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Stephen C. Landers
Troy State University

Scott W. Phipps
Weeks Bay National Estuarine Research Reserve

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Ciliated Protozoan Colonization of Substrates From Weeks Bay, Alabama

STEPHEN C. LANDERS AND SCOTT W. PHIPPS

Ciliated protozoan colonization of artificial substrates was examined during a 12-mo period from Jan. to Dec. 2001 in Weeks Bay, Alabama. Artificial substrates (glass slides) were suspended in the water at three locations in the Bay for a period of 1 wk/mo, and the population density of the stalked peritrich *Vorticella* was determined. Environmental data collected during the colonization period were compared with the population results. There was a positive correlation between colonization and water temperature at two sites and between phosphate and colonization at one site. Additionally, a negative correlation was determined between colonization and dissolved oxygen at two sites, between colonization and nitrate at one site, and between colonization and pH at one site. The protozoan assemblage varied from month to month, although the overall dominance of *Vorticella* was maintained. Although the size of the population of *Vorticella* is likely influenced by a number of different variables, we believe that temperature and bacterial abundance are the likely controlling factors.

Ciliated protozoans are important components of marine ecosystems, particularly as consumers of bacteria and other protists (Fenchel, 1987; Capriulo, 1990). Studies of marine planktonic protozoans are numerous, although reports focusing on the ecology of sessile attached ciliates are less common. These studies typically involve the use of artificial substrates such as glass slides, fiberglass plates, panels, and polyurethane foam substrates. The substrates are submerged for a specific time period for collection of protozoans, which are enumerated or identified in the laboratory. Studies of this nature include reports from the Caspian Sea (Agamaliyev, 1974), Ostend Harbor in Belgium (Persoone, 1968), Mobile Bay in Alabama (Jones, 1974), the lagoon of Venice (Coppellotti and Matarazzo, 2000), Quibray Bay in Australia (Anderson, 1995), Visakhapatnam Harbor in India (Bharati et al., 2001), and Dauphin Island, Alabama (Beech, 2001; Beech and Landers, 2002).

The above reports addressed a number of ecological issues regarding sessile protozoa, ranging from seasonal biofilm formation (assessed by optical density measurement) to taxonomic studies of colonizing protists. Marine sessile protists were used for water quality assessment in a recent study comparing two locations in Visakhapatnam Harbor, one location in an inner harbor region near a sewage outfall and another closer to the open sea (Bharati et al., 2001). That study reported differences in flagellate and ciliate assemblages and noted the presence of *Vorticella* near the sewage outfall. The findings supported the use of protists

as indicator species for evaluating pollution effects and reported that the dominance of bacterivorous protists such as *Vorticella*, *Monas*, and *Tetrahymena* was an indicator of aquatic stressed conditions (Bharati et al., 2001). Recent findings in a study of sessile protozoans from Dauphin Island, Alabama, have shown that sessile ciliates there undergo a strong season cycle, with populations varying considerably from month to month (Beech and Landers, 2002). That study reported that the two most dominant genera were *Vorticella* and *Zoothamnium*. This current study aimed at examining the genus *Vorticella* in a nearby estuary each month during an entire year for comparison with the Dauphin Island data. Further, through the use of electronic data loggers, we hoped to analyze more precisely the effects of environmental influences on *Vorticella*. These data will be useful in determining whether *Vorticella* can be reliably monitored in estuarine ecosystems and whether this genus can be used as a meaningful biological indicator throughout the year or only during specific seasons of the year.

MATERIALS AND METHODS

Protozoans were collected onto glass microscope slides, using plastic slide boxes whose sides had been cut away to allow for water flow (Jones, 1974; Beech and Landers, 2002). The slides were cleaned with isopropanol before they were loaded into the boxes. The boxes then were hung from dock pilings and let into the water at a depth that kept the slides submerged even at low tide. The boxes were kept

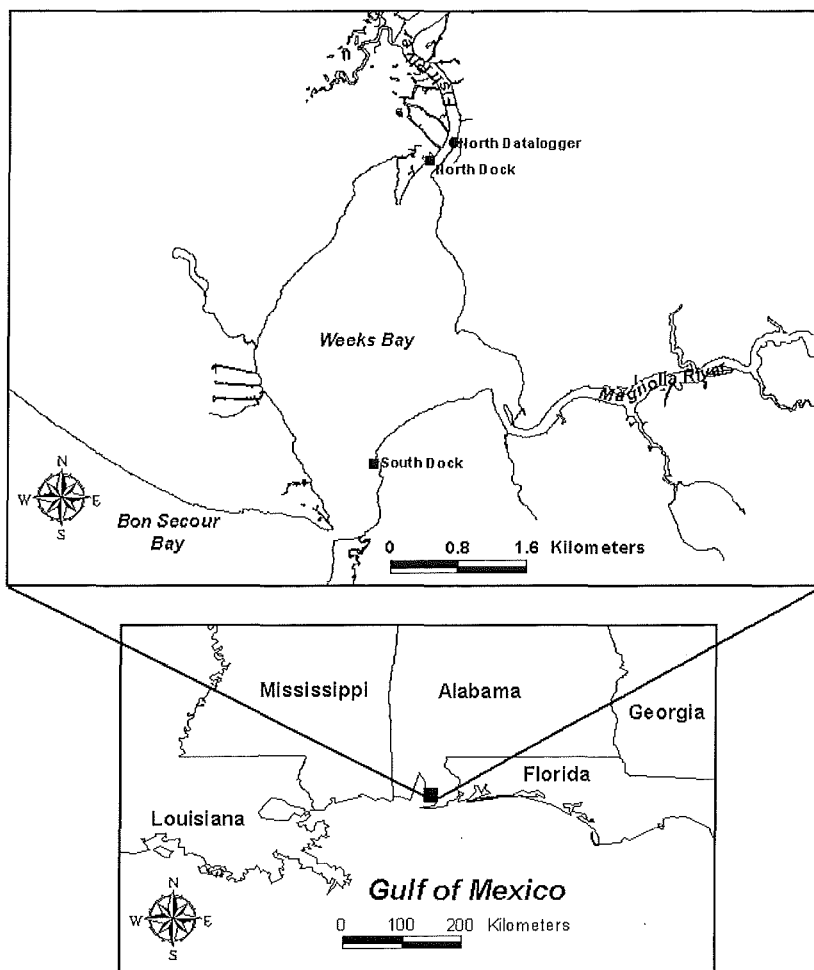


Fig. 1. Weeks Bay, Alabama.

suspended in the water for 7 d at three locations (Fig. 1): 1) the shallow end of a boat dock near the mouth of the Fish River and U.S. Highway 98, 2) the deep end of the same boat dock, and 3) the end of a boat dock at the southeast end of the Bay. These three sites were designated North Dock Shallow (NDS), North Dock Deep (NDD), and South Dock (SD), respectively. Slides at NDS and SD were approximately 0.5 m below the water surface at mean low tide and 20 cm from the bottom sediment. Slides at NDD were approximately 1.0 m below the water surface at mean low tide and 50 cm from the bottom sediment. Slides were submerged and collected at all three sites on the same days. Sampling periods were chosen each month to avoid consecutive collections (last week of 1 mo and first week of the next) and to avoid the lowest predicted low tides.

Two electronic data loggers (YSI 6600 Data-sonde[®]) were located near the protozoan collection sites: one approximately 200 m north of the NDD and NDS sites and one located at the SD site (Fig. 1). The data loggers recorded temperature, turbidity, salinity, pH, and dissolved oxygen (DO) every 30 min during the 7-d collection period. Data from these readings were averaged to obtain a representative value for the week. Water samples were collected at each site during the beginning and end of the colonization period and were used to measure nitrate (NO_3^-) and phosphate levels (Clesceri et al., 1998). These two values were averaged to obtain a representative value for the week.

After collection, the slides were fixed and stained for permanent preparations (Galigher and Kozloff, 1971; Beech and Landers, 2002). After the mounted slides (stained with hematoxylin and fast green) were dry, a 0.5-cm² area

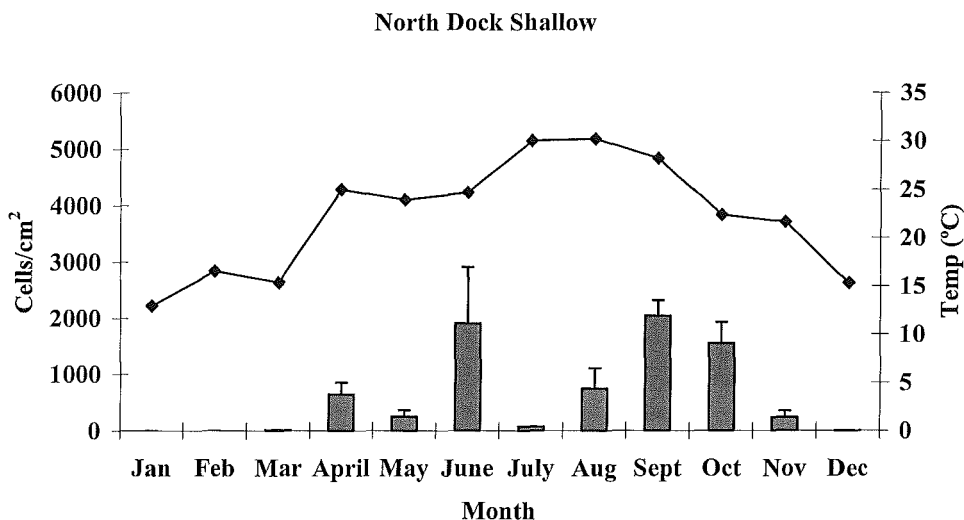


Fig. 2. Monthly population densities (bars) and temperatures (line) for NDS. Each bar represents the average of three slides colonized for a 7-d period that month. The SE is indicated for each month.

was marked on top of the coverslip with a marker for protozoan counts. *Vorticella* spp. were counted manually using a Nikon Alphaphot-2 light microscope. Ciliates were identified using Jones (1974) and Lynn and Small (2000) and were not identified to species because of the number of cells counted each month. Three stained slides from each site were examined every month. Some extra slides were studied fresh to facilitate identification of the ciliates. Population size is expressed as cells per square centimeter.

Statistical correlations between population

and environmental data were determined using the Spearman correlation on SPSS 10.1®.

RESULTS

Colonization for each site is shown in Figures 2–4. The general trend at each site was for *Vorticella* populations to increase as water temperature increased during the year.

At the two north dock sites (NDD and NDS), *Vorticella* populations did not exceed 500 cells/cm² until April (NDS) or May (NDD). At that time, their numbers rose greatly and contin-

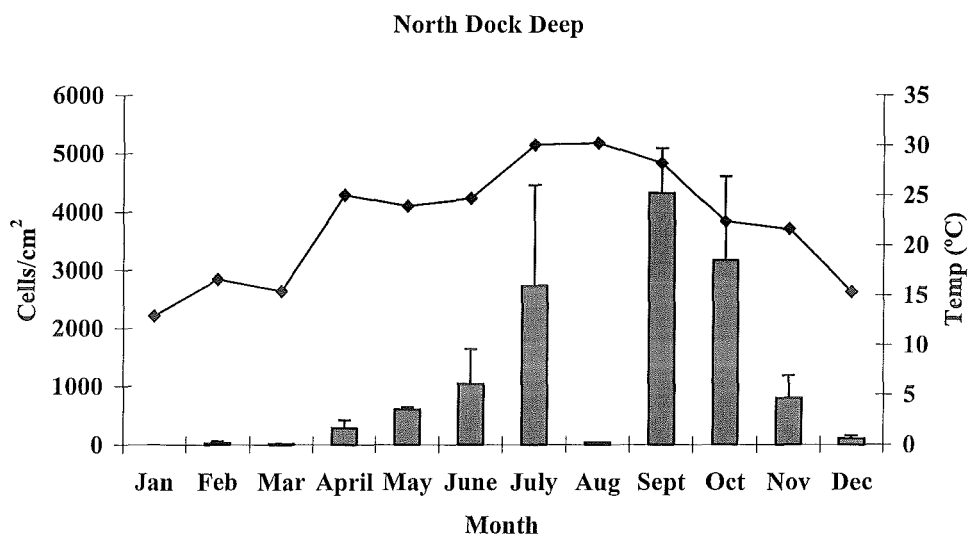


Fig. 3. Monthly population densities (bars) and temperatures (line) for NDD. Each bar represents the average of three slides colonized for a 7-d period that month. The SE is indicated for each month.

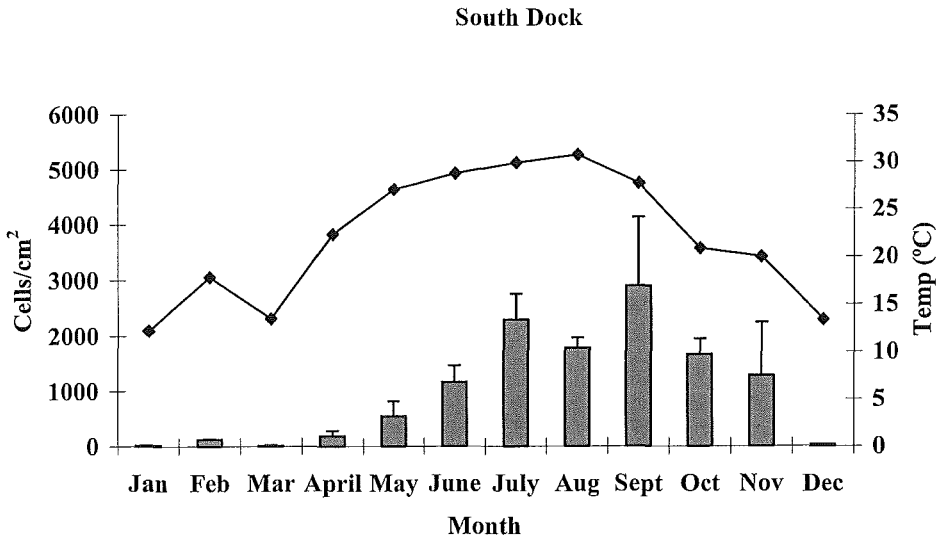


Fig. 4. Monthly population densities (bars) and temperatures (line) for SD. Each bar represents the average of three slides colonized for a 7-d period that month. The SE is indicated for each month.

ued to rise during the summer, eventually reaching a high of 4,321 cells/cm² in Sep. at NDD. Both sites experienced a midsummer crash, in July at NDS and in Aug. at NDD. This decline did not correlate to any identifiable environmental or biotic event (snail grazing, silt, etc.). As temperatures declined, colonization decreased to 5 cells/cm² (NDS) and 108 cells/cm² (NDD) in Dec. At the south dock site (SD), *Vorticella* density rose above 500 cells/cm² in May and remained high throughout summer and fall. At all sites, the ciliate assemblage changed from month to month throughout the study, with more genera present during the warmer months. Table 1 shows the sessile ciliates reported from all sites combined.

Statistical analysis (Table 2) indicated a positive correlation between sessile peritrichs and

temperature at SD and NDS and between population and total reactive phosphate (TRP) at SD. Additionally, a negative correlation was obtained between population and DO at the two shallow sites, NDS and SD, and between NO₃⁻ and population at SD. A negative correlation was obtained between colonization and pH at NDS.

DISCUSSION

This study has demonstrated a seasonal cycle of *Vorticella* colonization on artificial substrates in Weeks Bay, Alabama. A goal of this work was to establish the annual trends of *Vorticella* in this small estuary and to determine the extent to which this organism varies in abundance throughout the year. It is important to have

TABLE 1. Common sessile ciliates recovered from glass slides at Weeks Bay, AL. Presence of each genus is indicated by a "+."

Genus	Jan.	Feb.	March	April	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.
<i>Acineta</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Chaetospira</i>				+	+	+	+	+	+			
<i>Cothurnia</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Ephelota</i>	+	+	+	+	+		+	+	+	+	+	+
<i>Epistylis</i>						+	+		+	+		
<i>Lernaeophrya</i>			+	+	+	+						
<i>Pyxicola</i>				+	+	+	+					
<i>Metafolliculina</i>				+	+				+			
<i>Stentor</i>			+	+	+	+	+	+	+			
<i>Vorticella</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Zoothamnium</i>	+	+	+	+	+	+	+	+	+	+	+	+

TABLE 2. Statistical correlations between population and environmental factors at Weeks Bay locations.

Site	Temperature	DO	Turbidity	Salinity	TRP	NO ₃	pH
NDD	$r = 0.566$	$r = -0.524$	$r = 0.112$	$r = 0.042$	$r = 0.136$	$r = -0.465$	$r = -0.207$
	$P = 0.055$	$P = 0.080$	$P = 0.729$	$P = 0.897$	$P = 0.672$	$P = 0.128$	$P = 0.519$
NDS	$r = 0.692^*$	$r = -0.741^{**}$	$r = 0.573$	$r = -0.231$	$r = -0.407$	$r = -0.189$	$r = -0.581^*$
	$P = 0.013$	$P = 0.006$	$P = 0.051$	$P = 0.471$	$P = 0.190$	$P = 0.555$	$P = 0.047$
SD	$r = 0.811^{**}$	$r = -0.706^*$	$r = -0.147$	$r = 0.112$	$r = 0.813^{**}$	$r = -0.645^*$	$r = -0.364$
	$P = 0.001$	$P = 0.010$	$P = 0.649$	$P = 0.729$	$P = 0.001$	$P = 0.023$	$P = 0.245$

* Correlation is significant at the 0.05 level.

** Correlation is significant at the 0.01 level.

this information if *Vorticella* is to be used as a bioindicator of environmental changes or pollution effects in any future studies. We targeted *Vorticella* because of the results from the Dauphin Island study (Beech and Landers, 2002), in which *Vorticella* had a seasonal cycle and was a dominant protist colonizing glass slides. In that study, in the months when populations were over 100 cells/cm², *Vorticella* was the most abundant ciliate in 28 of 36 collections (Beech, 2001). A 7-d collection period was used to allow a direct comparison of the data with those from the Dauphin Island study, which also used a 7-d collection period (Beech and Landers, 2002). The senior author (SCL) determined that this time period was optimal for the Mobile Bay region, allowing for some colonization of ciliates during the coldest months but not overwhelming the glass substrates and making them unusable in summer.

Additionally, this study has examined the abundance of *Vorticella* in relation to a number of environmental factors. The correlation between colonization and water temperature at SD and NDS was expected and supports data from earlier reports (Agamaliev, 1974; Beech and Landers, 2002). The correlation between colonization and temperature at the NDD site does not meet the a priori observed significance level of 0.05 but still appears to follow the trend at the other sites ($r = 0.566$, $P = 0.055$). However, in a study of biofilm formation on fiberglass panels, *Vorticella* was absent during most seasons but was abundant in winter (Anderson, 1995). The other positive correlation we report is between colonization and TRP at SD. This may be a seasonal consequence, because TRP correlates with temperature at this site ($r = 0.757$, $P = 0.004$). The absolute values recorded for TRP were very low at this site, varying from 0.01 to 0.05 mg/liter. Because of these low values, the correlation between colonization and TRP may have little significance. A correlation between total phosphorus and planktonic ciliate concentration has been reported earlier in Lake Erie (Hwang and Heath, 1997).

A number of negative correlations with colonization were found. The negative relationship between colonization and DO occurred at the same sites where temperature correlated positively with colonization. This is not surprising because one would expect DO levels to decline as water temperature rises. Spearman correlations between DO and temperature support this assumption because the two variables were negatively correlated at both data logger sites (north data logger, $r = -0.748$, P

= 0.005; SD data logger, $r = -0.937$, $P < 0.001$). The negative correlation between colonization and NO_3^- at SD is more difficult to interpret. As with the TRP data, the absolute values for NO_3^- at SD were very low, ranging from 0.00 to 0.36 mg/liter and averaging 0.08 mg/liter. These low values make the significance of the NO_3^- correlation suspect. The Weeks Bay watershed is primarily agricultural, with terrestrial sources providing most of the nitrogen for the Bay. Our data revealed a typical trend, with a higher average level of NO_3^- at the north dock (0.28 mg/liter at NDD, 0.24 mg/liter at NDS), near the input of water from the Fish River, and a lower level of NO_3^- at the south dock.

Surprisingly, no correlation was found between colonization and turbidity, which was reported at Dauphin Island (Beech and Landers, 2002). Similarly, there was no correlation between colonization and salinity, which varied from 0.39 to 20.47 ppt at NDD and NDS and from 1.8 to 22.48 ppt at SD. Perhaps this is the result of our not identifying *Vorticella* to species in this study. It is possible that estuarine species were replaced by freshwater species during periods of high rainfall and low salinity, which would not be reflected by our data.

We are confident that seasonal temperature changes have a major influence on *Vorticella* colonization. Another component of the ecosystem, food availability, is probably a controlling factor as well. *Vorticella* is a bacterivore–detritivore (Persoone, 1968; Pratt and Cairns, 1985), and its colonization ability is likely linked with bacterial abundance. Bacterial abundance has been studied in Mobile Bay (Griffin, 1998) and was shown to correlate to water temperature on a seasonal basis. In a study of two sites at Visakhapatnam Harbor in India, the submerged slides at the site near a sewage outfall revealed the presence of *Vorticella*, whereas the site 6 km downstream and closer to the open ocean did not. The temperatures at both sites were similar (31 C and 30 C), so nutrient or food availability was likely the factor contributing to the protozoan faunal differences.

We did not identify any natural or unnatural events that could provide insight into the mid-summer population crashes observed at the two north dock sites. At NDS, the population crashed from 1,909 cells/cm² in June to 69 cells/cm² in July. However, in June the data varied from 24 cells/cm² to 3,436 cells/cm². Similarly, at NDD the population crashed from an average of 2,733 cells/cm² in July to 39 cells/cm² in Aug. However, in July the data var-

ied from 12 cells/cm² to 5,936 cells/cm². At both sites, there was an extreme variability from slide to slide before the crash, as shown by the error bars in Figures 2 and 3. During the June–Aug. period, the protist populations have an obvious patchiness and variability, which may have contributed to the anomalous findings. Unfortunately, most of our environmental data for NDS and NDD sites were recorded from a single datalogger nearby and not exactly at each site, so no correlations could be determined that would also help explain the changes at each site.

An analysis of protistan colonization with regard to all sessile ciliate genera was another goal of this project. Colonization density was low at all sites until April, at which time the ciliate density increased. A number of genera appeared in April that were not common during the colder months (Table 1). In April at NDD and NDS and in May at SD, the slides became associated with mats of silt and debris. *Stentor* was abundant in this silty environment, which created numerous tubes within the silt and greatly changed the habitat. Later during the year, the silt-*Stentor* habitat was not reliably present, although *Vorticella* populations were relatively high throughout the warmer periods.

In summary, we have documented the seasonal changes in *Vorticella* colonization and have shown that temperature changes correlate with population changes. Studies on sessile protozoa need to take into account this seasonality if *Vorticella* is to be used as a bioindicator of environmental or pollution effects. Colonizing protozoans have been used in environmental assessments of freshwater ecosystems (Stewart et al., 1986; Yun-Fen et al., 1986) and can be applied to marine and estuarine ecosystems if baseline levels of the protozoan fauna are established. Future studies on protozoan colonization by our laboratories will include bacterial concentration determination and will focus on environmentally stressed ecosystems.

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- (SCL) DEPARTMENT OF BIOLOGICAL AND ENVIRONMENTAL SCIENCES, 210A MSCX, TROY STATE UNIVERSITY, TROY, ALABAMA 36082; AND (SWP) WEEKS BAY NATIONAL ESTUARINE RESEARCH RESERVE, FAIRHOPE, ALABAMA 36532. Send reprint requests to SCL. Date accepted: December 30, 2002.