

**PIONEER COLONIZATION AND
INTERACTION OF PHOTOSYNTHETIC
AND HETEROTROPHIC
MICROORGANISMS ON AN ARTIFICIAL
SUBSTRATE OF POLYURETHANE FOAM IN
E. J. BECK LAKE, ILLINOIS**

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ABSTRACT

The accrual of pioneer microbial inhabitants on an artificial substrate of polyurethane foam in a freshwater ecosystem during a 22 day sampling period was an approximately linear event. Compared to the fast and sometimes fluctuating arrival of photosynthetic algae, the colonization by protozoa was slow and steady. However, there was greater diversity for the photosynthetic algae than for the heterotrophic protozoa. Artificial substrates harvested toward the end of the sampling period yielded more phylogenetically advanced organisms and perhaps signified a transition to a more complex stage in the colonization sequence.

INTRODUCTION

Use of artificial substrates to monitor aquatic conditions has increased over recent years (Weitzel, 1979; Henebry and Cairns, 1980; Cairns, 1982). The colonization process on artificial substrates, since it mimics similar naturally occurring processes, serves as an "island model" for studying the immigration rate and interaction of colonists on natural substrates in the aquatic environment (Cairns, Kuhn, and Plafkin, 1979; Cairns and Henebry, 1982; Osman, 1982). Synthetic polyurethane

foam (PF) is widely used as a substrate due to its superior collecting ability and low cost (Bamforth, 1982). This investigation examined the initial colonization process by charting the accumulation of some pioneer photosynthetic algae and heterotrophic protozoa on PF substrates for selected days during a 22 day period. Water surrounding the substrates was also sampled to provide an indication of the number and diversity of microorganisms immediately available for substrate colonization.

MATERIALS AND METHODS

E.J. Beck Lake, located near Des Plaines, Illinois, occupies approximately 38 acres and is managed by the Forest Preserve District of Cook County as a recreational area. In 1958, the lake was excavated to a depth of 23 feet to provide a source of fill for an interstate highway located immediately to the east (Forest Preserve bulletin, 1971). The water sources for the lake are ground water, highway run-off, and rain. The lake maintains a stocked population of fish.

The sampling site is located in the northwest corner of the lake. This area has a relatively calm surface due to screening by tall trees and other vegetation lining the shore. A dense growth of *Typha* along the shoreline limited the access to the area and diminished the likelihood of vandalism.

Sampling was performed during late October and early November of 1983. The artificial substrates were PF blocks measuring 5.0 by 6.5 by 7.5 cm (Cairns and Ruthven, 1970). The blocks were tied and tightly cinched in the middle with a piece of string; long ends were left to allow for attachment of an anchor and marker float (Bamforth, 1982). Each PF substrate was anchored with a stone approximately 10 lbs in weight. The stone was placed in a plastic bag and tied securely to one end of the string. These anchors were attached 60 cm from the PF substrates. This method assured a consistent positioning of the naturally buoyant PF substrates from the lake bottom. The distance of the PF substrates from the surface varied as the lake level changed during the sampling period: the distance below the surface was never less than 10 cm nor more than 20 cm. The other end of the string was attached to a styrofoam fragment which acted as a floating marker. Eight of these devices were deposited in a line 9 to 12 m from the shore and 3 m apart.

On each day of sampling, air and water temperatures were recorded using a "Testrite" brand thermometer (Japanese Instrument Co.). Water pH was tested with pHYdrion strips (Micro Essential Lab., N.Y.). Water surrounding the PF substrates was sampled by plunging a capped 200 ml sample bottle into the water adjacent to the PF substrate to be harvested. When the bottle reached the depth of the substrate, the cap was removed allowing the bottle to fill. The cap was then replaced underwater. Once these procedures were completed, the PF substrate was harvested. Upon removal from the water, the substrate was squeezed by hand until approximately 100 mls of fluid was collected in a 200 ml bottle. All samples were examined microscopically within the next hour.

In the laboratory, the sample bottles were shaken gently by hand. A 0.25 ml aliquot was removed from each bottle and diluted with equal amounts of 1% methyl cellulose to aid in observation. The enumeration of species was performed at 100X using an ocular lens fitted with a Whipple grid. Counts were adjusted for dilution. Organisms were identified with the aid of standard references (Ward and Whipple, 1959; Prescott, 1978; Jahn, Bovee, and Jahn, 1979).

The determination of the number of organisms per milliliter was accomplished by

using the "Lackey Drop Microtransect Counting Method" as modified below (Lackey, 1938; *Standard Methods*, 1976, p. 1027). Statistical treatment of the data involved the establishment of confidence intervals based on 1 standard error or deviation (Snedecor and Cochran, 1956, chapters 2, 3, and 17; Mendenhall, 1967, chapter 8).

$$\frac{C \times TA}{A \times S \times V} = \text{Number of organisms / milliliter}$$

where:

- C = actual number of organisms counted,
 TA = total area under coverslip (22 by 22 mm = 484 mm² times the number of drops of sample examined),
 A = area counted in microscope field at 100X; using the Whipple grid, 42 squares were counted in each field; each square is 200 μm on a side, so the entire field of 42 squares is 200 by 200 by 42 = 1.68 × 10⁶ μm² since 1 mm² = 1 × 10⁶ μm²,
 S = number of fields of 42 squares each that were counted; up to 60 such fields were examined for each sample,
 V = total volume in mls examined; 1 drop = 0.05 ml.

RESULTS

Environmental monitoring was performed at the time the samples were taken and indicated an air temperature range of 6° to 13°C, a water temperature range of 9° to 10°C and a consistent water pH of 6.5.

Results of microbial sampling appear in Table 1. Figures 1 and 2 represent the numbers of organisms found at each sampling over the period of the study. Identification of organisms collected on the PF substrates and from the surrounding water yielded a total of 14 genera representing 11 families and 2 kingdoms (Table 1). Only 1 genus (*Spirogyra*) present in the surrounding water did not appear on the PF substrates during the sampling period. Although the majority of collected organisms were photosynthetic, considerable variation existed in the families and genera present. Most photosynthetic organisms were from the Family Naviculaceae, with *Navicula* being the most prevalent. As time progressed, the filamentous blue-green algae such as *Oscillatoria* and *Anabaena*, and elongate diatoms such as *Fragilaria* and *Synedra* increased in number. This occurrence was noted particularly after day 9.

Natural vegetational substrates (*Typha*) within 9 m of the sampling area yielded additional types of organisms including 1 genus from the Kingdom Monera, 4 genera from the Kingdom Protocista, and 2 genera from the Kingdom Animalia. These data are not included in Table 1 nor are they reflected in the totals represented by Figures 1 and 2.

Figure 1 shows that the colonization of organisms on the PF substrates over the 22 day sampling period was approximately linear. It increased with each sample reaching a level nearly 12 times the average number of organisms present in the surrounding water during the same period of time. There was a sharp contrast between the relatively stable number of organisms present in the surrounding water (an average of 3.29 over the sampling period) and the progressive increase to 9 genera of organisms present on the PF substrates. This nearly threefold increase in diversity was largely the result of colonization by photosynthetic organisms (Fig. 2). At the end of the sampling period, autotrophic organisms were 20 times more numer-

ous than the heterotrophic organisms found on the same PF substrate. Moreover, the number of photosynthetic genera (8) found on the PF substrate for day 22 was nearly 5 times greater than the heterotrophic ones (average, 1.75) that colonized the substrates over the entire period of sampling.

DISCUSSION

The upward trend in the accrual of photosynthetic organisms on the PF substrates shown in Figures 1 and 2 corresponds to the initial "noninteractive" colonization stage described by Cairns and Henebry (1982). Since no plateau is reached during this period, it is assumed that the final equilibrium of the number and diversity of organisms was not reached (Cairns *et al.*, 1979).

The inhabitants present probably are pioneers comprising part of the initial colonization stage. The 7 genera found on nearby natural substrates and the 1 genus present in the surrounding water did not appear on the PF substrates. This suggests to us that additional colonization would likely occur given more time. According to Cairns *et al.* (1979), this happens because the natural environment contains a "source pool" of potential colonists.

Colonization of PF substrates by heterotrophs was a conservative event in comparison to colonization by photosynthetic organisms. Figure 2 shows only a slow rate of increase of heterotrophic organisms, both in number and diversity after day 3. This compares favorably with the results of Cairns *et al.* (1979) and Cairns and Henebry (1982) where a colonization equilibrium is reached within 3 days in similar aquatic environments. We believe it is likely that an equilibrium has occurred for the heterotrophs because there is a decrease in the number and diversity of those organisms recovered at day 22. However, further testing involving longer time periods would be required to confirm this.

The decrease in the number of photosynthetic organisms on day 5 (Fig. 2) was most likely due to a heavy rain on day 4 which caused the lake level to rise with accompanying disturbances of surface water. Evidently, these disturbances did not affect the variety of organisms available for colonization since the progressive increase in diversity continued for these organisms. These events also appeared to have little effect on the number and diversity of the heterotrophs found on the PF substrate for day 5 (Fig. 2).

Cairns and Ruthven (1970) pointed out that such "fluctuations" could be merely the result of sampling and counting errors. Care was taken in this study to minimize these problems; the fluctuations found on days 5 and 22 did not adversely alter the overall colonization trends and were not statistically significant as reflected by the standard error bars in Figs. 1 and 2.

The diversity of organisms colonizing the PF substrates after day 9 widened to include complex filamentous and elongate varieties of microbes. The accumulation of more advanced photosynthetic organisms increases the surface area on the PF substrates thus making it possible for even greater amounts of debris and other organisms from the surrounding water to collect on the substrates. Both autotrophs and heterotrophs eventually benefit from the greater diversity and accumulated debris since havens are created for additional subsequent colonization. The early autotrophic colonists prepare and modify the PF substrate habitat for later arriving heterotrophic protozoa (Cairns *et al.*, 1979). With increasing colonization, more abundant food sources like bacteria and algae become available to the heterotrophs (Bam-

forth, 1982). This stage of colonization is a necessary prelude to the next developmental stage where later immigrant species interact with the already established microcommunity (Cairns and Henebry, 1982).

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Table 1. Taxonomy, source and trophic categories of collected organisms.*

Kingdom	Family	Genus	Source	Trophic Category
Monera	Nostocaceae	<i>Anabaena</i>	W S	P
	Oscillatoriaceae	<i>Oscillatoria</i>	S	P
Protoctista	Naviculaceae	<i>Navicula</i>	W S	P
		<i>Pinnularia</i>	W S	P
		<i>Gyrosigma</i>	W S	P
	Fragilariaceae	<i>Synedra</i>	S	P
		<i>Fragilaria</i>	S	P
	Coscinodiscaceae	<i>Cyclotella</i>	S	P
	Hydrodictyaceae	<i>Pediastrum</i>	S	P
	Volvocaceae	<i>Volvox</i>	W S	P
	Zygnemataceae	<i>Spirogyra</i>	W	P
	Amphileptidae	<i>Litonotus</i>	S	H
Bodonidae	<i>Bodo</i>	W S	H	
Diffugiidae	<i>Diffugia</i>	W S	H	

*The taxonomy follows Margulis and Schwartz (1982), Prescott (1978), Corliss (1979), and Jahn, Bovee, and Jahn (1979). The sources of collected organisms are identified by W (surrounding water) and S (PF substrate). Trophic categories are identified by P (photosynthetic) and H (heterotrophic).

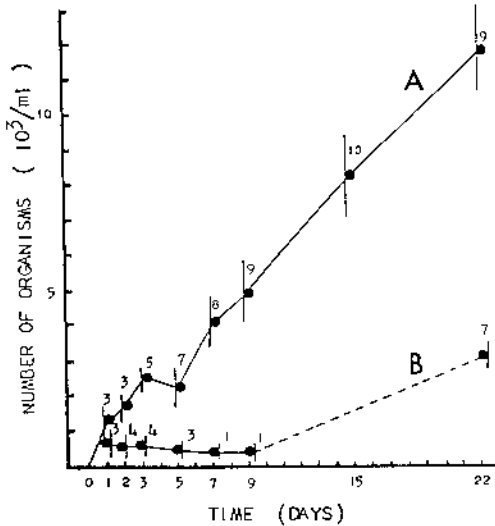


Fig. 1. Combined photosynthetic and heterotrophic colonization of the PF substrates (A) compared to the numbers of these organisms in the surrounding water (B). Since water data was unavailable for day 15, an extrapolation between days 9 and 22 is indicated by a dashed line. Numerals appearing above the data points refer to the daily total of genera represented by the plotted numerical totals. Vertical bars represent one standard error of the mean.

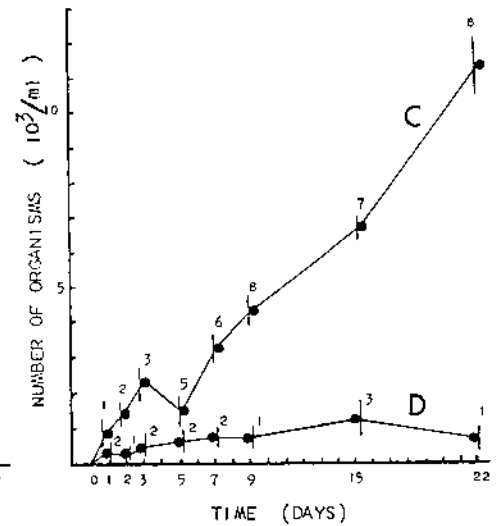


Fig. 2. The colonization of PF substrates by photosynthetic (C) vs. heterotrophic organisms (D). Numerals above data points and bars are as in Fig. 1.