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Environmental Factors affecting the Settlement and Growth of the Freshwater Bryozoan, *Fredericella Indica* (Ectoprocta), In One Harbor of Southern Lake Michigan

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ABSTRACT

Important factors which affect the settlement and growth of the freshwater bryozoan, *Fredericella Indica* (Ectoprocta) in one harbor in southern Lake Michigan are identified in this paper. Plastic plates are attached at two different depths on randomly chosen dock posts in Michigan City, Indiana. Weekly photographs of these plates are analyzed to determine the proportion coverage of *Fredericella Indica* on these plates. Also, a number of environmental variables including lake water temperature, pH level, secchi reading, nitrate level, total phosphate level, water hardness, amount of ammonia, oxygen level, amount of total dissolved solids in the water and water conductivity, are all measured on a weekly basis. A statistical analysis reveals that the date of measurement, post location, length of post, depth of plates, as well as lake temperature, nitrate level and the phosphate level are the most important factors that affect the settlement and growth of *Fredericella Indica* on the plastic plates. Interactions between various explanatory factors in the study are also found to be significant. A discussion is given which centers on the significance of, in particular, the depth of the plates and the temperature of the water on the settlement and growth of *Fredericella Indica*.

INTRODUCTION

Numerous studies have investigated the dynamics and interactions of the sessile benthic communities inhabiting rocky intertidal, coral reef, estuarine, and deep-sea vent habitats (Connell 1961, Dayton 1971, Paine 1971, Jackson and Buss 1975, Menge 1976, Osman 1977 and 1998, Buss 1979, Jackson 1979, Garrity and Levings 1981, Paine and Levin 1981, Gaines and Roughgarden 1985, Witman 1987, Bence and Nisbet 1989, Possingham and Roughgarden 1990, Alexander and Roughgarden 1996, Brown and Swearington 1998, Leonard *et al.* 1998, Rittschof *et al.* 1998, Barnes and Arnold 1999, Fairfull and Harriott 1999, Smith and Witman 1999, Menge *et al*. 1999, Barnes and Dick 2000). These studies have resulted in a body of knowledge and insight into the direct/indirect biotic and abiotic interactions and stresses occurring within these marine communities (Osman and Whitlatch 1998, Menge and Olson 1990).

For over a century, the ecological dynamics in the marine environment, and other communities, such as those observed in terrestrial habitats, have been studied (Cowles 1899, Elton and Miller 1954, Connell 1961 and 1983, Hughes 1996). These studies and the resulting general theories and mathematical models have done much to explain and increase our understanding of the environmental factors controlling and supporting biological communities (Vance 1984, Roughgarden *et al.* 1985, Hughes 1990, Farrell 1991, Dennis *et al.* 1995, Tanner *et al.* 1996, Dial and Roughgarden 1998). However, even with these basic understandings, there is little known about the occurrences and interactions of similar benthic organisms found within the freshwater communities, particularly the Great Lakes region of the United States (Ricciardi and Reiswig 1993 and 1994, Lauer and Spacie 1996, Lauer 1997, Alix and Scribailo 1998). This lack of understanding in benthic communities was found to be problematic where accidental introductions of exotic organisms such as the freshwater mussel, *Dreissena polymorpha* have occurred (Moyle and Light 1996). Exotic organisms can substantially modify their environment, creating extreme stresses on the native organisms (Griffiths 1993, Mills *et al.* 1994, Nalepa *et al.* 1998, Simon *et al.* 1998, Lauer *et al*. 1999). Many potential negative impacts cannot be delineated due to a lack of historical data, particularly on sessile benthic organisms in freshwater (Karatayev *et al.* 1997, Ricciardi *et. al* 1997).

Bryozoans (Bryozoa), commonly known as "moss animals" because of their plant-like appearance, inhabit most benthic habitats (Barnes and Ruppert 1991, Stiling 1996, Smith 2001). There are at least 5,000 known species of bryozoans worldwide, although only 50 of these species are known to inhabit freshwater systems (Margulis and Schwartz 1988, Smith 2001). Bryozoans are found in both lentic and lotic waters of varying degrees of eutrophication (Banta and Backus 1991). These animals are colonial and may be sessile or motile depending on the species and life stage. They can attach to a variety of substrates, including other sessile benthic organisms (Smith 2001).

Biogeographical information on bryozoans in North America has been limited to the distribution and general ecology of these organisms (Barnes and Lauer 2003, Ricciardi and Reiswig 1994, Wood 1989). In addition, bryozoan settlement and colonization studies have been restricted to laboratory cultures (Wood 1989). Moreover, little is known about the settlement and growth patterns of specific species, such as *Fredericella indica* (Annandale 1909). However, it is known that *Fredericella Indica* can grow in water temperatures less than 4° C and exhibits both adherent (attached to substrate) or erect (branched) colonial forms (Ricciardi and Reiswig 1994). It has also been found to be a major food source for several species of game fish and crustaceans, such as crayfish, thus suggesting a possible importance to conservation agencies and commercial fisheries (Ricciardi and Reiswig 1994).

Personal observations, surveys, and previous studies have shown that colonies *Fredericella Indica* were common within the Lake Michigan harbor basin at Michigan City, Indiana (Barnes; unpublished). In addition, colonies of this species readily attach to and grow on artificial substrate surfaces, including PVC plates, suspended vertically (Barnes; unpublished). Therefore, this species affords unique opportunities to perform investigative scientific studies, manipulations, and quantitative measurements on larval settlement, colonization patterns, seasonal growth, and competitive interactions.

There are three sections in the paper. The first section describes the materials and methods used to not only quantify the recruitment and colonizing patterns of the bryozoan species, *Fredericella Indica*, inhabiting the harbor basin located at Michigan City Indiana, but also to measure the environmental conditions controlling this organism's settlement and growth. The statistical analysis is given in the second section. The last section gives both a summary and discussion of the results.

MATERIALS AND METHODS

Plastic plates are attached at two different depths on randomly chosen dock posts in Michigan City, Indiana. Weekly photographs of these plates analyzed to determine the proportion of the plates covered by *Fredericella Indica*. A number of environmental variables including lake water temperature, pH level, secchi reading, nitrate level, total phosphate level, water hardness, ammonia, oxygen, total dissolved solid amount and conductivity, are all measured on a weekly basis. The Michigan City harbor study area, test plates and environmental analyses are described in greater detail below.

Study Area

The study area was located in the harbor basin located at Michigan City, IN harbor (L: 41° 43.549'N, Lo: 86° 54.431'W), on the southeastern shoreline of Lake Michigan. It has one entrance/exit point that opens into the mouth of Trail Creek as it empties in Lake Michigan. The study was conducted from May 21, 2002 to August 26, 2002 (15 weeks or 103 days; Julian calendar: day 135 to day 238). The mean water depth of the harbor basin was determined on August 16, 2000 by using 23 depth readings randomly selected from sites within the harbor. Water depth measurements were determined using a portable fishfinder (Apelco 265©, Raytheon Electronics, Lexington, MA). The mapping coordinates of each site were determined with the aid of a handheld global positioning instrument (GPS III©, Garmin International, Inc., Olathe, KS). The average water depth of the harbor was five meters. Depth within the basin did not vary substantially over the summer months in prior years (Barnes personal observation). Therefore, only a single set of measurements were collected.

Test Plates

Twenty four individual plastic (polyvinyl carbonate: PVC) settlement plates, measuring 15cm x 10cm x 0.6cm. were placed at both the one-meter depth and two-meter depth from the lake surface on 12 randomly selected pier posts using black plastic electrical ties on May 13 and 14, 2002 (Osman 1977). No settlement plates were placed deeper from the lake surface than two meters as preliminary studies done in 1997 revealed bryozoan settlement and coverage was less than 1% at water depths greater than two meters. Selection of the specific sites from the original site data set was performed using a random number sequence generated using a calculator (TI-83©, Texas Instruments Incorporated, Dallas, TX). The sample sites were re-designated as plate sites "A" through "L" and each plate was photographed weekly beginning May 15, 2002 and continuing until August 26, 2001 using underwater macrophotography similar to the techniques described in Hughes (1996) and Smith and Witman (1999).

Photographs were digitized using a computer scanner and scaled under a grid overlay using computer software (Sigma Scan Pro© 6.0, SPSS, Inc., Chicago, IL). Aerial coverage of colonizing bryozoans was determined by counting the number of times the organism was observed under the crosshairs of the grid (point-cross analysis) and converting the resulting value to a percent of the total area of the individual Plates (Hughes 1996, Smith and Witman 1999). Only newly settled colonies of bryozoans occurring on the plates were quantified and any spreading or “branching” of established colonies outside the area of the plate, but extending into the photographs, was omitted. Photo scaling errors were avoided using mechanical means (stationary framer) which fixed camera distance and angle so that uniform photographic data were collected from all plates.

Photographs and samples of bryozoan colonies were used to identify species settling and growing on the plates. Colonies on the plates were not disturbed during sampling. Rather, species sampled for identification were taken from areas adjacent the plates. Identification of bryozoans was accomplished using dissecting and compound light microscopes and comparing their characteristics to current taxonomic keys (Ricciardi and Reiswig 1994, Smith 2001, Wood 2001).

Environmental Analyses

Environmental variables in the study area were measured weekly. These tests were done at both one-meter and two-meter depths and in conjunction with the photographing of the 24 settlement plates. A total of five sampling sites were randomly chosen from the original 24 pier sites using the same protocol used in the selection of the photo plate sites; these same five sites were used throughout the study period. Mean values of these five values were calculated for each sample period. For the chemical analyses, lake water was collected using a two-liter capacity, Van Dorn sampler at both one-meter and two-meter depths. These samples were mixed in a bucket on location and homogenous sub-samples were extracted into 500mL plastic bottles for later analyses (Figure 3). All analyses were accomplished using methodologies found in Standard Methods (1998) and included the following ten tests: water temperature (°C), secchi disk (m), water hardness (mg/L of CaCO3), ammonia (mg/L), nitrate (mg/L), total phosphate (mg/L), dissolved oxygen (mg/L), pH (s.u.), conductivity (mS/cm3), total dissolved solids (TDS; mg/L).

All chemical tests, except the analyses for dissolved nutrients (e.g. nitrate, phosphate, etc.) were performed on-site using a Conductivity/TDS meter (Model 44600, Hach Company, Loveland, CO). Dissolved oxygen was measured using a digital meter (YSI© Model 85, YSI, Incorporated, MA). For the dissolved nutrient tests, water samples were collected using the same methodology described above, placed in a cooler, and transported to the lab for final analyses on a spectrophotometer using spectra-colorimetric techniques (Spectronic® Genesis™ 8 UV/Visible, Standard Methods 1998).

STATISTICAL ANALYSES

The data for this study is naturally broken into two different data sets, where one is called the physical data set and the other is called the environmental data set. Statistical analyses are applied to these two data sets. A comparison is made between the 2002 data and previous preliminary data collected in 1997. The statistical software package, SAS, is used in all of the statistical analyses.

Analysis of physical data set

The analysis of variance (ANOVA) statistical analysis described here shows that all three of the main explanatory factors in the physical data set, including date of measurement of the percent plate coverage by *Fredericella Indica*, dock post location and depth of the PVC plates below water surface on the dock posts, significantly affect the settlement and growth of *Fredericella Indica* on the PVC plates. Interaction factors between the main explanatory factors in the study are also found to significantly influence this bryozoan's growth rate. The model correlation is $r^2 = 0.926$, which, since it is close to the maximum value of one (1), indicates all of the main and interaction factors, together, describe the growth rate of *Fredericella Indica* extremely well.

The analysis of variance (ANOVA) table for the physical data set is given below. Every two weeks of data are merged into one to create enough degrees of freedom for the analysis and, as a consequence, factor "date2w" is used instead of the factor "date" in the analysis. In addition to the main explanatory factors, "date2w", "depth" and "post", there are also a number of interaction factors, including, in particular, the two-factor interactions, "date2w x depth", "date2w x post" and "depth x post". The arc sine transformation is applied to the % plate coverage measurements because this improves the ANOVA (Neter et al., p 773, 4th Edition, 1996).

Table 1: ANOVA of physical data set

Source	DF	Sum of Squares	Mean Square	F Value	P-value
Model	191	123.8298455	0.6483238	105.13	<.0001
date2w	7	91.91346662	13.13049523	2129.14	<.0001
depth	1	2.71312174	2.71312174	439.94	<.0001
post	11	10.70998057	0.97363460	157.88	<.0001
date2w*depth	7	1.45662601	0.20808943	33.74	<.0001
date2w*post	77	10.22173432	0.13274980	21.53	<.0001
depth*post	11	2.13592559	0.19417505	31.49	<.0001
date2w*depth*post	77	4.67899062	0.06076611	9.85	<.0001
Error	1608	9.9166134	0.0061670		

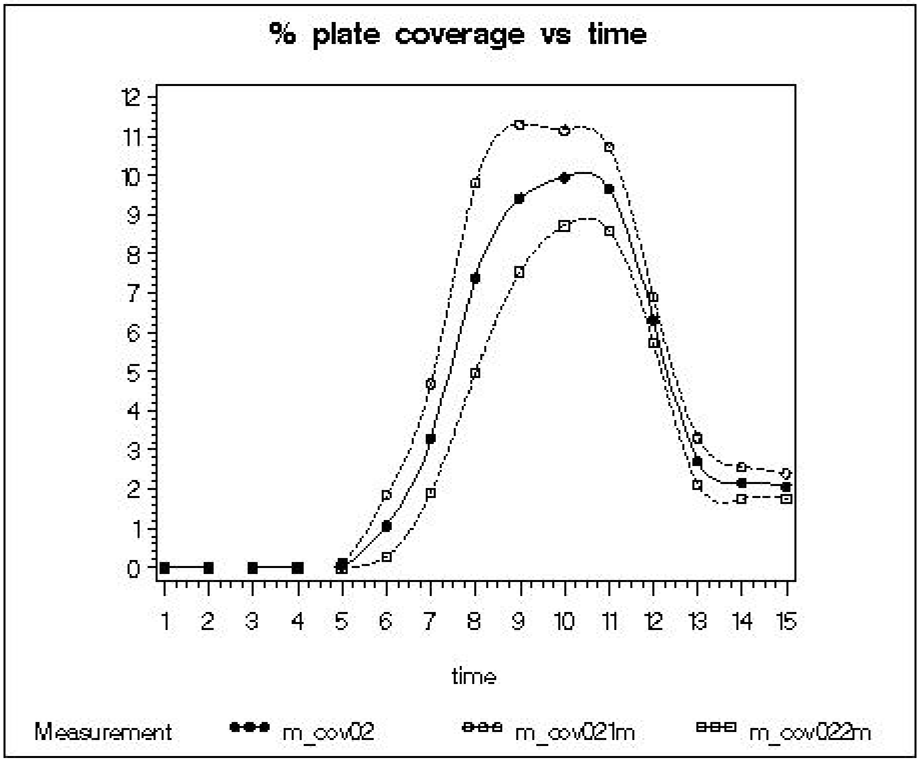
Corrected Total	1799	133.7464589

On the one hand, as shown in the ANOVA table, all of the main explanatory factors, date2w, depth and post, for bryozoan growth are highly significant (p-value < 0.0001). This means that *Fredericella Indica* growth is different throughout the season; plotted data shows this difference involves a bloom and die-out period. This also means there is difference in this bryozoan growth at 1 meter below the lake surface as compared to bryozoan growth at 2 meters below the lake surface; in particular, plotted data shows greater bryozoan growth occurs at 1 meter, than at 2 meters. Finally, this indicates this bryozoan growth is different at the different post locations.

On the other hand, not only are all main factors, date2w, depth and post, highly significant, but also all of the two-factor interaction factors, date2w x depth, date2w x post and depth x post, are also highly significant (p-value < 0.0001). These three pairwise significant interactions bring up a number of interesting issues.

Consider the significant date2w x depth pairwise interaction. As shown in Figure 2, the upper curve of the three curves is the % *Fredericella Indica* coverage of the plates which are attached one meter below the lake surface level on the selected posts over the study period; the lower curve is the % coverage of the plates which are attached two meters below the lake surface level on the selected posts over the study period and the middle curve is the average of the upper and lower curves. The test dates, between May 15th, 2002, and August 26th, 2002, are replaced by the numbers 1, 2… 15. In this case, although there is a higher percentage of bryozoan growth on all dates during the study on the plates attached at 1 meter below lake level, rather than on the plates attached at 2 meters below lake level, the *amount* of difference between the two percentages varies over the course of the season. In particular, there is a greater spread in the % plate coverages in the middle of the season, on dates 7 through to 11, than at any other time in the season. It appears that once the growth of this bryozoan starts in earnest, those in the upper warmer more nutrient-filled water level of the water column spread more rapidly than those in the lower colder less nutrient-filled levels of the water column.

Figure2: Various % coverage over the season



Consider the significant date2w x post pairwise interaction. In this case, there is a higher percentage of *Fredericella Indica* growth occurring on the plates on dock posts I and L throughout the peak (dates 7 to 12) season, except for at dates 10 and 11, when the plates on dock post D have the greatest % plate coverage by these bryozoans. In other words, during some periods of the season, these bryozoans grow well at particular dock post locations, but, during other parts of the season, bryozoans grow well on other different dock post locations. This suggests the conditions beneficial for this bryozoan growth move from one post location to the other throughout the season. One possible reason for these transient beneficial conditions could be boat traffic in the harbor.

Finally, consider the significant depth x post pairwise interaction. As shown in Table 2, there is a higher percentage of *Fredericella Indica* growth on the plates attached on posts at 1 meter below surface level, rather than on the plates attached on posts at 2 meters below surface level, expect for at posts I and L, where the reverse is true. A closer study of posts I and L reveal they are both in high traffic areas close to the lake break wall in the Michigan City harbor and so it is conceivable that churned up sediment as well as a mixed temperature water column in these locations caused this reversal.

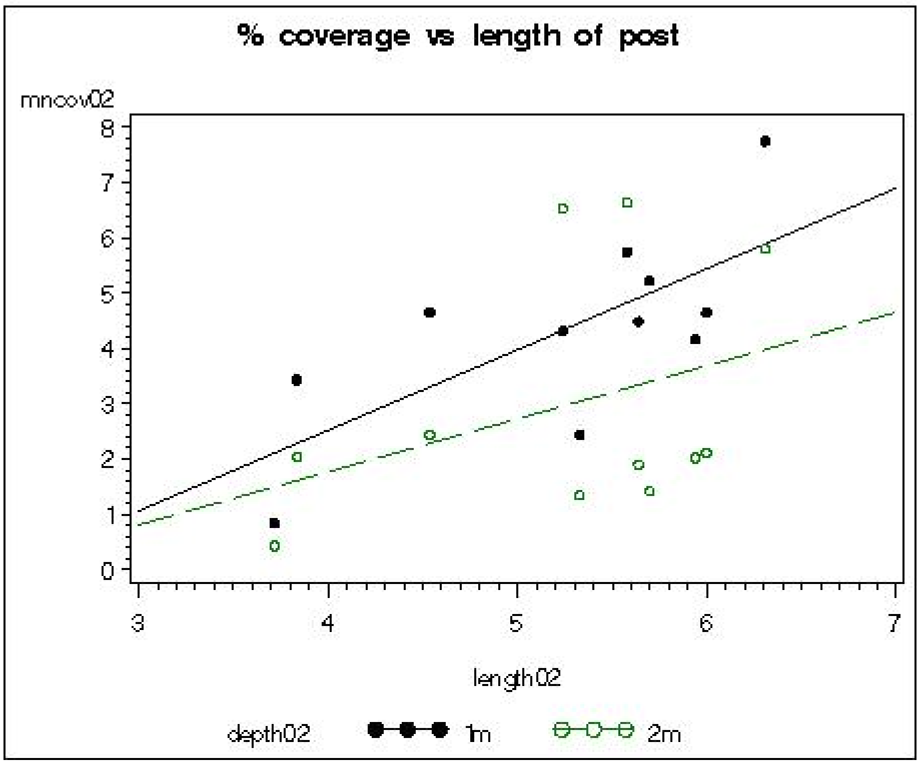
Table 2: depth x post interaction

	A	B	C	D	E	F	G	H	I	J	K	L

1m	0.85%	3.44%	4.66%	7.75%	4.65%	2.32%	5.22%	4.49%	4.32%	2.44%	6.02%	5.75%
2m	0.43%	2.05%	2.43%	5.80%	2.12%	1.89%	1.42%	1.91%	6.53%	1.34%	2.17%	6.64%

Related to the depth x post pairwise interaction, is the plot in Figure 2 which indicates that the % *Fredericella Indica* coverage increases for increasing length of post. That is, the % coverage is greater for plates attached to longer posts, or, in other words, for plates further from the lake bottom. It seems as thought that these bryozoans, found at similar depths of water, grow faster the farther away from the lake bottom they are.

Figure 2: % coverage versus post length



Summary

The *Fredericella Indica* growth rate is different throughout the season, involving a bloom and die-out period. There is greater growth rate at 1 meter below the lake surface, than at 2 meters below the lake surface. The growth rate is different at the different post locations. Significant interactions between these three main factors could be due to boat traffic in the harbor. Indeed, the correlation of the model without interactions, $r^2 = 0.822$, is not too much smaller than the correlation of the model with interactions, $r^2 = 0.926$.

Residual plots, not shown here, indicate the assumptions necessary to use this model to fit the data are somewhat suspect. In particular, a slight upward trend in the standardized residual versus observed arc sine coverage plot indicates a variable is missing or that another transformation is required to alter the model to better fit the data. Also, a non-linear normal probability plot indicates non-normal error when the error should, in fact, be normal. Neither one of these concerns about the residuals invalidates the analysis, but would need to be addressed in any future analyses of the data. In addition, all factors are treated as fixed factors, rather than random factors. Some analysis has been done which assumed "depth" and "post" are repeatedly measured on "date" and that "post" and "date" are random factors, but, in the end, these complications did not seem to help or significantly change the results of the study as conducted here.

Analysis of environmental data set

The two linear regression analyses and the one time series analysis given in this section demonstrate that of the ten environmental variables, the three most important variables that affect the settlement and growth of *Fredericella Indica* on the PVC plates are lake temperature, nitrate level and the phosphate level.

The environmental data set is given in Table 3 below. For example, on June 11th, 2002, the average % plate coverage by *Fredericella Indica* is 0.054%, the average temperature is 20.80 degrees Celsius, and the pH level is 7.878 and so on. On the one hand, the average % plate coverage of 0.054% is determined by averaging all twenty-four % plate coverages observed for dock posts A, B, C, D, E, F, G, H, I, J, K and L, at both the one meter and two meter depths on June 11th, 2002. On the other hand, the average environmental variable temperature of 20.80, for example, was determined by averaging the five temperatures measured at five post test site locations chosen at random from the twelve in the study (where each of these five measurements are themselves the average of two measurements taken at one meter and two meters) on June 11th, 2002. The other environmental variable averages are determined in a similar way.

Table 3: Environmental data set

Date	Cov	Environmental variables									
		temp	pH	secchi	oxygen	ammonia	nitrate	phosphate	hardness	conduct	TDS
May 15	0.000	13.14	7.366	0.26	7.38	0.484	0.077	0.050	120.4	0.371	0.183
May 21	0.000	12.52	7.480	1.36	7.58	0.471	0.073	0.049	162.6	0.515	0.255
May 28	0.000	15.98	7.720	2.66	4.94	0.422	0.062	0.075	163.0	0.569	0.292
June 04	0.000	17.44	7.902	1.56	7.92	0.415	0.254	0.047	130.8	0.444	0.227
June 11	0.054	20.80	7.878	3.34	8.58	0.558	0.184	0.329	143.2	0.475	0.239
June 18	1.071	21.12	7.854	3.16	7.56	0.713	0.169	0.334	158.6	0.518	0.264
June 25	3.300	24.38	8.200	2.56	4.81	0.619	0.082	0.396	133.6	0.413	0.207
July 02	7.392	23.20	8.226	2.90	8.28	0.385	0.154	0.300	113.8	0.358	0.180
July 09	9.433	26.72	7.728	2.86	7.40	0.640	0.067	0.261	143.2	0.440	0.221
July 16	9.946	25.10	8.214	5.00	6.44	0.451	0.067	0.319	124.6	0.375	0.188

July 23	9.663	25.78	7.988	0.58	6.48	0.445	0.084	0.102	135.2	0.452	0.227
July 30	6.325	25.16	8.292	0.92	8.42	0.383	0.083	0.236	166.2	0.459	0.230
Aug 06	2.721	21.00	7.916	3.36	9.03	0.380	0.060	0.327	159.8	0.357	0.178
Aug 13	2.163	19.80	7.636	0.76	7.97	0.374	0.071	0.251	134.2	0.363	0.181
Aug 26	2.083	20.26	7.688	1.00	8.02	0.377	0.072	0.125	154.8	0.361	0.184

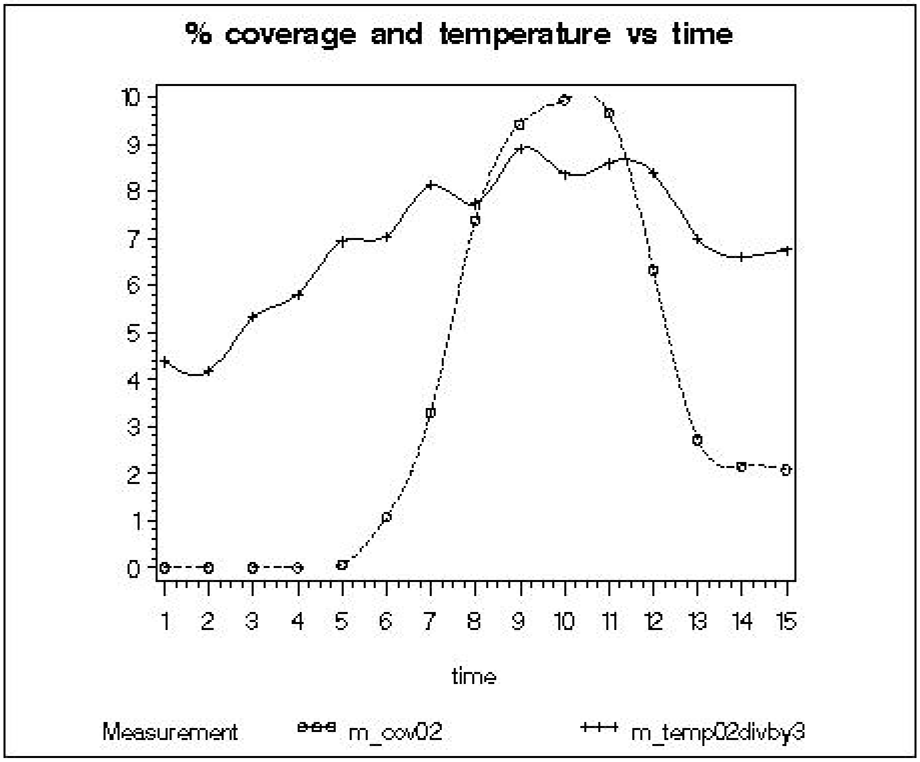
Three analyses of this data set are discussed. The first analysis involves applying a linear regression analysis that does not include either interactions between or time lags for the environmental variables on the environmental data set. The second and third analyses involve looking into, respectively, interactions between and time lags for the main environmental variables. The logit transformation is applied to the % plate coverage measurements because this improves the linear regression (Example 4.6.1, pages 128-135, McCullagh and Nelder, 2nd Edition, 1989).

Linear regression analysis of the environmental data set, without interactions or time lagged variables

All ten environmental predictor variables are more likely than a model with only a few of these variables to fit the data better, to better describe the growth rate of *Fredericella Indica*. However, a model with a lot of variables is more complicated, harder to interpret, than a model with a few variables. The main goal in the analysis, then, is to select the smallest set of environmental variables that describes the growth rate of *Fredericella Indica* as best as is possible. It is discovered that two environmental variables, temperature and nitrate levels, are chosen on the basis of this analysis as the best environmental variables to describe the growth rate of *Fredericella Indica*.

The linear regression relationship between the growth rate of *Fredericella Indica* and the two environmental variables, temperature and nitrate levels, is given by: % coverage by *Fredericella Indica* = a temperature + b nitrate + c, where a and b are parameters associated with each environmental variable and c is the regression intercept. The correlation of this model is $r^2 = 0.8702$. The plot in Figure 3 below demonstrates that the % plate coverage by *Fredericella Indica* is directly related to the environmental variable, temperature: the % plate coverage increases (or decreases) when the temperature increases (or decreases). The scale of the temperature measurement is altered (divided by 3) so that both % plate coverage and temperature measurements can be plotted with one another on the same graph.

Figure3: mean percentage plate coverage and temperature



Correlation analysis of the regression, % coverage by *Fredericella Indica* = a temperature + b nitrate + c, demonstrate that, of the two environmental variables, temperature is the most important influence on the % plate coverage by *Fredericella Indica*. In particular, the % coverage variable is found to be significantly (p-value < 0.01) correlated with temperature, but not correlated with nitrate, although the variables temperature and nitrate are uncorrelated with one another.

Residual plots, not shown here, indicate the assumptions necessary to use this regression model to fit the data are a little worrying. In particular, similar to the ANOVA analysis, there is a slight upward trend in the standardized residuals versus observed logit coverage plot indicating a variable is missing or that a transformation is required in the model. However, the linear normal probability plot of the residuals does indicate the error is normal, as it should be, to allow the regression model to properly fit the data.

Analysis of the environmental data set, with interactions

The environmental data set is analyzed using a regression model where the % coverage is regressed on not only the three main environmental variables, temperature, nitrate and TDS, but also the three pairwise interactions: % coverage = a temperature + b nitrate + c TDS + d (temperature x nitrate) + e (temperature x TDS) + f (nitrate x TDS) + h. On the one hand, similar to the previous ANOVA analysis, the three pairwise interactions are investigated here to determine if the three main predictors act together in a non-additive way on the % plate coverage by *Fredericella Indica*. On the other hand, whereas the pairwise interactions proved to be of significant interest in the ANOVA analysis, here in the regression analysis, they do not. Although the correlation for the linear regression model with interactions, $r^2 = 0.9495$, is a slight improvement over the model without interactions, $r^2 = 0.9061$, all of the t-statistics for the tests of whether *any* of the parameters associated with the main predictors and interactions are zero are *in*significant, indicating $a = b = c = d = e = f = h = 0$. That is, none of the environmental variables significantly influence % coverage by *Fredericella Indica* according to this t-statistic diagnostic test. As a consequence of this analysis, it was clear that the % plate coverage by *Fredericella Indica* is best described using the main environmental variables, temperature and nitrate alone, without any pairwise interactions.

Analysis of the environmental data set, with time lags

Using the Schwarz's Bayesian criterion (SBC) criterion as a measure of how well a time series model fits the data, one particularly good fitting model, with $SBC = -24.08$, is of the form: % coverage (at time t) = a temperature (at time t) + b phosphate (at times t-1 and t-2) + c nitrate (at times t, t-5 and t-6) + d. In other words, temperature and nitrate influence the bryozoan growth rate immediately, phosphate influences the bryozoan growth rate after a time lag of one and two weeks and nitrate influences the bryozoan growth rate after a time lag of five and six weeks. This finding indicates that a change in phosphate, for example, indirectly, rather than directly, influences bryozoan growth rate. A chain reaction of intermediate environmental variables must occur before the initial change in phosphate finally influences the *Fredericella Indica* growth rate itself. These statistical results are corroborated by mean treatment plots for all environmental variables similar to the plot given in Figure 3 above.

The assumptions necessary to use the various time series models used to fit the data here are satisfied. The residual autocorrelations and crosscorrelations are all statistical insignificant (p-value < 0.05), indicating the time series are, as they should be, stationary. Also, the t-statistics associated with the environmental parameters in the various models are significant indicating that these parameters are, in fact, significantly influencing the bryozoan growth rate.

Summary

Of the ten environmental variables investigated in this study, three influence the *Fredericella Indica* growth rate more than the others: temperature, nitrate, and phosphate. The temperature environmental variable acts on this bryozoan's growth rate immediately, the phosphate environmental variable acts on this bryozoan's growth rate after a delay of a week of more and the nitrate environmental variable act both immediately and after a delay of a few weeks or more.

Combined Physical-Environmental data set

When combined, the physical variables, depth, post (length) and date, dominate the environmental variables: water temperature, pH level, secchi reading, nitrate level, phosphate level, hardness, ammonia, oxygen, total dissolved solids and water conductivity. That is, the physical factors appear to be more important than the environmental variables, in influencing the *Fredericella Indica* growth rate. However, this finding might well be a consequence of the manner in which the two data sets were combined, rather than physical factors actually being more influential than the environmental variables when describing bryozoan growth rate.

The combined physical-environmental data set is analyzed using a regression model where the % coverage is regressed on both the physical factors, depth, post (length) and date, and also the environmental variables: water temperature, pH level, secchi reading, nitrate level, phosphate level, hardness, ammonia, oxygen, total dissolved solids and water conductivity. There is no one best way to combine these two different data sets. However, it is decided to assign every % of plate coverage measurement by *Fredericella Indica* to every environmental variable observation on every date of the study. Consequently, there is a total of 2 depths x 12 posts x 5 environmental test sites x 15 dates = 1800 data points. Based on different linear regression selection procedures, the three physical variables, depth, post and date are consistently chosen from the entire combined collection of physical factors and environmental variables, as the best predictors to describe the *Fredericella Indica* growth rate.

Statistical analysis of 1997 data set and comparison to 2002 study

In the summer of 1997, a similar but smaller precursor study to the one conducted in 2002 was undertaken to again identify how each of the physical and environmental variables influenced the bryozoan growth rate. In spite of the many differences, an attempt is made to compare the 48 useable points in the 1997 data set with the comparable 1440 useable points in the 2002 data set. The main results are given below.

1. Although the % plate coverage peaked at about the same time in each season, the % plate coverage in 1997 was much greater, peaking at 100% coverage, than the % plate coverage in 2002, peaking at around 20%.
2. A greater proportion of each plate was covered at two meters by *Fredericella Indica*, than at one meter in 1997, which is essentially the reverse of the results of the 2002 data analysis.
3. In 1997, an analysis of the physical data set revealed all main factors, depth, post and date, were significant, but all interaction factors including depth *x* post, depth *x* date, post *x* date and depth *x* post *x* date were either non-significant or only mildly significant. In 2002, recall, all effects, main and interaction, are significant, although, as explained above, the interactions do not change the results concerning the main factor.

4. An analysis of the environmental data set revealed that the important environmental variables included temperature, secchi, oxygen and ammonia in 1997, as compared to, recall, the variables temperature, nitrate and phosphate in 2002. It is interesting to note that the one variable common to both analyses is the variable temperature.
5. A second type of bryozoan, *Lophopodella carteri*, appeared on the plates used in the 1997 study that did not appear in the 2002 study.

On the one hand, there are a number of similarities between the 1997 and 2002 studies. The data collection procedure for the 1997 study proceeded pretty much in the same way as the data collection procedure for the 2002 study. In 1997, like in 2002, metal (not plastic) plates are attached to various dock posts in the Michigan City harbor. In both studies, pictures are taken of these plates on a weekly basis and later analyzed to determine the proportion of plate coverage by F. indica. Also in both studies, a number of environmental measurements are taken on a weekly basis.

On the other hand, there are a number of big differences between the 1997 and 2002 studies that, really, make them incomparable. The main big difference between the 1997 and 2002 studies is related to the dock posts: the posts used in the 1997 study are not the same posts used in the 2002 study. All nine (9) posts used in the 1997 study are from one dock, the 300 dock---the same 300 dock that had posts C and D in the 2002 study. In other words, all of the posts in the 1997 study are "close to" posts C and D in the 2002 study. The 300 dock was replaced by a new 300 dock after 1997 and, in particular, the wooden posts were replaced by metal posts. In other words, even though all of the posts in the 1997 study are close to posts C and D in the 2002 study, the posts are made of different material in the two different years. Furthermore, at the same time the 300 dock was replaced, the lake bottom sediment associated with this dock was dredged. This dock was the last dock replaced in the harbor. The other docks had been replaced and the associated lake bottom dredged in the harbor in previous years.

SUMMARY OF RESULTS AND DISCUSSION

The date of measurement, post location, length of post, depth of plates, as well lake temperature, nitrate level and phosphate level are the most important factors that affect the settlement and growth rate of *Fredericella Indica*. After reviewing the results of the study, a discussion is provided which explores the implications of these results. The discussion centers on the significance of the depth of the plates and the temperature of the water on the settlement and growth of *Fredericella Indica*.

Review of study results

The *Fredericella Indica* growth rate is different throughout the season, involving a bloom and die-out period. There is greater growth rate at 1 meter below the lake surface, than at 2 meters below the lake surface. The growth rate is different at the different post locations. Significant interactions between these three main factors could be due to boat traffic in the harbor.

Of the ten environmental variables investigated in this study, three influence the *Fredericella Indica* growth rate more than the others: temperature, nitrate, and phosphate. The temperature environmental variable acts on this bryozoan's growth rate immediately, the phosphate environmental variable acts on this bryozoan's growth rate after a delay of a week of more and the nitrate environmental variable act both immediately and after a delay of a few weeks or more.

When combined, the physical variables (depth, post and date) dominate the environmental variables (water temperature, pH level, secchi reading, nitrate level, phosphate level, hardness, ammonia, oxygen, total dissolved solids and water conductivity). That is, the physical variables are more important than the environmental variables in describing the growth rate of *Fredericella Indica*. However, this might well be a consequence of the manner in which the two data sets were combined, rather than physical factors actually being more influential than the environmental variables when describing bryozoan growth rate.

In spite of the difficulty in comparing the small 1997 study with the large 2002 study, it was discovered that the environmental variable temperature was significant in both the 1997 and 2002 studies.

Implication of study results: depth

An environmental variable that affected % plate coverage by *Fredericella Indica* was depth. Coverage by this species of bryozoan was significantly less at two meters compared to coverage at one meter. These results suggest that, although there was equal space on the settlement plates at the two depths, settlement and growth were inhibited at two meters. It is not known why this phenomenon occurred. One possibility is that temperature differences between the two depths led to differential settlement and growth. However, water temperature profiles within the harbor during previous years revealed that the water temperature only varied by approximately one degree Celsius between the surface and the first four to five meters of depth within the harbor basin. Additionally, normal lake stratification in southern Lake Michigan was unlikely to play a role because primary thermoclines are usually observed at approximately five meters or deeper during the summer months well below the depth of the sample plates (Horne and Goldman 1994, Barnes personal observations).

Bryozoan settlement (percent coverage) at two meters was reduced whereas the length of time the colonies remained on the plates after settlement was not. This suggested that larval recruitment was inhibited by factors other than temperature. For example, interspecific competitive interactions, such as interference by other sessile benthic organisms (Vance 1984), might have inhibited bryozoan settlement relative to water depth. Although this study did not investigate the settlement patterns of bryozoans beyond the two-meter depth, numerous studies have shown colonization by the exotic freshwater mussel, Dreissena polymorpha (zebra mussels), on hard substrata increases with water depth (Smit et al.1993, Stanczykowska and Lewandowski 1993, Lauer 1997, Barnes unpublished). Therefore, since both zebra mussels and bryozoans are filter-feeding organisms requiring hard substrata to colonize and both F. indica and zebra mussels inhabit the harbor habitat in close proximity, a form of exploitation competition, often observed in marine benthic communities, for suspended food particles, may have occurred between these species (Paine 1971, Buss 1979, Nandakumar and Tanaka 1997, Osman and Whitlatch 1998, Barnes and Dick 2000, Sepkoski et al. 2000). Moreover, zebra mussels may be inadvertently feeding on the planktonic larvae of F. indica (predation) thus causing a reduction in the abundance below two meters (Lauer et al. 1999).

A third possible reason for the reduction in settlement relative to depth observed in this study is that larvae of this species of bryozoan could be photophilic, similar to some marine bryozoan species (Cancino et al. 1991), and negatively respond to the reduced levels of light that are common with increased depth (Nybakken 2001).

Implication of study results: temperature

Water temperature showed the single greatest influence on bryozoan colonization serving as a cue to initiate larval settlement of *Fredericella Indica*. When the surrounding water temperature rose above 20°C, colonization and growth of F. indica increased. The distinct occurrence of settlement on all the plates at the same time by *Fredericella Indica* suggested that numerous planktonic larvae of this species were present in the water column. This supports the hypothesis that spawning of *Fredericella Indica* may also be temperature dependent. Other freshwater organisms, such as zebra mussels have used this same reproductive strategy in order to increase the survival of offspring (Garton and Haag 1993, Neumann et al. 1993, Sprung 1993). These findings are also evident in marine environments where temperature has played an important role in regulating spawning and larval recruitment of benthic invertebrates, including bryozoans (Gaines and Roughgarden 1985, Brown and Swearington 1998, Rittschof et al. 1998, Fairfull and Harriott 1999, Okland and Okland 2000). However they do contradict findings from a recent study in Norway where F. indica was absent in lake waters 19°C or above (Okland and Okland 2001). This environmental difference/preference could be indicative of ecotypic variation within the genome of this species (Futuyma 1998).

Summary

The findings of this study provide the first record of how various environmental variables impact the colonization and growth of the freshwater bryozoan, *Fredericella Indica*. This study has also shown that freshwater benthic communities may be as dynamic as those found in the marine environment, showing distinct changes and interactions. Although the results of this study raise additional questions about the ecological interactions occurring within freshwater habitats these questions will be better answered by future investigations into several areas including continuing measurements of successional change (multiple seasons), competitive interactions, planktonic larvae and recruitment, and habitat and water quality indicators (Index of Biotic Integrity: I.B.I.; Banta and Backus 1991) to name a few.

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