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Review paper

Good Reasons and Guidance for Mapping Planktonic Protist Distributions

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Abstract. Analysing the spatio-temporal distribution of protistan microplankton has faced both conceptual and technical difficulties. However, recognition of a need to study planktonic patchiness, application of a major geology-based methodology (geostatistics) to ecology, and advances in computational technologies have widened the interest in this topic and made it more assessable. This review provides methodological and conceptual guidance on the application of geostatistics to microplankton analysis, using ciliates as example model organisms. Applying geostatistical analysis (and complementary methodologies) to the distribution of ciliate and phytoplankton reveals that attributes of their populations and assemblages (e.g. abundance, biomass, production, diversity) are patchily distributed at multiple spatial-scales in different aquatic environments, and these change over time. Data examined from several environments and scales exhibit distinct patterns of patches regarding their shape, density, and structure; these data can then be used to suggest a behavioural niche-separation of ciliates and the influence of patchiness on the rate processes of food webs. Throughout the review, basic guidance is provided for interpreting where, when, and why planktonic ciliate are so distributed, and directions for work on patchiness is offered, including a guide to the main literature on the topic. This should, therefore, be a useful stepping-stone for researchers interested in the impact of patchiness on protistan ecology, regardless of the environment.

Key words: Patchiness, spatio-temporal distribution, geostatistics, planktonic ciliate.

INTRODUCTION

Although space is the scenery where ecological interactions occur, its explicit incorporation into the study of planktonic ecosystems has faced impediments related to: the complex nature of fluid environments; the necessity for indirect observation of organisms; and the lack of appropriate statistical methods and compu-

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tational tools. However, there is now a growing number of ecological studies where the spatial component is explicitly recognized and studied using spatial-statistics that have been modified for plankton ecology (Franklin and Mills 2007, Bulit *et al*. 2003). Here I review these methods and provide direction for their application to the study of spatial and temporal heterogeneity (patchiness) of planktonic protists.

As early as in the 70s, Levin (1974, 1994) recognised that patchiness results from the interaction between physical and biotic processes and occurs at literally every scale, in both terrestrial and aquatic environments. In this review the word "patchiness" is used

to describe the distribution of organisms aggregated in space or time, as opposed to random distributed. Implications of such patchy distributions include the relationships between trophic levels in food webs and predator-prey interactions (Folt *et al*. 1993), but also see Table 1. For instance, recently, patchiness of food (i.e. distribution and density) has been shown to regulate predator-prey relationships in marine pelagic environments and results in bottom up forcing with higher effect at higher trophic levels (Benoit-Bird and McManus 2012, Benoit-Bird *et al*. 2013). Other processes such as the exchange of material and organisms across boundaries and the concentration of different resources are also mediated by patchiness. Moreover, patchiness provides protection, increases mating opportunities, and facilitates reproduction and genetic transfer between populations (Marquet *et al*. 1993). Clearly, patchiness is a key process that requires study in all environments, including the pelagic realm.

In fact, spatial and temporal heterogeneity are more the rule than the exception for plankton, and recognising this is fundamental for understanding population dynamics and the organization and stability of communities (Pinel-Alloul *et al*. 1995, Mehner *et al*. 2005). An issue with such phenomena, however, is that patchy data generally do not lend themselves to identification by traditional statistical analysis, as data will generally be neither randomly nor uniformly distributed; this leads to problems during statistical analysis, as the assumption of independence between samples is violated. As ecologists have become more aware of the importance of the spatial components of the phenomena they study, and as the number of statistical and computational tools available for quantifying these processes has increased, explicit consideration of spatial structure in aquatic studies has become more common (Franklin and Mills 2007).

Specific to this review, different communities and populations of protists, including benthic foraminifera and diatoms (Ferraro *et al*. 2006, Kienel and Kumke 2002), soil ciliates and amoebae (Acosta-Mercado and Lynn 2002, Mitchell *et al*. 2008), phytoplankton (e.g. Montagnes *et al*. 2010a), and planktonic ciliates (Bulit *et al*. 2003, 2004; Montagnes *et al*. 1999) are patchy in their distributions. Here I review how the patchiness of protists can be assessed using geostatistics, an established method of spatial statistical analysis. In doing so, I indicate how this analysis can be combined with other methods, to reveal how ecological processes act on spatial patterns.

The methods I present have been demonstrated to be useful in both terrestrial and in aquatic environments for microbial and macroscopic organisms (Fortin and Dale 2005, Franklin and Mills 2007). However, to illustrate these applications, and in keeping with this special issue on microplankton, I use planktonic ciliates, as they play a key role in pelagic food webs (Pierce and Turner 1992, Weisse 2006, Sherr and Sherr 2008). Moreover, planktonic ciliates have been intensively studied (e.g. abundance, biomass, production, diversity) in a range of environments and exhibit characteristic spatial patterns that may be used to interpret patterns displayed by other protists. Additionally, ciliate populations often rapidly fluctuate, in both space and time, with populations displaying boom-bust events coupled with spatial patches; thus they provide a tool to assess temporal and spatial variation over short periods.

In summary, the main goals of this review are to: 1) draw attention to the potential of geostatistics analysis for spatio-temporal studies; 2) offer guideline on how to proceed with sampling design and analysis of data to explore patchiness; 3) illustrate how other statistical tools can be used in combination with geostatistics to assess the reasons for patchiness and; 4) outline problems that can arise and propose future work.

THE GEOSTATISTICAL APPROACH

Geostatistics allows us to: assess the spatial structure of a variable (e.g. abundance, biomass, production, diversity); estimate spatial and temporal scales of variation; map variables for visual interpretation; estimate error associated with distributions; and combine the above information with other methods, such as multiple regression, principal component analysis, and multidimensional scaling, to infer processes associated with patchiness.

At a basic level, the method is relatively simple. As indicated above, aggregated (or patchy) data are by definition neither randomly nor uniformly distributed; rather, they are autocorrelated, with measured variables (e.g. abundance) more similar to each other the closer the sampling sites (Legendre and Legendre 1998, Ettema and Wardle 2002). Geostatistics specifically takes advantage of this autocorrelation (Legendre 1993). Originally developed to evaluate the distribution of minerals for the mining industry (hence its name), geostatistics was first applied to ecological problems in

Table 1. Type of questions related to planktonic protists patches that geostatistics might answer, with indications of basic software and references mostly related to geostatistical analysis.

Problem	Procedure, analytical tool	Some available free software[1]	Selected references
Methodological			
1. How should sampling be conducted (sample number, grid size, grid shape, spatial layout) to detect and characterize a patch?	Pilot sampling using different sample spacing. Based on geostatistical analysis, sampling points can be added where kriging error is higher and omitted where error is lower	– Geo-EAS (V-K-2D[2]) – S-GeMS (V-K-C-3D) – R (ST) – Variowin (V, 2D)	Atkinson and Tate (2000) Fortin and Dale (2005) Goovaerts (1997) Isaaks and Srivastava (1989) Webster and Oliver (2007), Yfantis *et al.* (1987)
2. How to determine the appropriate sampling scale to assess planktonic patches?	Pilot sampling, sampling at different scales, nested sampling at different scales	– Geo-EAS (V-K-2D) – S-GeMS (V-K-C-3D) – R – Variowin (V, 2D)	Atkinson and Tate (2000) Armstrong (1998) Bellehumeur *et al.* (1997) Fortin (1999) Webster and Oliver (2003)
3. At what spatial scale does patchiness occur?	Sampling design at different scales, geostatistical analysis (variography, kriging)	– Geo-EAS (V-K-2D) – S-GeMS (V-K-C-3D) – R – Variowin (V, 2D)	Bulit *et al.* (2004) Franklin and Mills (2007)
Analytical			
4. Are patches uniform in shape, or are they extended in a preferred direction, and what causes this?	Directional variograms, variography, kriging, for environmental and biotic components	– Geo-EAS (V-K-2D) – GSLIB (V-K-C-3D-S) – R – Variowin	Isaaks and Srivastava (1989) Rossi *et al.* (1992)
5. Is it necessary to examine three-dimensional patchiness?	3D variography, 3D kriging	– GSLIB (V-K-C-3D-S) – S-GeMS (V-K-C-3D) – SADA (V-K-3D) – R	Keenan *et al.* (2007)
6. How can patches be followed in time?	Geostatistical analysis: spatiotemporal variogram, temporal variogram and kriging, time series analysis, autocorrelation function	– Gstat (ST) – R (ST) – BMELib comm	Cressie and Wikle (2011) Diggle (1990) Kyriakidis and Journel (1999) Sedda *et al.* (2010)
Mechanistic			
7. How do can forces that shape patchiness be identified (i.e. why are patches formed)?	Variography, kriging combined with complementary methods: regression, ordination, etc.	– R – S-GeMS (V-K-C-3D) – Variowin	Franklin and Mills (2007)
Ecological impact			
8. To what extent is patchiness of populations related or attributable to environmental patchiness?	Spatial relationships among variables: multivariate geostatistics, cross variograms, co-kriging (also useful for an under-sampled variable correlated with a well-sampled variable)	– Agromet (V-K-C-2D) – GeoPack (V-K-C-2D) – Gstat (V-K-C-3D) – R – S-GeMS (V-K-C-3D)	Chilès and Delfiner (1999) Goovaerts (1997) Wackernagel (1988)
9. Does patchiness of protists have an effect on organisms of other functional groups?	Combining geostatistical analysis of organisms belonging to different functional groups		Robertson (1995)
10. To what extent does patchiness influence food web dynamics? To what degree does food webs display patchiness?	Spatial analysis of organisms of different trophic levels		Benoit-Bird *et al.* (2012, 2013)

[1] A description of free and commercial software can be found at http://www.ai-geostats.org/bin/view/AI_GEOSTATS/WebHome (G. Dubois 2011, October 31, retrieved July 13, 2013). [2] Keys: V: variography, K: kriging, C: co-kriging, S: simulation; 2D/3D: dimensions, ST: spatiotemporal analysis

the 1980s and has became the underlying tool of several standard mapping programs (e.g. Surfer, SPlus, GS+) that are routinely used by plankton ecologists. However, like many analytical software packages, such tools rarely allow researchers to carefully assess their data; by their simplicity they may constrain analysis, forcing the user to follow set procedures and possibly not obtain the most from hard earned data sets (Table 1). Developing a fundamental understanding of the underlying principles of geostatistics allows researchers to, at the worst, recognise the limitations of standard programs and, at best, write code for their own analysis, using programs such as R, to tailor the analysis to their needs. This may be demonstrated using planktonic protists as an example.

Sampling: Requirements and strategies

To assess patchiness of planktonic protists, a sampling design is needed to: 1) determine the size of a patch, which might indicate, for instance, the distribution of resources; 2) quantify the distance between patches, as this could indicate the distance that consumers must move to encounter prey; 3) describe the uniformity of patches and the presence of "cores" that might suggest their origins; and 4) investigate the spatial orientation of patches, which might indicate their dispersal by environmental factors such as currents and winds. The geostatistical analysis that assess these attributes uses the variance of the studied variable (e.g. abundance) as a function of distance between sampling locations; this is based on the intuitive assumption that neighbouring sample values are more similar to each other than those far apart (i.e. they are more autocorrelated and will have a small variance). Underlying this phenomenon of spatial autocorrelation are, of course, biological processes, such as reproduction, growth, predation, and parasitism which impose further shape on the spatial structure; however these will be considered later.

Data for geostatistical analysis can be continuous or categorical. The former provide a measurement on a continuous scale, and most data used by ecologists belong to this type; e.g. characterizing patchiness of ciliate biomass or abundance across a defined basin to assess food web dynamics (Montagnes *et al*. 2010a). However, to obtain insight into the ecological structure of ciliate assemblages, it might be appropriate to designate them to trophic groups (e.g. autotrophs, mixotrophs, heterotrophs, bacterivores, algivores, carnivores) or size classes, related to availability to predators; these data are categorical. Clearly, the resolution and the number of categories will influence the ability to assess patchiness. Consequently, I would recommend that researchers determine taxa to the highest possible resolution. Then, patchiness of categorical groupings (such as trophic levels) could be assessed based on the knowledge of the involved group. By combining both approaches through geostatistics this may, in fact, provide a novel direction in plankton ecology, one that to my knowledge has not been explored (Table 1).

Clearly, samples must be collected over a defined region, ideally, but not necessarily, forming a regular grid (Fig. 1a); using a sampling scheme with fixed separation between observations (systematic sampling) generally allows more accurate estimates than random sampling, but irregular sampling can be adequately analysed. Different geometric forms of sampling grids have been proposed and evaluated (Yfantis *et al.* 1987). Sampling in aquatic environments may also include three dimensions, as in the case of data collected with acoustic echosounders (Kaltenberg and Benoit-Bird 2013), and specific software to deal with 3D data sets is available (Table 1). Furthermore, sampling should, ideally, be designed with a geostatistical analysis in mind, but many data sets may lend themselves to analysis, even if this was not so. One essential consideration for the design of field sampling is the spatial scale for detecting the pattern and its underlying process: the spatial structure that is ultimately identified depends on the chosen scale; obviously, it cannot be smaller than the smallest distance between samples or larger than the extent of the study (Legendre and Legendre 1998).

It follows then that when possible, a pilot study is needed, to determine dimensions of the sampled area, variability in different directions, and number of samples. Some authors (e.g. Webster and Oliver 2007) consider that a minimum of 150 data points across a landscape is required for a reliable estimation of the variogram (the key component of geostatistics, see below), while others support smaller data sets, as often occurs in petroleum reservoirs, when data are difficult to obtain and interpret (Goovaerts 1999). In fact, if the sampling interval (distance between samples) is within the range of the spatial pattern, fewer sampling points (20–30) may capture the essence of the pattern (Fortin and Dale 2005).

Occasionally, multiple scales of study may be needed to reduce effort but still identify patchiness, as patches may occur at different scales. For instance in a large landscape, focused fine-scale sampling would identify small patches while coarse-scale sampling would re-

kriging

Gaussian

exponential

spherical

$$\hat{\gamma}(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} \left[(z(x_i) - z(x_i + h)) \right]^2$$
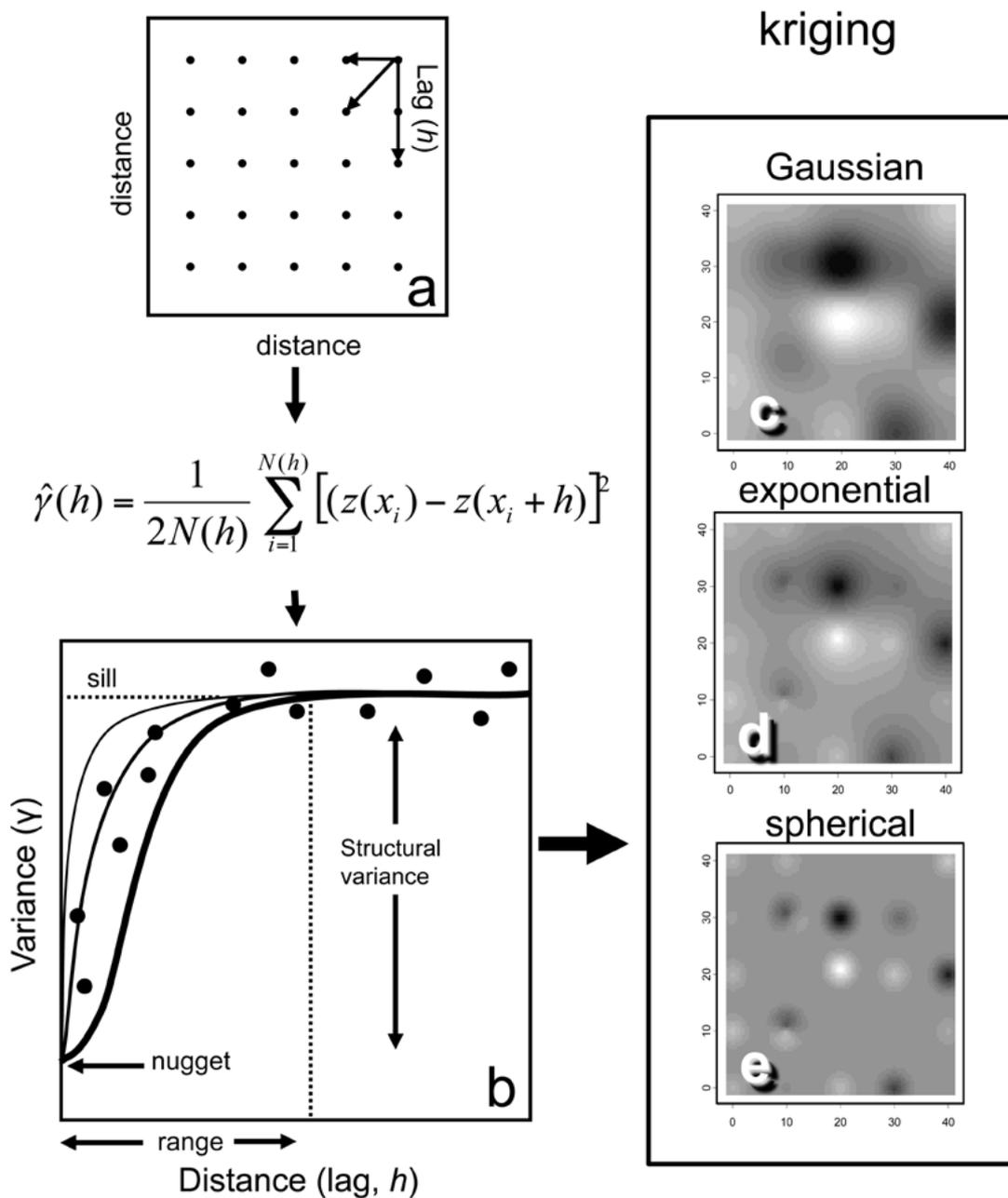
Fig. 1. A schematic description of establishing a variogram, modelling a function, and producing maps by kriging. Samples (e.g. to determine ciliate abundance) are collected at points of a sampling grid (a). Variance estimates of ciliate abundances at points separated by a common distance (lag, *h*) are calculated using the equation (explanations in the text); this is repeated for each lag (three examples of lags are illustrated in a). Each variance estimate is then plotted against its respective lag to produce an empirical variogram (points in b). Then, a model is fit to the variogram data (lines in b), and the model is used to predict abundance at unsampled points and to characterize patches. The parameters of the variogram models are the nugget, the range, and the sill (see text for their interpretation). Three models are the most common: the Gaussian, spherical and exponential (thick, medium, and thin lines, respectively, in b). Models are used to map ciliate abundance by the kriging procedure, with each model producing different predicted distributions (c, d, e): the spherical and exponential produce "fuzzier" images than the Gaussian.

veal larger patches; the fine scale need not be applied across the entire landscape. This can be illustrated by a study on the distribution of the bloom forming mixotrophic ciliate *Myrionecta rubra* (= *Mesodinium rubrum*) as a case study. This study used a sampling scheme of hierarchically nested squared grids (of side 4, 40, and 200 m, see Fig. 7 in Bulit *et al.* 2004). In doing so, it demonstrated that changes of one order of magnitude (20 to 250 cells ml^{-1}) existed in a 16 m^2 area, and that differences of ~ 3 orders of magnitude (1 to 700 cells ml^{-1}) were evident in the 40,000 m^2 region. Patches of a maximum size of 170 m, with abundance continuously increasing toward a core located off centre within the sampling area, were detected only when data of the three scales were simultaneously examined, by incorporating them in the analysis.

The size of a sample, the spacing among sampling points, and the extent of the area sampled will be restricted by time, effort, and cost. One approach to being economical with time and effort is to apply theoretical models of spatial variation and previous knowledge of the pattern or process of interest to choose an optimal sampling scheme. To my knowledge this approach has not been applied to planktonic protists, but there is now sufficient data available to guide future studies (Bulit *et al.* 2003, 2004, 2009, 2011, 2013; Montagnes *et al.* 2008, 2010a), and the reader is directed to Dolan and Stoeck (2011) for concepts and directions on how to apply these data.

Analyzing data: An introduction to the variogram, its modelling and mapping

Geostatistical analysis is based on a very simple concept: samples sites that are close together will have similar values (e.g. abundance). This can be demonstrated using the example of the distribution of the abundant, small (15 µm) planktonic ciliate *Lohmaniella oviformis* (from Bulit *et al.* 2003). For instance, we might assume that water samples that are collected 1 m apart will have similar numbers of *L. oviformis*, while those 100s meters apart will be more likely to differ. If we compare all pairs of samples that are collected at a small distance, say 1 m, to one another (distance is termed a "lag" in geostatistics) and calculate an "average" variance for these pairs, the variance should be small. In contrast if the lag was large, say 100 m, we might obtain a high variance when comparing all the pairs of samples. Eventually though, regardless of the size of the lag, the variance will not increase further; it becomes asymptotic (Fig. 1b).

How then is this variance calculated? For a single lag (e.g. abundance values for all points separated by 10 m), differences are summed, squared, and divided by twice the number of pairs (Eq. 1). This process is repeated for each lag, and each of the variance estimates is then plotted against the lag to produce an "empirical variogram" (points in Fig. 1b). The variance (*h*) for the whole range of distance intervals *h* is determined as,

$$\hat{\gamma}(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} \left[(z(x_i) - z(x_i + h))^2 \right] \tag{1}$$

where $N(h)$ is the number of observation pairs separated by lag h, $z(x_i)$ is the value of the variable of interest (e.g. abundance) at any one point, and $z(x_i + h)$ is its value at lag h from $z(x_i)$. One modification to the analysis is to minimize biases due to "border effects" of the sampling area and the subsequent reduction in the number of pairs, by only using lags that are less than half the maximum distance of the sampling area; furthermore, all points of the variograms should include > 30 data pairs (Isaaks and Srivastava 1989). This again aids in sampling design (see above).

Once data are analysed by the above method, they should ideally follow a pattern similar to that depicted in Fig. 1b. Then, the next step is to assess the shape of the empirical variogram. To this end a model (function) is fit to the variogram points (lines in Fig. 1b); these models ultimately allow us to predict values at unsampled points of the sampling area, to interpolate for mapping the distribution (e.g. abundance), and to characterize patches. Regardless of the model chosen, features of patches can be indicated by the three fundamental and extremely useful parameters of variogram (Fig. 1b): 1) the "range" or distance between the smallest sampled spacing and where the data become asymptotic indicates the mean extent or size of patches; 2) the "sill" or asymptote provides the total variability of the data in the sampling region and, 3) the "nugget" (a term denoting the geological roots of geostatistics) or *y*-intercept indicates the difference in abundance between two samples at an infinitely small distance from each other, either due to natural variation (e.g. real ciliate patches at a smaller scale than the one sampling), experimental error (e.g. different results in replicated counts of ciliates in Utermöhl chambers), or both. Then, the variability indicated by the sill is composed by the nugget variability and by the structural variability, which is the portion explained by the fit model.

Patches may differ in their characteristic distributions, and thus there may be different models that need to obey certain numerical properties and best fit the data of the empirical variogram. Three commonly used models are the spherical, exponential, and Gaussian functions (Fig. 1b, c, d, e,). The spherical model reaches the sill value at the specified range while the exponential and Gaussian models approach the sill asymptotically. The Gaussian model has a parabolic behaviour at the origin appropriate to represent very smoothly varying properties. The spherical and exponential models, with a linear behaviour at the origin, are adequate for representing attributes (e.g. abundance) with a higher level of short-range variability (Isaaks and Srivastava 1989, Goovaerts 1997, Armstrong 1998). Deciding which model best fits the data is an issue beyond this review, but it can be a combination of visual assessment, statistical procedures, and ancillary knowledge of the researcher; for more detailed guidance see Goovaerts (1997).

The next step is to map the patchiness, by predicting values (e.g. ciliate abundance) between the places where they were sampled. Using a process called "kriging" (after D. G. Krige), the selected model is then used to interpolate between sampling points to predict and to map the distribution (Fig. 1 c, d, e; Goovaerts 1997). Based on this analysis, Bulit *et al.* (2003) quantitatively defined a planktonic ciliate patch as the region where abundance is above the cut-off of the upper quartile, based on kriging predictions. This working concept of a patch allows researchers to delimit patches on maps and characterize distributions.

Finally, to complete the procedure, it is useful to estimate the confidence in the predicted distributions, providing an indication of the precision of boundaries and distributions. To this end, error-maps associated with kriging predictions can be calculated (for the case study of *L. oviformis* see Fig. 2c). Again, the details of this procedure are beyond the scope of this review, and the interested reader is directed to Goovaerts (1997).
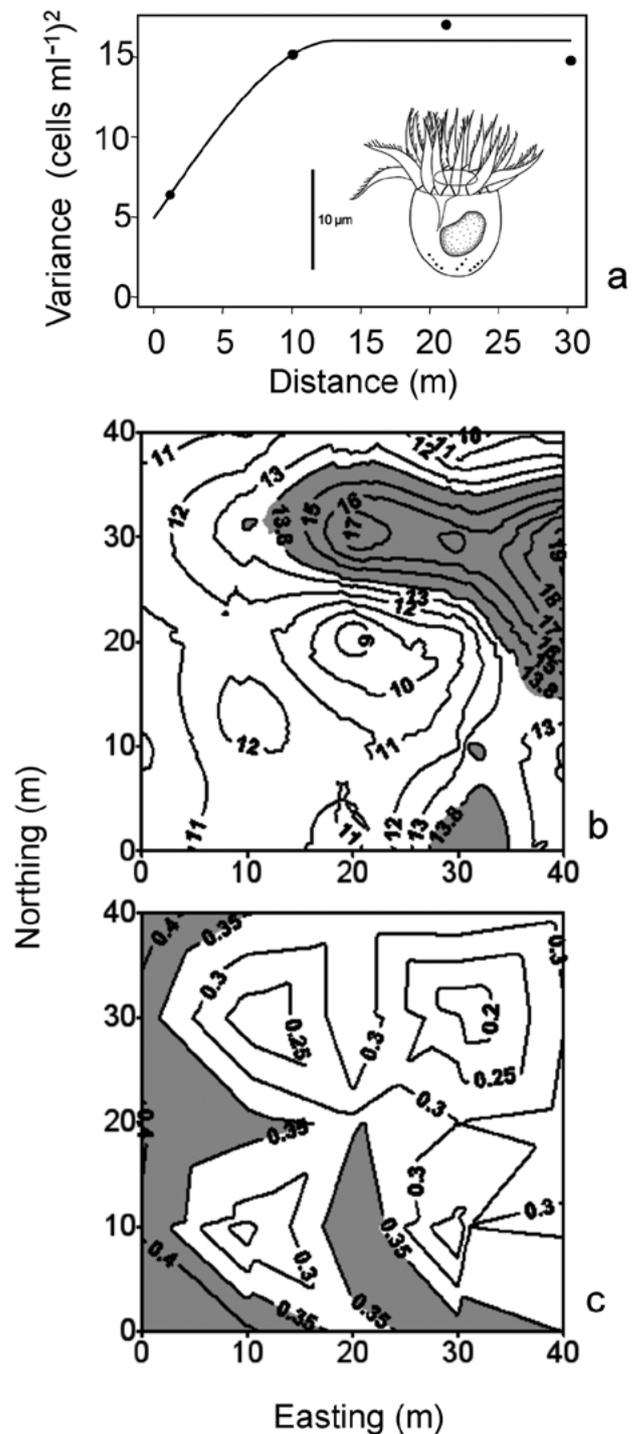
Fig. 2. Geostatistical analysis of *Lohmaniella oviformis* (inset in a) abundance (cells ml^{-1}) produces: a) the variogram, b) the kriging map, and c) a map of the coefficient of variation (CV). A spherical model (a, line) is fit to the empirical variogram (a, points); the points account for different number of pairs of abundance averaged on a class distance (lag). Only half of the maximum distance was calculated and represented to avoid the edge effect, where there are fewer sampling points (see text). The model (a, line) is used to predict abundance at unsampled points and to assess characteristics of patches. The model is also used to map patches of *L. oviformis* abundance (b, grey areas) using the kriging interpolator; a patch is operationally defined as abundance in the upper quartile. On the CV map (c), grey areas (with lower abundance and closer to edges) have the highest coefficient of variation of the estimated distribution.

All the above steps can be done using a range of statistical packages (see Table 1 for specific indications). Readers who plan to pursue this form of analysis are strongly encouraged to consult Cressie (1993), Goovaerts (1997), Diggle *et al*. (1998), Webster and Oliver (2007) and Table 1 for a more detailed list of appropriate literature.

Interpretation of results

Analysis and modelling of the variogram: Using the process described in the previous section allows the attributes of patches and spatial distribution to be characterized, as each component of the variogram will reveal distinct features of patches. This may be demonstrated, once again, by using the planktonic ciliate *L. oviformis* from Bulit *et al*. (2003, Fig. 2) as a case study.

First, the best fit to the empirical variogram (i.e. points, Fig. 2a) of *L. oviformis* data was made by a spherical model (i.e. line on Fig. 2a) and a nugget effect (i.e. a y-intercept > 0 at an infinitesimally small lag). The presence of a nugget indicates that at a distance below 1 m (the smallest sampled interval) there may be further small-scale patches that were not observed, and we may ultimately need to sample at a higher resolution, possibly at cm-scale, to understand the patchiness of this ciliate. The combination of a spherical model with a small range and the nugget effect then indicates that there are many small patchy areas, with high and low abundance across the sampled area (Fig. 2b). In contrast, if the model that best fit the data had been Gaussian, then this would indicate small organised patches across the sampled landscape, whilst if the modelled variogram had been exponential, then this would indicate patchiness across the sampled landscape was between the first two predictions (Fig. 1c, d, e). Knowing that this ciliate consumes small phytoplankton, we might conclude from the predicted patchiness of *L. oviformis* that its prey are equally patchy and that it may exhibit rapid, small scale predator-prey dynamics forming small blooms in localised regions (but see Temporal variability and periodicity, below).

As indicated above, the sill (Fig. 1b) is composed of two parts: the nugget and structured variability. For *L. oviformis* (Fig. 2a) the nugget accounted for 30% of the total variability; this is variability due to "noise" that we cannot detect. In contrast the structured variability, literally defining the structure of patches (represented by the difference between sill and nugget), accounted for 70%. A large nugget, as in this case, indicates diffuse patches, while a small one indicates well-structured, tight patches across a landscape (Dalthorp *et al*. 2000). We can then predict that patches of *L. oviformis* are somewhat "fuzzy," possibly suggesting dispersal following a population bloom. From this analysis, we, therefore, have now a better understanding of the distribution of *L. oviformis*, not only in our sampled area but across the lagoon. This will allow us to make predictions of how patches may be encountered or may spread and how this ciliate may act as a grazer of patches of prey and as food for other higher trophic organisms.

For *L. oviformis* the range (see Fig. 1b) was ~ 10 m (Fig. 2a), which predicts the mean size of discrete patches across the sampled landscape (Fig. 2b). Again, we may predict that these small patches arise from rapid population growth, as small ciliates can have generation times of hours in warm waters (Montagnes 1996). Moreover, the behaviour of *L. oviformis*, which is able to rapidly swim and reorient itself, might have contributed to accumulation in patches (Bulit *et al*. 2003). Geostatistical analysis thus provides observational limits for these patches at small scale that have generally not been proposed, and allows ecologists, with an understanding of the ciliate's biology, to infer potential causes for patchiness.

Finally, another ecologically important component, not outlined in the sections above, that can be obtained from the variogram, is a directional component of patches. For example, in aquatic ecosystems winds are likely to elongate patches of plankton, and this characteristic can be detected by calculating variograms for different orientations (see Rossi *et al*. 1992, Table 1). Analysis of *L. oviformis*, however, indicated that there was no directional component (the variogram was "omnidirectional", Bulit *et al*. 2003). We can, therefore, conclude that although there can be onshore and offshore winds in the lagoon, conditions, in this case, were sufficiently stable during the sampling to not influence the patch direction and shape.

Prediction mapping and associated error: Once the variogram has been established, the next step is to generate a prediction map (Fig. 2b) and an estimation of its error (Fig. 2c). The variogram model is used to predict abundance at unsampled locations by ordinary "kriging", a linear prediction method (for details see Goovaerts 1997), and then maps of abundance (Fig. 2b) can be produced to assess the distribution across the sampled region; e.g. for *L. oviformis* (Fig. 2b) the patch (as defined above) covers 23% of the total explored area.

However, like any statistical estimates there is error associated with prediction of the spatial distribution. For the case of *L. oviformis*, error was mapped as the coefficient of variation (CV), to indicate the precision of the estimated distribution (Fig. 2c). Here, the CV ranged from ~ 15 to 40%; as long as the CV is < 100%, we can conclude that outlying or erratic values are rare or nonexistent (Isaaks and Srivastava 1989). From this analysis we can be reasonably confident of our predictions regarding *L. oviformis* patchiness.

Issues associated with geostatistical analysis

Temporal variability and periodicity: Once patchiness of abundance has been detected and characterized, a further challenge is to assess temporal dynamics of patchiness, related to environmental and biological forcing. A complementary approach to geostatistical analysis is the study of temporal changes in patchiness (similar approaches have been applied to the transmission of trypanosomiasis using space-time covariation, Sedda *et al*. 2010). Such analysis will clearly require intensive sampling and effort to simultaneously characterize patchiness in both time and space. An alternate, simpler, approach may be to couple discrete measurements of spatial patchiness, as described above, with time-series data (Berryman and Turchin 2001), collected at a fix locality at regular time intervals over a long period. As with the spatial-autocorrelation, described above for spatial patches, many natural variables occurring over time are correlated; i.e. data that are temporally close together are more similar than those more distant. This property can then be employed to study population dynamics, periodicity of plankton production, and other fluctuating processes.

The use of such time-series data may be illustrated by another case study: temporal dynamics of *Cyrtostrombidium* sp., an easily recognised planktonic ciliate that forms small-scale spatial patches in a coastal lagoon (Bulit *et al*. 2013). Abundance data were collected weekly at a single, representative point for ~ 1 year. Data were then analysed using an autocorrelation function (ACF; Diggle 1990) that describes the correlation of data between successive observations with defined time intervals or temporal lags (cf. spatial lags for geostatistics, above). ACF was calculated between all pairs of *Cyrtostrombidium* abundance over the 57 weeks of the study, and then coefficients were plotted against time. Visual inspection of these data indicated temporal patchiness: positive spikes at lags 2, 3, and 4 indicate a short-term periodicity of ~ 3 to 4 weeks

(Fig. 3). Combined with the geostatistical analysis that indicated patches across the lagoon were ~ 30 m in size and a Gaussian model best fit the variogram, indicating abundance constantly increased inside patches (see Bulit *et al*. 2013), it was possible to predict that these patches represented ~ 1 month-long blooms of *Cyrtostrombidium*. This work then allowed us to make predictions regarding the population dynamics of this species, including the impact of parasites of the ciliate and the role of conjugation by the ciliate, both of which were observed during the study. Interpretation of these biological phenomena combined with the recognition of spatial and temporal patchiness and a knowledge of environmental factors (salinity, temperature, etc.) led us to speculate that biological factors rather than longer-term environmental changes drove *Cyrtostrombidium* population dynamics (Bulit *et al*. 2013).

Interactions of geostatistics with other methods for ecological studies: Multiple regression analysis is another useful tool that can be combined with geostatistics, as it can be modified to incorporate spatial and temporal correlation of data. For instance such methods have been used to explain the seasonal variability of ciliate diversity (rather than abundance of single species, as illustrated above). In this case, the analysis indicated that diversity increased due to changes in the physical environment, when the local region shifted from freshwater to brackish (Bulit *et al*. 2009).
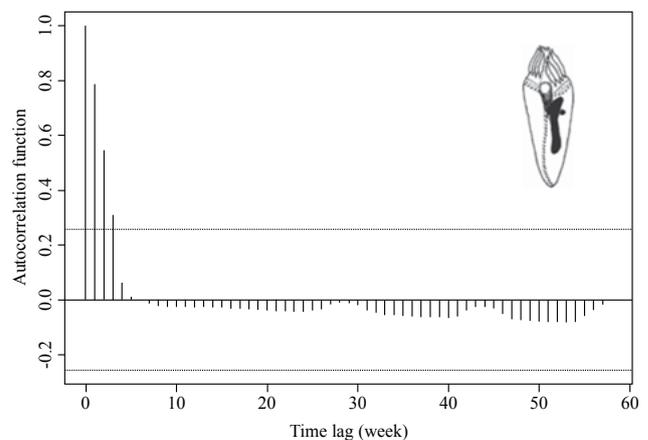
Fig. 3. A time series of *Cyrtostrombidium* sp. (inset) abundance during ~ 1 year at a fix point in a coastal lagoon. The autocorrelation function indicates positive spikes for weeks 2, 3 and 4 suggesting a persistence of *Cyrtostrombidium* bloom for ~ 1 month. Horizontal dashed lines indicate the ~ 95% confidence interval for the significance of each autocorrelation value.

More detailed information on the factors that impact diversity patches can then be obtained by combining geostatistics with principal component analysis (Lepš and Šmilauer 2003). In the above case where the distribution of ciliate diversity was studied (which again was in a coastal lagoon), the first principal component indicated a hydrodynamic axis with strong influence of salinity associated with the influx of marine waters. The second principal component indicated that more transparent waters of marine origin are associated with patches of higher ciliate diversity (see Fig. 3 in Bulit and Díaz-Ávalos 2009). In another case study that investigated the effects of seasons and biogeographical regions on the distribution of ciliate abundance in the open Atlantic Ocean (Irminger Sea, Montagnes *et al*. 2010a), geostatistical analysis was complemented with multidimensional scaling (MDS, Honarkhah and Caers 2010). By computing similarities between patterns of diversity at defined distances, this analysis allowed the authors to conclude that seasonal differences in ciliate taxonomic composition and abundance did not relate to previously described hydrographic zones in the region, supporting the concept of biological forcing of patchiness, rather than by large scale oceanographic processes.

These examples demonstrate how geostatistics may be combined with other techniques to allow further analysis, to reveal more detailed information associated with ecological principles that may influence the formation of patches (Table 1). Inevitably as microplankton researchers embrace this methodology and recognise its potential, they will be encouraged to expand on these and develop a suite of tools to assess temporal and spatial heterogeneity in the plankton.

REVEALING ECOLOGICAL IMPLICATIONS OF PATCHINESS

Here I offer several examples of why the analyses described above should be relevant to microplankton ecologists. In doing so, I also present some new avenues for the application of spatial statistics to protist ecology. These examples are not exhaustive; rather I hope they will stimulate researchers to consider the wider applications of geostatistics.

Patchiness depends on the organisms

Above, for *Lohmaniella oviformis*, I illustrated spatial patchiness and argued why it may form patches from an ecological perspective, but this is not typical of all ciliates. For instance, when studying the distribution of a range of several ciliates across a 1,600 m^2 area, the variogram of abundance of the small bacterivorous ciliate *Pleuronema* sp. indicated a pure nugget effect; i.e. the abundance of *Pleuronema* was randomly distributed and did not form patches over the scale of 1–40 m (Fig. 4; Bulit *et al*. 2003). Likely, given its bacterivorous mode of nutrition (Fenchel 1987), *Pleuronema* will form patches around detrital clumps, on the order of microns to millimetres in size. Thus, a substantially smaller sampling scheme would be needed to detect patches of such bacterivores. This highlights the point that to assess the patchiness of protozoa, and ciliates in particular, there is a need to understand their biology.

Patchiness of ciliate diversity contributes to ecological theory

There is a substantial body of literature on plankton diversity (e.g. Hutchinson 1961, Margalef 1978, Irigoien *et al*. 2004). However, the spatial structure of planktonic diversity has been less well investigated, although its study is a challenge that should contribute to the development of ecological theory (Mackas *et al*. 1985, Levin 1992, Leibold and Norberg 2004, Chave 2013).
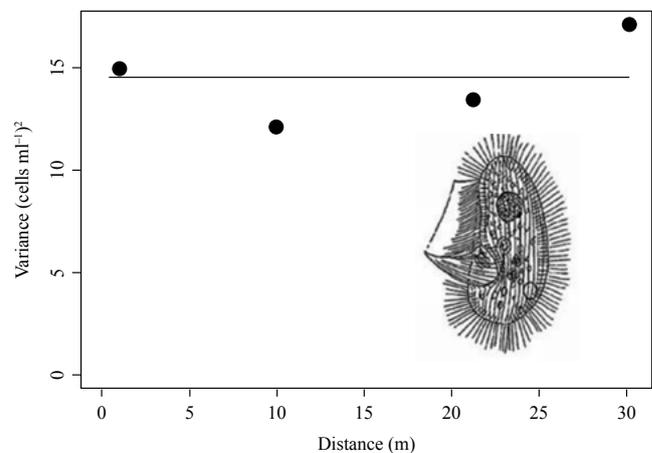
Fig. 4. A variogram for the ciliate *Pleuronema* sp. (inset) abundance. The best fit to the data (points) provided a pure nugget model; i.e. the distribution is random at the measured scale (40 m), with no observed patchiness.

Ciliates may be useful here: by applying geostatistics to characterize the spatial distribution of the Shannon diversity index of the ciliate assemblages in a model system (yet again, a coastal lagoon), Bulit *et al.* (2009) recognised distinct patterns of diversity patchiness at scales from 5 m to 3 km and then were able to suggest seasonal drivers of the distributions.

The general issue of patchiness of diversity at an ecosystem level may be most apparent in aquatic environments, where movement of water accelerates spatial heterogeneity promoting a higher diversity of micro-environments that favour species coexistence (Ettema and Wardle 2002). However, geostatistics is not limited to aquatic environments and has also been applied to diversity of soil ciliates in tropical forests, thereby widening the ecological scope of this approach (Acosta-Mercado and Lynn 2002). There is evidently substantial opportunity for the continued application of geostatistics to diversity studies of a range of protists.

Scales of patchiness are characteristic of different ecosystems and give insight on further ecological aspects

This point may be axiomatic, but it bears emphasising. The study of patchiness at different scales requires appropriate sampling designs, aimed at collecting information at recognised scales of interest (Pinel-Alloul and Gadhouani 2007). Following this reasoning, as indicated above, a multiscale approach revealed that for the mixotrophic ciliate *Myrionecta rubra* patches range from 10 to 10^3 m in a coastal lagoon (Fig. 5a, b, c). In a further study of *M. rubra* patches in the open ocean (Irminger Sea; Montagnes *et al.* 2008), it was revealed that patches were organized at an oceanic mesoscale, on the order of ~ 100 km, with higher abundance in spring (Fig. 5d). Furthermore, patchiness of *M. rubra* aided in understanding other aspects such as patchiness of cryptophytes (the ciliate's potential prey and symbiont) and the regional dominance by *M. rubra*, which functions as a primary producer and as a heterotroph, emphasizing the importance of this species within the microbial food web (Montagnes *et al.* 2008).

Additionally in a companion study to the one on *M. rubra*, Montagnes *et al.* (2010a) examined patchiness of abundance, biomass, and production of the total ciliate assemblage at basin scale of the Irminger Sea. This aided in assessing the flow of matter and energy through the food web and thus the role of microzooplankton in this region. For instance, kriging maps revealed the dominance of < 10 μm phytoplankton

and indicated that patches of phytoplankton biomass and total ciliate abundance coincide, suggesting a potential predator-prey relationship (Fig. 6; Montagnes *et al.* 2010a).

Patchiness should be incorporated into food web models

Following from the previous section, it should be clear that there is a need to incorporate patchiness of planktonic ciliates, and other microplankton, into pelagic food web models. *Cyrtostrombidium* may be considered here as a simple case-study example, of how patchiness may be incorporated into food web models. This oligotrich ciliate forms patches that last for 3 to 4 weeks (Fig. 3) and reaches numbers up to 100 ml^{-1} (Bulit *et al.* 2013). In this case, using geostatistical analysis and other ancillary data, we predicted the potential grazing impact of this ciliate to be 0.8–5.8% of total primary production on average, but up to 18–130% when *Cyrtostrombidium* patches and blooms occurred (Bulit *et al.* 2013). Thus, ciliate patches may act as hotspots for trophic transfer, both as consumers of phytoplankton and by acting as prey for zooplankton and fish larvae (Montagnes *et al.* 2010b).

Such patchiness could then be incorporated into trophic models at different scales using group or species-specific patterns rather than assuming populations are evenly distributed in space (Kareiva 1990). In this direction, researchers have experimentally studied the cascade of planktonic patchiness (Seymour *et al.* 2009) and combined experimental and fieldwork to estimate aggregations of prey, predators, and consumption rates (Menden-Deuer and Fredrickson 2010, Majaneva *et al.* 2013). Finally, recent findings regarding patchiness as driver of trophic relationships open new avenues to combine different methods (e.g. acoustic measurements and geostatistical analysis, Kaltenberg and Benoit-Bird 2013) to gain insight on these aspects. Once the need is recognised, spatial methods of study should be used to complement modelling efforts. Thus, I strongly support the direction of including geostatistical analysis in future modelling works, to assess the impact of microzooplankton patchiness.

CONCLUSIONS AND PERSPECTIVES

This work has indicated how geostatistics analysis can offer insights into the interpretation and characterization of spatial patchiness. Using ciliates as model
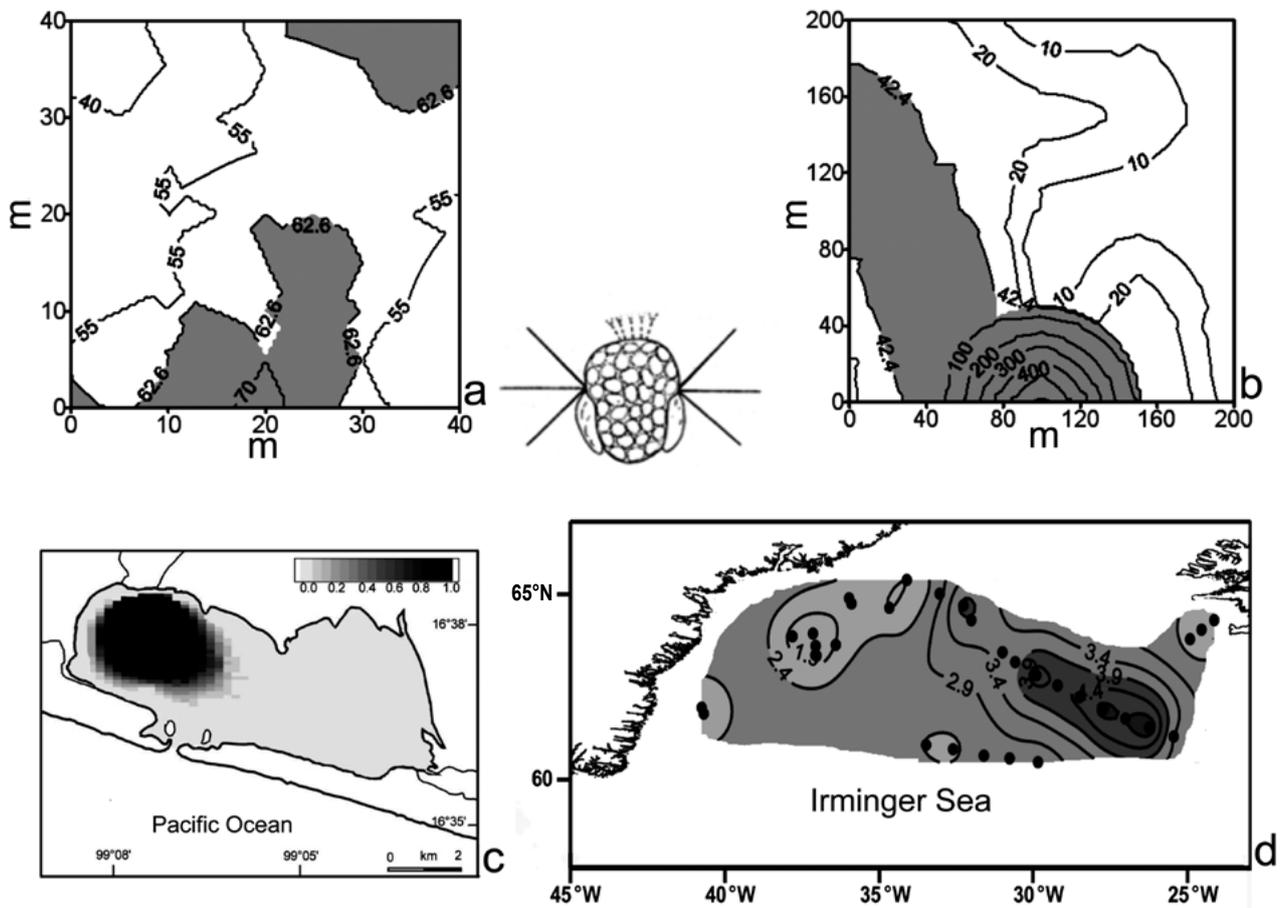
Fig. 5. Variability of patches of *Myrionecta rubra* (inset) abundance (cells ml^{-1}) at different scales and in different environments. (a–c) patches in a coastal lagoon at: (a) a small sampling area of 40 × 40 m; (b) a larger sampling area of 200 × 200 m; (c) at the lagoonal scale (~2 km, length). (d) patches of ~100 km length in the Irminger Sea, North Atlantic.
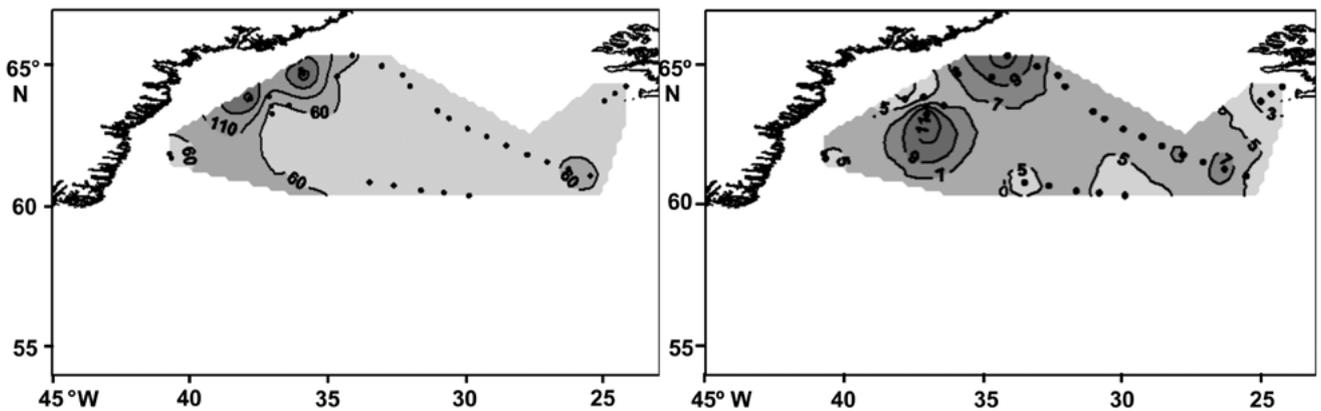
Fig. 6. Patches of total phytoplankton biomass (ng C ml^{-1}, left) and total ciliate abundance (cells ml^{-1}, right) in the Irminger Sea, North Atlantic. The spatial coincidence indicates a potential prey-predator relationship.

organisms, it is clear that ecological attributes of their populations and assemblages such as abundance, biomass, production, and diversity are patchily distributed at multiple scales and in different aquatic environments. Different populations of ciliates exhibit distinct patterns of patches regarding shape, density, and structure, indicating a behavioural niche-separation of protists, and the influence of rate processes on spatial distributions. Protists patchiness has been characterized both in aquatic and terrestrial environments, indicating it is a generalized property of their distribution. Moreover, spatial patchiness has temporal counterparts, represented by short blooms and their subsequent demise. Although spatial patchiness and temporal blooms are short-term episodic events, they must be considered in trophic models to more accurately calculate transfer of matter and energy.

The study of planktonic spatial heterogeneity at large scales has also aided in the development of other concepts, such as the role of metacommunities (Leibold *et al*. 2004) and metaecosystems (Loreau *et al*. 2003). This conceptual progress in ecological thinking and in cross-collaboration among researchers of different fields (e.g. ecology, statistics, molecular biology, evolution) has stimulated new questions regarding patterns and scales (Massol *et al*. 2011, Chave 2013) and methods for their study, with the support of technological advances in computational power and other techniques, as molecular tools.

Above, I have provided a brief and, hopefully, compelling introduction to geostatistics. I now strongly encourage microplankton ecologists to embrace the more advanced geostatistical methods, not necessarily outlined in this work. As an example, the joint spatial variability between organisms, between environmental variables, and then between organisms and environmental variables can be quantified using specific tools (e.g. cross-variogram, and factorial kriging analysis; Rossi *et al*. 1992). Also, when dealing with very expensive and/or time consuming data, multivariate geostatistics analysis can be applied, using a few available data complemented by more abundant data on a targeted, correlated variable that is cheaper and/or easier to obtain (Goovaerts 1997). There is thus a wide range of methods available to microplankton ecologists. We should not be intimidated by their apparent mathematical complexity. My own experience developed through close collaboration with statisticians, and as a parting comment, I highly recommend this interdisciplinary path.

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