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Ecology of the Red-Tide Dinoflagellate *Ceratium furca*: Distribution, Mixotrophy, and Grazing Impact on Ciliate Populations of Chesapeake Bay

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ABSTRACT. *Ceratium furca* is a primarily photosynthetic dinoflagellate also capable of ingesting other protists. During 1995 and 1996, we documented the abundance of *C. furca* in Chesapeake Bay and determined grazing rates on prey labeled with fluorescent microspheres. Abundance usually remained below 20 cells ml^{-1}, although the species was capable of localized late-summer blooms (≤ 478 cells ml^{-1}) in the more saline lower to mid-Bay region. Feeding rates ranged from 0 to 0.11 prey $dinoflagellate^{-1}$ h^{-1} or from 0 to 37 pg C $dinoflagellate^{-1}$ h^{-1} and were highest at lower salinities. Clearance rates averaged 2.5 ± 0.35 μl $dinoflagellate^{-1}$ h^{-1}. Impact of *C. furca* feeding on prey populations was higher in the lower Bay, averaging 67% of *Strobilidium* spp. removed d^{-1}. Ingestion rates were positively correlated with prey abundance and dissolved inorganic nitrogen, but negatively with salinity, depth, dissolved inorganic phosphorus, and inorganic P:N ratio. Daily consumption of prey biomass by *C. furca* averaged 4.6% of body carbon, 6.5% of body nitrogen, and 4.0% of body phosphorus, with maximal values of 36, 51, and 32%, respectively. Thus, the ability to exploit an organic nutrient source when inorganic nutrients are limiting may give *C. furca* a competitive advantage over purely photosynthetic species.

Key Words. Bloom formation, ingestion rate, nutritional benefits, *Strobilidium* spp.

OVER the last few decades, the frequency, magnitude and geographic extent of red-tide outbreaks produced by toxic and non-toxic dinoflagellates have increased (Anderson 1997; Hallegraeff 1993). Yet, the factors leading to initiation and persistence of these dinoflagellate blooms remain only partly understood. Several mechanisms have been suggested to play important roles. Physical factors, such as winds, currents, tidal flows, and density gradients, may act to concentrate cells in specific areas (Steidinger 1973). The ability to form cysts is thought to be another important factor, providing seed populations for blooms (Steidinger and Vargo 1988). In addition, upwelling of nutrient-rich water, enhanced organic and inorganic nutrient loading, high incident photosynthetically-active radiation (PAR), and reduced grazing pressure have been used to explain formation and persistence of dinoflagellate blooms (Gallegos, Jordan, and Corell 1992; Huntley 1982; Loftus, Subba Roa, and Selinger 1972; Paerl 1988).

More recently, increased attention has been given to the mixotrophic behavior so prevalent among many red-tide dinoflagellates. Mixotrophs gain their nutrition through a combination of photosynthesis and uptake of dissolved or particulate organic material. Supplementing their nutrition in this manner could give red-tide dinoflagellates a competitive advantage over strictly photosynthetic organisms, since phagotrophy would enable the mixotrophic species to acquire nutrients and carbon when these essential growth factors are limiting (Li, Stoecker, and Coats 2000a, b; Nygaard and Tobiesen 1993; Sanders, Porter, and Caron 1990). This ability to take advantage of additional nutrient sources may thus contribute to bloom formation and persistence (Smayda 1997; Stoecker et al. 1997).

In Chesapeake Bay, USA, mixotrophy is prevalent among many of the photosynthetic dinoflagellates capable of bloom-formation, including *Akashiwo sanguinea* (= *Gymnodinium sanguineum*), *Ceratium furca*, *Gyrodinium uncatenum*, *Gyrodinium galatheanum*, and *Prorocentrum minimum* (Bockstahler and Coats 1993a, b; Li et al. 1996; Smalley, Coats, and Adam 1999; Stoecker et al. 1997). However, feeding rates have only been determined for a few of these species. Bockstahler and Coats (1993b) and Li, Stoecker, and Coats (2001) estimated feeding and impact on prey populations by *A. sanguinea* and *G. galatheanum* using a 'gut clearance/gut fullness' approach (Dolan and Coats 1991; Kopylov and Tumantseva 1987). In this approach, digestion rates determined by following the disappearance of phagocytized prey over time were used in conjunction with data on "gut content" to estimate ingestion rates of specimens collected from the field or from laboratory cultures. This approach is attractive as no handling or incubation of the dinoflagellate is needed to estimate ingestion rates. However, it is very labor-intensive, and several assumptions are required. For example, digestion rate must be independent of "gut content," and ingestion and digestion must be in equilibrium (i.e. steady-state conditions).

The presence of food vacuoles in *C. furca* of Chesapeake Bay has been reported previously (Bockstahler and Coats 1993b; Li et al. 1996), although neither study determined feeding rates for the species. Recently, however, Smalley, Coats, and Adam (1999) investigated feeding in field populations of *C. furca* by employing a method using 1-μm fluorescent microspheres to label prey. Microspheres were added to natural plankton assemblages and were rapidly ingested by a variety of planktonic organisms including small flagellates, ciliates, dinoflagellates, ebriids, and amoebae. While *C. furca* did not ingest microspheres directly, labeled prey were phagocytized and could easily be detected within the dinoflagellate using epifluorescence microscopy. Ingestion rates were then determined by following the appearance of labeled food vacuoles in *C. furca* over time. Analysis of food vacuole content and potential prey availability indicated that *C. furca* preyed mainly on choreotrich ciliates of the genus *Strobilidium*, 10–40 μm in diameter. This method has several advantages for the study of mixotrophy in large, slow-feeding dinoflagellates, such as *C. furca*. Live prey found in natural water samples are used, and surface characteristics of prey presumably remain unaltered. No addition of prey is necessary, and only a minimum amount of handling of the sample is required. Incubation experiments can last for six hours, allowing sufficient time for the appearance of labeled food vacuoles in predators with low feeding rates.

In the present study, we used the method described in Smalley, Coats, and Adam (1999) to document the spatial and seasonal patterns of mixotrophy in *C. furca*. During monthly survey cruises along the main axis of Chesapeake Bay, we determined the distribution of *C. furca* and ciliate prey and investigated the potential role of physical, biological, and chemical factors in regulating *C. furca* distribution and feeding patterns. In addition, we estimated grazing impact on ciliate populations and calculated the potential nutritional contribution of ingested prey to *C. furca*.

MATERIALS AND METHODS

Field sampling. To determine the temporal and spatial aspects of *Ceratium furca* abundance and mixotrophy, surface

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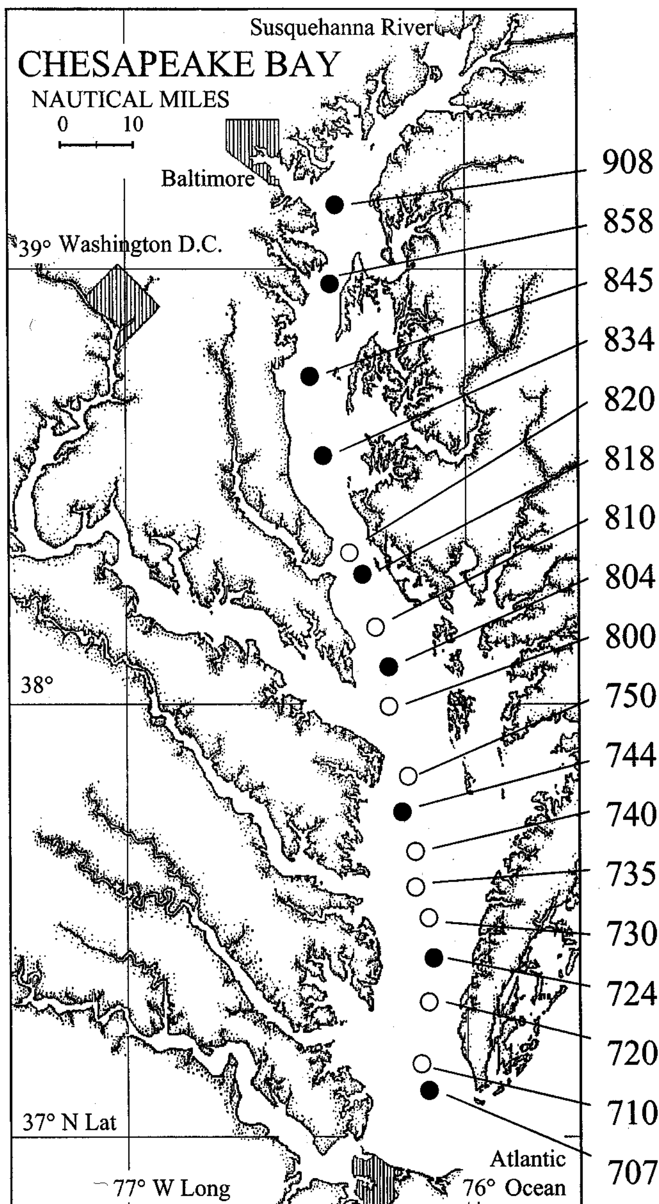

Fig. 1. Map of Chesapeake Bay showing routine (●) and additional (○) stations occupied during cruises in 1995 and 1996. Routine station designations from north to south are: 908 (39° 08′ N, 76° 20′ W); 858 (38° 58′ N, 76° 23′ W); 845 (38° 45′ N, 76° 26′ W); 834 (38° 34′ N, 76° 26′ W); 818 (38° 18′ N, 76° 17′ W); 804 (38° 04′ N, 76° 20′ W); 744 (37° 44′ N, 76° 11′ W); 724 (37° 24′ N, 76° 05′ W); 707 (37° 07′ N, 76° 07′ W). Additional station locations from north to south are: 820 (38° 20′ N, 76° 21′ W); 810 (38° 10′ N, 76° 15′ W); 800 (38° 00′ N, 76° 14′ W); 750 (37° 50′ N, 76° 07′ W); 740 (37° 40′ N, 76° 06′ W); 735 (37° 35′ N, 76° 11′ W); 730 (37° 30′ N, 76° 07′ W); 720 (37° 20′ N, 76° 07′ W); 710 (37° 10′ N, 76° 08′ W). In the text, stations 845 to 908 are referred to as upper (northern) Bay, 750 to 834 as mid-Bay, and 707 to 740 as lower (southern) Bay.

water samples were collected at routine stations along the main axis of Chesapeake Bay (Fig. 1) during June through September 1995 and July through September 1996. Additional samples were taken at intermediate stations and at various depths when the need or opportunity arose (Fig. 1). At each station, vertical CTD (conductivity, temperature, depth, plus fluorescence) and Niskin bottle casts were made during daylight hours. Duplicate water samples for inorganic nutrient analysis (i.e. nitrate and nitrite, ammonia, dissolved inorganic phosphate) were collected at routine stations, filtered, and stored frozen until analysis (Technicon Autoanalyzer II, Bran and Luebbe detector, TAOS software; Technicon Engineering Inc., Atlanta, GA), as described in Stoecker et al. (1997).

Feeding by *C. furca* on ciliate prey was determined at all stations and depths where *C. furca* densities exceeded ~ 0.25 cells ml^{-1}. Ingestion rates were determined as described in Smalley, Coats, and Adam (1999). Briefly, whole water containing *C. furca* was spiked with 1-μm fluorescent latex microspheres (Polysciences Inc., Warrington, PA) at a final concentration of 0.5–1.0 × 10^7 ml^{-1}. The water was incubated in polycarbonate bottles (250-ml to 2-liter bottles, depending on *C. furca* density) in a flowing seawater bath on deck. The waterbath was covered with a screen that reduced the incoming light intensity by approximately 65%. Twenty- to 40-ml aliquots were withdrawn and immediately preserved in modified Bouin's fixative (Coats and Heinbokel 1982) for subsequent determination of ciliate abundance. After approximately six hours of incubation, 100 ml to 2 liters of the sample were fixed in modified Bouin's solution for analysis of *C. furca* density and ingestion rates.

Ceratium furca densities and number of food vacuoles per *C. furca* cell were determined by placing each sample in a Zeiss settling chamber (5 to 50 ml, depending on *C. furca* densities). After allowing sufficient time for settling, the entire chamber was scanned at 200× to 400× on an inverted microscope equipped with epifluorescence optics (Zeiss Axioscope; 450–490 nm excitation). Cell densities were obtained by determining the total number of cells per chamber. The number of labeled food vacuoles per cell was recorded for the first 100 specimens encountered. Hourly ingestion rate was then calculated by dividing the mean number of food vacuoles per *C. furca* by incubation time. This approach was justified as Smalley, Coats, and Adam (1999) found a linear increase in food vacuoles per cell over at least six hours, indicating that food vacuoles were retained during this time period. To enumerate and identify ciliates, 20- or 40-ml quantitative Protargol stain (QPS) preparations were made (Montagnes and Lynn 1987) from each sample where *C. furca* feeding had been determined. Ciliates present in arbitrarily selected microscope fields (1,000×, Zeiss Axioscope) were enumerated and identified to genus until 100 cells had been encountered or an equivalent of 4 ml whole-water sample analyzed. Specimens belonging to the genus *Strobilidium* were measured using a calibrated ocular micrometer, and cell volume was calculated using the appropriate geometric formulae.

Impact of *Ceratium furca* grazing on prey populations. Impact of *C. furca* grazing on prey populations was expressed as daily removal of ciliate standing stock by *C. furca* as a proportion of prey abundance, assuming constant feeding over a 24-h period (i.e. no diel variations):

$$\% \text{ prey population removed } d^{-1} = 100 \times (24 \times I \times (C.\ furca\ ml^{-1}))\ /\ (\text{prey } ml^{-1}),$$

where I is the ingestion rate determined for *C. furca* (labeled prey *C. furca*$^{-1}$ h^{-1}). Values for ciliate and *C. furca* abundances and *C. furca* ingestion rate were obtained as described above.

Potential contribution of mixotrophy to *Ceratium furca* nutrition. Estimates of potential contribution of ingested carbon (C), nitrogen (N), and phosphorus (P) to *C. furca* are given as the daily ingestion relative to cellular content of *C. furca*:

$$\% \text{ ingested body C, N, or P} = 100 \times 24\ h \times I \times (\text{cellular C, N, or P content of prey})/\text{cellular C, N, or P content of } C.\ furca.$$

Daily ingested C, N, and P by *C. furca* were calculated assuming constant feeding over a 24-h period. We further assumed that *C. furca* fed on ciliates of the genus *Strobilidium* only (see Smalley, Coats, and Adam 1999).

Cellular C, N, and P contents of prey were estimated from biovolume measurements of *Strobilidium* sp. in QPS preparations. To convert ciliate biovolume to C, N, and P biomass, we determined C, N, and P densities for a *Strobilidium* sp. isolated from the Choptank River, a Maryland tributary of Chesapeake Bay (cultures obtained from Matt Johnson). Cells were grown in 7 practical salinity units (psu) estuarine water at 20 °C and fed the flagellate *Isochrysis galbana*. We measured cell vol. of live, 2% acid Lugol's-fixed, Bouin's-fixed, and QPS- treated ciliates and determined the extent of cell shrinkage upon fixation and QPS treatment. Mean cell vol. of live *Strobilidium* sp. was obtained with a Coulter Multisizer II electronic particle counter (Coulter Corporation, Miami, FL) interfaced with AccuComp particle characterization software (Coulter AccuComp Version 2.01, 1998). Cell vol. of Lugol's-fixed, Bouin's-fixed, and QPS specimens were determined by measuring width and length of 50 specimens with a calibrated ocular micrometer and calculating cell vol. using the formula for a prolate spheroid.

Cellular C, N, and P contents of *Strobilidium* sp. were determined for cultures with low *Isochrysis galbana* density. To correct for C, N, and P contributed by remaining prey, ciliate-free samples were obtained by removing ciliates from flagellate prey by gentle gravity filtration through a 5-μm Nucleopore filter. Triplicate samples of both ciliate-containing culture and ciliate-free filtrate were collected on precombusted Whatman GF/F filters for CHN (20 ml) and particulate P (20 ml) analyses. Cellular C and N were analyzed with an EAI CE-440 Elemental Analyzer (Exeter Analytical, Inc., North Chelmsford, MA), and particulate P was determined as soluble reactive P following high temperature combustion and hydrochloric acid digestion (Anderson 1976). The C, N, and P contents of *Strobilidium* sp. were corrected for remaining prey by subtracting the values obtained for the ciliate-free samples from those obtained for the unfiltered culture. *Strobilidium* sp. abundance was determined microscopically (100×) after Lugol's fixation by enumerating all specimens found in eight Palmer-Maloney chambers. Flagellate density was obtained by examining randomly chosen microscope fields (400×) of six Sedgwick-Rafter chambers until 100 cells had been encountered for each chamber. We calculated C, N, and P densities of *Strobilidium* sp. by dividing the C, N, and P contents by cell vol.

Cellular C, N, and P contents of *C. furca* were determined for cultures isolated from Chesapeake Bay by D. Wayne Coats. Replicate cultures were maintained at 20 °C and 150 $\mu E\ m^{-2}\ s^{-1}$ on a 14:10 h light:dark cycle in modified f/2-Si medium (Guillard and Ryther 1962). This medium was prepared using 15 psu Chesapeake Bay water supplemented with various combinations of nitrate and phosphate, ranging from 7.5–870 μM NO_3 and 0.06–25 μM PO_4. Over a 30-day period, 50-ml aliquots were repeatedly removed from each bottle for determination of cell density and particulate phosphorus and CHN analysis. Samples were washed three times (GF/F filtered, autoclaved 15 psu seawater) using gentle gravity filtration through 8 μm Nucleopore filters to remove bacteria and cell debris. *Ceratium furca* densities were determined microscopically after Lugol's fixation (2% final concentration) by examining successive microscope transects (100—200×) of triplicate Sedgwick-Rafter chambers until 100 cells had been counted for each chamber. Samples for CHN (20 ml) and particulate P (20 ml) analyses were collected, processed, and analyzed as above.

To estimate the relative importance of ingested prey C to photosynthetic C uptake, we used an average photosynthetic rate of 39 ± 1.1 pg C *C. furca*$^{-1}$ h^{-1} (GWS., unpubl. data) obtained for *C. furca* isolated from Chesapeake Bay and maintained in culture as described above. This rate was assumed to be representative of average photosynthetic rates in the field. Furthermore, photosynthetic C uptake was estimated over a 14-h photoperiod, while feeding was assumed to continue throughout the night.

Statistical analyses. All statistical analyses were performed using SigmaStat Version 2.0 (SPSS Inc.). As most data failed tests for homogeneity of variance or normality, non-parametric tests were performed for the majority of analyses. Spearman rank order correlation was used to relate abundance and feeding of *C. furca* to a variety of biological (*C. furca* and prey abundance, in vivo fluorescence), chemical (nutrient concentrations), and physical factors (salinity, temperature, depth). For mean comparison, Kruskal-Wallis ANOVA on ranks was conducted in most cases, unless the data met assumptions for one-way ANOVA. Means are reported ± standard error of the mean (SE).

RESULTS

Ceratium furca was present in Chesapeake Bay during all cruises in 1995 and 1996 (Fig. 2A, 3A). In both June (data not shown) and July 1995 (Fig. 2A), *C. furca* densities were low (≤ 7 ml^{-1}), and the species was restricted to the southern Bay (salinity ≥ 15 psu). By August, a localized peak in surface density of *C. furca* was evident at station 750, and the species extended further into the mid-Bay (salinity ≥ 13 psu). A more pronounced peak in cell density was evident in September at a similar location (station 800), reaching a surface density of 324 *C. furca* ml^{-1}. Densities remained low to the north and south of station 800, and cells were not found in waters below 18 psu. In July 1996, *C. furca* was more abundant (up to 19 ml^{-1}) and extended further into the mid-Bay region (salinity ≥ 10 psu) than in 1995 at that time of year (Fig. 3A). By August, a localized bloom had developed at station 730, with surface cell density reaching 480 ml^{-1}. *Ceratium furca* was also abundant to the south of this bloom, but did not extend into waters with salinities below 10 psu. The bloom had dissipated by September, and cells remained restricted to ≥ 10 psu waters. Median *C. furca* densities during cruises in 1995 and 1996 were not significantly different from each other (Table 1).

Strobilidium spp. densities and ingestion of labeled prey by *C. furca* were very low at all stations examined in June (data not shown), July, and September 1995 (Fig. 2A, B), with no clear spatial pattern emerging. In August 1995, *Strobilidium* spp. were slightly more abundant than in previous and subsequent months, especially in the mid- and upper Bay. A peak in ingestion rate at station 818 coincided with highest *Strobilidium* spp. density. During cruises in 1996, median *Strobilidium* spp. and total ciliate densities and ingestion rates of *C. furca* were significantly higher than in the previous year (Table 1), again mainly in the mid- to upper Bay (Fig. 3A, B). In July, peak *Strobilidium* spp. density and maximum *C. furca* ingestion rates coincided with high *C. furca* abundance in ~ 11–12 psu waters. By August, the *Strobilidium* spp. peak reached 50 ml^{-1} and had shifted northward following the 11 psu isohaline. Ingestion rates were also higher in the mid-Bay, where *C. furca* densities were lower. In September, *Strobilidium* spp. remained more abundant in the mid-Bay, north of maximum *C. furca* densities. Ingestion rates exhibited a similar pattern, but in addition to increased rates in the mid-Bay, they were also higher south of the *C. furca* density peak in the lower Bay.

Impact of *C. furca* feeding on *Strobilidium* spp. standing stock was highly variable among stations and months (Fig. 2B, 3B), and no significant difference was found between median impact during the summer months of 1996 and 1995 (Table 2). Impact remained below 50% *Strobilidium* spp. removed d^{-1} for

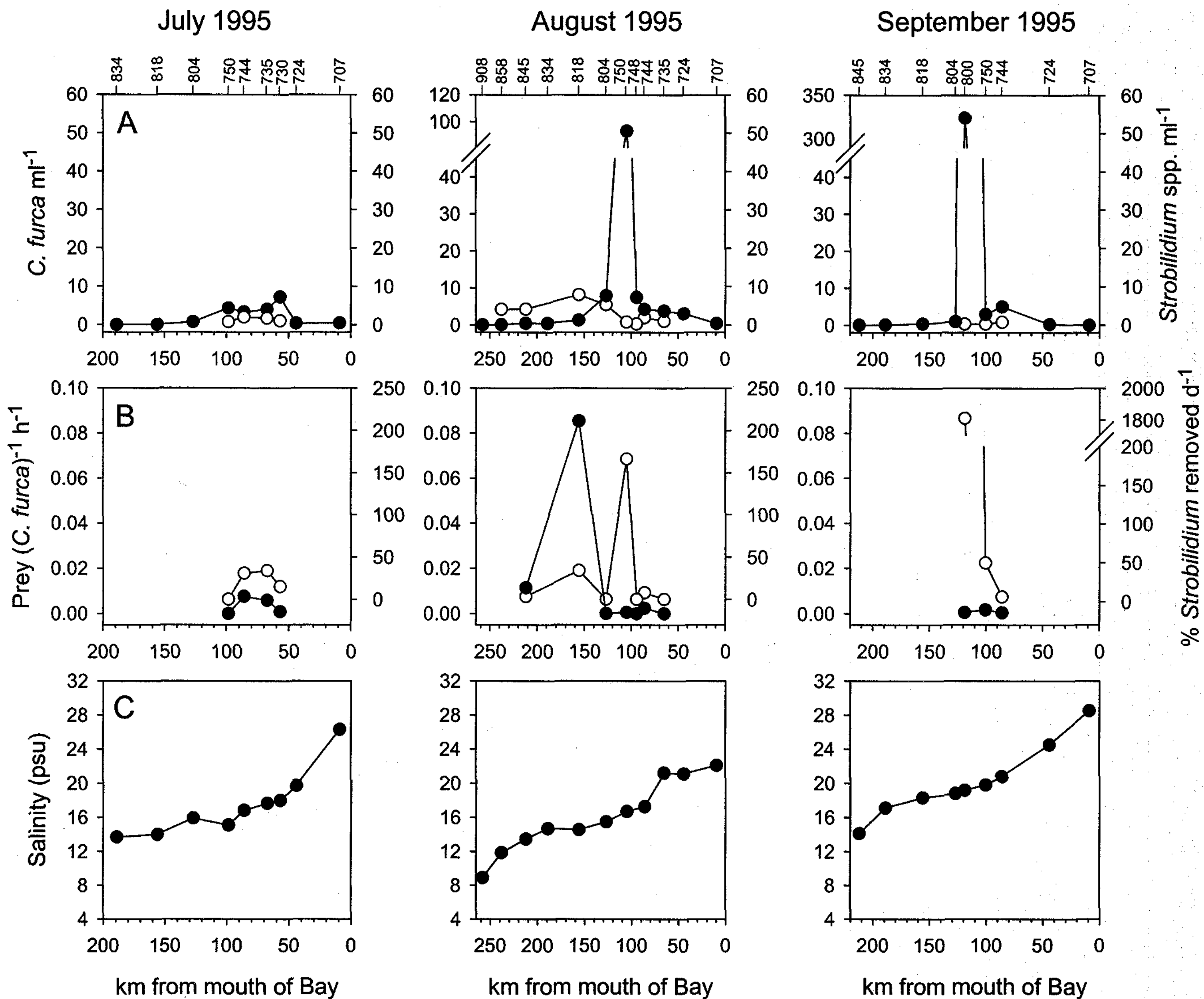

Fig. 2. **A.** Abundance of *Ceratium furca* (●) and *Strobilidium* spp. (○) in Chesapeake Bay during cruises in 1995. **B.** Ingestion of labeled prey by *C. furca* (●) and impact on *Strobilidium* spp. populations (○). **C.** Surface salinity. Station designations are indicated at the top of the panels (see Fig. 1 for station locations).

most of 1995, except for two stations in the lower to mid-Bay (750 in August, 800 in September; Fig. 2B). At these stations, low prey abundance coincided with high *C. furca* densities. Despite low feeding rates, this combination resulted in some of the highest theoretical values (≤ 1,800%) of *Strobilidium* spp. removed d^{-1} observed during our study period. In July 1996, maximum impact was again found in the lower to mid-Bay region where *C. furca* was most abundant (Fig. 3B). By August, peak impact had shifted to the lower Bay, where ciliates were less abundant, and this pattern persisted into September.

Combining data from cruises during both years revealed some interesting trends (Fig. 4). Densities of *C. furca* tended to be highest at intermediate (~ 15 psu) salinities, while *Strobilidium* spp. were most abundant at lower salinities (< 14 psu). Ingestion rates were also highest at these lower salinities where prey were abundant. Conversely, impact on *Strobilidium* spp. populations tended to be highest at higher salinities (~ 18 psu), coinciding with an increased relative abundance of *C. furca*.

Several biological, physical, and chemical factors were examined for association with abundance and ingestion rate of *C. furca*. Only one factor, in vivo fluorescence, showed a significant negative correlation with *C. furca* density ($r = -0.317$, $n = 84$, $p = 0.003$). While no relationship between *C. furca* abundance and salinity was found when data for both years were combined, a significant positive correlation was evident when only data for 1996 were considered ($r = 0.385$, $n = 44$, $p = 0.010$). Cells were most abundant at salinities ranging from 15 to 22 psu in 1995 and from 11 to 22 psu in 1996 and were restricted to waters above 10 psu throughout the study period (Fig. 2, 3). Ingestion rate, on the other hand, was negatively correlated with salinity ($r = -0.671$, $n = 65$, $p < 0.001$), suggesting that feeding was higher in the mid-Bay, where *C. furca* was near its lower salinity tolerance limit, but where prey were more abundant (Fig. 4). Strong positive correlations were found between total ciliate and *Strobilidium* spp. abundance and ingestion rate ($r = 0.577$, $n = 64$, $p < 0.001$ and $r = 0.806$, $n = 64$, $p < 0.001$, respectively). Depth exhibited a negative correlation with ingestion rate ($r = -0.354$, $n = 66$, $p = 0.004$),

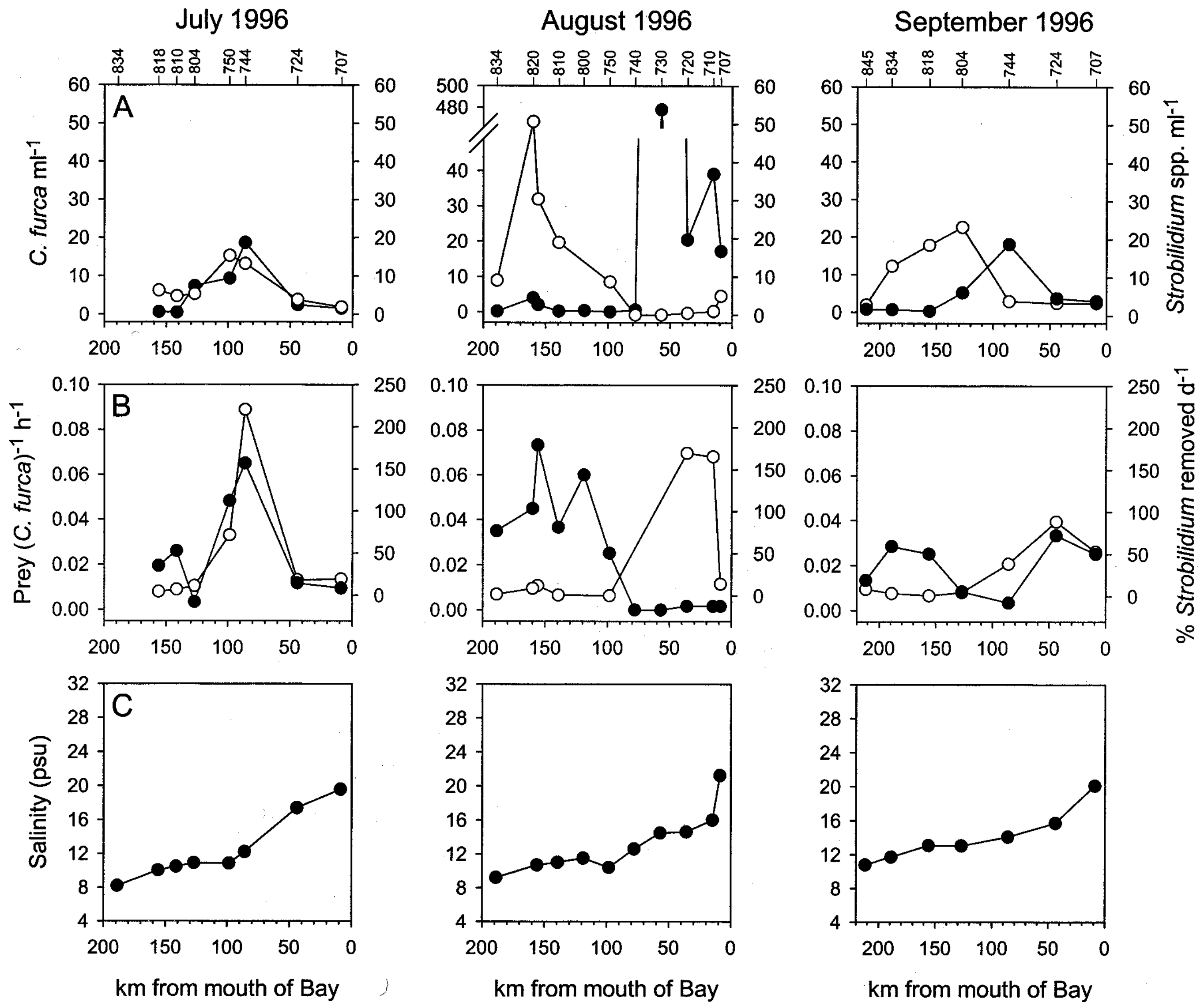

Fig. 3. **A.** Abundance of *Ceratium furca* (●) and *Strobilidium* spp. (○) in Chesapeake Bay during cruises in 1996. **B.** Ingestion of labeled prey by *C. furca* (●) and impact on *Strobilidium* spp. populations (○). **C.** Surface salinity. Station designations are indicated at the top of the panels (see Fig. 1 for station locations).

Table 1. Abundance of *Ceratium furca,* total ciliates, and *Strobilidium* spp. and ingestion and clearance rates of *C. furca* from Chesapeake Bay.

	Abundance			Feeding	
	C. furca ml^{-1}	Ciliates ml^{-1}	*Strobilidium* spp. ml^{-1}	(Prey *C. furca*$^{-1}$ h^{-1})	(µl *C. furca*$^{-1}$ h^{-1})
June–Sept. 1995:					
Mean ± SE	12.4 ± 8.31 (ns)	32 ± 5.1*	1.9 ± 0.43*	0.007 ± 0.0042*	2.1 ± 0.65 (ns)
Median	1.3	21	1.4	0.0007	0.9
Range	0–324	7–77	0–7.8	0–0.085	0–10.9
July–Sept. 1996:					
Mean ± SE	17.8 ± 11.13 (ns)	63 ± 5.3*	11.3 ± 1.83*	0.026 ± 0.0044*	2.7 ± 0.4 (ns)
Median	1.6	53	8.6	0.01	0.4
Range	0–478	6–136	0–51	0–0.11	0–10.8
Total:					
Mean ± SE	14.9 ± 6.82	53 ± 4.2	8.9 ± 1.45	0.02 ± 0.003	2.5 ± 0.35
Median	1.4	50	4.0	0.008	2.0
Range	0–478	6–136	0–51	0–0.11	0–10.9

ns = no significant difference ($p > 0.05$), * $p < 0.001$ between average values for 1995 and 1996 (Kruskal-Wallis ANOVA on ranks).

Table 2. Estimated impact of *Ceratium furca* grazing on prey populations of Chesapeake Bay.

	Percent prey standing stock removed d^{-1}		
	Ciliates	<20 μ Choreotrichids[a]	*Strobilidium* spp.
June–September 1995:			
Mean ± SE	2.1 ± 0.97 (ns)	4.4 ± 1.55 (ns)	115 ± 94.6 (ns)
Median	0.50	1.5	6.6
Range	0–18	0–24	0–1810
July–September 1996:			
Mean ± SE	9.4 ± 3.49 (ns)	22 ± 7.6 (ns)	45 ± 12.9 (ns)
Median	1.0	3.1	8.5
Range	0–106	0–218	0–375
Total:			
Mean ± SE	6.8 ± 2.31	16 ± 5.0	67 ± 31.3
Median	0.9	2.5	7.9
Range	0–106	0–218	0–1810

ns = no significant difference ($p > 0.05$) between average values for 1995 and 1996 (Kruskal-Wallis ANOVA on ranks).
[a] Order Choreotrichida, defined in accordance with Small and Lynn (1985) to include the suborders Tintinnina, Strombidinopsina, and Strobilidiina.

indicating that *C. furca* fed more heavily in surface than in deeper waters. In addition, significant positive correlations of ingestion rate with NH_4 (r = 0.349, n = 49, p = 0.014), dissolved inorganic nitrogen (DIN; r = 0.372, n = 49, p = 0.009), and dissolved inorganic phosphorus concentrations (DIP; r = −0.311, n = 49, p = 0.030) were evident (Fig. 5A, B). Concentrations of NO_3 + NO_2 were not correlated with feeding (n = 49, p = 0.153). The ratio of DIP:DIN exhibited a strong negative correlation with feeding rate (r = −0.477, n = 49, p < 0.001; Fig. 5C). Feeding increased as DIP:DIN ratios deviated farther from the Redfield ratio of 1:16.

Potential contribution of mixotrophy to *Ceratium furca* nutrition. Cellular C, N, and P contents of cultured *C. furca* grown at various nutrient concentrations averaged 2460 ± 25 pg C $cell^{-1}$, 427 ± 4.7 pg N $cell^{-1}$, and 94 ± 2.0 pg P $cell^{-1}$. Average C, N, and P contents of *Strobilidium* sp. were 126 ± 17.7 pg C $cell^{-1}$, 31 ± 1.3 pg N $cell^{-1}$, and 4.2 ± 1.63 pg P $cell^{-1}$, with C:N, C:P, and N:P ratios (molar) of 4.8, 78, and 16.3, respectively. Mean cell vol. of live *Strobilidium* sp. was 1680 ± 1 μm^3. Volumes of Lugol's-fixed, Bouin's-fixed, and QPS-treated specimens decreased significantly compared to that of live cells (F = 13.1, df = 199, p < 0.001), shrinking by 24, 45, and 48%, respectively. This resulted in different conversion factors for biovolume to C, N, and P biomass, depending on which fixative or stain was used. While live cells contained 0.08 pg C μm^{-3}, 0.018 pg N μm^{-3}, and 0.0025pg P μm^{-3}, the conversion factors for QPS-treated cells were 0.14 pg C μm^{-3}, 0.035 pg N μm^{-3}, and 0.0048 pg P μm^{-3}.

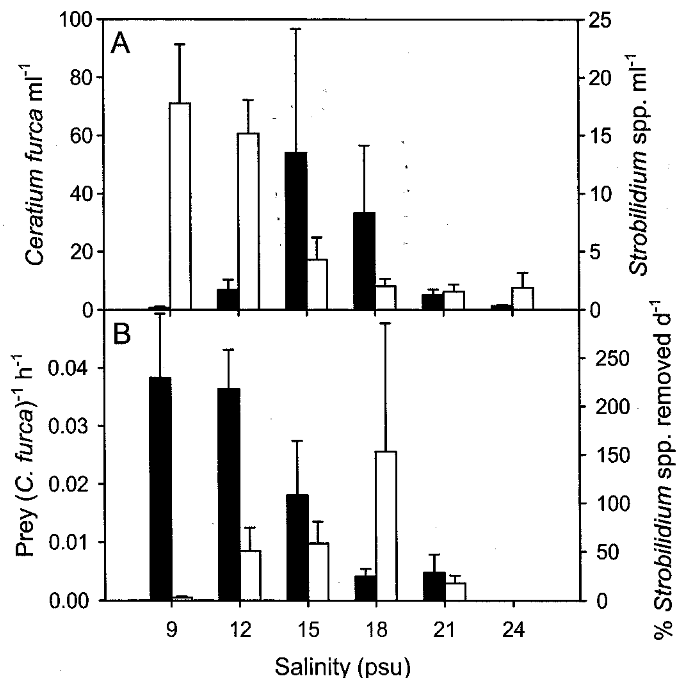

Fig. 4. **A.** *Ceratium furca* (■) and *Strobilidium* spp. (□) abundance in relation to salinity in Chesapeake Bay. **B.** Ingestion of labeled prey by *C. furca* (■) and impact on *Strobilidium* spp. populations (□) in relation to salinity. Mean ± SE were derived from combined data for 1995 and 1996.

Mean ingestion of prey C, N, and P by *C. furca* is shown in Table 3, while daily ingestion relative to cellular content of *C. furca* and the fraction of heterotrophic C uptake relative to total C uptake are presented in Table 4. During our study period, potential contribution of prey C, N, and P to *C. furca* was higher in the mid- to upper Bay, where *C. furca* abundance was low but feeding rate was high. Ingestion of prey C, N, and P and thus potential contribution of phagotrophy to *C. furca* nutrition was significantly higher during cruises in 1996 than in 1995 (Table 3, 4).

DISCUSSION

Ceratium furca was present in Chesapeake Bay throughout our study period, usually at densities ≤ 20 ml^{-1}. However, the species was capable of localized late-summer bloom formation in the lower to mid-Bay region, reaching densities of 324 ml^{-1} and 478 ml^{-1} in September 1995 and August 1996, respectively. *Ceratium furca* seemed to be restricted to ≥ 10 psu waters and was most abundant at or above 15 psu, and at temperatures ranging from 24 to 27 °C. A similar distribution pattern of *C. furca* in Chesapeake Bay was observed by Bockstahler and Coats (1993a) for 1988. They reported peak *C. furca* densities in the mid-Bay for July at salinities of 13–16 psu, with decreasing abundance at lower and higher salinities. Earlier studies mainly restricted to the lower Bay also documented the occurrence of *C. furca*, which was most abundant in summer and fall (Cowles 1930; Marshall 1980) at salinities of 16–22 psu and temperatures of 23.9–24.6 °C (Mulford 1963).

Ceratium furca is a cosmopolitan species, and reports of its salinity and temperature optima vary greatly. For example, in

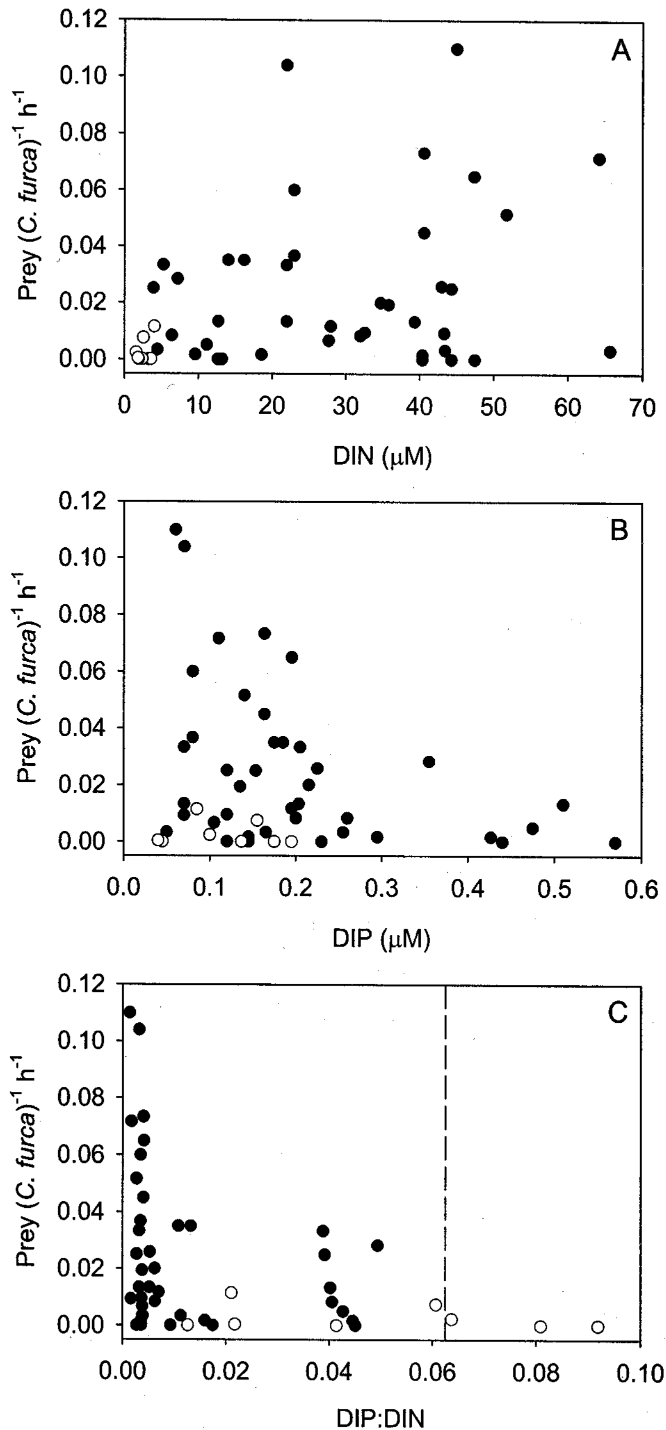

Fig. 5. Relationship between ingestion of labeled prey by *Ceratium furca* and dissolved inorganic nitrogen (A), dissolved inorganic phosphorus (B), and the DIP:DIN ratio (C) for 1995 (○) and 1996 (●) in Chesapeake Bay. Dashed line represents Redfield P:N ratio (1:16).

Laguna de Sontecomapan, Mexico, *C. furca* was detected in waters with salinities ranging from 13–35 psu, and temperatures from 30–34 °C (Guerra-Martínez and Lara-Villa 1996), while Nordli (1953) found most rapid growth of this species at 20–25 psu and 20 °C. These observed variations may be due to different physiological tolerance with regard to salinity and temperature among various strains or subspecies of *C. furca*.

Table 3. Estimated ingestion rates of prey carbon (C), nitrogen (N), and phosphorus (P) by *Ceratium furca.*

	Ingestion		
	(pg C *C. furca*$^{-1}$ h^{-1})	(pg N *C. furca*$^{-1}$ h^{-1})	(pg P *C. furca*$^{-1}$ h^{-1})
June–Sept. 1995:			
Mean ± SE	1.2 ± 0.68*	0.29 ± 0.167*	0.04 ± 0.023*
Median	0.2	0.05	0.01
Range	0–13.5	0–3.3	0–0.45
July–Sept. 1996:			
Mean ± SE	6.3 ± 1.26*	1.5 ± 0.31*	0.21 ± 0.042*
Median	4.0	.10	0.13
Range	0–37	0–9.1	0–1.24
Total:			
Mean ± SE	4.7 ± 0.94	1.2 ± 0.23	0.16 ± 0.031
Median	1.68	0.4	0.06
Range	0–37	0–9.1	0–1.24

* $p < 0.001$ comparing average values for 1995 and 1996 (Kruskal-Wallis ANOVA on ranks).

Alternatively, factors other than or in addition to salinity and temperature may influence *C. furca* distribution. In our study, *C. furca* abundance was positively correlated with salinity during cruises in 1996. Furthermore, the species was not found below the 10 psu isohaline, suggesting that salinity limited the northward expansion of *C. furca* during the summer of 1996. In 1995, however, no significant correlation between *C. furca* distribution and salinity was evident. In addition, the northern limit of the *C. furca* population coincided with waters ranging in salinity from 13 to 18 psu. Thus, the peak *C. furca* abundance at ~ 15 psu reported in this study probably reflects an interplay of salinity tolerance and other factors, such as inorganic and organic nutrient supply or grazing pressure.

A profound effect of river flow on the dynamics of phytoplankton biomass and production has been demonstrated for Chesapeake Bay at both seasonal and annual scales (Fisher et al. 1992, 1999; Harding 1994; Malone et al. 1988, 1996). Riverine input is the major source of freshwater and external nitrogen to the Bay. During winter and spring, maximum freshwater flow provides high nitrogen concentrations, pushing the system towards P limitation. When freshwater runoff decreases in summer, N becomes limiting to algal growth. Nutrient limitation also varies over spatial scales due to freshwater inflow with high N:P and seawater inflow with low N:P (Fisher et al. 1999). Consequently, P limitation is more pronounced in low salinity regions, while N limitation increases at higher salinities.

During our study period, mean annual flow of the Susquehanna River, the major source of freshwater to the Bay, was much lower in the drought year of 1995 (68×10^6 m^3 d^{-1}) than in 1996 (156×10^6 m^3 d^{-1}; data from US Geological Survey, Conowingo, MD). Consequently, salinity at our sampling sites was reduced significantly during cruises in 1996 compared to 1995 (13.5 ± 0.60 vs. 17.9 ± 0.65; $F = 19.584$, $df = 64$, $p < 0.001$), while nitrogen input and N:P increased by an order of magnitude (2.5 ± 0.32 vs. 29.2 ± 2.59 μM N and 30.1 ± 7.64 vs. 206.0 ± 26.02 N:P, respectively). This trend was observed Bay-wide (Li, Stoecker, and Coats 2000a; Smith 2000) and had a profound impact on phytoplankton community structure and production (Smith 2000). While dinoflagellates together with cyanobacteria dominated the summer phytoplankton assemblage in 1995, diatoms were important throughout the summer of 1996 (Smith 2000). In our study, we found that *Strobilidium* spp. and total ciliate abundance were significantly higher in 1996, possibly due to an increase in small flagellate or bacterial

Table 4. Potential contribution of prey carbon (C), nitrogen (N), and phosphorus (P) to *Ceratium furca* nutrition.

	Estimated C, N and P specific ingestion			Hetero/Photo[a] C uptake (%)
	% body C d^{-1}	% body N d^{-1}	% body P d^{-1}	
June–Sept. 1995:				
Mean ± SE	1.2 ± 0.66*	1.6 ± 0.94*	1.0 ± 0.59*	5 ± 3.0*
Median	0.2	0.3	0.2	0.8
Range	0–13	0–18.6	0–11.5	0–60
July–Sept. 1996:				
Mean ± SE	6.2 ± 1.23*	8.7 ± 1.74*	5.4 ± 1.07*	28 ± 5.6*
Median	3.9	5.5	3.4	18
Range	0–36	0–51	0–32	0–164
Total:				
Mean ± SE	4.6 ± 0.92	6.5 ± 1.30	4.0 ± 0.80	21 ± 4.2
Median	1.6	2.3	1.4	7.4
Range	0–36	0–51	0–32	0–164

[a] Hetero = heterotrophic, Photo = phototrophic; a photosynthetic rate of 38.8 pg C $cell^{-1}$ h^{-1} over a 14-h photoperiod was assumed to estimate phototrophic C uptake.

* $p < 0.01$ comparing average values for 1995 and 1996 (Kruskal-Wallis ANOVA on ranks).

prey. Interestingly, we did not observe a significant increase in the density of *C. furca* in 1996 compared to 1995 despite the higher nutrient concentrations. Increased grazing pressure may have prevented *C. furca* from reaching higher densities. Alternatively, *C. furca* may not have been able to capitalize on the increased inorganic nitrogen available to the plankton community. In the Arabian sea (Cochin Backwater, India), *C. furca* densities peaked at low nitrate concentrations and low N:P ratios, while the diatom *Biddulphia sinensis* predominated at higher N:P ratios when nitrate was more plentiful (Qasim, Bhattathiri, and Devassy 1973). The authors attributed this to the low half-saturation constants for N and P uptake measured in their isolate of *C. furca*. Similarly, the increased importance of diatoms throughout the summer of 1996 in Chesapeake Bay may have prevented *C. furca* from taking advantage of the additional inorganic nitrogen.

The unusually high N:P ratios in 1996 also raise the possibility of P limitation of *C. furca* growth or biomass. This hypothesis is particularly intriguing in light of two observations. First, cellular C:P and N:P ratios of *C. furca* cultures were lower than expected from the Redfield ratio (75 ± 2.4 and 10.9 ± 0.28 vs. 106 and 16, respectively). Similar ratios have been determined for certain other dinoflagellates and indicate a high relative P content compared to other phytoplankton species (Li, Stoecker, and Coats 2000b; Nielsen 1996; Sakshaug et al. 1984). Second, we observed significant negative correlations of ingestion rate with DIP and DIP:DIN, but a positive correlation with DIN. Feeding in *C. furca* may thus be stimulated by low inorganic nutrient concentrations (especially low DIP) and/or N:P ratios that deviate significantly from optimal values. In support of these observations, we found that nutrient additions to field samples of *C. furca* reduced feeding, while ingestion was stimulated by N or P limitation in laboratory cultures of the same dinoflagellate (Smalley and Coats 2000; Smalley, Coats, and Stoecker 2001). This pattern would be expected for type IIA mixotrophs (i.e. primarily photosynthetic organisms that phagocytize in response to limiting dissolved inorganic nutrients (Stoecker 1998)), and has been documented for some photosynthetic dinoflagellates (Li, Stoecker, and Coats 2000a, b; Stoecker et al. 1997). Our findings thus suggest possible P limitation of *C. furca* throughout the summer of 1996, conceivably due to the high riverine N input observed that year. In addition to stimulating feeding in 1996, this P deficiency could have led to the observed increase in feeding at lower salinities, where higher N:P ratios would be expected.

Feeding in *C. furca* may depend not only on inorganic nutrient concentrations and N:P ratios but also on prey abundance. We observed a strong positive correlation between ingestion rate and prey density. In addition, increased feeding at lower salinities coincided with higher ciliate densities at these stations. This relationship may also partially explain the significantly higher ingestion rates in 1996 than in 1995, as ciliate and *Strobilidium* spp. abundance was higher in 1996 as well.

Ingestion and clearance rates reported here for *C. furca* fall within the range determined for other mixotrophic and heterotrophic dinoflagellates. The large mixotrophic dinoflagellate *Akashiwo sanguinea* (= *Gymnodinium sanguineum*) ingested ciliates at a rate up to 0.06 prey $individual^{-1}$ h^{-1} and 29.6 pg C $individual^{-1}$ h^{-1}, with clearance rates between 0 and 5.8 μl $individual^{-1}$ h^{-1} (Bockstahler and Coats 1993b). On the other hand, the much smaller mixotrophic dinoflagellate *Gyrodinium galatheanum* exhibited lower feeding rates, with maximal rates of 0.011 prey $individual^{-1}$ h^{-1} or 0.5 pg C $individual^{-1}$ h^{-1} (Li, Stoecker, and Coats 2001), and with a maximal clearance rate of 0.01 μl $individual^{-1}$ h^{-1}. Because our feeding estimates are based on uptake of prey labeled with fluorescent microspheres only, reported ingestion rates could underestimate feeding if a significant proportion of prey did not take up the label. However, this problem is minimized as *C. furca* fed primarily on small choreotrich ciliates of the genus *Strobilidium*, which were readily labeled within 15 min of microsphere addition (Smalley, Coats, and Adam 1999). If feeding on other choreotrich taxa was significant, clearance rates reported here could be overestimated by a factor of up to 5 as they were calculated assuming feeding on *Strobilidium* spp. only.

A synthesis of feeding in heterotrophic and mixotrophic dinoflagellates in relation to body size is presented in Fig. 6. As reported previously (Hansen, Bjørnsen, and Hansen 1997; Strom 1991), heterotrophic dinoflagellates showed a significant decrease in specific ingestion and clearance rates with increasing body size (Fig. 6). This trend was not observed for mixotrophic dinoflagellates, although data on feeding was limited to a few species. While maximal feeding rates of the larger mixotrophs fell within the range reported for heterotrophic dinoflagellates, the smaller mixotrophic species tended to have lower specific ingestion and clearance rates compared to heterotrophic dinoflagellates of similar size. However, this apparent increase in specific ingestion and clearance rates with increasing body size was not statistically significant ($n = 7$, $p = 0.080$ and $n = 5$, $p = 0.296$, respectively). Whether the observed trend is an

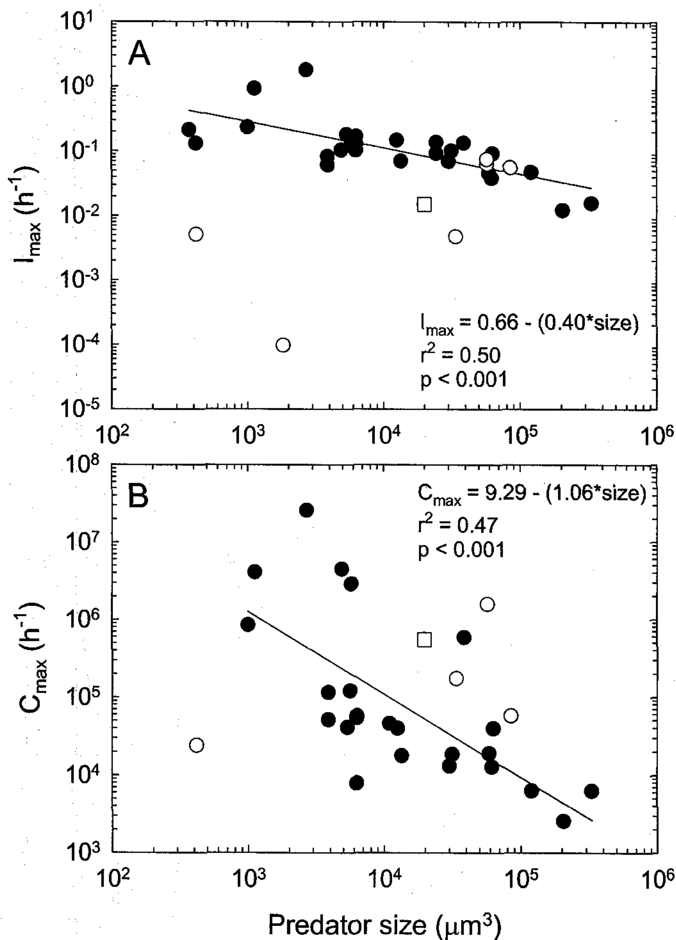

Fig. 6. Maximum carbon-specific ingestion (A) and volume-specific clearance rates (B) of heterotrophic (●) and mixotrophic (○) dinoflagellates and *Ceratium furca* (□) as a function of predator size. Data on specific ingestion rates (prey C ingested per predator C) and clearance rates (vol. cleared per predator vol.) were collected from the available literature. Where feeding rates were reported in terms of prey or μl per predator only, specific rates were calculated using data on prey and predator vol. and C contents reported in the same paper if possible, or in another paper by the same authors. Feeding data were corrected for temperature differences using a Q_{10} of 2.8 (Hansen, Bjørnsen, and Hansen 1997). Linear regression was conducted considering heterotrophic dinoflagellates only. Values were collected from the following literature: Archer et al. 1996; Bjørnsen and Kuparinen 1991; Bockstahler and Coats 1993b; Buskey 1997; Buskey, Coulter, and Brown 1994; Goldman, Dennett, and Gordin 1989; Hansen 1992, Hansen and Nielsen 1997; Jacobson and Anderson 1993; Jeong and Latz 1994; Jeong et al. 1999; Legrand, Granéli, and Carlsson 1998; Li, Stoecker, and Coats 2001; Skovgaard 1996, 1998; Strom 1991; Strom and Buskey 1993; Weisse and Kirchhoff 1997.

inherent characteristic of mixotrophic nutrition or just an artifact of limited sample size cannot be discerned at this time, and more data on mixotrophic feeding are clearly needed to draw firm conclusions.

Grazing impact of *C. furca* on prey populations was highly variable in space and time, but tended to be highest in areas where high *C. furca* abundance coincided with low prey densities (i.e. in the lower Bay). Although median impact on total ciliate standing stock was negligible (0.9% removed d^{-1}), *C. furca* appeared to exert significant grazing pressure on its preferred prey, small *Strobilidium* spp. (median impact 7.9% removed d^{-1}). Under bloom conditions, *C. furca* theoretically was capable of removing in excess of 100% of ciliate standing stock d^{-1} and in excess of 1,800% *Strobilidium* spp. standing stock d^{-1}. These findings indicate that *C. furca* can at times play a major role in regulating ciliate and especially *Strobilidium* spp. populations. Similar values were reported by Bockstahler and Coats (1993b) for the mixotrophic dinoflagellate *A. sanguinea* from Chesapeake Bay. In 1988, this dinoflagellate removed up to 162% of small (≤ 20 μm) oligotrichs d^{-1} (median value 17.4%) and 24.5% of total ciliate standing stock d^{-1} (median value 5.1%). As *C. furca* and *A. sanguinea* often co-occur in Chesapeake Bay, these two species could exert significant combined grazing pressure on ciliate populations, but may also directly compete with each other for the same prey.

Potential contribution of prey C, N, and P to *C. furca* averaged 4.5% of its body C, 6.5% of its body N, and 4.0% of its body P d^{-1}, with maximal daily ingestion of 36%, 51%, and 32% body C, N, or P, respectively. These values are similar to or higher than values reported for other primarily photosynthetic mixotrophic dinoflagellates. For example, *A. sanguinea* ingested an average of 2.5% of its body C d^{-1} (maximal value 12%) and 4.0% of its body N d^{-1} (maximal value 19%; Bockstahler and Coats 1993b). Daily ingestion of cryptophyte biomass by *Gyro. galatheanum* was lower, averaging only 0.5% of its body C, 0.6% of its body N, and 0.9% of its body P, with maximal values of 13%, 13%, and 21%, respectively (Li, Stoecker, and Coats 2001).

The potential contribution of prey C, N, and P to *C. furca* nutrition depends on the conversion factors used to convert prey vol. to biomass. As *C. furca* primarily feeds on small ciliates of the genus *Strobilidium* (Smalley, Coats, and Adam 1999), we determined C, N, and P densities for a small *Strobilidium* sp. and used these values in our calculations. Carbon to vol. ratios for various ciliates have been determined before, either experimentally or based on theoretical considerations (Fenchel and Finlay 1983; Finlay and Uhlig 1981; Putt and Stoecker 1989; Verity and Langdon 1984), and range from 0.06–0.22 pg C μm^{-3}. The C density of the *Strobilidium* sp. determined here falls within this range, although it is only about half of the C density determined by Putt and Stoecker (1989) for other aloricate oligotrich ciliates. The C:N ratio of *Strobilidium* sp. (4.1 wt:wt) is also similar to that reported by Putt and Stoecker (1989) for *Strobilidium spiralis* (3–4) and Verity and Langdon (1984) for various tintinnids (4.1–4.7). As data on the P content of individual ciliates has largely been lacking, it has usually been estimated assuming a N:P ratio of 16 (Redfield ratio). To our knowledge, the P content reported here for *Strobilidium* sp. represents the first such measurement on a ciliate species. We measured C:N and C:P ratios below the Redfield ratios of 6.6 and 106, respectively, indicating that *Strobilidium* sp. was relatively C-poor or N- and P-enriched. On the other hand, we observed a N:P ratio of 16.3, which lends support to the use of the Redfield ratio to estimate ciliate P content in the absence of available data.

Laboratory cultures of *C. furca* isolated from Chesapeake Bay exhibit a doubling time of ~ 4.5 at 20 °C and 15 psu (GWS., unpubl. data). Using this value in conjunction with the ingestion rates determined here, and assuming a gross growth efficiency of 40% (Fenchel 1987), *C. furca* could meet an average of 8% of its C, 12% of its N, and 7% of its P requirements for reproduction through phagotrophy (maximal values 65% C, 92% N, and 57% P). These values are considerably higher than those reported for *A. sanguinea* (up to 15% of its N requirement; Bockstahler and Coats 1993) or *Gyro. galatheanum* (up to 10% of its C, 11% of its N, and 17% of its P requirement; Li, Stoecker, and Coats 2001). *Ceratium furca* may thus rely

more heavily on ingested organic nutrients than other primarily photosynthetic dinoflagellate species.

Although acquisition of carbon does not seem to be the driving force behind phagotrophy in primarily photosynthetic dinoflagellates, such as *C. furca, A. sanguinea*, and *Gyro. galatheanum* (Bockstahler and Coats 1993b; Li, Stoecker, and Coats 2000b), it is likely that ingested C will be at least partially utilized by the cell. Assuming an average photosynthetic rate of 39 pg C $cell^{-1}$ h^{-1} (GWS., unpubl. data) over a 14-h photoperiod, heterotrophic C ingestion by *C. furca* averaged 21% of photosynthetic C uptake, with a maximal value of 164%. However, these calculations do not consider the proportion of C that was actually digested and assimilated, thus the actual contribution of ingested prey C to *C. furca* is likely to be lower. In addition, the photosynthetic rate above was determined for photosynthetically grown laboratory cultures, which may not be representative of mixotrophically growing field populations, thus introducing an error of unknown direction and magnitude. Nevertheless, the data indicate that *C. furca* is capable of supplementing photosynthetic C-uptake by ingesting a substantial amount of organic C through phagotrophy.

The ability to exploit an organic nutrient source may give *C. furca* a competitive advantage over purely photosynthetic species, especially when inorganic nutrients are limiting. When grown phototrophically, *C. furca* exhibited a doubling time of 4.5 d. Mixotrophic growth rates of *C. furca* have never been determined. However, we can estimate the theoretical increase in growth rate due to mixotrophy if we make the following assumptions: mixotrophically growing *C. furca* exhibit the same photosynthetic rates and cellular C content as under phototrophic growth conditions; *C. furca* ingest prey at the maximum rates determined in the field; and the gross growth efficiency is 40%. Under these conditions, the additional C gained through phagotrophy would increase the C available for reproduction by a factor of ~ 1.65, which would reduce doubling time by ~ 40%, resulting in one doubling every 2.7 d. To date, only a limited number of studies have looked at the link between phagotrophy and enhanced growth in photosynthetic dinoflagellates. Skovgaard (1996) reported that growth rates of *Fragilidium subglobosum* were considerably higher when cultures were fed *Ceratium tripos* compared to growth in unfed cultures, especially at low growth irradiance. Similarly, Li, Stoecker, and Adolf (1999) found that growth rates of *Gyro. galatheanum* almost tripled when the dinoflagellate was provided with cryptophyte prey. These dinoflagellates may thus be capable of rapid growth in nutrient-limited environments by supplementing nutrition through phagotrophy, which could aid in bloom formation and persistence. However, to what extent ingested organic compounds are assimilated by the mixotroph and how they are allocated between cellular pools is not known and deserves further attention.

In the present study, we have documented the distribution of *C. furca* and its prey in Chesapeake Bay and have shown that *C. furca* is capable of ingestion rates comparable to those reported for other mixotrophic dinoflagellates. At times, the dinoflagellate had a significant impact on prey populations, especially on small ciliates of the genus *Strobilidium*. In addition, the potential contribution of ingested prey C, N, and P to nutrition and growth of *C. furca* could be substantial. We identified several factors that could potentially influence feeding in this dinoflagellate, including inorganic nutrient concentrations and nutrient ratios. Future research will explore in more detail the role that these variables play in the regulation of feeding.

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