

Seasonal distributions of the photosynthesizing ciliates *Laboea strobila* and *Myrionecta rubra* (= *Mesodinium rubrum*) in an estuary of the Gulf of Maine

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ABSTRACT: The mixotrophic ciliate *Laboea strobila* was present year-round in the surface waters of the Damariscotta estuary, Maine, USA. In contrast, the abundance of the autotrophic ciliate *Myrionecta rubra* showed a strong seasonality and was absent during most of the summer and autumn. Non-loriccate species usually dominated ciliate abundance and biomass, but tintinnids were occasionally dominant. *L. strobila* constituted up to 15% of the total ciliate biomass (including *M. rubra*) while *M. rubra* biomass exceeded the combined biomass of all other ciliates on several dates. The maximum biomass observed for *M. rubra* was ca 35 $\mu\text{g C l}^{-1}$. When *M. rubra* was present, its estimated contribution to primary production was usually greater than that of *L. strobila*. Up to 23% of total community photosynthesis was attributed to these 2 ciliates even though red water blooms of *M. rubra* were not observed.

KEY WORDS: Ciliates · Mixotrophy · *Laboea* · *Myrionecta* · Primary production

INTRODUCTION

The presence of chloroplasts in marine ciliates has been documented numerous times since their incidence was first described by Lohmann (1908). *Myrionecta rubra* (= *Mesodinium rubrum*) is the most widely known of the photosynthetic marine ciliates due to its historically recorded occurrences as a red tide organism. *M. rubra* contains a reduced endosymbiotic cryptomonad, does not ingest particulate matter, and is generally acknowledged as an obligate phototroph (Taylor et al. 1971, Smith & Barber 1979, Crawford 1989). *Laboea strobila*, like several other species in the Strombidiidae, retain photosynthetically functional plastids from ingested algal cells. These ciliates require algal food as a source of plastids and other nutritional factors (Stoecker et al. 1987, 1988).

Both *Laboea strobila* and *Myrionecta rubra* have world-wide distribution in coastal waters (Taylor et al. 1971, Lindholm 1985, Stoecker et al. 1987, Bernard & Rassoulzadegan 1994). Plastid-retaining species (mixotrophs), including *L. strobila*, can constitute a substantial fraction of the ciliates in some coastal and open ocean waters (Stoecker et al. 1987, Putt 1990, Sime-Ngando et al. 1992). Relatively little is yet known, however, about the abundances of mixotrophic ciliates or their contribution to community photosynthesis. Likewise, the role that non-bloom occurrences of *M. rubra* play in community primary production has received little attention. This is despite reports of exceptionally high chlorophyll *a* concentrations and primary production rates during red water events caused by *M. rubra* (Lindholm 1985, Crawford 1989).

Recently, single cell isolation techniques showed that maximum photosynthetic rates (P_{max}) for *Laboea strobila* and *Myrionecta rubra* were in the range of those for phytoplankton (Stoecker et al. 1988, 1991).

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Single cell determinations of photosynthetic rates can be combined with abundance or biomass information to estimate the total primary production of the ciliate community (Stoecker et al. 1989). This study investigates the seasonal distribution of *L. strobila* and *M. rubra* in the Damariscotta estuary, Maine, USA, and uses literature-derived photosynthetic rates to calculate the potential contribution of these 2 ciliates to the total primary production.

METHODS

Three sampling stations were located at approximately 10 km intervals along the axis of the Damariscotta estuary (43° 56' N, 69° 35' W). These correspond to Stns 1, 2 and 3 (from the mouth toward the head of the estuary) in Sanders (1987). Water samples were collected from just below the surface using a 4.5 l Van Dorn bottle. Two Van Dorn casts were combined and a 3.5 l subsample was preserved with acidic Lugol's iodine (1% final concentration). Samples were concentrated for Utermöhl counts of ciliates using 3 sequential settling steps of 72 h duration each. From 70 to 90% of the remaining sample volume was removed by aspiration at each step for a final concentration factor of approximately 500. The total number of ciliates enumerated per subsample ranged from 100 to >2100; replicate counts typically varied by 5 to 15%. Ciliate biovolumes were calculated from length and width measurements by assuming cells conformed to common geometric shapes. Biomass was estimated using a volume-to-carbon conversion factor of 0.19 pg C μm^{-3} for cells preserved with Lugol's iodine (Putt & Stoecker 1989).

Primary production was determined monthly (September 1981 through March 1982) from the incorporation of $^{14}\text{C-NaHCO}_3$ (Strickland & Parsons 1972). Surface water was incubated for 3 h in 300 ml BOD bottles (3 light bottles, 1 dark) under 'cool-white' fluorescent light (ca 100 $\mu\text{E m}^{-2} \text{s}^{-1}$). Temperature was maintained with flowing water to within 2°C of the surface water. Radioactivity was measured using liquid scintillation counting. In order to estimate the contributions of the ciliates *Laboea strobila* and *Myrionecta rubra* to community photosynthesis, literature-based rates determined for isolated cells incubated at an irradiance of 100 $\mu\text{E m}^{-2} \text{s}^{-1}$ were multiplied by their abundance (Stoecker et al. 1989). Rates of 85 and 465 pg C fixed ciliate $^{-1} \text{h}^{-1}$ were used for *M. rubra* and *L. strobila*, respectively (Stoecker et al. 1989).

RESULTS AND DISCUSSION

Laboea strobila was detected at one or more stations on every sampling date during the study. It was observed at Stn 2 (mid-estuary) on all sampling dates except one, but had more sporadic occurrences at the mouth and head of the estuary (Table 1). The maximum abundance of this species in the Damariscotta was 775 cells l^{-1} (8.23 $\mu\text{g C l}^{-1}$) at Stn 2 during September. *L. strobila* generally did not exceed 15% of the total ciliate biomass (Fig. 1). The biomass of *L. strobila* was greater than that of tintinnid ciliates on several occasions (Fig. 1), but even when the biomass of the obligate phototroph *Myrionecta rubra* was excluded, it did not exceed 20% of the total ciliate biomass. The abundances of *L. strobila* in this study are similar to those observed in July on Georges Bank and the Gulf

Table 1. Abundances (no. l^{-1}) of *Laboea strobila*, *Myrionecta rubra* and other ciliates in the Damariscotta estuary, Maine, USA. Dashes indicate species not observed. nd: no data. Dates given as Month/Day

	1981									1982					
	5/7	6/17	7/9	8/11	8/31	9/15	10/13	11/17	12/10	1/12	2/11	3/4	3/25	4/15	5/13
Stn 1															
<i>L. strobila</i>	40	2	4	-	20	-	-	-	15	55	10	10	120	30	90
<i>M. rubra</i>	-	-	-	-	nd	-	-	-	690	2150	4100	4140	4060	575	9750
Other ciliates	4500	1160	2100	130	nd	300	10200	345	1050	1820	2100	3430	7330	6740	13000
Stn 2															
<i>L. strobila</i>	30	5	-	1	60	775	30	2	50	10	5	25	20	20	100
<i>M. rubra</i>	-	-	-	-	85	700	-	-	5740	3720	2900	4330	5170	4800	37000
Other ciliates	12130	3730	1170	4880	5820	29400	1750	495	4360	2770	2780	6110	8140	20200	18900
Stn 3															
<i>L. strobila</i>	5	90	1	1	3	15	6	4	-	7	-	-	-	-	375
<i>M. rubra</i>	-	-	-	-	nd	275	50	-	2640	910	110	1780	510	11620	17250
Other ciliates	18280	4400	8960	9245	nd	8925	6300	635	13900	875	420	9650	3335	35740	8690

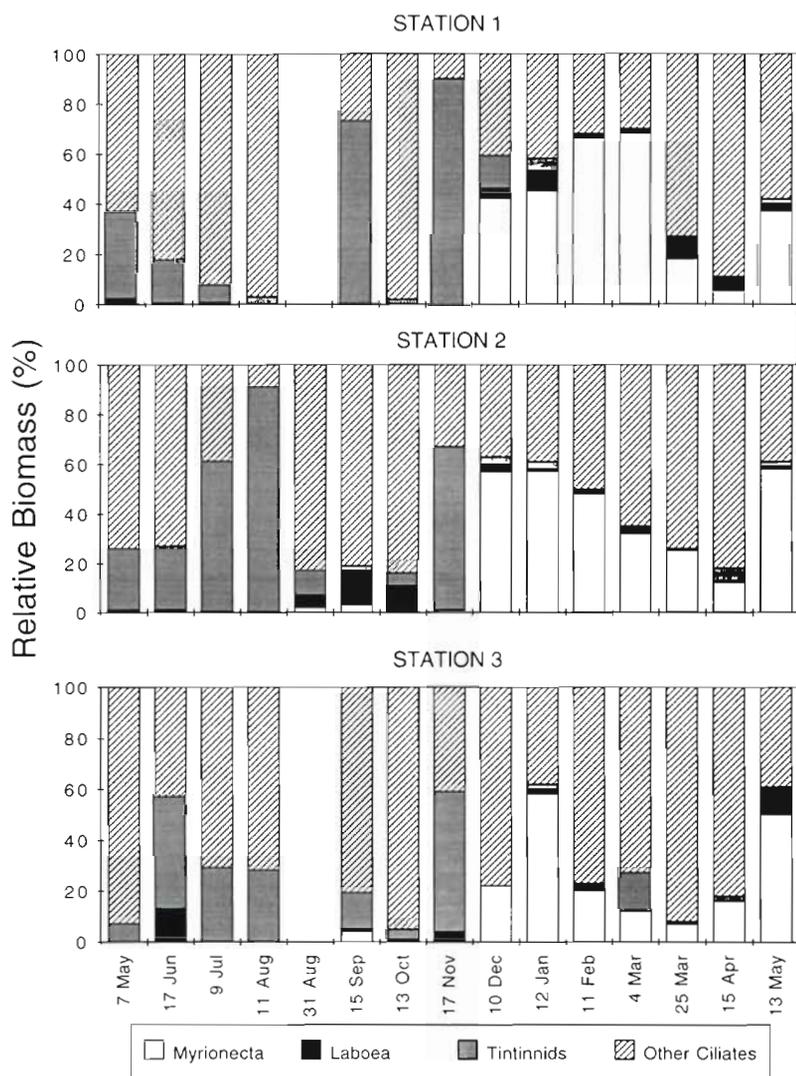


Fig. 1. Relative biomass of the autotrophic ciliate *Myrionecta rubrum*, the mixotrophic ciliate *Laboea strobila*, other non-loricated ciliates, and tintinnid ciliates in the Damariscotta estuary, Maine, USA

of Maine, and in July–August in the Iceland, Greenland and Barents Seas (Stoecker et al. 1989, Putt 1990). In Long Island Sound, USA, *L. strobila* was observed on 6 of 7 days in the spring and early summer and exceeded 1000 cells l^{-1} at some depths during mid-June (McManus & Fuhrman 1986). McManus & Fuhrman (1986) found that the occurrence of *L. strobila* in Long Island Sound was coupled with that of *M. rubra*. This was not the case during seasonal studies in the Damariscotta estuary or in the Mediterranean Sea (Table 1; Bernard & Rassoulzadegan 1994).

In contrast to *Laboea strobila*, the distribution of *Myrionecta rubra* had a strong seasonality in the Damariscotta estuary. *M. rubra* was present at all stations between December and April, and generally

absent in June through November (Table 1, Fig. 1). This agrees generally with the observations of Montagnes & Lynn (1989) who observed *M. rubra* throughout the year around the Isle of Shoals, Maine, but noted very low abundances during the summer and fall. In contrast, biomass peaks and/or red water due to *M. rubra* were observed during the summer in the Mediterranean Sea and in Southampton Water, UK (Kifle & Purdie 1993, Bernard & Rassoulzadegan 1994). Although *M. rubra* biomass in the Damariscotta estuary often exceeded that of the phagotrophic ciliates (Fig. 1), *M. rubra* populations in the Damariscotta estuary never approached the bloom conditions that produce red water phenomena; more than 200 000 *M. rubra* cells l^{-1} are required to give seawater a detectable red coloration (McAlicie 1968). Red water conditions due to *M. rubra*, including late summer and early fall blooms, have been reported in close geographical proximity to the Damariscotta estuary, and may even be regularly occurring events in the coastal waters of Maine (Powers 1932, McAlicie 1968, White et al. 1977). It is likely, however, that *M. rubra* red water is localized spatially and/or temporally (White et al. 1977, Montagnes & Lynn 1989).

The factors that regulate abundances of phototrophic ciliates are not well known. Light is obviously a requirement for these autotrophic and mixotrophic ciliates, and *Myrionecta rubra* and *Laboea strobila* will often accumulate near the surface at light levels that are apparently optimum for photosynthesis (Smith & Barber 1979, McManus & Fuhrman 1986, Stoecker et al. 1988). But turbulence can modify both lateral and vertical aggregations (Crawford 1989). Because vertical stability of the water column is likely an important factor allowing *M. rubra* to reach very high abundances, turbulence may have reduced the probability of observing red water in the Damariscotta estuary. Differences in abundances of both *M. rubra* and *L. strobila*, as well as tintinnids, were generally negligible when samples from the surface, 7 m and near-bottom (22 m) were compared at Stn 2 (results not shown). Tidal mixing is strong the Damariscotta, especially in this section of the estuary

(McAlice 1977), and undoubtedly contributed to the vertically well-dispersed populations.

Relatively high nutrient concentrations may be required to sustain blooms of *Myrionecta rubra* (Smith & Barber 1979, Lindholm 1985). Levels of $\text{NO}_2 + \text{NO}_3$ in the Damariscotta tend to be highest in the winter and early spring (McAlice 1979, Revelante & Gilmartin 1987) when *M. rubra* abundances also were greatest (Table 1). Temperature could also affect abundances of photosynthetic ciliates. *M. rubra* has been reported in a wide range of temperatures. However, maximum numbers of *M. rubra* tend to be associated with temperatures above, but close to, 15°C (Taylor et al. 1971). In the Damariscotta, temperatures exceeded 15°C only between June and October (Sanders 1987). Thus, optimum temperatures and the high nutrient levels needed to support blooms of *M. rubra* may not have occurred simultaneously on a frequent basis in this estuary. The direct importance of dissolved nutrients and temperature to *Laboea strobila* and other mixotrophic species has not been studied.

Both top-down and bottom-up predator-prey interactions may also play a role in population control of photosynthetic ciliates. Since ciliates are ingested by a variety of aquatic organisms (Sanders & Wickham 1993), predation (top-down control) can reduce abundances. The presence of suitable amounts and types of

prey/plastids (bottom-up control) could also limit some mixotrophic ciliates (i.e. those that retain ingested plastids). It is likely that multiple environmental factors, including light intensity, turbulence, temperature, nutrient level, and predator-prey interactions, act together to encourage or limit blooms of photosynthetic ciliates.

Both *Myrionecta rubra* and *Laboea strobila* can account for measurable contributions to community photosynthesis. The highest rates of community primary production determined during this study were in early spring at Stns 2 and 3 (8.35 and 9.98 $\mu\text{g C l}^{-1} \text{h}^{-1}$, respectively). The maximum rate at Stn 1 (6.13 $\mu\text{g C l}^{-1} \text{h}^{-1}$) occurred in September (Table 2). During periods when community photosynthesis was high, *M. rubra* and *L. strobila* generally accounted for only a few percent of the total primary production in the Damariscotta. At Stn 1 in January and Stn 2 in February, however, $\geq 20\%$ of the community primary production could be attributed to *M. rubra* (Table 2). Photosynthesis by *L. strobila* contributed a very small part of the planktonic primary production. In September when *L. strobila* was most abundant, it was estimated to have fixed 0.36 $\mu\text{g C l}^{-1} \text{h}^{-1}$ which represented 6% of the community photosynthesis (Table 2). By comparison, McManus & Fuhrman (1986) estimated that *L. strobila* contributed about 2% of the total photosynthesis dur-

Table 2. Total measured primary production and calculated estimates of primary production for the mixotrophic ciliate *Laboea strobila* and the photosynthetic ciliate *Myrionecta rubra* in the Damariscotta estuary, Maine, USA. The percent of total primary production attributed to each ciliate species is listed in parentheses for dates when total primary production was determined. Primary production reported as $\text{ng C l}^{-1} \text{h}^{-1}$. Dashes indicate species not observed. nd: no data. Dates given as Month/Day

	1981								1982				
	5/7	6/17	7/9	8/31	9/15	10/13	11/17	12/10	1/12	2/11	3/4	4/15	5/13
Stn 1													
Total production	nd	nd	nd	nd	6700	5370	2600	1690	1200	1760	4000	nd	nd
<i>L. strobila</i>	19	<1	<2	-	-	-	-	7	25	5	5	14	42
									(2%)	(<1%)	(<1%)		
<i>M. rubra</i>	-	-	-	nd	-	-	-	58	182	349	78	49	829
								(3%)	(15%)	(20%)	(2%)		
Stn 2													
Total production	nd	nd	nd	nd	6130	4330	3790	3720	1460	2510	8350	nd	nd
<i>L. strobila</i>	13	2	-	28	360	13	1	21	5	2	12	9	47
					(6%)	(<1%)	(<1%)	(<1%)	(<1%)	(<1%)	(<1%)		
<i>M. rubra</i>	-	-	-	7	60	-	-	490	316	246	389	407	3150
					(<1%)			(13%)	(22%)	(10%)	(5%)		
Stn 3													
Total production	nd	nd	nd	nd	1340	nd	2400	1900	1160	1700	9980	nd	nd
<i>L. strobila</i>	2	42	<1	1	6	3	2	-	3	-	-	3	174
					(<1%)		(<1%)		(<1%)				
<i>M. rubra</i>	-	-	-	nd	23	4	-	224	77	9	395	416	1470
					(2%)			(12%)	(7%)	(<1%)	(4%)		

ing a spring cruise in Long Island Sound. Estimates of non-bloom production of *M. rubra* generally range from <1% to ca 10% of the primary production (Lepänen & Bruun 1986, Montagnes & Lynn 1989, Stoecker et al. 1991), but *M. rubra* accounted for >70% of the community primary production during one experiment in a small estuary (Stoecker et al. 1991). *L. strobila* and *M. rubra* together accounted for 1 to 7% of total photosynthesis (up to >90% of the photosynthesis by microplankton) at several stations in the Gulf of Maine during July (Stoecker et al. 1989).

The amount of primary production that is attributable to ciliates in the Damariscotta estuary may be greater than the calculations in Table 2 indicate. Other ciliates, in particular several *Strombidium* spp., are known to sequester chloroplasts and several have substantial photosynthetic rates (Jonsson 1987, Stoecker et al. 1987, Laval-Peuto & Rassoulzadegan 1988, Putt 1990). Numerous *Strombidium* were observed in the Damariscotta estuary during this study, but their abundances were not recorded separately as were those of *Laboea strobila* and *Myrionecta rubra*. Thus, no estimate of the photosynthetic contribution was possible. However, Stoecker et al. (1989) estimated that *L. strobila* and *M. rubra* accounted for only about 50% of the biomass of ciliates with chlorophyll in shallower waters of the Gulf of Maine. Even if there were smaller proportions of these other photosynthetic ciliates in the Damariscotta than in the Gulf of Maine, it is likely that they augmented the photosynthetic contribution of *L. strobila* and *M. rubra* to community primary production.

There are several factors that could affect the estimates of primary production presented here for *Laboea strobila* and *Myrionecta rubra*. Light intensity has a major effect on photosynthetic rate, and both higher and lower rates than those used here have been determined for these 2 species (Jonsson 1987, Stoecker et al. 1988, 1989). The rates used to calculate the photosynthesis of the ciliates were chosen to approximate photosynthetic rates expected at the light levels used for measuring total primary production. Thus, actual field rates likely varied from my estimates due to changing light levels, but proportions of total measured primary production attributed to ciliates (Table 2) should not be considerably different. Likewise, low nutrient concentrations could reduce photosynthetic rates in both ciliates and phytoplankton. For *M. rubra* variations in size could affect the estimates of their photosynthetic contribution. The rate used in my calculations ($85 \text{ pg C cell}^{-1} \text{ h}^{-1}$) was for cells 30 to 50 μm in length, but smaller *M. rubra* may have a reduced per cell rate at a given light intensity (Stoecker et al. 1991). Two distinct size classes of *M. rubra* have previously been reported to occur either

together or in different seasons (Lindholm 1985, Montagnes & Lynn 1989, Bernard & Rassoulzadegan 1994), and 2 size classes of *M. rubra* always co-occurred in the Damariscotta samples. Assuming that photosynthetic rate is proportional to biomass, the primary production reported for *M. rubra* is affected substantially (reduced) only for May 1992, when the small size class represented approximately 50 to 60% of the enumerated cells at all 3 stations.

Smith & Barber (1979) emphasized that the contribution of *Myrionecta rubra* blooms to the productivity of coastal ecosystems was greatly underestimated. It is becoming apparent that even if red water phenomena are not observed, *M. rubra* and several species of mixotrophic ciliates frequently are abundant and can make up a large proportion of the ciliate standing stock in coastal waters (Crawford 1989, Stoecker et al. 1989). Thus, the importance of photosynthesis by *M. rubra* and other ciliates during non-bloom periods also has been underestimated. The few determinations of primary production by ciliates during non-bloom conditions indicate that a sizable proportion of community photosynthesis, especially in the microplankton size-fraction favored by zooplankton predators, can sometimes be attributed to these protists. Furthermore, ciliates, including *M. rubra*, are ingested by a variety of zooplankton and shellfish (Lindholm 1985, Sanders & Wickham 1993), so it is probable that autotrophic and mixotrophic ciliates make an important contribution to coastal food webs. To understand the true role of photosynthetic ciliates in microbial food webs, future studies are needed to determine how they interact with other trophic levels, and how environmental parameters such as light, nutrients, temperature and food affect phototrophy and phagotrophy by these ciliates.

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LITERATURE CITED

- Bernard C, Rassoulzadegan F (1994) Seasonal variations of mixotrophic ciliates in the northwest Mediterranean Sea. *Mar Ecol Prog Ser* 108:295–301
- Crawford DW (1989) *Mesodinium rubrum*: the phytoplankter that wasn't. *Mar Ecol Prog Ser* 58:161–174
- Jonsson PR (1987) Photosynthetic assimilation of inorganic carbon in marine oligotrich ciliates (Ciliophora, Oligotrichina). *Mar microb Food Webs* 2:55–68
- Kifle D, Purdie DA (1993) The seasonal abundance of the phototrophic ciliate *Mesodinium rubrum* in Southampton Water, England. *J Plankton Res* 15:823–833
- Laval-Peuto M, Rassoulzadegan F (1988) Autofluorescence of marine planktonic Oligotrichina and other ciliates. *Hydrobiologia* 159:99–110

- Leppänen JM, Bruun JE (1986) The role of pelagic ciliates including the autotrophic *Mesodinium rubrum* during the spring bloom of 1982 in the open northern Baltic proper. *Ophelia* (Suppl) 4:147–157
- Lindholm T (1985) *Mesodinium rubrum* — a unique photosynthetic ciliate. *Adv aquat Microbiol* 3:1–48
- Lohmann H (1908) Untersuchung zur Feststellung des vollständigen Gehaltes des Meeres an Plankton. *Wiss Meer-sunters*, Kiel 10:129–370
- McAlice BJ (1968) An occurrence of ciliate red water in the Gulf of Maine. *J Fish Res Bd Can* 25:1749–1751
- McAlice BJ (1977) A preliminary oceanographic survey of the Damariscotta River estuary, Lincoln County, Maine. Maine Sea Grant Tech Rep TR-13-77, NOAA, Washington, DC
- McAlice BJ (1979) Hydrographic and nutrient data — Damariscotta River estuary, Lincoln County, Maine. Maine Sea Grant Tech Rep TR-43-79, NOAA, Washington, DC
- McManus GB, Fuhrman JA (1986) Photosynthetic pigments in the ciliate *Laboea strobila* from Long Island Sound, USA. *J Plankton Res* 8:317–327
- Montagnes DJS, Lynn DH (1989) The annual cycle of *Mesodinium rubrum* in the waters surrounding the Isle of Shoals, Gulf of Maine. *J Plankton Res* 11:193–201
- Powers PBA (1932) *Cyclotrichium muenieri* sp. nov. (Protozoa, Ciliata); cause of red water in the Gulf of Maine. *Biol Bull* 63:74–80
- Putt M (1990) Abundance, chlorophyll content and photosynthetic rates of ciliates in the Nordic Seas during summer. *Deep Sea Res* 37:1713–1731
- Putt M, Stoecker DK (1989) An experimentally determined carbon:volume ratio for marine 'oligotrichous' ciliates from estuarine and coastal waters. *Limnol Oceanogr* 34:1097–1103
- Revelante N, Gilmartin M (1987) Seasonal cycle of the ciliated protozoan and micrometazoan biomass in a Gulf of Maine estuary. *Estuar coast Shelf Sci* 25:581–598
- Sanders RW (1987) Tintinnids and other microzooplankton — seasonal distributions and relationships to resources and hydrography in a Maine estuary. *J Plankton Res* 9:65–77
- Sanders RW, Wickham SA (1993) Planktonic protozoa and metazoa: predation, food quality and population control. *Mar microb Food Webs* 7:197–223
- Sime-Ngando T, Juniper K, Vézena A (1992) Ciliated protozoan communities over Cobb Seamount: increase in biomass and spatial patchiness. *Mar Ecol Prog Ser* 89:37–51
- Smith WO Jr, Barber RT (1979) A carbon budget for the autotrophic ciliate *Mesodinium rubrum*. *J Phycol* 15:27–33
- Stoecker DK, Michaels AE, Davis LH (1987) Large proportion of marine planktonic ciliates found to contain functional chloroplasts. *Nature* 326:790–792
- Stoecker DK, Putt M, Davis LH, Michaels AE (1991) Photosynthesis in *Mesodinium rubrum*: species-specific measurements and comparison to community rates. *Mar Ecol Prog Ser* 73:245–252
- Stoecker DK, Silver MW, Michaels AE, Davis LH (1988) Obligate mixotrophy in *Laboea strobila*, a ciliate which retains chloroplasts. *Mar Biol* 99:415–423
- Stoecker D, Taniguchi A, Michaels AE (1989) Abundance of autotrophic, mixotrophic and heterotrophic planktonic ciliates in shelf and slope waters. *Mar Ecol Prog Ser* 50:241–254
- Strickland JDH, Parsons TR (1972) A practical handbook of seawater analysis. Fisheries Research Board of Canada, Ottawa
- Taylor FJR, Blackbourn DJ, Blackbourn J (1971) The red-water ciliate *Mesodinium rubrum* and its 'incomplete symbionts': a review including new ultrastructural observations. *J Fish Res Bd Can* 28:391–407
- White AW, Sheath RG, Hellebust JA (1977) A red tide caused by the marine ciliate *Mesodinium rubrum* in Passamaquoddy Bay, including pigment and ultrastructure studies of the endosymbiont. *J Fish Res Bd Can* 34:413–416

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