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Characterization of microbial communities in minimal-exchange, intensive aquaculture systems and the effects of suspended solids management

Andrew J. Ray [a,*], Gloria Seaborn [b], John W. Leffler [c], Susan B. Wilde [d], Alisha Lawson [a], Craig L. Browdy [e]

[a] Waddell Mariculture Center, South Carolina Department of Natural Resources, 211 Sawmill Creek Rd., Bluffton, SC 29910, USA
[b] Center for Coastal Environmental Health and Biomolecular Research, NOAA, 219 Fort Johnson Rd., Charleston, SC 29412, USA
[c] Marine Resources Research Institute, South Carolina Department of Natural Resources, 217 Fort Johnson Rd., Charleston, SC 29412, USA
[d] School of Forestry and Natural Resources, The University of Georgia, Athens, GA 30602, USA
[e] Novus International, 5 Tomotley Ct., Charleston, SC 29407, USA

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ABSTRACT

Minimal-exchange, intensive culture systems require little, if any, water exchange and have high animal stocking densities. Intensive nutrient inputs lead to an abundant community of microorganisms. These microbes are partially contained within suspended "biofloc" particles and contribute to water quality maintenance and provision of supplemental nutrition to the culture species. Optimal function of minimal-exchange, intensive systems is likely dependent on the structure of the microbial communities within them. This document offers a short review of microbial groups important for intensive marine aquaculture and descriptions of three methods for quantifying their abundance. The document also describes an experiment during which these methods were used to monitor the effects of partial biofloc removal on microbe abundance. The first method uses light microscopy, with the option of epifluorescence, along with a ranking system to enumerate the abundance of microbial taxa. The second method exclusively uses epifluorescence to illuminate chlorophyll and cyanobacteria pigments. Images are taken of each fluorescing group of pigments and processed using image analysis software to quantify the respective abundance of the two pigment types. Using the third method, changes in bacterial abundance were determined by gas chromatographic measurement of bacteria-specific fatty acids in solvent extracted water column lipids. Using these techniques, it was determined that removing solids from the culture water significantly ($P \leq 0.01$) reduced the abundance of nematodes, rotifers, cyanobacteria, and bacteria. Understanding microbial composition and the effects that management protocols have on that composition may help system managers make better informed decisions.

1. Introduction

1.1. Minimal-exchange, intensive systems

Minimal-exchange, intensive aquaculture systems offer an environmentally attractive means of shrimp and fish production, allowing for high density culture and little or no water exchange. With high animal density comes intensive nutrient input in the form of feed. When water is not exchanged with surrounding water bodies, expensive nutrients from feed are retained within the system. In minimal-exchange, intensive systems excess nutrients are assimilated and mineralized by a dense microbial community in the water column, thus alleviating potential toxicity (Avnimelech, 2006; Bratvold and Browdy, 1998; Ebeling et al., 2006; Ray et al., 2009). The microorganisms not only remove excess nutrients, but have been implicated in nutritional provision for animals, including shrimp and tilapia, that can result in improved growth rate, feed conversion ratio (FCR), and weight gain (Azim and Little, 2008; Burford et al., 2004; Moss and Pruder, 1995; Wasielesky et al., 2006).

1.2. Microorganism groups

Important microorganism groups in minimal-exchange, intensive aquaculture systems include algae, zooplankton, and bacteria. Algae utilize toxic total ammonia nitrogen (TAN), as well as less dangerous nitrate-nitrogen and phosphate compounds to construct cellular structures such as proteins and sugars. Various forms of algae, most notably diatoms, are nutritious and can benefit shrimp production by contributing qualities such as essential amino acids and highly unsaturated fatty acids (Ju et al., 2009; Moss et al., 2001). However, potentially harmful algae can also be found in aquaculture systems. One group that has been problematic for shrimp culture is cyanobacteria, also known as blue-green algae. Some cyanobacteria are capable of producing toxins that may diminish shrimp growth or directly cause mortality (Alonso-Rodriguez and Paez-Osuna, 2003; Zimba et al., 2006).

* Corresponding author. Gulf Coast Research Laboratory, 703 East Beach Dr., Ocean Springs, MS 39564, USA. Tel.: +1 843 367 9407.
E-mail address: AndrewJRay@gmail.com (A.J. Ray).

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Zooplankton consume algae and bacteria and can play an important role in the transfer of nutrients from primary producers to secondary consumers. Zooplankton such as rotifers can contribute significantly to the protein and energy requirements of shrimp (Focken et al., 1998). However, zooplankton also consume oxygen and can cause a reduction in alkalinity through respiration.

Two functional categories of bacteria are primarily responsible for water quality maintenance in minimal-exchange, intensive systems: heterotrophic ammonia-assimilative and chemoautotrophic nitrifying bacteria (Ebeling et al., 2006; Hargreaves, 2006). The heterotrophic group removes TAN from the water column to build cellular proteins. Nitrifying bacteria acquire energy through the oxidation reactions of TAN to nitrite-N and nitrite-N to nitrate-N, the latter of which is much less toxic than its predecessors in this sequence. Both the bacterial assimilation and nitrification processes consume oxygen and reduce alkalinity, thereby often requiring the supplementation of those two components.

A portion of the microbial community in minimal-exchange, intensive culture systems is contained on or within particles, commonly called biofloc (Fig. 1A). Biofloc is composed of a variety of microorganisms, uneaten feed, feces, and detritus, and the particles are kept in suspension with water propulsion and aeration. Biofloc offers numerous ecological advantages for microbes, including protection from predators, direct access to nutrients, and necessary substrate area (De Schryver et al., 2008). Biofloc is thought to provide a packaging of microbial proteins and nutrients that is directly accessible to culture animals (Avnimelech, 2009; Burford et al., 2004). Biofloc technology (BFT) can be considered a culture technique in which water quality is maintained and *in situ* animal feed is simultaneously produced in the form of biofloc particles (Crab et al., 2007). Although biofloc can be beneficial, some level of control over the concentration of particles is likely required for optimal system performance. Ray et al. (2010) demonstrated that shrimp biomass production (kg m^{-3}) was increased 41% when biofloc concentration was managed through the use of external settling chambers. They also showed a 60% reduction in nitrate-nitrogen concentration and a 61% reduction in phosphate concentration when biofloc concentration was managed.

To optimize system function, a beneficial microbial community should be developed and sustained. This requires knowledge of microbial community composition and the ability to monitor changes. By investigating microbial composition, inferences may be made of the underlying reasons for differences in community structure and the effects of management techniques, such as biofloc removal, on that structure. Monitoring of microorganisms may help characterize opportunities for supplemental nutrition, and help managers recognize the occurrence of problematic organisms, thereby guiding informed management decisions.

The purpose of this document is to describe three techniques that can be used to characterize microbial communities in minimal-exchange, intensive aquaculture systems. To illustrate these techniques in a practical application they were used in an experiment that explored the effects of removing suspended solids (biofloc) on microorganism abundance.

1.3. Visual microscopy abundance quantification

In this case, abundance quantification describes a system of ranking the relative abundance of organismal groups. Organisms are categorized and ranked according to a predetermined scale, similar to the previous work of researchers. Newall et al. (2006) used such a scale to rank the abundance of diatoms in a river system. DeVantier et al. (1998) described trained researchers who used a subjective scale to rank macro-invertebrate abundance.

The current document explores the use of an abundance quantification system to describe the relative abundance of six microorganism categories important and common to intensive marine aquaculture systems: chlorophytes (green algae), diatoms, dinoflagellates, nematodes, rotifers, and cyanobacteria. The abundance of organisms belonging to these categories was assessed using light and fluorescence microscopy with live samples. The categories used were selected based on the authors' experience with shrimp culture systems at the Waddell Mariculture Center (WMC) in Bluffton, South Carolina, USA. Other categories may be needed, depending on location and endemic biota. Broad classifications such as these save time, minimize the amount of taxonomical training required by the analyst, and can be less susceptible to biases (DeVantier et al., 1998).

This technique can be performed with an ordinary compound light microscope, although epifluorescence technology is advantageous. Many of the cyanobacteria and algal cells in intensive culture systems are small and become concealed by particles in the water, making visualizing and discerning between these two groups difficult. Epifluorescence can illuminate the chlorophyll-a pigment, contained by both eukaryotic algae and cyanobacteria, and can fluoresce the cyanobacteria pigment phycocyanin exclusively. This facilitates ease of observation and differentiation between eukaryotic algae and cyanobacteria. Kastovska et al. (2007) described using epifluorescence in this manner to discern between the two groups. Epifluorescence functions by powering a broad-spectra mercury light bulb; filters are then used to select the specific wavelengths of light that cause chlorophyll pigments, cyanobacteria pigments, or stains such as 4′6-diamidino-2-phenylindole (DAPI), a DNA stain, to fluoresce. An advantage that epifluorescence has over other pigment analyses is that many types of filters are available to discern a variety of biological components, not limited to pigments.

An inverted compound microscope allows the observer to look up, through a larger sample than would be possible with an ordinary compound microscope. This technology allows the microorganisms in a relatively large sample (3 mL in this case) to settle down onto one plane. The three-dimensional biofloc particles that are common in intensive culture systems are better visualized using an inverted scope because the sample is not compressed onto a flat slide.

1.4. Epifluorescence with image analysis quantification

This procedure utilizes the epifluorescence technology described above, except that rather than using live samples, images of fluorescing chlorophyll (chlorophyll-a) and cyanobacteria pigment (phycocyanin) are captured using preserved samples on flat slides. An image of fluorescing chlorophyll is taken, and an image of cyanobacteria pigment is then taken in the same location on the slide. This enables a direct comparison between chlorophyll (including cyanobacteria) and cyanobacteria pigment exclusively. The details of wavelengths used for pigment excitation and fluorescence emission are given in Section 2.1. To determine the relative abundance of fluorescing pigments in each image, the pictures are processed using the computer program ImageJ.

Researchers have used this technique in other applications. Selinummi et al. (2005) processed DAPI-stained images with image analysis software to quantify bacterial cells. Verity and Sieracki (1993) outlined the general procedures for measuring plankton biomass using this methodology.

1.5. Bacterial fatty acid assessment by gas chromatography

In this method, gas chromatography with mass spectrometry (GC–MS) is used to separate, identify, and quantify fatty acids (FAs) derived from the lipid component obtained by solvent extraction of biofloc. The sum of odd and branched chain fatty acids thus obtained serves as a measure of the relative abundance of bacteria. Odd and branched chain fatty acids, produced primarily by both aerobic and anaerobic bacteria, are widely accepted as biomarkers for bacteria and sums are frequently used to estimate bacterial contributions; examples