We describe remarkable diurnal changes in the morphology of the planktonic dinoflagellate *Ceratium ranipes*. The species is distinguished by the unusual appendages, known as toes or fingers, protruding from its horns. Varieties have been described based on the characteristics of the fingers. We discovered that cultures, maintained on a 12:12 photoperiod, when examined during the dark period were composed of ‘finger-less’ cells. Monitoring of isolated cells revealed a diurnal cycle of distinct changes in morphology with daytime cells showing appendages, well-stocked with chlorophyll, and the absence of the fingers at the end of the photoperiod. Fingers are absorbed at the end of the light period and re-grown at the end of the dark period. Sampling the Bay of Villefranche, we found the distinct morphologies in a natural population at night and during the day.

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Key words: morpho-species; circadian; diurnal; diel; chloroplast; infraspecific; taxonomy.

Introduction

The pelagus is a habitat which appears to harbor a seemingly unreasonable number of forms, “the paradox of the plankton” (Hutchinson 1961). Further complicating the paradox is the fact that the relationships between morphologically-defined species and genetic species are far from clear with regard to planktonic protists (Dolan 2005). The discovery of cryptic species (e.g., De Vargas et al. 1999; Slapeta et al. 2006) has led to the suggestion that the genetic, or true species diversity may be even greater than the apparent diversity based on the morphological recognition of species. Conversely, diversity maybe less than we think because different morphological forms may not represent different species. Here we report on the latter case.

Among the planktonic algae, the marine dinoflagellate *Ceratium ranipes* is well-known for its remarkable appendages, termed either toes, or here, as fingers. The species was described by P. T. Cleve in 1900 from material collected near the Azores by ships of opportunity. He described it as...
a “rare and remarkable form” (Cleve 1900). Although found in low abundances, it is relatively common in tropical and subtropical oligotrophic waters throughout the world oceans (e.g., Matrai 1986; Taylor 1976; Tunin-Ley et al. 2007; Weiler 1980). Forms of Ceratium ranipes with fingers of different length or without fingers have long been reported and termed varieties (see Fig. 1). The distinct morphotypes, sometimes spatially and temporally separated, and sometimes co-occurring, suggest the existence of a species complex, or forms with thermal preferences (Sournia 1967). The existence and possible causes of infraspecific taxa or varieties in dinoflagellates, and in particular with regard to Ceratium have been discussed in some detail (e.g., Taylor 1976). However, we found quite by accident an explanation which, to our knowledge, has never been considered. We discovered that Ceratium ranipes shows a highly unusual diurnal polymorphism; it has a day morphology with fingers and a night morphology without fingers. Thus, the different morphologies, thought to represent varieties, are exhibited by the same individual.

Results

Clonal cultures of Ceratium ranipes, started from individual cells isolated from the Bay of Villefranche-sur-Mer, were grown in K medium in an illuminated culture chamber with a 12 h photoperiod, set to a dark period of 18:00-06:00. Growth rates among isolates were slow and variable, with an average generation time of about 3 days. The photo-period program was lagged over a few days to a dark period of 22:00-10:00 so that ‘night’ cells could be examined for cell division stages during regular working hours. While no obvious peak in division rates were found in ‘night’ cells, cultures were composed of cells without fingers and when examined later in the afternoon, after a few hours of illumination, the same cultures were composed of cells with ‘fingers’. In our cultures, the fingerless night forms appeared to be more active, constantly in motion, compared to the lethargic daylight forms.

Individual cells were isolated in well-plates and periodically examined over a 24 h period. Ceratium ranipes shows a highly unusual diurnal polymorphism; it has a day morphology with fingers and a night morphology without fingers. Thus, the different morphologies, thought to represent varieties, are exhibited by the same individual.

Figure 1. Example from Balech 1988 of the morphological variability in Ceratium ranipes which has been reported. He noted that the rare ‘fingerless’ forms (1 from ‘lamina’ or plate 62 above) could be recognized as C. ranipes with facility (Balech 1988; p. 142).

Figure 2. Images of a single pair of Ceratium ranipes after 10.5 h in darkness (04:30), 2.5 h of illumination (08:30), 6.5 h of illumination (12:30) and after 6 h of darkness (22:00).
Ceratium ranipes showed formation of appendages beginning at the start of the light period and loss at about 2 h into the dark period (Fig. 2). Observations of isolated cells at the end of the dark period showed that finger formation took about 2-3 h and monitoring individuals at the end of the light period showed re-absorption took 2-3 h as well.

Fully formed fingers were well-stocked with chloroplasts, based on chlorophyll auto-fluorescence (Fig. 3). When stained with Calcofluor White (Fritz and Triemer 1985), the fingers showed fluorescence similar to the thecal plates (Fig. 3) but we were unable to clearly discern the borders of the thecal plates. Cells scheduled to experience darkness lose chlorophyll in the appendages about an hour before the dark period (Fig. 4).

Monitoring cultures every 2 h over a 24 h period showed that cell transformations were not absolutely synchronized among individuals. While all cells were without fingers after 4 h in the dark period, a small portion of cells showed no fingers throughout the light period (Fig. 5) and a marked variability was evident in the numbers and lengths of fingers between individuals.

Finger absorption, monitored in single individuals over the first 3.5 h of the dark period (Fig. 6) showed distinct stages. Complete finger absorption took about 2 h and well-defined fingerless horns, such as depicted by Balech (Fig. 1) were shown after about 3.5 h in darkness.

Figure 3. A formalin-preserved specimen of the ‘finger’ daytime morphology viewed with transmitted light, and with the insets showing chlorophyll autofluorescence and fluorescence after the addition of Calcofluor White of the same cell. Note that the ‘fingers’ show strong chlorophyll autofluorescence and Calcofluor fluorescence.

Figure 4. A formalin-preserved specimen of the ‘end of the day’ transitional morphology viewed with transmitted light, and with the inset showing chlorophyll autofluorescence of the same individual. Note that the ‘fingers’ lack strong chlorophyll autofluorescence.
Finger formation, followed in single individuals, also showed distinct stages. The earliest stages appeared to be of the longest duration (Fig. 7).

With regard to the structure of the fingers, it may be noteworthy that in preparing material for SEM observations we found that washing in distilled water un-fixed cells, or cells fixed in non-buffered formalin, resulted in finger loss. Examination of the fine-structure of fully-formed ‘fingers’ with SEM (Fig. 8) showed a rigid appearance, similar to thecal plates with many pores. In cells absorbing the fingers, the surface characteristics of the fingers appear similar (Figs 9—12). We noted the appearance of a ‘sleeve’ at the base of the horn, similar to that shown in Balech’s figure of a cell with short fingers (see Figs 1 and 9). Figures 9—12 are ordered in what we presume to be a sequence of finger absorption, based on the observations of a live individual documented in Figure 6.

Figure 5. Time-course changes in the portion of Ceratium ranipes cells showing fingers in a culture examined at 2 h intervals over a 24 period of 12 h in the light and 12 h in the dark. For each time point, 50-130 cells were examined.

Figure 6. Finger absorption in a single individual beginning at the start of the dark period (T=0) and then after 11 minutes of darkness (T=11), 68 minutes of darkness (T=68), 88 minutes of darkness (T=88), 117 minutes of darkness (T=117) and 211 minutes of darkness.
An SEM micrograph of a fingerless night form is shown in Figure 13 and resembles the figure of Balech (Fig. 1) and the stage shown in Figure 6 of a cell after 3.5 h of darkness. SEM observations of cells from the beginning of the light period are shown in Figures 14—17 in what we presume to be a sequence of finger formation based on the observations of a live individual documented in Figure 7.

Figure 7. Finger formation in a single individual beginning after 35 minutes of light (T=35), 97 minutes of light (T=97), 9 minutes of light (T=99), and 180 minutes of light (T=180).

Figure 8. SEM micrograph of a Ceratium ranipes from the light period. Note the rigid appearance of the fingers.

Figure 9. SEM micrograph of a Ceratium ranipes from the end of the light period. Note the shortened fingers and the sleeve appearance at the end of the horn, similar to that depicted in the drawings by Balech (Fig. 1).
Among a natural population in the Bay of Villefranche, we examined the morphologies of cells found at the end of the night and mid-day. *Ceratium ranipes*, while rare (14 in the pre-dawn sample and 10 in the day sample), conformed roughly, but not completely, to the pattern of cultured cells. For the pre-dawn sample in which we expected to find all cells without fingers, 10 of the 14 *C. ranipes* were fingerless. The late afternoon sample, in which we expected to find all cells with fingers, 6 of the 10 daytime cells observed showed fingers.

The possible role of fingers in altering the sedimentation rate of *Ceratium ranipes* cells was examined by estimating sinking rates of cells from cultures with and without fingers. We estimated sedimentation rates of preserved cells and found that cells without fingers sink faster at a rate of about 1 m h\(^{-1}\) compared to 0.6 m h\(^{-1}\) for cells with fingers.

**Discussion**

The fact that morphological variability occurs in dinoflagellates is well recognized (Taylor 1987). For example, the occurrence of seasonal variability in *Ceratium* morphologies has been known since at least the 1950s (Nielsen 1956). However, to our knowledge, this is the first report of diurnal changes in the gross morphology of a dinoflagellate. Interestingly though, individual elements of this phenomenon have been previously described in other dinoflagellates. The first observed is most likely that of the autotomy and regeneration of ‘horns’ in many species of *Ceratium*, described by Kofoid (1908, 1909) which he deduced to occur in *Ceratium* from observations made on preserved natural populations. Kofoid (1908) assumed that horns were ‘lost’, as opposed to broken, to alter the sedimentation rate, or floatability, of individual cells. The possible occurrence of autotomy in *C. ranipes* was noted by Taylor in his monograph of Indian Ocean Dinoflagellates as an explanation of the distinct morphologies found (Taylor 1976). Gross morphological change in individual dino-
flagellate cells has also been documented, in the form of the reversible effects of turbulence on the morphology of *Ceratocorys horrida* (Zirbel et al. 2000). Cells exposed to turbulence within hours lose the large spines typical of the species and exhibit small spines; in cells removed from turbulence, long-spines reappeared within a day (Zirbel et al. 2000). And finally, a large variety of diurnal or circadian rhythms, both physiological and behavioural, are known from dinoflagellates (Sournia 1975). These range from synchronous nighttime cell division in different species, including *Ceratium* (e.g., Chang and Carpenter 1994; Heller 1977; Weiler and Chisholm 1976; Weiler and Eppley 1979; Weiler and Karl 1979), to bioluminescence in a number of different genera (e.g., Sweeney and Hastings 1957; Kelly and Katona 1966), the expansion and contraction of chloroplasts in *Pyrocystis noctiluca* (Hardeland and Nord 1984), and diurnal migration through the water column in many species (e.g., Garcés et al. 1998; Macintyre et al. 1997), including *Ceratium* (Baek et al. 2009; Olsson and Granéli 1991). Thus, diurnal rhythms, reversible changes in morphology, and autotomy have been described separately but occur together in a single remarkable phenomenon in *Ceratium ranipes*.

Our attempts to vary culture conditions, and previous reports of culture failure with continuous light (Brand and Guillard 1981), suggest that a dark period is obligatory for *Ceratium ranipes*. Unfortunately, we can only speculate as to the ‘why’ of daily appendage growth and loss, a process which must involve a considerable cost. Kofoid (1908) suggested that autotomy in *Ceratium* was a means primarily of altering the cell surface to volume ratio in order to vary sinking speed. Indeed *Ceratium ranipes* without appendages may sink somewhat faster. We estimated sedimentation rates of preserved cells and found that cells without appendages sink at about 1 m h\(^{-1}\) compared to 0.6 m h\(^{-1}\) for cells with appendages. However, these rates were estimated for dead cells and living cells appear to...
exhibit different swimming speeds or patterns. Furthermore and most importantly, the possible value of sinking a few meters in the water column at night is unclear. *C. ranipes* is generally found at depths below 50 m (Taylor 1976). To our knowledge, there are no data showing diel vertical migration in *C. ranipes*.

A possible explanation for finger loss is that the cells, becoming smaller without appendages, enter a size-refuge from the larger planktonic predators present in the upper water column at night. As in other systems, in the N.W. Mediterranean Sea, a variety of relatively large planktonic omnivores and carnivores migrate from deeper waters into the surface layer to feed at night (e.g. Raybaud et al. 2008). These relatively large organisms (>500 μm) are found in the surface layer at night in concentrations several orders of magnitude greater than during the daylight hours. It is possible that *Ceratium ranipes* cells without fingers are less subject to predation from such migratory zooplankton and planktivorous fish.

Testing of the hypothesis would involve logistically difficult ship-board experiments with migratory organisms.
us to suggest that the classic taxonomic monographs, which we sometimes peruse with skepticism with regard to the reality of the variety of forms depicted (e.g., Dolan 2006), may be the place to begin such an examination.

Methods

Clonal cultures of Ceratium ranipes, started from individual cells isolated from the Bay of Villefranche-sur-Mer, were grown in K (Keller et al. 1987) medium in an illuminated culture chamber at 19 °C with a 12 h photoperiod (1 h at 62 μE m⁻² s⁻¹ followed by 10 h at 135 μE m⁻² s⁻¹ followed by 1 h at 62 μE m⁻² s⁻¹) and a 12 h dark period. Thus, cultures were exposed to artificial dusks and dawns. Cultures are available on request.

Observations of individual cells were made using 2 ml well plates containing isolated individuals. Light microscopic observations were made with a Zeiss Axiophot inverted microscope equipped with epifluorescence and a Cannon G6 digital camera. The protocol of Fritz and Triemer (1985) was used for Calcofluor staining.

For scanning electron microscopy, we examined cells from the end of the dark period and cells from the end of the light period. Cells were preserved with a fixative based on that of Cachon et al. (1991); with the following final concentrations: glutaraldehyde 2.5%, formaldehyde 4%, Na-Cacodylate 0.2 M, pH 7.2, NaCl 0.1 M, sucrose 0.15 M. Fixed cells were then gently drawn down on a 0.2 Nucleopore filter, rinsed and desalinated using distilled water rinses then dehydrated using an alcohol series followed by dessication with Hexamethyldisilazane HMDS, and palladium gold coating. Cells were examined using a FESEM JEOL 6700F.

Plankton net tow samples from the Bay of Villefranche were taken an hour before dawn, and again in the afternoon on December 12, 2008. Net tows employed a 50 μm mesh phytoplankton net towed from 70 m depth to the surface at 43°41.10’N, 7°19.00’E. For each net tow, 1 liter of material, representing the entire net tow material, was preserved with Lugol’s and examined in its entirety in 10 ml aliquots dispensed into a scored counted plate using a binocular microscope at 20× total magnification.

The sedimentation rate of Ceratium ranipes cells was examined by estimating sinking rates of cells from cultures with and without fingers. Aliquots of ‘day’ and ‘night’ cultures were preserved with Lugol’s fixative, mixed into 500 ml of seawater and dispensed into 100 ml sedimentation chambers. Cells were allowed to settle for periods ranging from 5 to 50 minutes and the number of cells in the bottom of each chamber determined with an inverted microscope. Plotting elapsed time against % of total cells we estimated the time corresponding to 100% of the cells sedimented. Using the mid-point of the 100 ml sedimentation chamber as the average distance traveled, we calculated a sedimentation rate of preserved cells for the two cell morphologies in m h⁻¹.

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Figure 17. SEM micrograph of a Ceratium ranipes from the beginning of the light period. Note the rigid appearance of the fingers.

Another possibility is that of the night form as a dispersal morphology. The daytime morphology with extensive chloroplast-filled ‘fingers’ while perhaps well-adapted as a ‘shade species’ (e.g., Sournia 1982) swims very slowly and appeared to easily become tangled with other cells. More efficient directed motion, at night when the chloroplast-filled fingers are not needed, may explain their loss.

While it well known that clones of a protistan organism can display different morphologies, our results demonstrate that a single individual undergoes remarkable morphological changes over a circadian cycle. Thus, regardless of the adaptive value of the phenomenon, the possibility that other species could also show diurnal variability in morphology, explaining the existence of apparently distinct varieties, merits examination. Depictions of nearly all the different stages we found in the absorption and formation of fingers in Ceratium ranipes existed in the literature. This leads...
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