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PERSPECTIVE

Gephyrocapsa huxleyi (*Emiliania huxleyi*) as a model system for coccolithophore biology

Glen L. Wheeler[1] | **Daniela Sturm**[1,2] | **Gerald Langer**[3]

[1]The Marine Biological Association of the United Kingdom, The Laboratory, Plymouth, UK

[2]School of Ocean and Earth Science, University of Southampton, Southampton, UK

[3]Institute of Environmental Science and Technology (ICTA-UAB), Universitat Autònoma de Barcelona, Barcelona, Spain

Correspondence
Glen L. Wheeler, The Marine Biological Association of the United Kingdom, The Laboratory, Citadel Hill, Plymouth, Devon PL1 2PB, UK.
Email: glw@mba.ac.uk

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Abstract

Coccolithophores are the most abundant calcifying organisms in modern oceans and are important primary producers in many marine ecosystems. Their ability to generate a cellular covering of calcium carbonate plates (coccoliths) plays a major role in marine biogeochemistry and the global carbon cycle. Coccolithophores also play an important role in sulfur cycling through the production of the climate-active gas dimethyl sulfide. The primary model organism for coccolithophore research is *Emiliania huxleyi*, now named *Gephyrocapsa huxleyi*. *G. huxleyi* has a cosmopolitan distribution, occupying coastal and oceanic environments across the globe, and is the most abundant coccolithophore in modern oceans. Research in *G. huxleyi* has identified many aspects of coccolithophore biology, from cell biology to ecological interactions. In this perspective, we summarize the key advances made using *G. huxleyi* and examine the emerging tools for research in this model organism. We discuss the key steps that need to be taken by the research community to advance *G. huxleyi* as a model organism and the suitability of other species as models for specific aspects of coccolithophore biology.

KEYWORDS
algae, model, ocean acidification, phytoplankton

INTRODUCTION

The striking biomineralized structures of coccolithophores have held fascination for biologists ever since the first indications that they were derived from living cells (Sorby, 1861; Wallich, 1861). Coccolithophores are one of the most important groups of marine phytoplankton, contributing to the global carbon cycle as major primary producers but also through their ability to precipitate large quantities of calcium carbonate (Ziveri et al., 2023). It is estimated that there are around 300 species of coccolithophores in modern oceans, although only a handful are able to grow in laboratory culture (Probert & Houdan, 2004). This limitation has strongly shaped the development of model species. The vast majority of laboratory research into coccolithophore biology has involved two species, *Emiliania huxleyi* and *Chrysotila* (formerly *Pleurochrysis*) *carterae*. Recent phylogenetic studies have placed *Emiliania* within *Gephyrocapsa* (Bendif et al., 2023; Filatov et al., 2021), and so we will refer to this species as *Gephyrocapsa huxleyi* in the text below.

Both *G. huxleyi* and *Chrysotila carterae* exhibit robust growth in laboratory culture, in contrast to many other coccolithophore lineages. *Gephyrocapsa huxleyi* has emerged as the dominant coccolithophore model species primarily because of its ecological relevance (Westbroek et al., 1993; Figure 1). It is the most abundant coccolithophore species in modern oceans and displays a cosmopolitan distribution, forming large

Abbreviations: RNA-seq, RNA sequencing.

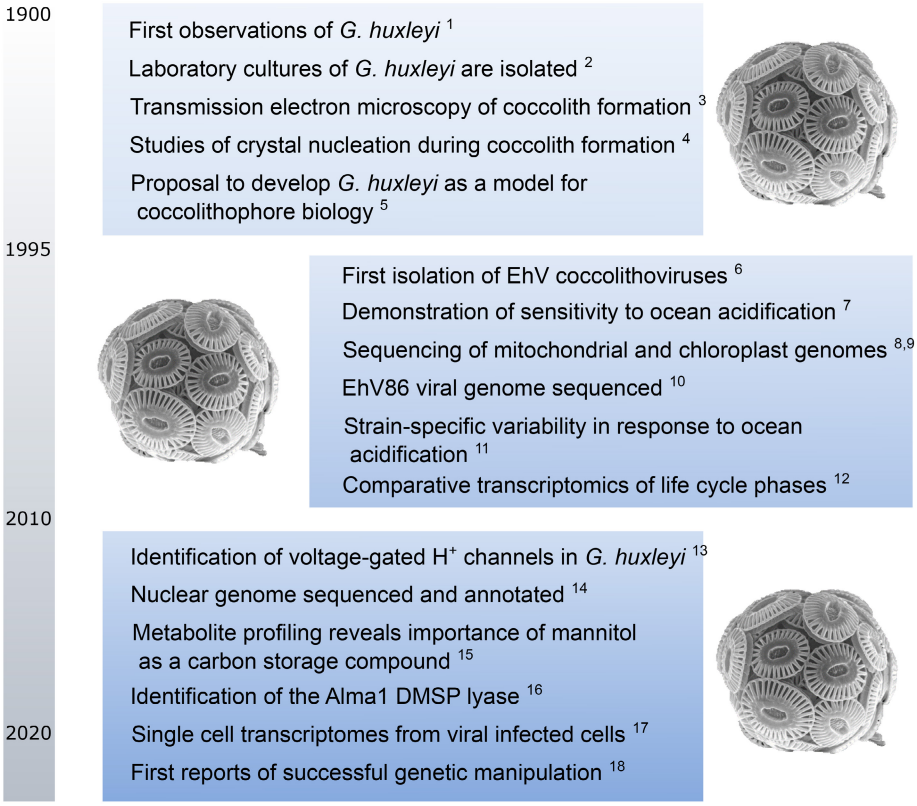

FIGURE 1 A timeline of *Gephyrocapsa huxleyi* research. A list of 18 key publications in *G. huxleyi* research. 1: Lohmann (1902), 2: Paasche (1962), 3: Wilbur and Watabe (1963), 4: Young et al. (1992), 5: Westbroek et al. (1993), 6: Bratbak et al. (1996), 7: Riebesell et al. (2000), 8: Sanchez Puerta et al. (2004), 9: Sanchez Puerta et al. (2005), 10: Wilson et al. (2005), 11: Langer et al. (2009), 12: von Dassow et al. (2009), 13: Taylor et al. (2011), 14: Read (2013), 15: Obata et al. (2013), 16: Alcolombri et al. (2015), 17: Ku et al. (2020), 18: Cai et al. (2021). Scanning electron microscopy images of *G. huxleyi* are shown. [Color figure can be viewed at wileyonlinelibrary.com]

blooms in both coastal and oceanic environments. In contrast, *C. carterae* is restricted to coastal environments and forms a much less prominent role in phytoplankton assemblages, although it remains an important model for specific aspects of coccolithophore biology (Kadan et al., 2021; Marsh, 1999).

GEPHYROCAPSA HUXLEYI AS A MODEL ORGANISM

Much research involving *G. huxleyi* has understandably focused on biological processes relating to calcification. Early studies using electron microscopy demonstrated cell ultrastructure associated with the developing coccoliths, such as the nature of the coccolith vesicle (Klaveness, 1972), and revealed the conserved nature of crystal nucleation during coccolith formation (Young et al., 1992). More recent studies have demonstrated the presence of calcium-rich organelles associated with the coccolith vesicle, although their role in the calcification process remains unclear (Sviben et al., 2016). Many isolates of *G. huxleyi* exhibit reduced calcification after prolonged growth in laboratory culture or have the lost the ability to calcify altogether. The ability to directly compare calcified and non-calcified *G. huxleyi* strains has been extensively exploited by researchers to examine how calcification influences cell physiology (Mackinder et al., 2011; Paasche, 1998).

Gephyrocapsa huxleyi represents an important focal point for research into the impacts of ocean acidification on calcifying organisms. Riebesell et al. (2000) demonstrated decreased calcification rates and defects in coccolith morphology in *G. huxleyi* cells exposed to elevated CO_2. Further studies identified strain-specific sensitivity to ocean acidification and determined the potential for adaptive evolution in *G. huxleyi* cultures maintained at elevated CO_2 for hundreds of generations (Langer et al., 2009; Lohbeck et al., 2013). *Gephyrocapsa huxleyi* is primarily sensitive to low seawater pH (rather than elevated CO_2; Bach et al., 2011), which is due to the requirement for unusual mechanisms for pH homeostasis (voltage-gated H^+ channels) in the calcification process (Taylor et al., 2011). Despite the importance of *G. huxleyi* as a model organism for calcification, relatively few specific gene products have been directly linked to the calcification process, and fewer still have been functionally characterized. Partial purification of coccolith-associated polysaccharides from *G. huxleyi* have enabled the identification of a

novel low-complexity protein in these extracts (GPA), although its role in the calcification process remains unknown (Corstjens et al., 1998). A recent proteomic analysis of purified coccoliths revealed a sub-set of 68 coccolith-associated proteins that represent excellent candidates for further study into the calcification process (Skeffington et al., 2023). The voltage-gated H^+ channel is perhaps the only specific gene product where a functional role in coccolith formation has been directly shown (Kottmeier et al., 2022).

Like all coccolithophores, *G. huxleyi* displays a haplo-diplontic life cycle (Frada et al., 2018). The diploid phase of *G. huxleyi* is non-motile and produces the characteristic heterococcoliths, while the haploid phase is motile and non-calcified. The two life cycle phases therefore provide an excellent model system in which to compare the molecular mechanisms and physiologies associated with these characteristics (Rokitta et al., 2011; von Dassow et al., 2009). There are several reports of life-cycle transitions in culture, primarily the appearance of motile haploid cells in cultures of diploid cells (Houdan et al., 2005), but the ability to reproducibly trigger these transitions on demand has remained elusive. Our inability to complete the life cycle of *G. huxleyi* in the laboratory remains a major obstacle to its development as a model organism, as it prevents the development of classical genetic approaches, such as mapping and complementation of mutants, which are available in other algal models, such as *Ectocarpus* and *Chlamydomonas* (Cock, 2023; Salome & Merchant, 2019).

Gephyrocapsa huxleyi has proven to be an important model system for understanding the biotic interactions of phytoplankton. Viral particles had previously been observed in many phytoplankton cells, but *G. huxleyi* represented the first system in which the infection cycle could be induced in culture and studied extensively (Bratbak et al., 1996). The coccolithoviruses are giant double-stranded DNA viruses belonging to the Phycodnoviridae. They exhibit a classic lytic infection cycle and contribute to the termination of large *G. huxleyi* blooms in nature (Wilson et al., 2002). Sequencing of the EhV86 viral genome revealed a remarkable number of genes (472), including an entire pathway for sphingolipid biosynthesis (Monier et al., 2009; Wilson et al., 2005), which was subsequently demonstrated to play a direct signaling role in the viral infection cycle (Vardi et al., 2009). *Gephyrocapsa huxleyi* has also proven to be useful in the study of wider microbial interactions. These studies are beginning to reveal the complexity of algal–bacterial interactions, such as the "Jekyll and Hyde" relationship between the α-proteobacterium *Phaeobacter inhibens* and *G. huxleyi* in which the bacteria initially promote the growth of the algae but ultimately kill them through the release of toxins as cell densities increase (Segev et al., 2016; Seyedsayamdost et al., 2011).

An important characteristic of the *G. huxleyi* metabolism is its ability to accumulate large amounts of the osmolyte dimethylsulphoniopropionate (DMSP), which is a precursor of the climate active gas, dimethyl sulfide (DMS; Steinke et al., 1998). The substantial DMSP lyase activity exhibited by some *G. huxleyi* isolates has enabled the use of protein purification techniques to resolve this activity. This approach led to the identification of Alma1, a novel DMSP lyase belonging to the aspartate racemase superfamily that is widespread among eukaryote phytoplankton and distinct from all known bacterial DMSP lyases (Alcolombri et al., 2015; Johnston et al., 2016). Another area of the *G. huxleyi* metabolism that has attracted much research attention is the production of lipids, including the production of omega-3 polyunsaturated fatty acids that are important for human health (Sayanova et al., 2011) and neutral long chain lipids such as the $C_{37–39}$ alkenones that have been utilized as a climate proxy by paleobiologists (Sawada & Shiraiwa, 2004).

RESOURCES FOR *G. HUXLEYI* RESEARCHERS

One of the primary resources for *G. huxleyi* research is a large collection of environmental isolates that are held in algal culture collections across the world. These strains have been isolated from multiple locations and exhibit significant differences in physiology and coccolith morphology. Strains that have been used extensively for laboratory experiments include CCMP1516 (used for genome sequencing), RCC1216/1217 (a haploid/diploid pair for comparisons of life-cycle phases), and CCMP373/374 (which show contrasting phenotypes for DMSP lyase activity and susceptibility to viral infection; Alcolombri et al., 2015 Bidle et al., 2007; Read et al., 2013; von Dassow et al., 2009). This phenotypic variability represents an important tool for *G. huxleyi* research, although accurate recording of strain ancestry and standardization of strain choice remain important considerations for the future development of *G. huxleyi* as a model organism.

The mitochondrial and chloroplast genomes of *G. huxleyi* were sequenced in 2004 and 2005, respectively (Sanchez Puerta et al., 2004, 2005), followed by a full nuclear genome assembly in 2013 (Read et al., 2013). The initial genome assembly of the diploid CCMP1516 strain (Emihu1) revealed a 142 Mb haploid genome that was GC-rich (65%) and dominated by highly repetitive regions (>64%). One notable feature of the *G. huxleyi* genome is the presence of many non-canonical intron splice junctions (GC-AG rather than GT-AG), which can interfere with de novo prediction of open reading frames. A refined transcriptome assembly, guided by a dataset of manually curated genes, led to a significantly improved transcript catalog

TABLE 1 Characteristics of model species for coccolithophore research. [Color table can be viewed at wileyonlinelibrary.com]

Species	Robust growth in culture	Degree of calcification	Calcification mutants identified[a,b]	Calcification in haploid phase[c]	Annotated genome[d]	Transcriptome available[e]	Reports of genetic transformation[f,g]	Environmental distribution
Gephyrocapsa huxleyi	+++	++	Yes	No	Yes	Yes	Yes	Cosmopolitan
Chrysotila carterae	+++	+	Yes	No	No	Yes	Yes	Coastal
Coccolithus braarudii	++	+++	No	Yes	No	Yes	No	Ocean
Calcidiscus leptoporus	++	+++	No	Yes	No	Yes	No	Ocean

Note: [a]Paasche (1998); [b]Marsh and Dickinson (1997); [c]Frada et al. (2018); [d]Read et al. (2013); [e]Faktorova et al. (2020); [f]Cai et al. (2021); [g]Endo et al. (2016). Shaded squares represent advantageous traits for model coccolithophore species.

(Feldmesser et al., 2014). This revealed that the vast majority of splice sites in *G. huxleyi* are non-canonical (GC-AT; 65%), contrasting with only 20% of non-canonical splice sites in the initial gene catalog. More recently, sequencing and assembly of the nuclear genome of *G. huxleyi* strain AWI1516 (derived from strain CCMP1516) using PacBio sequencing technology has resulted in a greatly improved genome assembly (Emihu2) with a haploid genome size of 98 Mb on 165 scaffolds (Skeffington et al., 2023). Forty percent of the predicted proteins in Emihu2 do not have hits to the Emihu1 proteome, which may be due to greater inclusion of proteins with a low complexity or biased amino acid composition than are typical for many biomineralization-associated proteins (Skeffington et al., 2023).

Gephyrocapsa huxleyi strains show a large variability in gene content (Read et al., 2013). This genomic diversity may be driven in part by the loss of genes associated with the haploid life cycle phase in many environmental *G. huxleyi* isolates (Bendif et al., 2023; von Dassow et al., 2015). Recent construction of phylogenies using genome-wide single-nucleotide polymorphisms have revealed three distinct clades within the *G. huxleyi* supercomplex that likely represent distinct species (Bendif et al., 2023). The phylogenies of nuclear, chloroplast, and mitochondrial genes exhibit significant incongruence, pointing to a convoluted inheritance of organellar genomes during introgressive hybridization between these diverging lineages (Bendif et al., 2015; Kao et al., 2022).

The availability of the *G. huxleyi* genome has facilitated the application of a range of omic technologies. Transcriptomic studies include examination of the cellular responses to nitrogen starvation and identification of calcification-related mechanisms through the manipulation of seawater calcium concentrations (Nam et al., 2020; Rokitta et al., 2014). An exciting development is the application of single-cell RNA-seq, which was used to profile transcriptomes from individual *G. huxleyi* cells throughout the viral infection cycle (Ku et al., 2020). Proteomic approaches have been used extensively in *G. huxleyi* to examine, for example, the cellular responses to warming (Dedman et al., 2023) and to identify processes that are affected by nutrient limitation (McKew et al., 2015; Shire & Kustka, 2022). Metabolomic studies have revealed the importance of mannitol as a carbon storage compound in *G. huxleyi* (Obata et al., 2013) and identified important metabolic differences between life-cycle stages during nutrient starvation (Wordenweber et al., 2018).

TOOLS FOR GENETIC MANIPULATION

One of the key obstacles to the future development of *G. huxleyi* as a model organism has been the

development of a robust system for genetic modification to enable the characterization of individual molecular mechanisms through protein localization and genome editing. *Gephyrocapsa huxleyi* does not grow well on solid media, which severely hampers the selection and isolation of individual transformed lines. Skeffington et al. (2020) demonstrated that a starch embedding method could be used to grow *G. huxleyi* on solid media, enabling the selection of individual colonies from a mixed population of cells. Recent reports of successful transformation of *G. huxleyi* using a promoter from the fucoxanthin chlorophyll-binding protein to drive the expression of a serine palmitoyltransferase from the EhV virus are encouraging (Cai et al., 2021). Successful transformation of other coccolithophores (*Chrysotila carterae*) and members of the Isochrysidales (*Tisochrysis lutea*) has also been reported (Endo et al., 2018, 2016). However, these remain isolated reports, and it remains to be seen whether these protocols can be readily transferred to other laboratories to support the research activities of the wider community. Community-wide approaches are needed to support the development of tools and techniques to aid genetic manipulation.

FUTURE PERSPECTIVES

Ease of laboratory culture combined with high ecological relevance have made *G. huxleyi* the most important model for many aspects of coccolithophore biology. Recent developments such as a better understanding of the mechanisms causing phenotypic diversity between strains and an improved genome assembly allowing refined gene model predictions represent important steps forward. However, the development of robust and efficient protocols for genetic transformation and the ability to complete the life cycle in the laboratory remain important priorities for the future development of *G. huxleyi* as a model organism.

Certain characteristics, particularly its small cell size, means that *G. huxleyi* is not an appropriate model for some laboratory techniques (Table 1). *Chrysotila carterae* remains an important model for calcification, supported by the availability of non-calcifying mutants (Marsh & Dickinson, 1997) and its much larger cell size, which has enabled high-resolution examination of coccolith formation using cryoelectron tomography (Kadan et al., 2021). Other important models for calcification include *Coccolithus braarudii* and *Calcidiscus leptoporus* (Avrahami et al., 2022; Langer et al., 2021). These large species exhibit less robust growth in laboratory culture than either *G. huxleyi* or *Chrysotila carterae* but calcify at a much greater rate and are amenable to cell physiology approaches, such as the patch-clamp technique, which has revealed novel aspects of coccolithophore membrane physiology (Taylor & Brownlee, 2003; Taylor et al., 2011). Understanding the biology of heavily calcified species is critical because although they are less numerous than *G. huxleyi*, they contribute a greater proportion of calcite export to the deep ocean (Hernandez et al., 2020). Improving our understanding of the wider physiological and ecological roles of calcification remains an important challenge in coccolithophore biology. Although it is likely that primary role of coccoliths is to act as a protective barrier around the cell (Monteiro et al., 2016), the significant morphological diversity of coccoliths between species suggests considerable diversification of their cellular roles. *Gephyrocapsa huxleyi* remains the species of choice for many applications, and it will certainly continue to develop as the premier model for coccolithophore biology, although the utility of other species, particularly in the study of calcification, will likely support the development of alternative model systems.

AUTHOR CONTRIBUTIONS

Glen L. Wheeler: Conceptualization (lead); writing – original draft (lead); writing – review and editing (lead). **Daniela Sturm:** Writing – original draft (supporting); writing – review and editing (supporting). **Gerald Langer:** Writing – original draft (supporting); writing – review and editing (supporting).

ORCID

Glen L. Wheeler https://orcid.org/0000-0002-4657-1701

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