Studies at the Great Wicomico River, VA

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Introduction

This is a summary report of studies undertaken at the Great Wicomico River, VA, a small tributary located on the northwestern shore of the Chesapeake Bay. Two floways were studied with different experiments over a period of about 1.5 years. On Floway 1, tests were carried out on algal growth and dynamics relative to different substrate types with an emphasis on comparing the traditional two-dimensional (2-D) design to a new three-dimensional (3-D) design. On Floway 2, two experiments were undertaken utilizing the 3-D screen: one on CO₂ additions to test its role as a limiting factor on algal growth and the other on a lipid trigger test. Nutrient analyses on the inflowing and outflowing waters were carried out on both systems and are reported here. Furthermore, the species composition of the algal communities and their dynamics are described herein.

Substrate Project, Floway #1

Prior to this study, ATS systems operating on canals, small streams and aquaculture and tertiary treatment applications, were dominated by filamentous green algae. Diatoms, of many species and genera, were important components, but not the over-riding dominants. In those cases, the principal diatoms were often epiphytic on the green filaments. Visually this is seen as a “browning” of the growing green biomass as it nears harvest, and the diatom filaments fill in the upper structure of the algal “forest.” Early in this investigation, it had become clear that on ATS systems employed on the larger freshwater rivers and estuarine waters of the Chesapeake Watershed, a diatom community, dominated by species of the filamentous genera Melosira, Berkeleya, Diatoma and Fragilaria, would self-establish on ATS floways. Green algal species were always present, in small quantities, and typically showed a spike in abundance in the spring, but on a yearly basis would provide only a small part of total biomass and nutrient removal.

The traditional substrate in the ATS system is a flat plastic screen. Many varieties of plastic screen have been employed, although a HDPE screen of 3x5 mm mesh is typical, a wide range of mesh size has been used. On the scale of the ATS floway and the enhanced algal community, these screens are 2-dimensional structures. The dominant diatom communities that occurred on river ATS systems quickly attach to these “standard” screens, but their filaments constantly “shear-off” in the moderate energy environment of an ATS, producing a lower standing crop and ultimately lower water remediation capabilities and by-product biomass. Diatom filaments have
an entirely different structure from that of typical green algal filaments. In the latter case, the
often massive cellulosic wall is continuous from cell to cell, usually with no break. Individual
cells can die without compromising the integrity of the green filament, at least in the short term.

Diatoms, on the other hand, are basically cellular in construction, and have an entirely different
kind of cell wall. The young, naked, diatom cell develops vesicles in its plasmollemma
membrane, which in turn secrete amorphous silica within a matrix of protein, polysaccharide and
lipid. When the rather complex silica frustule of typically four parts, two valves and two thin
encircling girdles (holding the valves together) is completed, the entire complex is encased in a
matrix of polysaccharide (sulphonated glucoromannan). The silica unit is the frustule; it is long-
lasting, and after the death of the cell, it can become fossilized, sometimes as extensive deposits
(diatomaceous earth). In filamentous diatoms, the frustules, and their individual cells are “glued”
together by polysaccharides, often at spines, or held together in a polysaccharide matrix that can
allow cell to cell sliding. Diatom unicells are often mobile, and can slide with a conveyor belt
like movement of the matrix along grooves in the frustules. In some genera, such as Berkeleya,
the large, “parenchymatous filament” is an extension of the polysaccharide sheath, in which the
individual cells are organized. Diatom cells can quickly attach to a substrate with the
polysaccharide “glue” of their wall, and that is why they are generally the first colonizers of new
surfaces.

Unlike green algal filaments (or red or brown algal filaments in sea water), diatom filaments are
basically fragile and subject to breakage in the energy-rich ATS environment. This experiment
was established primarily to determine how to prevent this loss, and principally involved
examining the efficacy of 3-dimensional screens/fabrics as support structures. A wide variety of
off-the-shelf, deep pile throw rugs, with 1-2 cm thick loose fibers, were tested along with special,
more open variants produced by the carpet company Interface. Open plastic fabrics used for soil
retention were also examined. Many provided an improvement in diatom retention over the 2-D
screens, but unfortunately were easily degraded by the solar UV. Some provided a significant
improvement in algal production, but facilitated the attachment of invertebrates which eventually
made harvest and processing difficult. However, several of our test screens were hand woven,
specifically with the purpose of structuring a growth environment with the limitations of diatoms
in mind. Braided Dacron fibers, 2 cm long were attached to a 5mm mesh basal screen. The
Dacron was employed because it would provide for minimal degradation under solar UV. The
braided fibers were used to provide maximum attachment surface for the diatoms. Two types,
one with a coarser braid (#14 on Figure 3) and the other with a fine, “hairy” surface (#17 on
Figure 3) were used. These were established in the central part of the ATS test floway. Standard
2-dimensional (2-D) ATS screens were arrayed both above and below for comparison. This
study was carried out on Floway #1 on the Great Wicomico River (Figure 1); the results are
reported herein.
Figure 1: Floway #1 (left) and Floway #2 (right) on the Great Wicomico River in the Central Chesapeake Bay. This is a mesohaline river, with salinities at about 12-15 ppt. Two separate, submersible pumps, established on the outer part of the flowways provide a flow of about 20 gpm to each. The pumps are on separate electrical circuits, with an automatic backup electrical generator. During the term of operation of these two flowways, no interruption of water flow has occurred, except during short term, non-drying harvests and maintenance.

Twenty-two months of operation of Floway #1 are shown in Figure 2. Samples were generally taken every seven days in the summer and 14 days in the winter. However, there was a small amount of variation due to weather, and during the spring and fall, when switch-over from one time-mode to the other occurred. Although the expected yearly cycle of biomass production is shown, with summer production about 5 times that of mid-winter production on both types of growth substrate, the 3-D screens show a consistently greater level of production that is highly significant. Largely following temperature and light, the 3-D screens produce at a level of about 2.5 X that of the 2-D screens. The yearly mean for 3-D screens was 36.9 g/m²/day, and that for the 2-D screens is 15.0 g/m²/day.
Separate graphs for the two different 3-D screens are shown in Figure 3. Production on the two screens follows each other closely, but the screen with the finely-braided and somewhat hairy surface tends to produce biomass at 15-20% higher than the screen with the coarser fibers. The difference is relatively small, and although the two screens are only one meter apart on the floway, some local environmental factor might be responsible. Certainly, the subject of the fine character of the 3-D fibers needs to be further investigated, as major improvements in production seem likely with this parameter.
Productivity of the two 2-D screens is shown in Figure 4. These screen sample areas were placed at about the 10% and 80% positions on the floway (most of the background on the floway without test screens was the standard 2-D screen that was used for testing). Again, in their productivity, these screens follow each other closely, likely mutually following changes in ambient light and temperature. The upstream screen shows a slightly higher mean productivity that could be the result of slight, downstream nutrient depletion, as seen on some floways; however, this difference is not significant in this case. Clearly, all four test screens are following the same environmental parameters, with only occasional anomalies; except for a single anomalous and slight overlap out of 67 samplings, the 3-D screens range from 2-3 times that of the 2-D screens.

![Figure 4: Biomass productivity on Floway 1, comparing 2-D screens placed at about the 10% and 80% positions on the floway.](image)

The harvest rates during the summer of 2010 showed some strong dips that are not characteristic of ATS floways. This happened when the PI (Adey) was incommunicado in the field in Labrador and technicians were running the system. In late August, when the PI for this project learned of these large dips, the harvest mode was shifted from the standard late afternoon harvest to an early morning harvest, and no further biomass production dips occurred. Floway temperatures are shown in Figure 5. At the first dip, the incoming river water temperature was about 27 C. When the dips were finally aborted, the temperature was about 29 C, falling from a peak of nearly 34 C. Approximately 1/1/2 hours is required to harvest this floway, when test screen sampling is being carried out, followed by a general floway harvest. A large part of the water on the floway is allowed to drain in this process to facilitate separation of the algal biomass from the entrained water. On very hot, sunny afternoons, the algae and remaining water, likely exceeds 50 C during the later stages of the harvest. These temperatures would be fatal to the remaining algal seed that is so important to biomass recovery after harvest, and are very likely responsible for the strong dips in biomass production during the summer of 2010.
As can be seen in Figure 2, biomass harvests in early June, 2011 on these 3-D screens had exceeded 90 g/m²/day; the river temperature at that time was 27°C. Several more samples will be taken to assure that these levels of production will be continued; however, by mid June, with no reduction of production apparent, and with water temperatures approaching 30°C, significant dips had not occurred. This season, all harvests have taken place in the early morning, so that sun warming of a drying floway is not a significant problem. If this hypothesis is correct, the round-topped dashed curve, shown in Figure 6 is the more likely form of the production curve. In this case, the yearly mean production on 3-D substrates is probably in the mid 40’s g/m²/day. This would provide a significant increase in the efficiency of both nutrient removal and algal biomass production in ATS systems employed on non-point-source waters. It seems likely that regardless of substrate type, day-time harvest ATS floways during the summer has been a significant factor in both biomass production variation and the reduction of yearly mean production.

Figure 5: Biomass productivity on Floway 1, comparing 2-D screens placed at about the 10% and 80% positions on the floway.
Figure 6: Hypothesized production during summer, with early morning harvest to avoid lethal heating of residual floway algae during harvest. In this mode of operation, it is estimated that yearly mean biomass production at this location will be about 45 g/m²/day.

**CO2 Project, Floway #2**

During the summer of 2010, a second ATS floway was constructed adjacent to the Floway #1 on the Great Wicomico River. This was done, in part to examine the potential role of CO2 in enhancing ATS algal production and to investigate the possibility of stack gas amelioration on large scale ATS systems. Floway #2 was similar to #1 in most respects; however, it was 80 feet long and had a slope of 2% (Floway #1 was 50 feet long with a slope of 1%) (Figure 1).

The primary difference between the two floways was the addition of 3-D substrate on the entire length of the floway (Figure 7). While, as described earlier, special hand-woven 3-D substrates were more than 2X as productive as the earlier used flat screens, the only available 3-D substrate at the time of construction was the Interface #2 type. Fortunately, this type was under test on Floway #1, so we understood its characteristics and its relationship to the optimal 3-D substrates.
Figure 7: Close-up of Interface screen showing the coarse, twisted fibers. There is a continuous, impervious plastic backing holding the fibers in place.

Coarser than the optimal 3-D substrates (#’s 14 and 17 on Figure 3) on Floway # 1, where it “produced” algal biomass at 79% of the optimal screens (for the same ten month period), the 3-D substrate used on Floway # 2 also had a non-porous basal mat. This allowed attachment of mussels and was labor intensive, requiring removal of young mussels after harvest. Nevertheless, the entire length of this 3-D substrate on Floway # 2 (78 feet) “produced” algal biomass at 74% of the mean of the optimal screens on Floway #1. This is an extremely important finding, as it demonstrates that the greatly increased production rates of the optimal 3-D screens are not a sampling artifact and likely will apply to very large ATS systems. Unfortunately, Floway # 2 was not fully operational until mid summer 2010, and lack of research funding required a shut down before the summer of 2011. Thus, a full data set is not available for the highly productive summer interval.

Floway #2 had two basal cross channels, one at 50% of the floway length and the other at 75%. The purpose of the channels was to allow the introduction of CO\textsubscript{2} with hollow fiber semipermeable membranes. In the third segment of the floway, some of the non-porous Interface substrate was removed and replaced with the standard flat screen so that hollow fibers could be run part way down the floway. The initial unit was installed by Bob Beitle of the University of Arkansas in the summer of 2010, and introduced 100 % CO\textsubscript{2} through manually-operated pressure regulators (Figure 8).

Figure 8: Hollow fiber membranes underlying 2-D screen. For the first experiment, these fibers were not only laid in the basal channel, they extended down a portion of the third quarter of the floway. In this segment, because the Interface 3-D screen had an impervious base (unlike the optimal 3-D screens of Floway #1), the 3-D screen was removed and replaced with open 2-D screen on part of that segment.

Once in operation, it was immediately apparent that introduction of CO\textsubscript{2} by this means was quite effective. However, using 100% CO\textsubscript{2}, with only manual valve controls, produced highly variable pH levels on the lower lengths of the floway (Figure 9). Also, a significant amount of CO\textsubscript{2} gas
was wasted, a single tank only lasting 3-4 days (in later experiments with pH controlled CO₂ injection, one of these same tanks would last several weeks). Often, this arrangement produced sharp drops in pH levels, well below that in the ambient waters. After several months of operation, the result was a sharp shift in algal community structure (Figure 10). The typical, highly productive filamentous diatoms, usually dominant on these flowways, shifted to a dominantly cyanobacterial (blue-green algal) community of less productivity. Also, by autumn, it was apparent that the hollow fibers were degrading, resulting in considerably more CO₂ use, mostly lost to the atmosphere, as shown by large bubble release. In November, the CO₂ supply was shifted to a 12% mixture, in an attempt to gain more control over pH. Although this reduced the pH drop, and allowed the community to shift back to one dominated by diatoms, even the larger CO₂ bottles would last only about a week due to the large loss to the atmosphere.

Figure 9: pH down Floway # 2, under manual operation, during the first few months of operation. Note that CO₂ was injected at 40-50 feet and again at 60 feet (75%). In the fourth quarter of the floway, after injection ceased, the pH began to recover rapidly due to algal photosynthesis. The rise rates are greater, under the same solar regime than on the upper part of the floway, where less carbon was available.
Figure 10. Algal communities on Floway # 2 at the 25% position (left) and the 85% position (right). No CO2 injection in the upper images; full CO2 injection in the lower-right image. Diatoms dominate in all images except the lower right, under low pH regime. Here, Blue-green algae (Cyanobacteria) have come to dominate the community.

As shown in Figure 11, during the entire period of operation, from mid-summer to December, production levels dropped progressively down the floway. CO2 injection appeared to reduce the drop, especially after the switch to 12% CO2 with the subsequent removal of extremely low pH levels. However, low winter levels of algal production in general prevented a conclusive assessment of the effects of CO2 injection. In December, it was decided to shut down the CO2 introduction experiment for the winter to concentrate on PUFA studies, while developing an automatic CO2 control system to prevent excessive quantities of CO2 from reducing pH below optimal levels for algal growth and biomass production. Also, since the hollow fibers were clearly not robust enough for an environmentally-exposed, production system, requiring weekly harvest, protection of the fibers or another means of injection was required. As can be seen on Figure 12, for the early segment of operation, even on average, CO2 injection (pH drop) exceeded that removed by the algae, and ideally, should have returned the pH to the level of River water (8.2-8.3) where it would exactly replace the CO2 taken out by the algal biomass.
Figure 11: Algal biomass production at positions down Floway # 2, as a function of CO2 introduction regime. Note the black bars represent winter runs without CO2 injection.

Figure 12: Mean Change of pH down Floway # 2 during the first phase experiment. Readings were taken from 0900 to 1500 hours weekly to bi-weekly. Note that when CO2 was injected during this period, discharge pH was lower than river/incoming pH; thus, on average it was in excess of the algal demands for CO2. When CO2 was not injected, daytime algal removal of CO2 constantly raised pH down the floway.

In the early spring of 2011, Bob Beitle delivered an entire pH/CO2 control system, consisting of a pH probe and control unit that allowed a set point for pH to control a mechanical valve on the CO2 delivery system (Figure 13). Also, because it seemed likely that broader spectrum nutrient depletion was in part responsible for the production decline down the floway, we concluded that a full stack gas, rather than pure CO2 would be more likely to reduce the decline. Unfortunately, none of the project members felt that a permit for using stack gas in this environment was likely
on the time scale of the project, nor was the University of Arkansas able to obtain the replacement hollow fibers for the injection system within the few months requirement.

Figure 13: Stainless steel injectors at the 50% injection trough. Note the short tube exiting the neoprene insulator just to the left of the CO2 tubes; this is the drip point for the nutrient solution. The 75% injection point is the same, although without nutrient injection.

In March, 2011, we were able to obtain stainless steel injectors used for delivering CO2 to carbonated beverages (Figure 13). With the pH controller, this allowed an optimum level of control of CO2 injection (Figure 14). At the same time, we installed a variable speed peristaltic/tubing pump to deliver a narrow spectrum of low level liquid nutrients to simulate a stack gas. The latter was installed under the floway at the 50% point; drip injection occurred at the same point as the 50% CO2 injectors (Figure 13). No nutrient injection was applied at the 75% CO2 injection point. This entire system was operational by early April, 2011.
Figure 14: Typical pH cycling intervals at the ends of the normal daytime spectrum of algal biomass and solar energy. The reference curve was taken with a separate pH meter that was calibrated before each run. This is the correct pH; thus the actual operating set point was 8.2 to 8.3, close to the average, daytime incoming river water. Ideally, this provides the proper level of CO2 injection, with all CO2 that enters the water column being taken up by the algae.

The carbonated beverage injectors (Figure 13) only lasted about one month in the corrosive salt water environment. The mixture of salt water in an alternatively highly acidic environment, as the CO2 cycled on and off and the sea water entered the injector chambers, proved too much for the stainless steel units. Initially, they became blocked. This was easily overcome by raising the pressure slightly, but after a few adjustments, the injectors started blowing out, destroying the fine bubble capability and dropping the pressure on the still working units. They were not expensive units and could have been easily replaced, but time was not available to do so. The entire Floway # 2 operation was shut down about June 1, 2011.
Figure 15: Biomass production, upstream vs downstream on Floway # 2 during the entire period of operation. Note that once algal production was fully developed (mid-summer 2010), at no time did biomass production downstream exceed that upstream until controlled CO$_2$ introduction and partial nutrient replacement was applied. As discussed in the text, the pre 75% position biomass production, having full available nutrients and 50% CO$_2$ introduction remained below that of downstream production.

Nevertheless, after eight months of operation, with the lower one quarter of the floway consistently producing at lower levels than the upper one half (37% lower), algal production on the lower quarter reversed for the period of successful CO$_2$ introduction with pH control, and produced at a level 24 % higher than the upper half (Figure 15). The rate of nutrient injection was approximately that of removal. In addition at the same interval, the pre-75% sample (which has the full effect of the nutrients that were available to the lower quarter of the floway, but a higher pH on average – ie., less available CO$_2$) produced less than either the upper or lower segments of the floway.

When both effective and controlled CO$_2$ and low level nutrient injection were applied consistently, the algae on the lower floway increased their production by 61%. This increase is primarily due to the CO$_2$ injection as we have shown. As we have also demonstrated, CO$_2$ control is easily attained that would allow simple replacement of the CO$_2$ removed by the algae during their photosynthetic processes (ie, CO$_2$ release to the atmosphere can be easily avoided). While the period of success was too short for sweeping conclusions, this experiment strongly suggests that a broad spectrum stack gas could be effectively ameliorated and ATS algal production increased (with concomitant increase of nutrient removal and by-product production). Also, it demonstrated that ATS systems being used in precipitation processes and dependent on set-point pH (phosphorus and heavy metal removal from non-point-source waters) can be easily controlled with CO$_2$ injection to optimize that removal.

**Nutrient Analyses**
Basic nutrient analyses for floways on the Great Wicomico River are shown in Table 1. The Great Wicomico River has only moderate nutrient levels during both Fall and Spring, and these levels are not likely limiting for the production levels seen on the 3-D screens of Floway # 1. Floway #1 actually shows a slight increase in TN in the spring, incoming over effluent, although not in the Fall; effluent values are all below river levels. Floway # 1 is only a 16m long floway, the majority of which was 2-D screen. In contrast, Nitrogen shows a significant drop down Floway #2 when it is operated without nutrient injection. Since this floway has a version of 3-D screen, and is 24m long, the average total production levels are approximately 4-5 X those on Floway #1.

As described in the text, in the final phase of the CO₂ experiment, nitrogen injection (at the 50% point on the floway), at approximately 0.03 mg/l DN, is close to the average Spring total drop (0.0202 mg/l) on the whole floway. A single sample taken at the beginning of the lower quarter of the floway at the same time as the general 5/16/11 nutrient samples, showed less DN than either incoming or outgoing samples. The intent of the injection was to replace nutrients lost on the upper three quarters of the floway, so that the only growth controlling factor was CO₂.

Table 1. Basic nutrient analyses for Floways on the Great Wicomico River

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<th>Mean Nitrogen (mg/l) (# samples)</th>
<th>Floway #1</th>
<th>Floway #2</th>
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Algal species analyses on Floway 1 and Floway 2

Floway 1

A total of 86 algal taxa over X sample dates, belonging to seven different phyla (Bacillariophyta, Chlorophyta, Cyanobacteria, Rhodophyta, Dinophyta, and Chrysophyta) were found on Floway 1. The most diverse phylum was Bacillariophyta (diatoms) with 77% of total taxa (66 of 86 taxa) found coming from this group, while also being the most abundant algal group on the system during the study period (53% of total relative abundance) (Figure 17). Chlorophyta (4 taxa) and Cyanobacteria (11 taxa) were the following most diverse and abundant groups, with 24% and 22%, of total relative abundance, respectively. The other phyla accumulated to roughly 1% of total relative abundance.

The most abundant taxa (followed by abundance values) were Berkeleya spp. (B. fennica, B. fragilis, and B. rutilans) (20%), Gloeothecae sp. (13%), Ulva intestinalis (10%), Ulothrix sp. (9%), Melosira spp. (M. moniliformis and M. nummuloides) (9%), Lyngbya cf. salina (7%) and Achrachetae sp. (7%). The other taxa occurring on the floway accounted for 2% or less of total abundance each.

The most frequent taxa (occurring >84% of samplings) were Achnanthes spp. (e.g. Achnanthes brevipes), Amphora spp., Berkeleya rutilans, Mastogloia spp., Melosira nummuloides, Staurostella sp., Navicula spp., and Grammatophora spp.

The periphyton growing on these engineered systems are dynamic. Diatoms are always present and abundant where different species become dominate during different times of the year. For example, Tabularia tabulata, Nitzschia sigmoidea, and N. sigma are most abundant from July to September, but when it becomes colder these species are not found, other taxa, such as Nitzschia nana, Thalassionema sp., and Thalassiosira sp. are found. However, as a group, chlorophytes tend to increase during late spring and late summer, reaching 70% of total abundance (Figure 18). The cyanobacteria were abundant in fall due to the large amount of two taxa, first, in mid-September, Gloeothecae sp. was dominating the periphyton and afterwards Lyngbya cf. salina was present.

Floway 2

On this floway, 98 algal taxa over X sample dates, belonging to seven different phyla (Bacillariophyta, Chlorophyta, Cyanobacteria, Rhodophyta, Dinophyta, and Chrysophyta) were found on Floway 2. The most diverse phylum was Bacillariophyta (diatoms) with 73% of total

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### Mean Year TP

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Sample dates: (Fall: 10/17; 10/23; 11/20, 2010; Spring: 3/12; 4/24; 5/23, 2011)
taxa (72 of 98 taxa) found coming from this group, while also being the most abundant algal group on the system during the study period (67% of total relative abundance) (Figure 19). Cyanobacteria (11 taxa) and Chlorophyta (5 taxa) were the following most abundant groups, with 24% and 7%, of total relative abundance respectively. The other phyla accumulate to roughly 2% of total relative abundance. Different than seen on Floway 1, there were 7 taxa of dinoflagellates compared to only 2 taxa on floway 1.

The most frequent taxa (occurring >85% of samplings) were *Achnanthes* spp. (eg. *Achnanthes brevipes*), *Amphora* spp., *Berkeleya rutilans*, *Grammatophora* spp., *Licmophora* spp., *Melosira nummuloides*, *Navicula* spp., and *Tabularia tabulata*.

The most abundant taxa were *Berkeleya* spp. (*B. fennica* and *B. rutilans*) (19%), *Lyngbya* cf. *salina* (19%), *Thalassionema* sp. (14%), *Melosira nummuloides* (7%), and *Ulva intestinalis* (6%). The other taxa occurring on the floway accounted for 3% or less of total abundance each.

Figure 20 illustrates how the periphytic community on this system is also dynamic. As in Floway 1, diatoms are always present, though there are changes in community structure occurring throughout the year. Cyanobacteria tend to have an increase from August to November (up to 60%) of total abundance, dominated by *Lyngbya* cf. *salina*. Although present several times during the year, chlorophytes have their highest abundance in the late springtime, reaching up to 40% of the total relative abundance. Both *Ulva intestinalis* and *Ulothrix* are responsible for this abundance.
Figure 17. Total cumulative relative abundance of periphyton growing on Floway 1.
Figure 18. Population dynamics of periphyton on Floway 1 between July 1 2010 and May 16 2011.
Figure 19. Total cumulative relative abundance of periphyton growing on Floway 2.
Figure 20. Population dynamics of periphyton on Floway 2 between July 3 2010 and May 26 2011.