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A NEW IN SITU METHOD FOR MEASURING SESTON UPTAKE BY SUSPENSION-FEEDING BIVALVE MOLLUSCS

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ABSTRACT The most commonly used methods for measuring the amount of seston removed from the water column (uptake) by populations of suspension-feeding bivalve molluscs involve taking discrete water samples followed by laboratory analyses. Here we describe a new method based on in situ fluorometry that provides rapid measurement of seston removal rates. The new system is comprised of two identical units, each consisting of an in situ fluorometer, data logger and peristaltic pump with plastic tube attached to a deployment device. The deployment device allows precise placement of the fluorometer probe and intake end of the plastic tube so that in situ fluorescence (chlorophyll a) can be measured and water can be sampled for seston analyses in the laboratory from the same height. The typical setup involves placing one unit upstream and the other downstream of the study area and sampling the water at periodic intervals. Changes in seston concentration are revealed in the field by the fluorometers, and the sampled water can be analyzed in the laboratory for various seston parameters. Comparisons of the in situ data with data from laboratory analyses of pumped water samples were made for three species at four study sites: the eastern oyster (Crassostrea virginica), hard clam (Mercenaria mercenaria) and blue mussel (Mytilus edulis). Comparisons of measured upstream versus downstream seston concentrations indicated significant (t-tests, P < 0.05) differences (uptake) for six of eight trials based on in situ fluorometry, but only marginally significant (P < 0.10) differences at two of the four trials using laboratory chlorophyll a measurements. These data demonstrate that compared with sampling methods requiring laboratory analyses, the new in situ method provides much more rapid quantitative assessments and may provide more accurate estimates.

KEY WORDS: bivalve, fluorometry, seston uptake, suspension-feeding, Crassostrea, Mercenaria, Mytilus

INTRODUCTION

Empirical and theoretical research demonstrates that populations of suspension-feeding bivalve molluscs can remove substantial amounts of suspended particulates (seston) from the overlying water column by their feeding activities (Ulanowicz & Tuttle 1992, Dame 1996, Newell et al. 2005, Haamer & Rodhe 2000, Cressman et al. 2003, Nelson et al. 2004). This has important implications for shellfish aquaculture and more recently for water quality management associated with shellfish restoration projects (Luckenbach et al. 1999, 2005, Thayer et al. 2005). Field studies on seston uptake typically involve laboratory analysis of discrete water samples obtained manually or by pumps (Dame & Libes 1993, Newell & Shumway 1993, Cressman et al. 2003, Nelson et al. 2004). This approach is effective and it allows the measurement of multiple water parameters. However, there is a need to develop in situ approaches that have the potential to greatly increase the spatial and temporal resolution of measurements of the feeding/seston uptake process.

A major impetus for development of the new method is the need for quantitative success metrics for constructed shellfish (mainly oysters) reefs that are part of ongoing restoration programs in many areas. These projects often emphasize the ecological functions of oysters, instead of or at least in addition to, their historical role as a commercial resource (Luckenbach et al. 1999, 2005, Brumbaugh et al. 2000, Coen & Luckenbach 2000). One of the major ecological functions of oyster reefs is their potential influence on water quality because of their filtration capacity (Dame 1996, Dame et al. 2001, French McCay 2003, Peterson et al. 2003). Field studies, however, typically have not demonstrated measurable seston removal by natural (Dame & Libes 1993, Wilson-Ormond et al. 1997) or restored (Nelson et al. 2004) oyster reefs. Cressman et al. (2003) is the only study we are aware of that measured substantial (up to 25%) removal of seston by small intertidal reefs. Hence, there is a need to critically assess this often-cited reason for restoring shellfish reefs (Coen & Luckenbach 2000, Luckenbach et al. 2005). How much of an impact on water quality should be expected from restored shellfish reefs?

Previous research has used in situ fluorometry to assess food availability to cultured bivalves (Grant & Bacher 1998) and phytoplankton biomass as part of broader ecological studies (Gregor et al. 2005). However, we are not aware of previous attempts to use in situ fluorometry to measure seston uptake by benthic organisms in the field. Here we describe a novel method for directly measuring uptake rates, and the results of field trials involving three bivalve species that compare measurements using the new in situ method with laboratory analysis of pumped water samples.

DESCRIPTION OF IN SITU FLUOROMETRY APPARATUS

Each apparatus consists of a fluorometer (Seapoint Sensors Model SCF,) with multimeter/datalogger (Extech Model 383,274), and peristaltic pump (Masterflex Model 7533) with 1-cm ID plastic tube attached to a custom-made deployment device (Fig. 1A, B, C). The fluorometer probe is placed within a 5-cm ID PVC pipe that is attached to the bottom plate. The probe and light shield are held in position by a hose clamp on the outside of the PVC pipe; loosening of the clamp allows the light shade and probe to be moved up and down on the pipe and secured at any height above the bottom plate. The bottom plate is designed to rest on the bottom so the fluorometer probe remains at the same height.
Figure 1. *New in situ* fluorometer and water sampling apparatus. (A) Complete apparatus (one of two identical units); (B) close up oblique view of fluorometer probe in deployment device; (C) close up face-on view; (D) deployment of two identical units over intertidal oyster reef in Florida.
throughout the measurement period, but the entire apparatus can be suspended at any height and moved up and down in the water column as needed. A tail fin attached to the bottom plate orients the probe so that water flows directly through the sensing chamber as it is lowered to the bottom (Fig. 1C). The bottom plate is constructed of 2-mm thick stainless steel sheet material and provides sufficient weight to hold the apparatus in position under most flow conditions. The fluorometer probe and the end of the water intake tube are placed at the same height so that comparisons can be made between in situ fluorescence (chlorophyll \(a\)) and laboratory analysis of pumped water. Total cost of each unit is about $5,000 (2006 US$).

The typical setup involves placing one apparatus upstream and another downstream of the study population and sampling the water at periodic intervals (Fig. 1D). To be sure the downstream apparatus is exposed to the same water mass as the upstream apparatus, a floating object is released periodically from the upstream position to show the ambient water flow path across the reef. Adjustments in location of either device, including raising the apparatus to allow the tail fin to reorient the probe with respect to water flow direction, can be made as needed. Changes in seston concentration caused by bivalve feeding (“seston uptake”) are revealed immediately by differences in the two fluorometer readings (upstream vs. downstream), and the sampled water can be analyzed for various parameters to verify the fluorometry and provide additional data on changes in seston characteristics.

**MATERIALS AND METHODS**

Populations of bivalve molluscs were studied at sites in New Hampshire (a blue mussel, *Mytilus edulis*, reef), Virginia (hard clam, *Mercenaria mercenaria*, beds at an aquaculture farm), South Carolina (a restored eastern oyster, *Crassostrea virginica*, reef), and mid-Atlantic Florida (several natural eastern oyster reefs). Table 1 summarizes the environmental characteristics of the study sites, and the measured seston uptake rates. Sites were chosen because they met two criteria: (1) water less than ~1.5 m deep and (2) water flow constrained laterally, or width-to-length ratio sufficient to minimize lateral transport across the width of the sampled area. Meeting these criteria would likely result in environmental conditions (e.g., well mixed water column) that would allow sampling at one height to be representative of the entire flow field.

The general protocol for field studies consisted of making repeated measurements of environmental conditions and changes in seston concentration upstream and downstream of each population of bivalves based on in situ fluorometry and laboratory analysis of pumped water samples (Table 1; Fig. 1C). Each of the fluorometer probes and water intake tubes were set at 5–10 cm above the bottom (either the top of the reef for mussels and oysters or the sediment surface for clams). A set of measurements (each consisting of 10–20 fluorometry readings recorded at 10-s intervals) was made at 10-min intervals for the duration of each deployment at each site (except for the South Carolina study, which consisted of single readings recorded at 5–15-min intervals). The peristaltic pumps required about 5 min to obtain each sample. Readings from the two fluorometers also were compared side-by-side at the beginning of each deployment and again after the last set of readings was taken to be sure they gave similar readings.

Pumped water samples were stored in the dark on ice and returned to the laboratory for filtration (Whatman GF/C or Gelman GF/F filters) within 6 h of collection; the filters were further pro-
cessed immediately in most cases, or frozen until processed. The New Hampshire, Florida and Virginia samples were analyzed spectrophotometrically using acetone extraction and standard techniques (APHA 1992). The South Carolina samples were analyzed fluorometrically following a modified EPA Method 445.0 procedure (Arar 1997, Rev. 1.2).

Bivalve densities were determined at most sites by sampling 5 to 10 of 0.16 m² quadrats, counting and measuring shell length or height of all live bivalves collected to the nearest mm using calipers. For the South Carolina oyster reef, data on bivalve size and density available from previous samples taken at that site were used. Flow length was the distance between the two fluorometers. Water depth was measured at 10-min intervals using a marked rod. Water flow speed was measured at the same height as the fluorometer probes were placed (5–10 cm above the bottom) with a Marsh-McBirney Model 201 electromagnetic current meter, with 10–20 replicate speeds recorded at 10-s intervals every 10 min for the duration of the measurement period. Near-surface water speed and flow direction were also estimated by releasing an orange at the upstream site and recording travel time to the downstream site. This provided a measurement of near-surface water flow speed to compare with the near-bottom measurements and insured that the sampling units were placed properly.

Data analysis consisted of comparisons of chlorophyll a concentrations, based on field data (in situ fluorometry) with laboratory analysis of pumped water samples. Data were analyzed graphically (scatterplots), and statistically (t-tests, regression and correlation) using SYSTAT version 10 (2000) software. For the in situ fluorometry data only, the t-tests were done using the means of the mean values for each 10-min observation period.

RESULTS AND DISCUSSION

A total of eight trials of paired upstream versus downstream in situ fluorometry measurements were taken at the four study areas; pumped water samples were taken for laboratory chlorophyll a analysis during four of the eight trials (Table 1). T-tests indicated significant differences between mean upstream and downstream fluorometry readings for six of the eight trials (Fig. 2), but only marginally significant (P < 0.10) differences at two of the four trials for laboratory measurements (Fig. 3). Both approaches, however, showed instances of eston uptake rates exceeding 25%.

A scatterplot of the full dataset comparing each in situ fluorometry reading with its corresponding laboratory chlorophyll measurement showed two distinct groupings (Fig. 4). Overall, this assessment indicated that the two measurement techniques yield comparable data, but their relationship is not simple.

Using In situ Fluorometry to Provide Rapid Measurement of Seston Uptake

As already noted, field studies on eston uptake typically have involved laboratory analysis of discrete water samples obtained manually or by pumps using various sampling protocols (e.g., Dame & Libes 1993, Judge et al. 1993, Newell & Shumway 1993, Cressman et al. 2003, Nelson et al. 2004). We obtained pumped water samples for laboratory analysis as means of “ground truthing” the in situ fluorometry data because in the long-term our goal is to rely as much as possible on the latter. Most applications of in situ fluorometry to date have been in the area of water quality monitoring (e.g., Gregor et al. 2005), in some cases (e.g., Grant & Bacher 1998) related to bivalve aquaculture. Our application essentially represents an ecological extension of the method, but it required some important modifications to off-the-shelf fluorometers. Ambient sunlight can strongly interfere with the sensor, so a large aluminum plate was added to shade the probe (Fig. 1). It was also necessary to construct a deployment apparatus that allowed precise placement of the probe vertically, and it automatically oriented the probe so the predominant water flow was directly through the sensor chamber. These modifications have resulted in an apparatus that is simple to deploy and appears to consistently yield reliable data.

In situ fluorometry could become a fast, effective and nondestructive approach to quantifying the impacts of shellfish populations on eston removal but it needs further development, including more comparisons with laboratory analysis of pumped water samples. One of the issues that will need to be settled for some applications concerns the relationship between the in situ fluorometry data and laboratory-determined chlorophyll a concentrations. Our data from four different areas showed two distinct groups, with substantial disparity between the two in the relationship between fluorescence and chlorophyll a concentrations determined by wet chemistry techniques (Fig. 4). Grant and Bacher (1998) also reported substantial disparities between the two approaches. Gregor et al. (2005) noted that differences between in situ fluorometric methods and laboratory methods should be expected when different analytical techniques (e.g., ethanol vs. acetone extraction) are compared and phytoplankton taxonomic composition varies. For our data, the pumped water samples were a composite taken over about 5 min, as compared with multiple fluorometry readings taken over about 2 min (see Methods section). Hence, although both types of samples were taken at approximately the same time, some of the differences (Fig. 4) could represent actual temporal variability in eston concentrations.

Another potential limitation of in situ fluorometry is that it does not provide data on components of the eston other than phytoplankton that can be important food items for bivalves. However, if chlorophyll a data alone are sufficient then in situ fluorometry represents a much more effective approach compared with analysis of pumped water samples because it provides rapid results, and if fluorometers are deployed at multiple heights in the water column, spatial variations in eston removal that are related to hydrodynamical factors can be assessed. For example, a fully mixed water column is not necessary for estimating uptake rates.

Seston Uptake by Oyster Reefs

As discussed earlier, the need to estimate the potential impact of constructed or restored oyster reefs on water quality has driven the development of the in situ fluorometers. The potential for bivalve shellfish to control phytoplankton populations in coastal areas such as San Francisco Bay was proposed over 20 y ago (Cloern 1982, Officer et al. 1982). Subsequent studies in the Bay documented a variety of ecological effects, including eston depletion, attributable to dense infaunal bivalve populations (e.g., Alpine & Cloern 1992). In a widely cited paper, Newell (1988) hypothesized that the historical depletion of oyster populations in Chesapeake Bay has been a major factor in water quality degradations and other ecological changes in the Bay (also see Heck 1987). Subsequent studies support this notion (Ulanowicz & Tuttle 1992, Newell 2004). Similar water quality impacts in some areas of the Great Lakes by the invasive zebra mussel have also been documented (Budd et al. 2001, Ackerman et al. 2001). Recent research in mesocosms has further characterized the role that sus-
pension-feeding bivalves can play in controlling phytoplankton populations (Cerrato et al. 2004, Porter et al. 2004). It seems reasonable to expect measurable water quality effects from restored oyster reefs, but empirical studies of the effects are needed.

Several field studies involving bivalves such as clams, mussels and other taxa have demonstrated substantial seston uptake and in some cases longer-term water quality changes caused by bivalve feeding and filtration (e.g., Alpine & Cloern 1992, Haamer 1996, Coen et al. 2000, Haamer & Rodhe 2000, Ackerman et al. 2001; see reviews by Dame 1996 and Dame et al. 2001). Field studies on oysters, however, typically have shown no measurable uptake or very little (Dame & Libes 1993, Wilson-Ormond et al. 1997, Nel-

Figure 2. Upstream and downstream in situ fluorometry measurements for all study sites. P values from t-tests; error bars show 1 SD.
son et al. 2004); Cressman et al. (2003) is the only field demonstration of substantial seston removal (up to 25% decreases in chlorophyll $a$) we are aware of for oyster reefs. In the present study, maximum measured seston uptake rates for the three bivalve species ranged from 27.8% for *Mytilus*, 37.4% for *Crassostrea*, to 62.3% for *Mercenaria*. The differences in rates mainly reflected differences in bivalve size and densities relative to water flow and water depth (Table 1). Hence, it seems reasonable to conclude that field studies on oysters should consistently yield quantitative measures of seston depletion if they are properly scaled. In any case, our new *in situ* device would allow rapid assessments for future studies on restored and natural oyster reefs.

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**Figure 3.** Upstream and downstream laboratory measurements of chlorophyll $a$ concentrations from pumped water samples for all study sites. $P$ values from *t*-tests.

**Figure 4.** *In situ* fluorometry compared with laboratory analysis of pumped water samples for all study sites.


