ACTA
PROTOZOOLOGICA

Acta Protozool. (2013) 52: 1–12
http://www.eko.uj.edu.pl/ap
doi:10.4467/16890027AP.13.001.0829

Insights on Short-term Blooms of Planktonic Ciliates, Provided by an Easily Recognised Genus: *Cyrtostrombidium*

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Abstract. Planktonic ciliates occasionally form brief rapid increases in numbers (blooms) that can be trophically important. Although model simulations and mesocosm studies indicate that blooms occur over 10 to 20 days, field data are rarely sufficiently detailed to reveal their occurrence and demise. Our data (collected over 57 weeks across a coastal lagoon) offer insights into the population dynamics of a single species, place these in the context of the entire ciliate assemblage, and provide guidance on what should continue to be examined. Specifically, to evaluate population dynamics we examine two species of *Cyrtostrombidium*, characterise temporal and spatial variation of their abundance, and relate these to abiotic phenomena and biological factors. This is also the first report of *Cyrtostrombidium* in a tropical coastal lagoon. Collectively our analysis reveals key aspects of the dynamics of this genus: 1) small-scale peaks in abundance are ~30 m in size and can persist for ~10–30 days, reaching a maximum of 100 cells ml^{-1}; 2) these increases are driven by biotic factors (revealed through autocorrelation analysis); 3) long-term trends are driven by the shift between dry and rainy seasons and by the periods of isolation of lagoon from the sea (revealed through multiple regression analysis); 4) blooms may at times control primary production; 5) conjugation, an ecologically important event, may be associated with blooms (at times 9% of population was conjugating); and 6) dinoflagellate parasitism, poorly described in oligotrichs, is potentially important in population demise. These results both reflect on how ciliates may behave in short-term events and should encourage the continued need for detailed observations of field samples at a high taxonomic resolution.

Key words: Bloom, conjugation, parasitism, patch, population dynamics, lagoon.

INTRODUCTION

Ciliates are trophodynamically active nano- and microplankton in marine environments in general (Sherr *et al.* 1986, 1988; Rassoulzadegan *et al.* 1988; Pierce and Turner 1992) and in tropical coastal lagoons in particular (e.g. Bulit *et al*. 2009). Seasonal protozoan cycles are apparently controlled by top-down processes, mainly by crustacean zooplankton predation (reviewed in Gifford 2007). However, on shorter time scales ciliates interact with a range of environmental and biotic changes, occasionally forming short blooms that may be trophically important (Montagnes 1996). There is,

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thus, a continued need to assess these episodic events associated with increases in populations. Although model simulations (e.g. Montagnes and Lessard 1999) and mesocosm studies (e.g. Gismervik *et al*. 2002) support the occurrence of short-term blooms of ciliate species over 10 to 20 days, field data are rarely sufficiently detailed to reveal their occurrence and demise in nature (although blooms have been captured in "snapshot" sampling, e.g. Andersen and Sørensen 1986, Dale and Dahl 1987, Reid 1987, Montagnes and Humphrey 1998). As there are few data on the extent of episodic population dynamics or biological phenomena associated with them, we provide extensive and intensive data on two species of one easily characterised but poorly examined genus; these data on *Cyrtostrombidium* provide unique and useful insights into short-term ciliate population dynamics.

Three *Cyrtostrombidium* species have been described (Lynn and Gilron 1993, Kim *et al*. 2002), and the genus occurs widely, being found in: the Caribbean Sea (Lynn and Gilron 1993); Plymouth Sound (Leakey *et al*. 1994); brackish waters of North Germany (Agatha and Riedel-Lorjé 1997); Chesapeake Bay (Coats and Revelante 1999); the Gulf of Naples (Modigh 2001); the northeast coast of Japan (Kim *et al*. 2002); southern Atlantic coastal waters (Barría de Cao *et al*. 2003, Pettigrosso 2003); the Bay of Biscay (Urrutxurtu *et al.* 2003); northeastern Japanese waters, as cysts (Ichinomiya *et al*. 2004); southern waters of Beagle Channel (Biancalana *et al*. 2007); a solar saltern of the Yellow Sea (Lei *et al*. 2009); and Helgoland Roads (Löder *et al*. 2012). In a recent work on global diversity of oligotrichs, Agatha (2011) describes a wide distribution of *Cyrtostrombidium* in marine and brackish waters, with the South Pacific and Indian Ocean being the only regions where it has not been found. Thus, there are good reasons to investigate and understand its population dynamics.

Although *Cyrtostrombidium* exists in other brackish waters (Agatha and Riedel-Lorjé 1997), it has not been observed in tropical coastal lagoons. These lagoons are a common feature of coasts (Lankford 1977), commercially important as they support fisheries, ecologically important as they harbour a variety of ecosystems and are highly productive (Kjerfve 1994), and rich in ciliate diversity and abundance (Bulit *et al*. 2003, 2004, 2009). This field study, therefore, fills a potentially important gap in our understanding of the protistan biota of these ecosystems.

Insights from field-data by their nature are often serendipitous, revealing new and important features. Our data present the change in total ciliate abundance over an extended period and offer insights into the occurrence of single species population dynamics. Specifically, we have identified two species of *Cyrtostrombidium*. In doing so, we were able to characterise temporal and spatial variation of their abundance, and, in part, relate these to abiotic phenomena and biological factors. This was done using a series of statistical techniques including geostatistical analysis to assess spatial variation (see Bulit *et al*. 2003, 2004), multiple regression to assess factors influencing blooms (e.g. Bulit *et al*. 2009), and autocorrelation to assess temporal persistence and periodicity of blooms (e.g. Bulit *et al*. 2004). The study also provides unique qualitative data that offer new evidence for two processes of ecological importance. First, we have observed a parasite (tentatively identified as a dinoflagellate) being a mechanism for the demise of an aloricate ciliate bloom. Second, we reveal that aloricate, planktonic ciliates conjugate at the end of blooms; this is a rejuvenating event that may occur when cells are starved as their food is depleted by grazing pressure (Lucchessi and Santangelo 2004, Lynn 2008).

MATERIALS AND METHODS

Study site, sampling, identification, and enumeration

Chautengo is a shallow, chocked lagoon on the Mexican Pacific coast, characterized by dominant wind forcing, with a surface of 36 km^2 that receives input from the Nexpa and Copala rivers (Fig. 1). The lagoon exhibits a seasonal pattern: during the dry season (November–May), riverine input is reduced, a sand bar forms across the opening to the ocean (see Fig. 1), evaporation increases, the lagoon level drops, and the lagoon becomes brackish. When rains concentrate at the beginning of the rainy season (June–October), the lagoon becomes less saline, the water level rises by ~2 m, and the sand bar is breached (for details see Bulit *et al*. 2004, 2009).

All samples were collected at 0.4 m with a 0.4 L van Dorn-type bottle, and were preserved with acid Lugol (2% v/v); at each deployment of the bottle only one sample was taken. Water salinity, temperature, and transparency were determined at each sampling site with a YSI 30 conductivity/temperature meter and a Secchi disk, respectively.

All ciliates (except the functional autotroph *Mesodinium rubrum*, which has been examined independently, see Bulit *et al*. 2004) were counted at 200 × and 400 × magnifications in a 5 ml Utermöhl chamber, using a Zeiss 25 CFl inverted microscope, and abundance was expressed as cells ml^{-1}. For *Cyrtostrombidium*, DAPI staining of the nucleus (Strüder-Kypke and Montagnes 2002) and protargol staining of Lugol's fixed cells (QPS, Montagnes and Lynn 1987, Skibbe 1994) revealed further morphological diagnostic

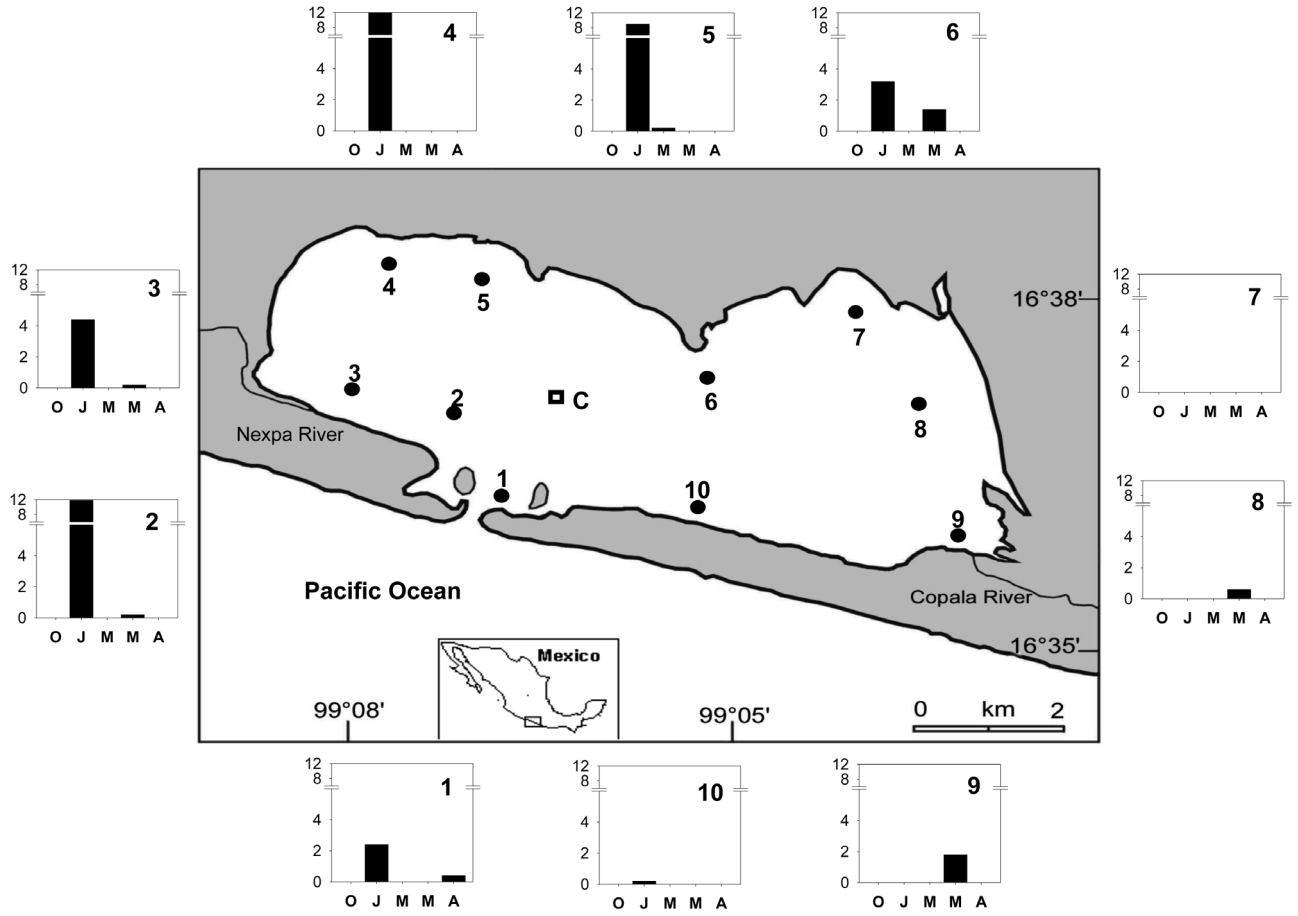

Fig. 1. Chautengo lagoon, México, indicating the location of 10 sites (black circles) where 5 seasonal samplings took place and the one grid site (C) where geostatistical analysis was conducted and long-term data were collected. Spatial distribution of *Cyrtostrombidium* abundance (as cells ml^{-1}) is presented as bar graphs at the 10 sites, over 5 months (October, January, March, May and August). For long-term data on site C, see Fig. 2.

features, the occurrence of parasites, and the occurrence of conjugation (that was then clearly recognisable in Lugol's fixed material). Note that these Lugol's fixed, QPS prepared samples were not of as high quality as those that might be obtained from cells fixed initially with Bouin's.

To identify species, morphometric data were compared with published data on *Cyrtostrombidium* taxonomy (Table 1, Lynn and Gilron 1993, Agatha and Riedel-Lorjé 1997, Kim *et al*. 2002). Morphometric data are presented as means and ranges, based on protargol stained specimens. Cell length, width, number of anterior polykinetids, ventral kinety length, macronucleus length and width, and length of the cytopharyngeal basket were measured. For trophodynamic analysis, *Cyrtostrombidium* cell length and width measurements were converted to volume, using appropriate geometric shapes; live volumes were estimated assuming that fixed cells shrank (see Jerome *et al*. 1993), and these were converted to carbon (see Menden-Deuer and Lessard 2000).

Spatial and temporal variability of *Cyrtostrombidium* and its relation to other ciliates

To assess lagoonal trends in the spatial distribution of *Cyrtostrombidium* abundance in dry and rainy seasons, 10 sites were sampled in October, January, March, May, and August (Fig. 1). Specifically, to address the influence of environmental factors on the spatial and temporal variability of *Cyrtostrombidium* abundance at the lagoonal scale, a multiple regression analysis was conducted on these data (Neter *et al*. 1990; S-Plus, MathSoft). Abundance values were $\log_{10}(x + 1)$ transformed, to ensure normality of data and account for zero values. The relative effect of temperature, salinity, transparency, season (dry, rainy), and sand-bar state (open, closed)

Table 1. Morphometrics and meristics of *Cyrtostrombidium* spp. Measurements were made on protargol impregnated specimens (µm) and are presented as means (range). APk: anterior polykinetids, Mac: macronucleus, G: girdle. Data were obtained from the written descriptions or figures (*) in the original work.

Species/Author	Cell length	Cell width	Mac length shape	Mac width	Cytopharynx length	APk number	Ventral kinety dikinetids	Anterior protuberance	Longitudinal break	Extrusomes
Cyrtostrombidium longisomum Lynn and Gilron 1993	49 (42–60)	15 (11–17)	15 (8–24) elongate, ovoid	4 (2.5–6)	7–10*	13 (12–18)	> 30*	conical*	present*	?
Cyrtostrombidium longisomum **n = 31 this work**	41.7 (30–56)	12.5 (10–13)	11.3 (7–16) elongate, ovoid	4.3 (3–6)	8.3 (6–12)	13 (12–14)	24 (15–33)	almost spherical*	?	insert above G
Cyrtostrombidium wailesi Lynn and Gilron 1993	32 (26–39)	12 (10–16)	8 (4–13) ovoid	4 (3–6)	5–8*	12	> 20*	conical*	none*	?
Cyrtostrombidium wailesi **n = 103 this work**	34.3 (23–42.5)	8.6 (6–10.5)	9 (6–14) ovoid	3.8 (2.5–7)	7.1 (3.5–12)	12 (10–14)	20.5 (16–25)	almost spherical*	present	insert above G
Cyrtostrombidium boreale Kim *et al*. 2002	120 (50–159)	39 (18–54)	53 (20–69) elongate large	10 (7–15)	27 (18–37)	14 (12–16)	82 (72–98)	conical*	present	?
Cyrtostrombidium spec. Agatha and Riedel-Lorjé 1997	62 (54–76)	218 (18–22)	(17–22) comma shaped	(4–8)	(14–22)	16	35 (17–56)	almost spherical*	none	insert above G

was assessed (collinearity was tested for and found not to be a significant issue). Season and sand-bar state were treated as categorical variables, and the other were considered continuous. Variables were tested for significance ($\alpha = 0.05$).

Small scale patterns in the spatial distribution of *Cyrtostrombidium* were assessed by analysing cell abundance at samples taken at 30 to 35 points of a 40 × 40 m^2 grid at site C (Fig. 1) on five occasions, during dry and rainy seasons (October, January, March, May, and September). Geostatistical techniques were applied to the abundance data collected from the grid; sampling points were separated by 10 m, with 10 additional points at 1 m distance added to provide better resolution at the small scale. For methodological details on this sampling grid and geostatistical analysis in general see Bulit *et al*. (2003); simply, the procedure modelled the spatial variability of abundance data at increasing distances, using the variogram tool (Rossi *et al*. 1992) and then applied the model to make predictions by interpolating and mapping the distribution of abundance on the entire grid, from the 30–35 collected data (i.e. the Kriging method; Goovaerts 1997).

To detect significant temporal trends in the variability of abundance, *Cyrtostrombidium* abundance from discrete samples collected at the central site (C) was assessed over 57 weeks. Autocorrelation analysis was applied to weekly abundance data: correlation between abundance values separated by 1, 2,…57 weeks (lags) was conducted (Diggle 1990, Dornelas *et al*. 2012). A positive autocorrelation for a given lag or time interval (i.e. from 1 to 57 weeks) indicates persistence of the current state (i.e. high or low abundance) and thus allows predictions about duration of blooms. Once patterns of blooms were detected they were compared with seasonal environmental influences.

Trophodynamic impact of *Cyrtostrombidium* on the food web

To estimate the potential grazing impact of *Cyrtostrombidium* on phytoplankton across the entire lagoon, we performed the following calculations, specifically in January when the ciliate was abundant and when we had obtained data for all sites around the lagoon (and estimates of primary production, see below). First, we determined the mean abundance of the ciliate (in this instance the assemblage was dominated by the smaller species, *C. wailesi*, Figs 1–3) and then determined ciliate biomass, as above. Carbon-based ciliate production (P, C m^{-3} d^{-1}) was estimated as P = biomass × ciliate abundance × ciliate growth rate, where growth rate (r) was predicted to range between 0.7 and 2.1 d^{-1} (i.e. one to three doublings per day, which is a reasonable range for oligotrichs that are provided with a surfeit of food and the warm temperatures experienced in the lagoon; see Montagnes 1996). Daily ciliate ingestion (I, C m^{-3} d^{-1}) was determined by assuming a gross growth efficiency (GGE) of 0.3 (Straile 1997): $I = P$ / GGE. Although we made no estimates of primary production in this study, estimates for December–January were ~1 g C m^{-3} d^{-1} (based on dissolved oxygen changes in 2-h incubations of light and dark bottles, made at sites 1 and 6; C. Bulit, unpublished data); this was used in our calculations. The impact of *Cyrtostrombidium* was then calculated as the percentage of carbon-based primary production predicted to be consumed by the ciliate population.

RESULTS

Identification of species, conjugating cells, and parasitized cells

Cyrtostrombidium longisomum and *C. wailesi* Lynn and Gilron 1993 were identified based on morphological features (Table 1; Fig. 3). For *C. wailesi*, pairs of cells were observed, where pairs were joined near the oral region (Fig. 3c, d); protargol stains clearly revealed that cells were joined in conjugation, and observation of Lugol's fixed, similarly paired cells were also deemed to be conjugating. Conjugating pairs of *C. wailesi* occurred during a population peak (~5 ml^{-1}) in March, prior to data presented in Fig. 2 (0.5 pairs ml^{-1}) and in December (1.4 and 3.4 pairs ml^{-1}), during the dry season, when *Cyrtostrombidium* bloomed (~100 ml^{-1}) at the central part of the lagoon (arrows on Fig. 2); up to 9% of the population were conjugating.

A presumptive parasitic dinoflagellate within *C. longisomum* was identified from the literature (Cachon 1964); up to four parasites occurred within cells, but more typically there was one or two (e.g. Fig. 3h). No parasites were observed in *C. wailesi*. Although observations from 2001 samples indicate that parasites sporadically occur mainly in May, unpublished observations of recent samples collected in March 2012 (by C. Bulit) reveal that up to 7 of 10 *C. longisomum* in one sample were infected.

Environmental characterization and lagoonal distribution of *Cyrtostrombidium*

Abiotic factors varied with time and space during the study. Salinity ranged from 0 to 34 (psu) in the rainy season and from 26 to 31 in the dry season; it was always higher near the open inlet and lower near the river mouths. Ephemeral stratification events (> 0.5 m) occurred during the mornings in the rainy season and when the sand bar was open; after midday the strong sea breeze mixed the shallow waters of the lagoon. Site depth varied from 0.4 to 1.9 m. Water temperature ranged from 26.5 to 31.6ºC during the surveys. Transparency (i.e. Secchi depth) varied between 0.3 and 1.5 m, with more transparent waters near the mouth of the lagoon.

Cyrtostrombidium abundance varied at the lagoonal level, ranging from 0 to 13.4 cells ml^{-1} and was higher in the west of the lagoon (Fig. 1). In rainy season months

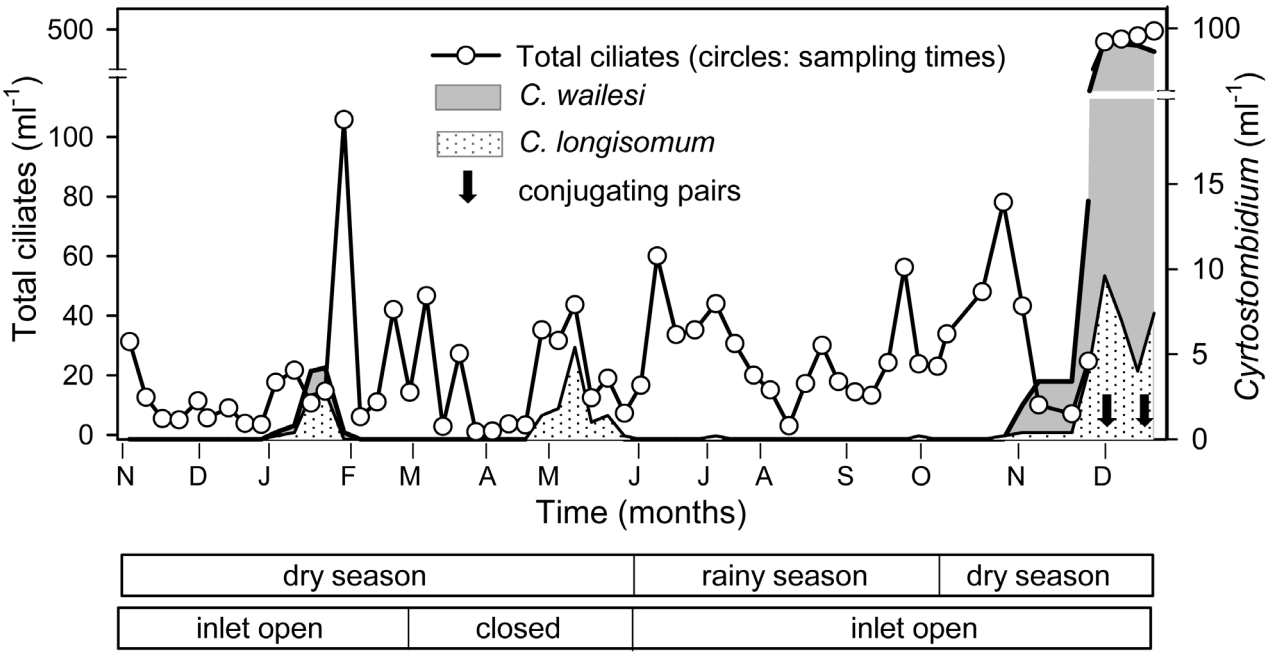

Fig. 2. Temporal variability in the abundance of the total heterotophic ciliate assemblage and the two *Cyrtostrombidium* species (ml^{-1}), with an indication of the occurrence of conjugating pairs (arrows), at site C (Fig. 1).

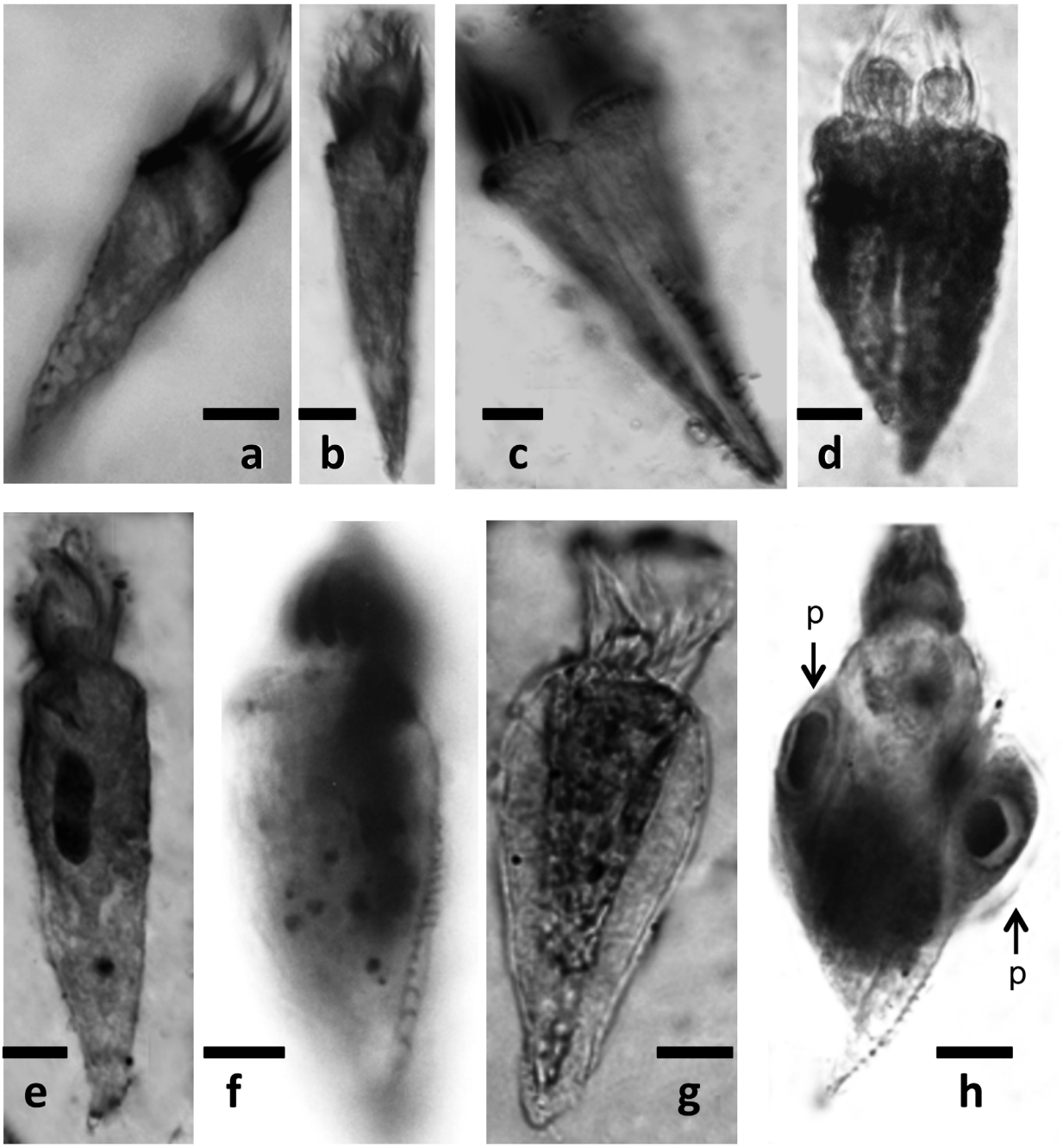

Fig. 3. *Cytostrombidium wailesi* and *C. longisomum.* **a**, **b** – Lugol's preserved, protargol impregnated examples of *C. wailesi*; **c** – Lugol's preserved, protargol impregnated, conjugating *C. wailesi*; **d** – Lugol's preserved, conjugating *C. wailesi*; **e** – Lugol's preserved, protargol impregnated examples of *C. longisomum*; **f** – protargol impregnated example of *C. longisomum*; **g** – Lugol's preserved *C. longisomum*; **h** – protargol impregnated example of *C. longisomum* with two parasites (**p**). Scale bars: 5 µm.

(October and August) *Cyrtostrombidium* did not occur at any of the 10 sampling sites. At this lagoonal scale multiple regression analysis indicated that *Cyrtostrombidium* abundance changed seasonally (temperature, transparency, and salinity were not significant), increasing during the dry season ($P = 0.003$) and when the sand bar was open ($P = 0.000$). This suggests that abiotic or biotic seasonal factors may have influenced abundance.

Small scale patches and temporal variability

At the small scale (i.e. the intensively sampled grid, site C, Fig. 1) *Cyrtostrombidium* occurred on two (January and May) of the five sampling periods. Geostatistical analysis revealed a patch of *Cyrtostrombidium* over a range of 30 m in May at the end of dry season (Fig. 4). In January there was a random distribution of cells abundance (i.e. no patches were identified at this scale).

Cyrtostrombidium abundance was highly variable at the central site of the lagoon, with periods of increase and decrease ranging from 0 to ~100 cells ml^{-1} (Fig. 2). Composition of population peaks of the two species differed through time: *Cyrtostrombidium wailesi* dominated in March 2000, comprising up to 98% of abundance of the genus. In January 2001 *C. wailesi* represented 60%. In May 2001 *C. longisomum* represented 80%. Finally, in December 2001 *C. wailesi* accounted for 90% (Fig. 2). Only in December did *Cyrtostrombidium* represent a large component (~20%) of the total heterotrophic ciliates (Fig. 2), when *C. wailesi* formed a distinct bloom.

Autocorrelation analysis plots the correlation between values of *Cyrtostrombidium* abundance at different times (weeks in this time series) as a function of the time difference. This analysis of time-series data of abundance revealed short-term positive temporal correlations at lags 2, 3, and 4 (i.e. time intervals of one, two and three weeks; Fig. 5). This indicates that abundance at a week (lag 2) is significantly correlated with abundance at one and two weeks later (lags 3 and 4) and statistically supports *Cyrtostrombidium* patches lasting for 3 weeks; this can be also qualitatively noted in Fig. 2, where populations increase and then decrease over 3 to 4 weeks.

Trophodynamic impact of *Cyrtostrombidium* on the food web

Cyrtostrombidium production was estimated to range from ~2.5 to 17.5 mg C m^{-3} d^{-1}, depending on the assumed growth rate (0.7 and 2.1 d^{-1}, see Materials and Methods). From this it was then established that on average, across the lagoon, ciliates would have consumed between 0.8 to 5.8% of the total primary production (i.e. combining highest and lowest predictions of ciliate growth). This wide range provides an indication that even when relatively abundant in January (Fig. 1) across the lagoon this genus would not have heavily impacted average phytoplankton production. However, applying a similar calculation to the bloom in December at the centre of the lagoon (C, Fig. 1), when *C. wailesi* reached ~100 ml^{-1}, the ciliate may have consumed between 18 to 130% of the primary production, and this bloom lasted for close to a month (Fig. 2).

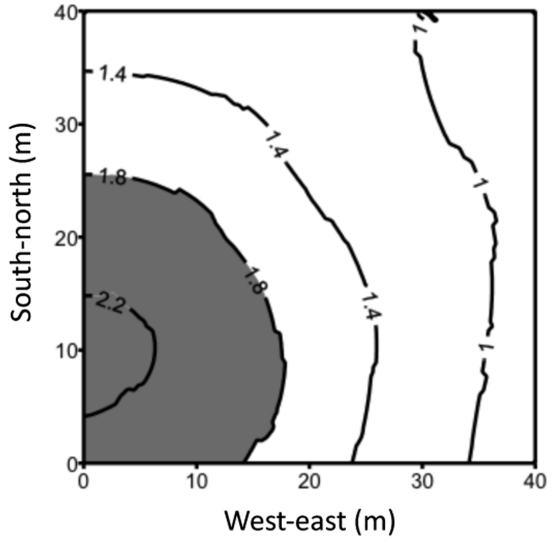

Fig. 4. Small-scale patch revealed by geostatistical analysis (~30 m, grey area) of *Cyrtostrombidium*, at the centre of the lagoon, in the dry season (January), when the inlet of the lagoon was open. Isolines indicate abundance (ml^{-1}).

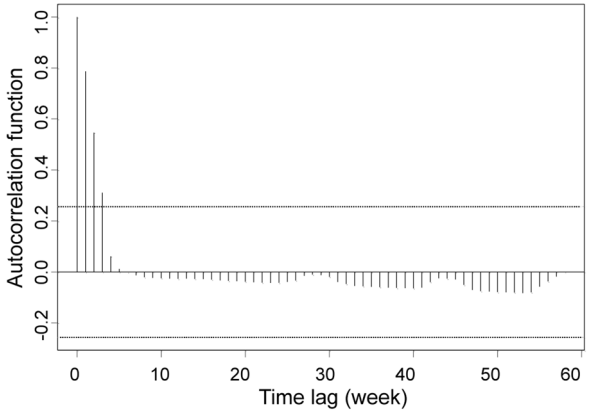

Fig. 5. Autocorrelation function of the weekly abundance of *Cyrtostrombidium* at site C. Horizontal dashed lines indicate ~95% CI for significance of each autocorrelation value. The first autocorrelation is 1 by definition; it is the correlation of a sample with itself. Correlation for lags (weeks) 2, 3, and 4 falls beyond the 95% CI, indicating a persistence of 3 weeks for a peak in abundance.

DISCUSSION

As indicated in the Introduction, despite its wide distribution, there is little basic taxonomic and ecological information on *Cyrtostrombidium* Lynn and Gilron 1993. Therefore, there is a gap in our knowledge about a potentially important taxon that can form blooms. Furthermore, this genus has distinct features (Fig. 3; Table 1) that allow it to be identified easily in Lugol's fixed material (a standard method for preserving field samples). Detailed data on its distribution and abundance can then provide species-specific trends associated with population dynamics that may also occur for other less recognisable ciliate species. Consequently, here we provide data on two species, *C. longisomum* and *C. wailesi* Lynn and Gilron, 1993 (Table 1; Fig. 3) and indicate the extent and potential causes of short-term increases in populations; these seem to be seasonal and appear to extend for ~3 weeks (Fig. 5), but possibly up to one month (Fig. 2). Below, we examine these and in doing so provide insights into population dynamics that may have significance for understanding planktonic ciliates in general.

Identification of species, conjugating pairs, and parasitism

The cells parameters of the two species analysed are very similar to the published ones by Lynn and Gilron (1993). Although there is also variability in some measurements (Table 1), based on the data we have and the limited amount of good descriptions of species in this genus (see Introduction), we concluded that the species identifications were appropriate. Likewise, few live (e.g. Montagnes *et al*. 1996) or protargol stained (e.g. Montagnes and Humphrey 1998) observations of conjugating planktonic ciliates have been recorded. Based on these (and our personal observations) we concluded that cells in our samples were conjugating.

Regarding our presumptive identification of the parasite, to distinguish between dinoflagellate infections in ciliates it is necessary to follow the life cycle of the parasite, which we were unable to do, and examine structural components of the cell that were not visible in our preparations (Coats 1988, Coats *et al*. 2012). Therefore, based on its general shape and structure revealed by protargol (Fig. 3h) we can only suggest that this is a dinoflagellate parasite. Given the wide spread occurrence of dinoflagellate parasites of ciliates (e.g. Coats and Bachvaroff 2013) this is a reasonable assumption, but clearly it requires further study when more material presents itself.

Temporal and spatial distribution and environmental influence on blooms occurrence

Small-scaled spatial patches of ciliates occur, on the order of 13 to 170 m (Bulit *et al*. 2003, 2004), and *Cyrtostrombidium* is no exception to this. The spatial characterization of the *Cyrtostrombidium* patch (~30 m) revealed at the end of the dry season provides further evidence of the possible spatial extent of population peaks that were detected by only single samples; i.e. weekly samples at the centre of the lagoon (Fig. 2) and samples around the lagoon that were taken on five occasions (Fig. 1). Specifically then, we can make predictions of the population dynamics of *Cyrtostrombidium*; e.g. patches increase the likelihood of encounter of congeners and thus conjugation (see Conjugation associated with blooms, below) and will impact on food web dynamics (see Trophodynamic impact of *Cyrtostrombidium* on the food web, below).

More importantly, by examining this easily recognisable genus, we can make some predictions regarding the spatio-temporal dynamics of ciliate populations. For instance, peaks in total ciliate abundance (Fig. 2) are potentially formed by single species blooms or a combination of a few species; in fact, another study that examined spatial (but not temporal) dynamics in this lagoon revealed that high ciliate numbers in December (see Fig. 2) can be partially attributed to ~10 m patches of the small ciliate *Lohmaniella oviformis* (Bulit *et al*. 2003). By considering this type of spatially structured population dynamics we now may be able to better understand and model the role of microbial food webs (see Menden-Deuer and Fredrickson 2010).

There is, therefore, a need to be able to predict the likelihood of increases in populations occurring. *Cyrtostrombidium* abundance was highly variable at the central site of the lagoon, with periods of abundance persisting for ~3 weeks (Figs 2, 5). This short-term persistence may suggest that biological factors (e.g. prey abundance, parasites), rather than longer-term environmental changes, are responsible for the periodicity in blooms (e.g. see Trophodynamic impact of *Cyrtostrombidium* on the food web and Bloom demise by parasitism?, below). In contrast, at the lagoonal scale (Fig. 1), multiple regression analysis indicated that *Cyrtostrombidium* abundance changed seasonally, increasing during the dry season and when the sand bar was open; this suggests that abiotic or biotic factors associated with seasonal changes and hydrodynamic processes that were not assessed (e.g. longer-lived metazoan preda-

tors, wind mixing, stratification) may dictate when a species (or genus) is likely to form blooms. Clearly, to understand and predict such dynamics there is a need for further detailed field sampling coupled with analysis similar to that which we have performed.

Trophodynamic impact of *Cyrtostrombidium* on the food web

Our predictions are based on upper and lower estimates of growth rate (see Materials and Methods) as better ones are lacking, but they generally indicate that when relatively abundant in January (Figs 1, 2) across the lagoon *Cyrtostrombidium* alone would not have heavily impacted average phytoplankton production, consuming ~1 to 6% of the production. Possibly, this is not surprising as we are only considering a single genus. However, during the December bloom at the central site (see Fig. 1) we predict that *C. wailesi* consumed 18 to 130% of the primary production, and as the December bloom lasted for close to a month, this single species may at times control productivity in localised patches. Furthermore, *C. wailesi* may have been responsible for removal of its food source, as its consumption was potentially > 100% of production. This would ultimately have lead to its starvation and the demise of the bloom, as has been predicted for other planktonic ciliates (see Banse 1982, Montagnes 1996, Strom and Morello 1998, Montagnes and Lessard 1998).

Consequently, our data support the notion that the short-term increases in total ciliate abundance in the lagoon (Fig. 2) may be boom-bust population dynamics of single or few species; i.e. biological phenomenon, as suggested in our analysis above (Fig. 5). Similar dynamics have been suggested for other ciliate species in this lagoon (Bulit *et al*. 2003) and may be a major driver of trophodynamics in general, as ciliates may consume much of the primary production and then be consumed by higher trophic levels, including mesozooplankton, fish, or parasites (Gifford 2007, Montagnes *et al*. 2010b, Coats and Bachvaroff 2013). Again, this supports arguments by others (e.g. Montagnes 1996, Menden-Deuer and Fredrickson 2010) that such population dynamics should be considered by ecosystem modellers, rather than treating ciliates as a single, uniformly distributed trophic unit.

Conjugation associated with blooms

Rarely, have conjugating naked planktonic ciliates been observed (or at least recorded) in natural samples, and to our knowledge they have never been documented at such a high percentage of the population in a single-species bloom (i.e. up to 9%). In contrast, examining a planktonic ciliate in the laboratory, Montagnes *et al*. (1996) supported the expected trend for ciliates to require conjugation to rejuvenate cell lines (see Bell 1988). The rare, but episodic conjugation events observed in this field study might also reflect the need for this ciliate to rejuvenate itself through conjugation.

Crowding of *Cyrtostrombidium* in a patch (up to ~100 ml^{-1}, Fig. 2) might promote encounters of complementary cells (Lucchesi and Santangelo 2004). Likewise, conjugation may have been stimulated by post bloom reduction of prey, as starvation stimulates conjugation (Lynn 2008). Similar arguments were made by Montagnes and Humphrey (1998), who noted that within a "red-tide" bloom of the chloroplast bearing planktonic ciliate *Strombidium lingulum*, ~0.3% of the population were conjugating. Clearly, the extent to which conjugation occurs and the genetic impacts on populations is important, as conjugation frequency is the main factor determining the genetic structure of ciliate populations (Doerder *et al*. 1995). This in turn may be a driver of ciliate evolution at ecological scales (see Carroll *et al*. 2007). We, therefore, encourage continued vigilance directed towards noting the occurrence of conjugating cells in field studies.

Bloom demise by parasitism?

From our data, we predict that parasitism may play an important role in controlling population dynamics of *Cyrtostrombidium* and, in extension, other naked planktonic oligotrichs. Although tintinnids and heterotrophic dinoflagellates are well known to be infected by dinoparasites (Park *et al*. 2004, Coats *et al*. 2012, Coats and Bachvaroff 2013), there are few indications that naked planktonic ciliates are prone to parasitism; an exception being the early observations of *Duboscquella caryophaga* acting as a parasite of *Strombidium*, "*Strombilidium*," and *Prorodon* (Cachon 1964). At present, we lack data on the ability of parasites to control *Cyrtostrombidium*. However, Coats and Heisler (1989) have predicted a significant impact of parasites on planktonic ciliate populations. For instance, *Duboscquella cachoni* induced mortality was estimates to removed 7 to 24% of the standing stock of the tintinnid *Eutintinnus pectinis* per day in eutrophic coastal waters, an effect comparable to predation by the dominant copepod grazer; likewise, *D. aspida* produced a somewhat lesser impact on populations of the tintinnid *Favella panamensis* (Coats *et al*. 1994). These data on ciliates parallel oth-

ers studies (Montagnes *et al*. 2008, Chambouvet *et al*. 2008) that indicate that parasites may control short term blooms of dinoflagellates. We, therefore, propose that the demise of blooms in the lagoon, and their potential periodicity (Fig. 2) may have been due to top-down control by parasites, rather than the typically applied explanation of food limitation (see Spatial and temporal variability of *Cyrtostrombidium* and its relation to other ciliates). Thus, this field study has provided further evidence that parasitism of ciliates may be an important pathway within the microbial loop (Coats and Bachvaroff 2013), and should stimulate careful observations in other systems that may reveal parasitism to be more common and critical in controlling trophodynamics.

SYNTHESIS

What then are the implications of the population dynamics of *Cyrtostrombidium* that we observed? First, these data support other conjectures that planktonic ciliates, which can have growth rates as fast, or faster, than their prey (Banse 1982, Montagnes 1996) should exhibit short-term blooms, where they remove their prey and then starve as prey levels drop below threshold levels (see Montagnes 1996) or become reduced by parasitism (as we suggest here). Such population peaks will not only occur spatially (e.g. around a lagoon, Fig. 1) but temporally (Fig. 2). Furthermore, there are indications that blooms will occur throughout the water column, if there is small-scale vertical structure of the water column (e.g. Montagnes *et al*. 1999, Menden-Deuer and Fredrickson 2010). Therefore, studies that hope to assess trophodynamics within water bodies should consider high resolution temporal and spatial sampling to elucidate population dynamics.

As indicated above, these small-scale population dynamics may, at times and in localised regions, be important in the transfer of energy through the food web, if they are grazed or parasitized, or they may be a sink of energy if the ciliates die due to starvation (Montagnes 1996). We might then speculate that blooms of other ciliates, as observed in this lagoon (Bulit *et al*. 2003, 2004) and elsewhere (see Introduction) will have similar impacts, and that the ciliate-dynamics within such water-bodies are composed of a series of bloom events, as single species, or a few species, encounter patches of suitable prey. It is through field data such as those we present here that such speculations can arise and the need for their assessment is emphasised.

However, there are clear limits to our study which reveal the need to extend the study of patches. For instance, planktonic patches are ephemeral by nature and can be dissipated by mixing processes (e.g. daily in Chautengo lagoon, see Materials and Methods). Biotic and abiotic processes likely contribute to the ephemeral formation and demise of patches at different scales (Bulit *et al*. 2004), and impose limits to the study and sampling schemes (e.g. in January, when no spatial structure of *Crytostrombidium* abundance was detected). Furthermore, in terms of detectability our sampling scheme can detect patches between 10 to ~70 m (for details on the methodology see Bulit *et al*. 2003), but it lacks resolution and spatial extent to recognise larger or smaller patches. Consequently, we support future, more intensive and extensive study of patches. Finally, although it is clear from our work that short-term and long-term blooms and seasonal cycles occur, we lacked key data to reveal why. We suggest then that future work in lagoons, and for that matter in most aquatic systems, should be interdisciplinary, including phytoplankton, zooplankton, parasites, and a range of abiotic factors, beyond those we were capable of measuring.

Acknowledgements. Thanks are given to the Editor, to John Dolan, and to an anonymous reviewer for their constructive comments on an earlier version of this paper.

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Received on 13th November, 2012; revised on 9th January, 2013; accepted on 12th January, 2013