MICROALGAL FUNCTIONAL TRAITS

Measures of morphological complexity of microalgae and their linkage with organism size

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Abstract In phytoplankton ecology the shape of microalgae appears predominantly as a categorical variable. Using shape-realistic 3D models of 220 microalgae we proposed and calculated six shape metrics and tested how these relate to each other and to the size of the microalgae. We found that some of the metrics are more sensitive to elongation, while others are related to multicellularity. We found a linear relationship between shape measures and the greatest axial linear dimensions of the microalgae. Our findings suggest that there is an evolutionary trade-off between the shape and size of the microalgae. It is important to stress that we found that surface area to volume ratio of the microalgae are hyperbolic functions of the length and volume for each shape. In our study, we demonstrated that the proposed shape metrics serve as suitable quantitative traits, and help to understand better how simple shapes evolved to more complex outlines.

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Introduction

Looking at algal samples under a microscope, the first thing that captures the attention of the observer is the enthralling richness of shapes, sizes and colours. These are not only aesthetic features, but are also represented as traits that serve highly important functions in the performance and operation of the algae in the aquatic environment. The colour of algae depends on the quantity and quality of a wide array of photosynthetic pigments that microalgae acquired by several endosymbiotic events during their evolution (Larkum & Vesk, 2003). The amount of the pigments in the cell and the range of wavelengths where the pigments have their optimal absorption can be measured and thus, quantified. To characterise the size of microalgae, several measures are available. The quantitative size-related metrics of microalgae, such as specific biovolume, the greatest axial linear dimension (GALD), the equivalent spherical diameter (ESD) or the surface area to volume ratio are relevant quantitative traits in ecological studies (Lewis, 1976). Values of these metrics determine the grazing tolerance (Carpenter et al., 1995), sinking properties (Padisák et al., 2003) or the light and nutrient acquisition of microalgae (Kruk et al., 2015).

A number of studies investigate the role of size in the photosynthesis (Banse, 1976; Cermeño et al., 2005), nutrient acquisition (Edwards et al., 2011), light exploitation (Key et al., 2010) or in grazing avoidance of phytoplankton (Branco et al., 2020). The importance of size has also been recognised in shaping the growth rate (Chisholm, 1992; Savage et al., 2004), the seasonality (Gaedke, 1992), the rarity (Görgényi et al., 2022) of the phytoplankton or in the structuring of planktic communities (Hillebrand et al., 2022). In contrast, there have been few attempts to investigate how shape of the microalgae relates to the issues mentioned above (Naselli-Flores et al., 2021). The shape is also a master trait. By modifying the surface area to volume ratio of the cells it exerts strong effects on the performance of physiological traits (i.e., photosynthesis, nutrient uptake requirements) (Litchman & Klausmeier, 2008). The shape of the cells also affects behavioural trait, like motility, because it determines how the cell moves in the highly viscous aquatic environment (Purcell, 1977; Naselli-Flores & Barone, 2011; Naselli-Flores et al., 2021).

The simplest way to characterise the shape of an object is comparison to well-known geometrical forms. The first classification of microalgal morphologies appeared in the early ages of limnology (Schütt, 1892; Grahn, 1912). Since taxonomy of microalgae was based on morphological characteristics of cells and colonies, the shape categories became widely accepted and used in microalgal research (Stanca et al., 2013). The need for quantitative phytoplankton analyses evoked biovolume and surface area estimation approaches during recent decades. These approaches are based on the comparison of shapes to various geometric forms (Hillebrand et al., 1999; Sun & Liu, 2003). During development of trait-based approaches in phytoplankton ecology, various morpho-functional groups have been defined for microalgae and the authors of these approaches (among others), also applied morphological categories for shape classification (Salmaso & Padisák, 2007; Kruk et al., 2010). Although, these approaches triggered important advances in phytoplankton ecology (Salmaso et al., 2015), they also have some shortcomings and limitations.

In a number of taxa, uncertainties emerge during shape classification. Several colonial species, such as *Coelastrum, Synura, Uroglena* spp. have dense sarcinoid shape in their early development stage, which turns to a vesicular structure when cell numbers increase. Several taxa e.g., *Ankistrodesmus, Asterionella,* and dozens of centric diatoms have both unicellular and colonial forms. The other shortcoming is that shape characterisation of microalgae results in categorical traits that are less suitable to reveal changes in environment—trait interactions (Lavorel & Garnier, 2002).

Recently, the numerical characterisation of microalgal shapes has developed. To characterise the roundness of quartz particles, Wadell (1935) proposed the use of sphericity, defined as the ratio of the surface area of an equal-volume sphere to the actual surface area of the particle. The inverse of this measure (the surface extension) was used by Ryabov et al. (2021) to describe the relationships among cell morphology, size and taxonomic diversity in unicellular marine phytoplankton assemblages. Applying automatic photo imagery Sonnet et al. (2022) measured various shape attributes (length, width, perimeter, etc.) on two-dimensional images of marine phytoplankton species to study seasonal changes in

cell complexity and chain formation. Based on size measurements on shape-realistic 3D images of microalgae, Borics et al. (2021) proposed species-specific surface area (C_S) and volume constants (C_V) to calculate biovolume and surface area of microalgae. Using these metrics, a two-dimensional morphospace was defined to study morphological diversity of various algal groups. These recent results suggest that the new techniques like automatic imaging and/or image analysis tools enables the numerical characterisation of microalgal shapes, giving the possibility to shift from qualitative morphological traits towards the use of quantitative ones. Analysis of quantitative morphological traits enables phytoplankton ecologists to gain a far more comprehensive understanding of how the various morphologies contribute to the assembly of phytoplankton under various ecological conditions.

To quantify morphological complexity, spheres serve as excellent reference objects. It is not only the simplest geometric form, but since the very first living creatures were also spherical (Koch & Koch, 2006), it is also a good selection from evolutionary point of view. Sphere has the smallest surface area per unit volume, thus any distortion of the sphere results in an increase in the surface to volume ratio. Therefore, the metrics that related even only tangentially to the surface area of an object can serve as good candidates to characterise morphological complexity. In this study therefore, we propose six quantitative shape measures for microalgal species.

Greatest axial linear dimension (GALD)/width ratio

The simplest among the selected metrics is the greatest axial linear dimension/width ratio, its value is 1.0 for the sphere (independently of the directions of measurements). At a rough scale this measure can describe several microalgal shapes, but practically it gives reliable results for bodies of revolution.

Relative elongation ($\mathfrak{R}$) i.e.: GALD/equivalent spherical diameter ratio

The equivalent spherical diameter (ESD) is a size measure, but if the greatest axial linear dimension (GALD) is compared to it, then it becomes a size independent shape measure called as Relative elongation ($\mathfrak{R}$) herein. Its larger value expresses the rate of difference from a sphere.

Relative surface area extension (RSAE)

Sphericity of an object (Ψ) expresses how spherical it is (Wadell, 1935). It is practically the ratio of the surface area of a sphere with the equivalent volume as the object to the surface area of the object. Its inverse is the relative surface area extension (RSAE), which is also increases if a shape moves away from a sphere.

Compactness (C)

We can define the compactness (C) of an object as a ratio of its surface area to its volume in a dimensionless way (Bribiesca, 2008).

Cs/Cv ratio

The shape-specific surface area (Cs) and volume constants (Cv) are those dimensionless metrics that can be used to calculate the surface area and volume of the objects (Borics et al., 2021). The ratio of these dimensionless metrics also expresses the compactness of the object and serves as a candidate shape metric.

Fractal dimension (FD)

Fractal dimension (FD) of the objects has often been proposed as a measure of shape complexity. Fractals are irregular, fractional dimensional objects, often exhibiting self-similarity patterns at various scales. This phenomenon has been observed in several natural objects such as snowflakes or tree branches. The fundamental theory of the fractal geometry stems from the coastal paradox, which suggests that the length of the coastline exponentially increases with the degree of cartographic resolution and with the length of the ruler we use to measure it. To analyse this pattern in action, the box counting procedure defines a dimension metric of the objects, referred here as fractal dimension.

In this study we investigate how the proposed shape metrics relate to each other and how they link to the size of microalgae. We also investigate how the surface area to volume ratios of the various shapes relate to each other and how these values change along the size (length and biovolume) scale. Supposing that the maximal linear dimension of an object is equal to one (GALD = 1.0) surface area to volume ratio (*A/V*) of the object is equal with its Cs/Cv shape measure. In any other case (GALD ≠ 1.0) A/V of the objects are

reciprocal function of their linear dimensions $A/V = Cs/Cv \times GALD^{-1}$. When changes in the A/V values are investigated in contrast to biovolume, changes in the exponent can be expected. Because the volume is the third power of the linear dimension of an object ($V = f\ l^3$), the expected formula is $A/V = Cs/Cv \times V^{-0.33}$.

We hypothesise that the metrics represent various aspects of morphology and show differences in their relationship to the size of microalgae.

Materials

To calculate the proposed shape measures we applied the following formulas:

1. Greatest axial linear dimension (GALD)/width (w) ratio (Eq. 1)

$$\frac{GALD}{w} \tag{1}$$

2. Relative elongation ($\Re$) i.e.: GALD/equivalent spherical diameter ratio (Eq. 2).

$$\Re = \frac{GALD}{ESD} = \frac{GALD}{\sqrt[3]{\frac{6V}{\pi}}}, \tag{2}$$

where V: volume of the object.

3. Relative surface area extension (RSAE) (Eq. 3).

$$RSAE = \frac{1}{\Psi} = \frac{A}{A_{sphere}} = \frac{A}{\pi^{1/3}(6V)^{2/3}}, \tag{3}$$

where A: surface area of the object,
A_{sphere}: surface area of a sphere of the same volume as the object.

4. Compactness (C) (Eq. 4)

$$C = \frac{A^{3/2}}{V} \tag{4}$$

5. Cs/Cv ratio (Eq. 5).

$$\frac{C_S}{C_V} = \frac{\frac{A}{GALD^2}}{\frac{V}{GALD^3}} = \frac{A}{V}GALD, \tag{5}$$

where C_S: shape-specific surface area constant,
C_V: shape-specific volume constant.

6. Fractal dimension (FD)

The FD is calculated as the exponential growth rate of the number of cubes covering the fractal object compared to the reciprocal of the side lengths of the cubes. In this paper, the fractal dimension of the surface of the microalgae is estimated, where we approximate this value with the slope of the regression line fitted on the data of the number of the unit cubes covering the surface and sequence of scaling of the unit cubes plot (Eq. 6). For the calculation of the value of the FD, each object was scaled to have a surface area of one unit size.

$$FD = \frac{\log(N(s))}{\log\frac{1}{s}}, \tag{6}$$

where $N(s)$: number of the cubes covering the surface. s: size ratio of cubes.

It is worth taking some notes. Firstly, although some microalgae might display fractal-like patterns, they are not regular fractals. Secondly, the theoretical lower bound for the FD of a surface is 2, but these values of the 3D models of the microalgae range from 1 to ~2.6. The reason is that the FD is assigned to the boundary of the object that is created by process of the 3D shell modelling. Thus, if the approximated FD of a surface (boundary) is strictly smaller than 2 then this shows that the object is reasonably thin and elongated with respect to the scales of approximation. Values higher than 2 show that the surface (boundary) is rough, striated at the scales of approximation.

Calculation of these metrics needs the basic size measures of the objects, e.g., length, width, surface area, volume and coordinates of the objects in a three dimensional Euclidean space. To calculate these size measures, we generated the shape-realistic models of 220 microalgal taxa using a free open-source 3D graphics application Blender 3.0.0 (Blender Foundation and Community, 2020) (Borics et al., 2021). The selected microalgae represented the most relevant shape and size classes in 20 main algal lineages (Supplementary Table 1).

Surface area and volume of the objects were measured applying the NeuroMorph software toolset (Jorstad et al., 2015). The selected taxa cover the most relevant forms of unicells and colonies (Borics et al., 2022). The value of fractal dimension was quantified using the VoxR package in R (v4.2.1; R Core Team, 2022), which is a free and open-source language and

environment for statistical computing and graphics. Using this software package we estimate the fractal dimension of the boundary of the 3D model of our algae by an algorithm calculating the number of cubes covering the object at five log 10 scales.

Analysing the size–shape relationships among the selected microalgal taxa, we plotted each form on a metric value–GALD plot. To describe the strength of the relationship we applied major axis regression. Values were log-transformed to improve normality.

Surface area to volume ratios (A/V) of the selected taxa were also studied along their size scale. As size measures we used the GALD and biovolume of the species.

Results

Relationship between the shape measures

The calculated shape measures are not independent from each other (Fig. 1). Since all measures aim to describe how the shapes differ from a sphere, they showed some resemblance. We found a deterministic relationship between C and RSAE (Eq. 7), namely,

$$RSAE = \frac{C^{2/3}}{\pi^{1/3}6^{2/3}}. \tag{7}$$

This indicates that these measures are only different mathematical representations of the same morphological attributes (Fig. 1j). In the most cases, the comparisons revealed increasing trends between the shape metrics (Fig. 1a–j) although with apparent bifurcation in the data points. The two branches of the fork-shaped data structure represent different shapes and organisations: i.e., (*i*) increasing elongation of cells (or colonies) and (*ii*) multicellular colonies with shorter GALD such as star-like, vesicular and dense coenobial structures (Table 1).

The monotonously increasing trend was not characteristic for the plots where fractal dimensions of the objects were compared to other shape measures (Fig. 1k–o). In these cases, decreasing trends were more characteristic. The reason for these patterns is that while the elongation indicates a gradual increase in the majority of shape metric values, in the case of FD the minimum value is 1.0 that represents an elongated, thin filament and increases towards the

sphere (FD = 2). Larger FD values are characteristic of spherical objects with projections.
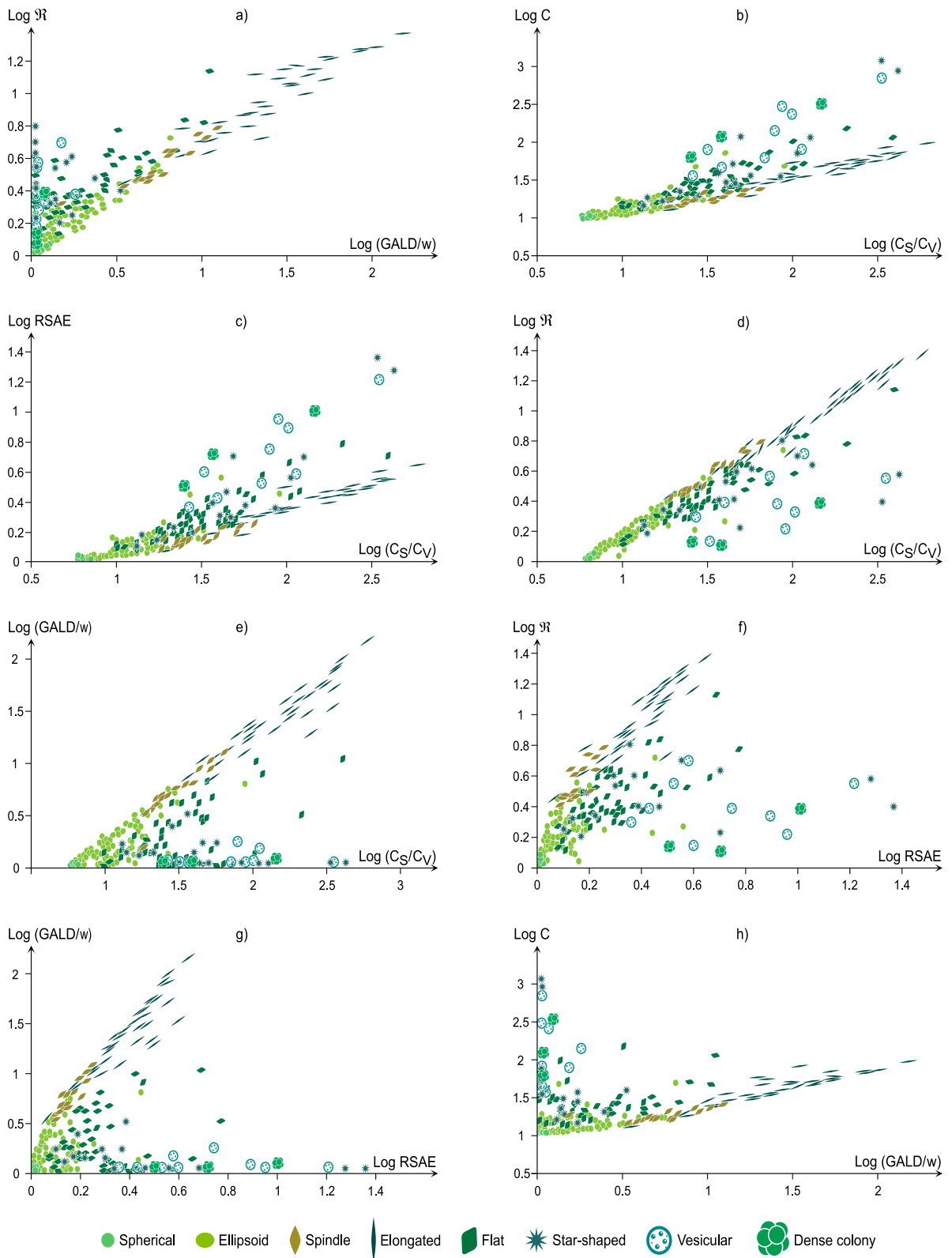
Relationship between the GALD and the shape measures

The GALD of organisms appeared to be an increasing function of most of the shape measures (Fig. 2). The exception is the FD–GALD relationship, where an opposite trend was detected. The strongest relationship was observed for the Cs/Cv value, while the fractal dimensions did not perform well in explaining the shape variation. The data were highly heteroscedastic with increasing variance as FD values increased. Because of the strong deterministic relationship between C and RSAE the position of each data point was almost identical in Fig. 2c, d with the exception that in the case of C a smaller scatter of data points could be observed along the x axis. The symbols of different shapes are clearly separated along the GALD scale, showing that morphology sets the boundary of their largest attainable size.

### Relationship between the size and the surface area to volume ratio

We studied the linkage between the *A/V* ratio and the size (length, biovolume) of the taxa. We found that *A/V* of the shape types can be approximated by proposed hyperbolic function; see Figs. 3 and 4. The large scatter of data points along the length scale indicate that *A/V* ratios of some large sized elongated or multicellular colonies can be as high as that of small spherical or ellipsoid forms. Although, the scatter of data was similar along the two explanatory variables, in the case of the biovolume the reciprocal relationship was more pronounced.

Discussion

Quantitative characterisation of the shapes of microalgae needs to consider ecologically relevant numeric shape attributes. The surface variation seemed to be the most promising shape attribute because the surface of microalgae and cyanobacteria provide many ecological and physiological implications (Borics et al., 2022). Surface area of the objects appears explicitly in the formulas of Cs/Cv, FD, C and RSAE,

Fig. 1 Relationships between the shape metrics of taxa at $\log_{10}$ scale
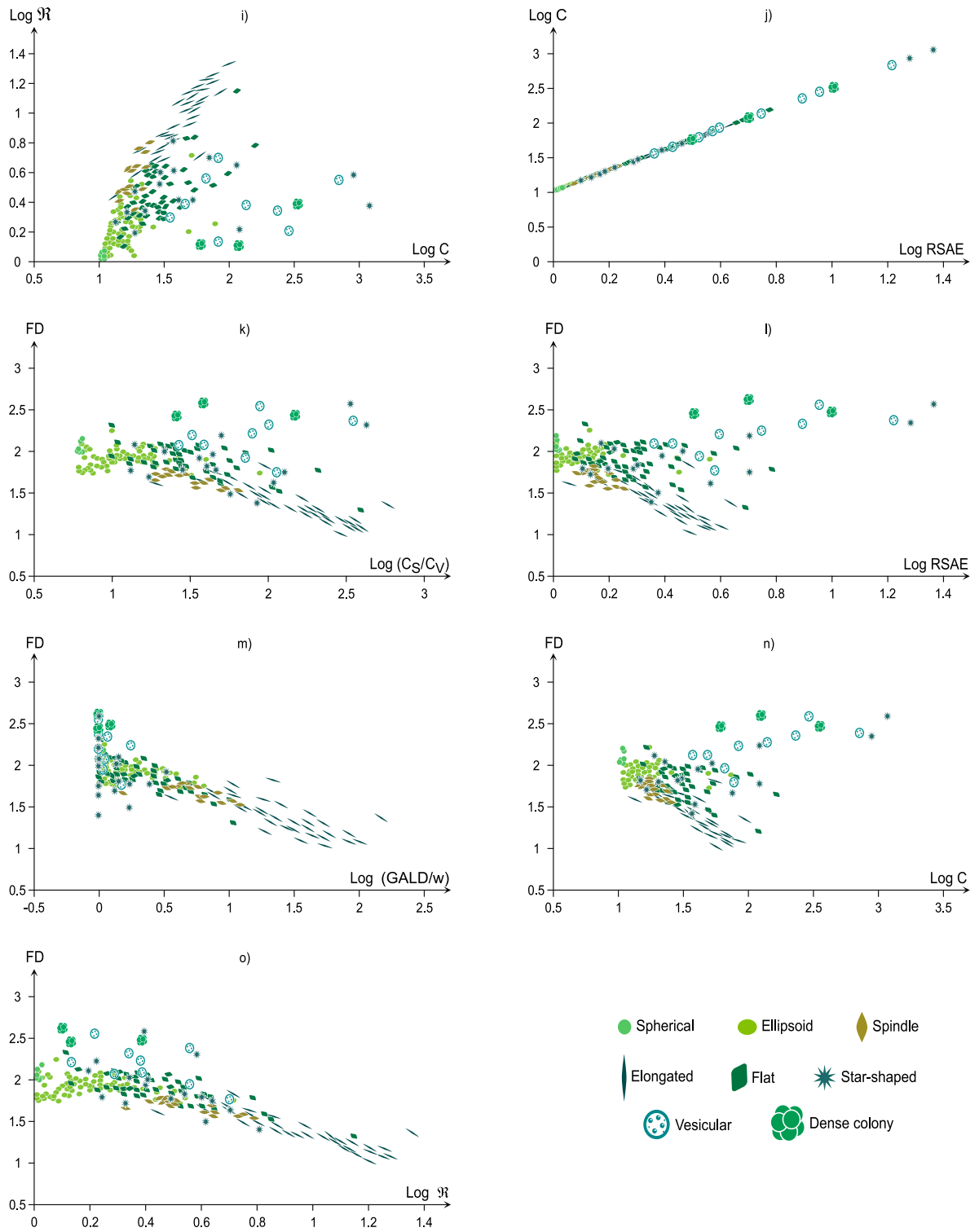
Fig. 1 (continued)

Table 1 Describing data structure of the different shapes of taxa in function of metrics

Compared shape measures	Slope values of the major axis regressions		Absolute values of differences between the slope values
	Elongated shapes	Other shapes	
log (Cs/Cv) vs. log C	0.48	1.12	0.64
log (Cs/Cv) vs. log RSAE	0.32	0.75	0.43
log (Cs/Cv) vs. FD	− 0.50	− 0.00	0.50
log (Cs/Cv) vs. log RE	0.71	0.48	0.23
log (Cs/Cv) vs. log (GALD/w)	1.09	1.31	0.22
log C vs. log RSAE	0.67	0.67	0.00
log C vs. FD	− 1.02	0.09	1.11
log C vs. log RE	1.49	0.28	1.21
log C vs. log (GALD/w)	2.28	− 1.01	3.29
log RSAE vs. FD	− 1.53	0.85	2.38
log RSAE vs. log RE	2.23	0.41	1.82
log RSAE vs. log (GALD/w)	3.42	− 1.52	4.94
FD vs. log RE	− 1.42	− 0.72	0.70
FD vs. log (GALD/w)	− 2.17	− 1.18	0.99
log RE vs. log (GALD/w)	1.54	1.68	0.14

and although not directly also in the other two measures, i.e., in the GALD/width ratio and in the $\mathfrak{R}$. For the latter two measures, the surface area is indirectly involved because the "reference" shape is represented by the sphere which has the maximum volume to its surface area. Direct comparison of the measures showed a bifurcation of the data points, which can be explained by differences in metrics' sensitivities. In the case of GALD/width ratio, FD, $\mathfrak{R}$, the elongation has a large impact on the metric values. In the case of the other three metrics, compact multicellular colonies also have higher values. When values of these metrics are plotted against the elongation sensitive ones, the bifurcation of data points becomes easily recognisable. Elongated cells constitute one branch of the fork. Elongation is a one-dimensional increase in cell size which considerably increases the *A/V* ratio compared to a sphere of equal-volume. Elongation is an ancient morpho-evolutionary process that had led to the development of fusiform creatures in the early ages of microbial evolution (Walsh, 1992; Oehler et al., 2014). This was observed in the case of recent bacteria that switch from bacillary to filamentous morphology to avoid predation by protists (Justice et al., 2008; Möller et al., 2012). This beneficial morphological adaptation occurs at the whole size range of microalgae from the smallest (*Nannokloster*

belenophorus Pasch*.,* Trebouxiophyceae ~ 3 μm³) (Komárek, 1983) to the largest ones (*Closterium acerosum* Ehr. ex Ralfs, Zygnematophyceae, ~ 10,000 μm³) (Coesel & Meesters, 2007).

The other branch of the fork is constituted by multicellular colonies. There is an apparent trade-off between light and nutrient acquisition and grazing resistance of phytoplankton species (Lampert et al., 1994; Lürling, 2021). The large size that gives a competitive advantage to microalgal species living under large grazing pressure, reduces their nutrient and light harvesting capabilities. By forming compact multicellular colonies, microalgae can reduce this trade-off, because they can maintain large surface area to volume ratios even in the case of large biovolumes (Lewis, 1976).

Shape—GALD relationships

Dependence of the size of microalgae on their shapes has been revealed by each shape metric—GALD relationship (Fig. 2). The best model performances were achieved using the Cs/Cv metric, where the highest R-squared values were obtained. In the plots of shape–GALD relationships the microalgae of various shapes occupied different regions. While compact single-celled forms were
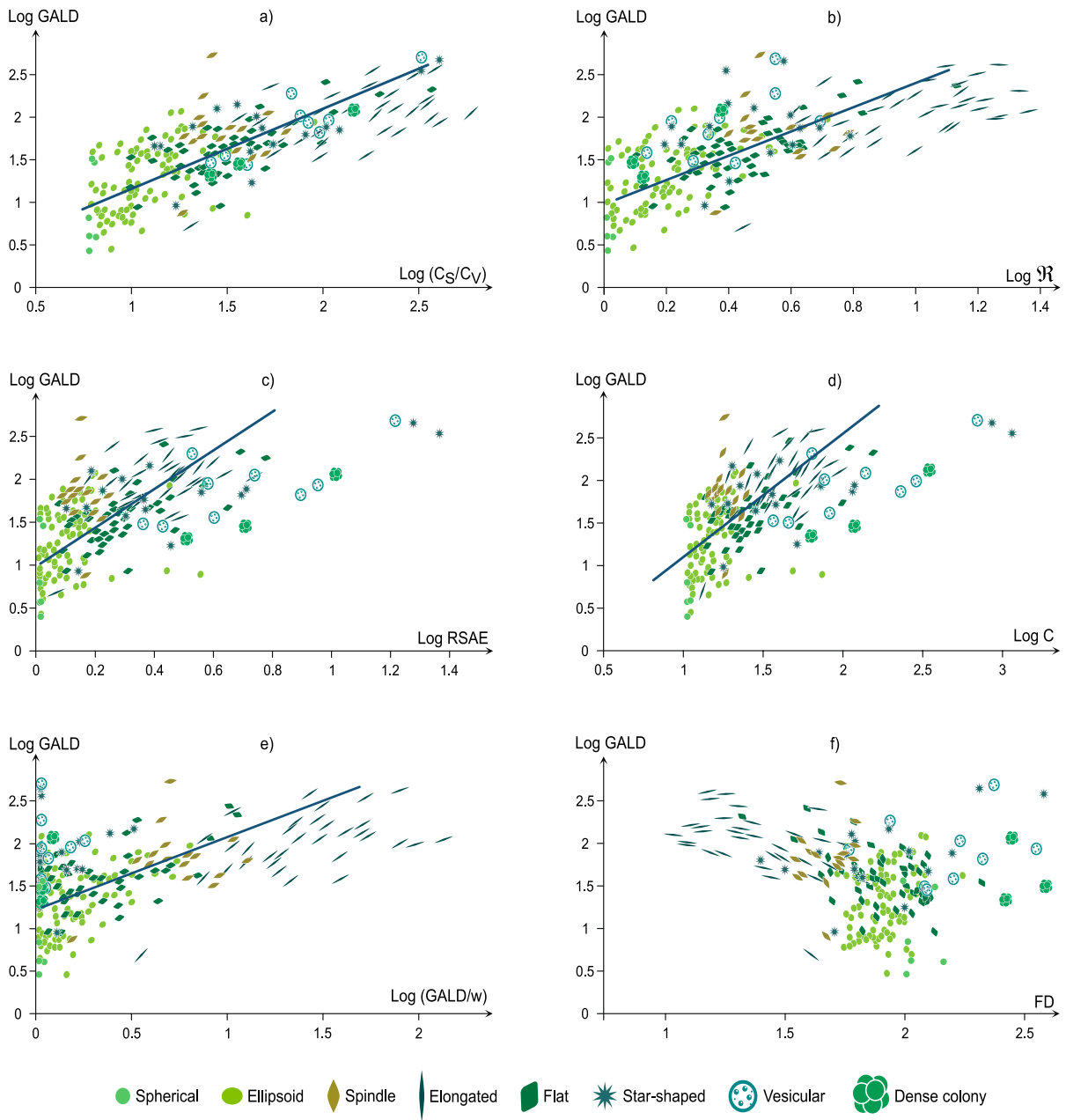
Fig. 2 Dependence of the size of taxa **a** on their shape-specific surface area constant/shape-specific volume constant (C_S/C_V) ratio **b** on their relative elongation ($\mathfrak{R}$) **c** on their relative surface area extension (RSAE) **d** on their compactness (C) **e** on their greatest axial linear dimension/width (GALD/w) ratio **f** on their fractal dimension (FD) at $\log_{10}$ scale

located only at small length values, elongated shapes were scattered almost along the whole length range. These results correspond well with that of former studies (Chisholm, 1992; Grover, 1989), which argue that elongation gives a competitive advantage to the elongated forms over spherical species of equal-volume. This advantage is due to the better light and nutrient acquisition, lower sinking velocity and diffusion limitation, and higher
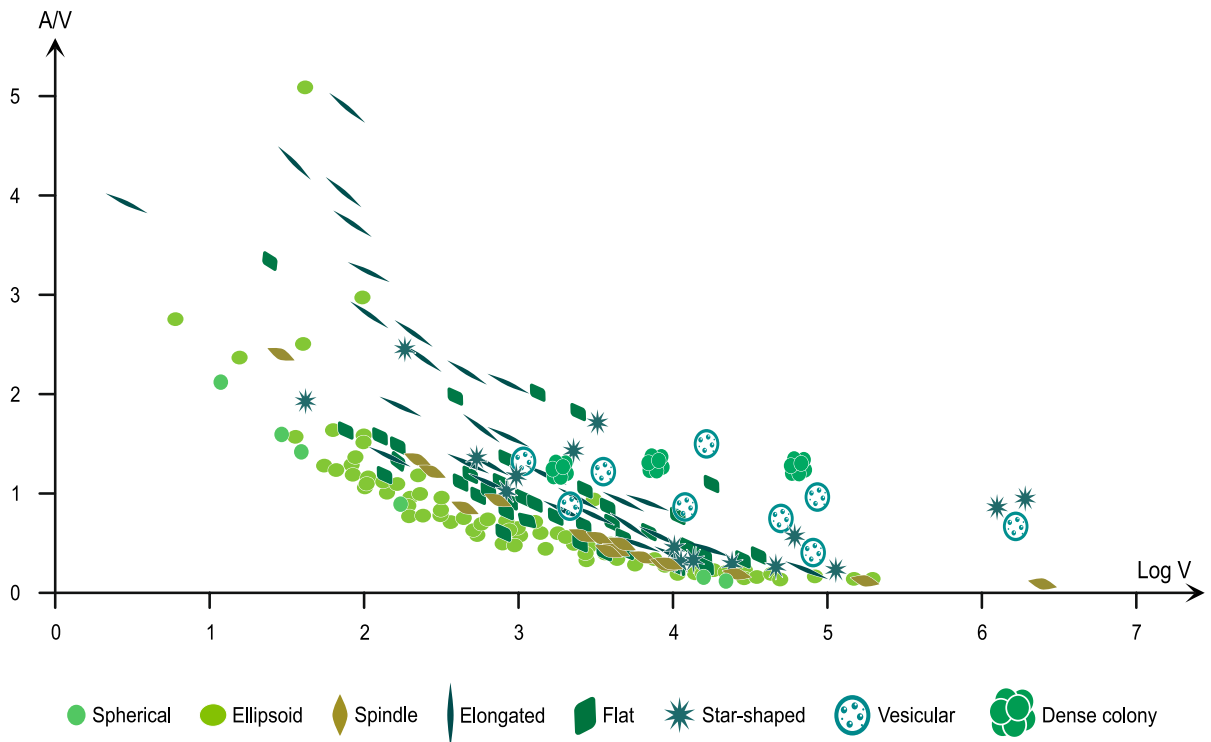
Fig. 3 Relationship between the volumes of taxa and their surface area/volume (A/V) ratios at $\log_{10}$ scale
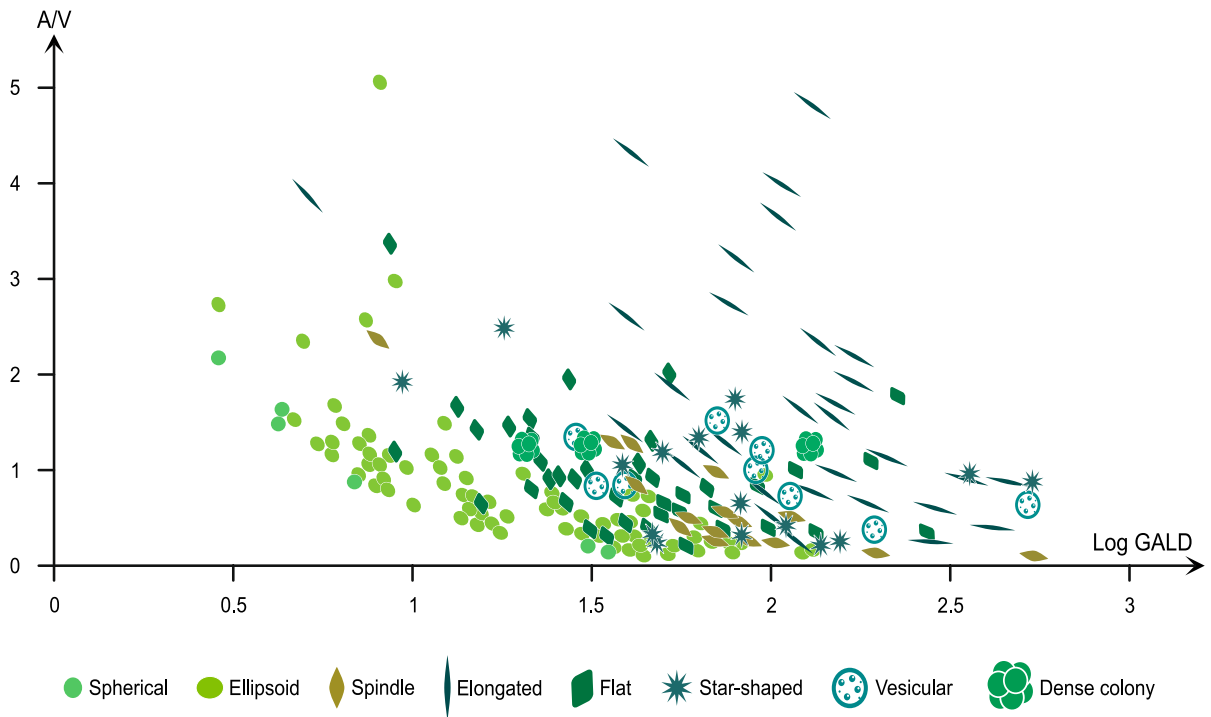
Fig. 4 Relationship between the greatest axial linear dimensions of taxa and their surface area/volume (A/V) ratios at $\log_{10}$ scale

grazing resistance of elongated forms (Padisák et al., 2003; Reynolds, 2006; Lürling, 2021).

Compact multicellular colonies also have several important adaptations, such as protection against intense illumination, buoyancy control, grazing resistance and good nutrient acquisition (Fulton & Paerl, 1987; Carlton & Paerl, 1989; Pettersson et al., 1993; Istvánovics et al., 1994; Reynolds, 2007). Surface area to volume ratios of compact multicellular forms can be as high as that of the elongated forms, but because of their smaller shape resistance, these taxa sink faster achieving a thinner concentration boundary layer (Kiorboe, 2008) and thus, a more effective nutrient acquisition.

We note here that the observed regularities in the size distribution of microalgal shapes are valid primarily for obligate photoautotrophic organisms, where the shapes, sizes and surface area to volume ratios are delicately balanced. High level of mixotrophy and especially phagotrophy fundamentally changes the rules of the game, because utilization of allochtonous organic compounds allows these taxa to survive with large biovolume and low A/V ratio (Borics et al., 2022).

We studied the maximum attainable size of microalgae in relation to their shapes. Shape and size are closely coupled traits and intuitively it is appealing to think that size controls the shape of microalgae. However, morphology of microorganisms evolved under tight control of complex genetic programs (Volke & Nikel, 2018), and in accordance with Bergman's rule (Bergmann, 1847) size of the organisms depends on the performance of shapes within the constraints of their habitats.

Size—Surface area to volume ratios

The inverse relationship between size and *A/V* ratio was apparent in the case of each shape category and both for length and biovolume (Figs. 3, 4). The exponents of the formulae fell within the expected ranges but were somewhat smaller than the theoretical values (length: − 1; volume: − 0.33), showing a less steep decrease in surface area along the size ranges.

Investigating the size dependence of A/V ratios in natural phytoplankton populations might occasionally result in different trends. Studying this relationship for marine phytoplankton (Southern Adriatic/Ionian Sea) Stanca et al. (2013) found similar patterns to ours in the case of most forms. However, there were considerable differences in the case of prisms, where the authors found increasing *A/V* ratios with organism size. Since theoretically this is not possible, the only explanation for this finding is that the applied shape categories were too general and contained shapes with highly different Cs/Cv shape constants. In this case, if the size range is not broad enough increasing tendencies might occasionally occur.

The high surface to volume ratios have several advantages, among the higher diffusion is one of the most important phenomena. Diffusion is the dominant transport mechanism at the size scale of microalgae because it governs the exchange of solutes between the cell and its surroundings (Karp-Boss et al., 1996). High A/V ratios confer competitive advantage to the species because of the higher diffusive flux of nutrients. However, the energy and material costs of creating a large outer cell envelope per unit volume can be high (Karp-Boss & Boss, 2016) and these costs might outweigh the gains. This should be the reason why small phytoplankton species ($<100~\mu m^3$) have simple subspherical or spheroid forms without useful but costly arms or protuberances of the cell envelope.

Concluding remarks

The aim of the present study was to provide mathematical formulas to numerically characterise the shape of microalgae. The sphere as a reference object directly or indirectly appears in each shape metrics. We are confident that the shape metrics shown above will facilitate the numeric description of the adaptive value of microalgal shapes as a function of environmental variables and serve as useful numerical traits of microalgae. This suggests the importance of the compilation of a trait database which contains the shape metrics for the microalgae species.

Author contributions VL, ET-K and GB developed the structure and wrote the manuscript. VL, IT, TK made the 3D models of microalgae. ZB, BB, ZV and BT carried out mathematical procedures. VL, ÁL, ET-K created the database containing the size measures of the objects and calculations. All

authors contributed to revisions substantially and gave final approval for publication.

Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical approval No ethical approval was required for the completion of this study.

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