawtooth) that better represent the light regimes occurring at industry scales.

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Data availability The database is available in the online resources.

Declarations

Competing interests The authors declare no competing interests.

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