

Molecular Ecology of Aquatic Communities

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Molecular Ecology of Aquatic Communities

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Preface

Over the past decade, molecular biology approaches have had a significant impact on many areas of biological sciences, including ecology. In 1997, a special session on the application of molecular techniques to aquatic communities was held at the American Society for Limnology and Oceanography Aquatic Sciences Meeting in Santa Fe, New Mexico. The focus of that session, and the collection of papers presented here, is that molecular information can be used to study the concepts involved in the interactions of species and individuals that are the basis for the features that we observe as aquatic communities.

In this volume, papers present approaches and perspectives that address interactions and relationships involved in community level characteristics. Molecular approaches have provided information on organisms at all trophic levels from prokaryotic microbes to fish and mammals, and including important ecosystem components such as viruses and plasmids. Researchers have applied these techniques over the globe, in diverse environments from hot springs to Antarctic lakes and Arctic ocean basins, from tropical and temperate seas to lakes and rivers.

It is hoped that this volume will integrate studies across subdisciplines, and provide a useful research and educational reference. More importantly, it is hoped that the philosophy of looking forward from what we have done with molecular tools, to what we can hope to do in the field of aquatic community ecology, will stimulate molecular ecology students and researchers to pursue new approaches and ask new questions, at the community level.

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Guest Editors



Molecular ecology of aquatic communities: reflections and future directions

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Abstract

During the 1980s, many new molecular biology techniques were developed, providing new capabilities for studying the genetics and activities of organisms. Biologists and ecologists saw the promise that these techniques held for studying different aspects of organisms, both in culture and in the natural environment. In less than a decade, these techniques were adopted by a large number of researchers studying many types of organisms in diverse environments. Much of the molecular-level information acquired has been used to address questions of evolution, biogeography, population structure and biodiversity. At this juncture, molecular ecologists are poised to contribute to the study of the fundamental characteristics underlying aquatic community structure. The goal of this overview is to assess where we have been, where we are now and what the future holds for revealing the basis of community structure and function with molecular-level information.

Introduction

Studies of freshwater and marine communities have played an integral role in the history and development of the science of ecology (Lindeman, 1942; Hutchinson, 1957; Paine, 1980). Ecology has matured during the past quarter century, with theoretical and quantitative developments in the description and modeling of populations, communities and ecosystems (Jones & Lawton, 1995). In parallel, the development of molecular biological techniques has spawned new ways of looking at organisms in the environment, assessing biological processes and activities (Zehr, 1998; Zehr & Hiorns, 1998), and studying population genetics and species distributions (Medlin et al., 1995; Vanoppen et al., 1995; Palumbi, 1996; Geller, 1998; Graves, 1998; Parker et al., 1998).

The trajectories of ecological theory and molecular biology technique development have converged during this decade, and the application of molecular techniques has begun to provide information relevant to ecological questions. Ecological studies have focused on different levels and scales ranging from individual

organisms to species, populations and ecosystems, and these different perspectives are now being integrated (Grimm, 1995). Given the complexity of ecosystems and ecological interactions, it could be questioned whether the extension of these studies to the scale of molecules has anything to offer the study of community and ecosystem ecology. Nonetheless, aquatic biology and ecology have already benefited from molecular approaches (for reviews, see Falkowski & LaRoche, 1991; Joint, 1995; Burton, 1996; Cooksey, 1998; Parker et al., 1998). The objective of this discussion is to develop a framework for integrating molecular biology into community ecology and community structure studies, thus making a link from spatial scales of molecules to ecosystems that may foster new avenues of ecological research.

Molecular biology contributions to aquatic ecology

Some of the fundamental concepts that have driven studies in aquatic ecology at the community and ecosystem levels are:

1. Energy flow and trophic dynamics (Lindeman, 1942),
2. Biogeochemical cycling of elements,
3. The 'niche' as the ecological hyperdimensional 'space' of an organism (Hutchinson, 1957),
4. Competition for resources (Tilman, 1982),
5. Food web structure including the 'microbial loop' (Pomeroy, 1974; Steele, 1974; Paine, 1980; Azam et al., 1983; Carpenter et al., 1985; Carpenter & Kitchell, 1988; Azam, 1998),
6. Interactions between species including herbivory, predation and symbiotic relationships, and
7. Community properties including diversity, stability and succession (MacArthur, 1955; Connell, 1961; May, 1972).

Although traditional ecological approaches have provided means to investigate these characteristics of communities, molecular biology has injected a new vitality into studies of some of these concepts. Molecular techniques provide information on the genetics, activities and capabilities of organisms at the most fundamental level. In the following discussion, we will provide some examples of areas where molecular approaches have contributed, and are likely to make contributions to ecological studies.

Biodiversity

A major contribution of molecular techniques has been to provide real measures of biodiversity of organisms at the species, population and community levels. Particularly with respect to microbial assemblages that were previously difficult to study due to constraints of culturability and nondescript morphology, nucleic acid sequence information obtained directly from natural communities has provided a new perspective on diversity in aquatic microbial communities and has led to the identification of major new groups of microorganisms (Murray et al., 1996; Ferrari & Hollibaugh, 1999; Nold & Zwart, 1998).

Molecular sequence information has provided for a number of new approaches for microbial ecology, by facilitating the design of oligonucleotide probes for determining the composition of natural assemblages with fluorescent *in situ* hybridization, and primers for polymerase chain reaction based approaches (Muyzer et al., 1993; Amann et al., 1995; Vanhannen et al., 1998).

Molecular information makes it possible to catalogue the distribution of 'species' and 'populations' (Medlin et al., 1995). This information is essential

for determining biological diversity and providing a framework for conservation strategies (Haig, 1998; Palumbi & Cipriano, 1998). At the microbial level, information on species-level diversity would be virtually nonexistent if not for the surveys of terrestrial and aquatic environments that have dominated molecular microbial ecology for the past decade (Pace et al., 1986; Pace, 1997; DeLong, 1998; Head et al., 1998; Methé et al., 1998).

Molecular techniques have also provided information on gene transfer among microorganisms in the environment (Ashelford et al., 1997; Williams et al., 1997; Jiang, 1998), with implications for their evolution, as well as the effects of introductions of new species and genetically-engineered organisms. Molecular approaches have provided means to investigate the ecological roles of viruses (Proctor, 1997; Scanlan & Wilson, 1999; Short & Suttle, 1999) and plasmids (Sobecky & Mincer, 1998; Sobecky, 1999). Much of the biodiversity efforts have remained at the cataloguing stage, with studies only recently beginning to detail the dynamics of individual species or phenotypes, or to use the information to ask classical ecological questions.

It is now possible to use the molecular sequence information and databases to develop probes for studying the dynamics of individual species or phylotypes (DiChristina & DeLong, 1993; Amann et al., 1995; Gordon and Giovannoni, 1996; Methé and Zehr, 1999), to use sequence information to calculate diversity indices (Watve & Gangal, 1996; Nubel et al., 1999), and to investigate relationships between microbial diversity and ecosystem attributes such as community stability. The sequence information can also be used as markers to aid in cultivation of specific groups, which ultimately is critical for understanding the physiological ecology of these organisms in the environment (Palleroni, 1997).

Population biology, biogeography and gene flow

The application of molecular approaches to studies of eukaryotes or macroorganisms has focused on population structure and evolutionary questions, on organisms ranging from picoeukaryotes to whales (DeLong, 1998). Molecular information has provided markers for identifying individuals, determining population structure and studying parentage (Coffroth & Lasker, 1998; Zuccarello et al., 1999), as well as documenting the dispersion of species and larvae in the ocean (Bucklin, 1995; France & Kocher, 1996; Bucklin et

al., 1999). Population structure data can be used to assess the effects of disturbances, such as the introduction of toxins and contaminants, on population diversity (Guttman 1994; Depledge 1996; Hebert & Murdoch 1996; Guttman & Berg 1998). The expression of stress proteins and other proteins provide the potential to identify environmental stressors prior to shifts in populations (Chen et al., 1999a; Karouna & Zehr, 1999). Molecular techniques have facilitated the identification of the larvae of species that are otherwise too small or nondescript to identify by traditional means (Burton, 1996), facilitating studies of gene flow and population dynamics (DeLong, 1998). This type of information can ultimately be used to study linkages in aquatic communities, such as the effects of predation and competition on population genetic structure. Currently, these studies are usually descriptive in that they generally do not relate the genetics of populations to the environmental basis for selection or fitness in the environment. However, this may be a rewarding, yet difficult, objective of future studies.

Productivity

Productivity and energy flow are the common measures of the performance of aquatic communities. Measures of microbial productivity are currently constrained to measuring 'community' rates, thus information is lost on the contribution of individual species to community productivity. Molecular approaches that target RNA or protein can provide specific assessments of productivity, growth or gene expression in specific groups of microorganisms (Kramer & Singleton, 1993; Pichard et al., 1996), sometimes at the single cell level (Chen et al., 1999b; Orellana & Perry, 1995). Measurements of phytoplankton primary productivity are made in bulk, whereas macrophyte primary productivity assays use individual plants. Molecular techniques provide the means to assay individual phytoplankton for proteins involved in carbon fixation (Orellana and Perry, 1995), growth and cell division (Lin et al., 1995) and to interrogate cells for nutritional or physiological status (LaRoche et al., 1993; Palenik & Koke, 1995; LaRoche et al., 1999; Scanlan & Wilson, 1999) and study the photosynthetic apparatus (Geider et al., 1993). This type of information can also be obtained from macroalgae or macrophytes, providing better information on their physiological status, growth and metabolism. It may be possible to obtain growth information for nonphotosynthetic eukaryotic organisms, including inverteb-

rates, by targeting developmental genes or measuring RNA/DNA ratios (Smerdon, 1998; Buckley et al., 1999). These tools now provide the potential for integrated community studies, to determine the effects of community structure on growth and productivity of species and individuals in populations.

Competition

Competition is one of the classic concepts in ecology. In contradiction to the prediction of basic competitive exclusion principles, the plankton of oligotrophic systems is more diverse than would be expected if the best competitor for the limiting nutrient grew the fastest and outcompeted other species. This diversity was described over thirty years ago as the "Paradox of the Plankton" (Hutchinson, 1961), and various explanations have been offered since then (Richerson et al., 1970; Siegel, 1998). A recent modeling study suggested that one possible explanation is that the outcome of competition is not predictable at the population level, but only by considering the effects of competition at the individual level (Siegel, 1998). Testing this conclusion requires analyses at the level of the individual and the use of molecular tools. As discussed above, several approaches have been developed for investigating the growth (Lin & Carpenter, 1995), productivity (Orellana & Perry, 1995) and physiological status (Palenik & Wood, 1998) of individual phytoplankton cells using microscopy or flow cytometry (Urbach & Chisholm, 1998; Collier & Campbell, 1999). Thus, molecular biology provides a tool for attempting such studies, even in microscopic species.

Biogeochemical cycles

Many of the critical steps in biogeochemical cycles are catalyzed by very specific groups of microorganisms, using specific enzymes. Molecular approaches have provided important inroads for the detection and characterization of microbes involved in biogeochemical processes, from natural elemental cycles such as nitrification and denitrification (Voytek & Ward, 1995; Voytek et al., 1999), nitrogen fixation (Zehr & Capone, 1996), sulfate reduction (Kane et al., 1993) or sulfur oxidation (Schramm et al., 1996; Gray & Head, 1999), to environmentally important transformations of anthropogenic xenobiotics such as metal compounds (Neilson et al., 1992; Nazaret et al., 1994; Sayler et al., 1995; Langworthy et al., 1998). Probes for specific metabolic pathways are particularly useful since they

can be used to determine redundancy within guilds involved in biogeochemical cycling, which may be an important factor in community or ecosystem stability.

Food web structure

The pathways of energy and nutrient transfer through different trophic levels is a fundamental characteristic of communities and ecosystems. Molecular and immunological techniques provide markers that can be used to determine the fate of individual organisms and to identify groups such as the heterotrophic nanoflagellates (Caron et al., 1999). Molecular tracers can provide information on trophic pathways (who eats whom). Immunological techniques were used to identify major food of fish larvae (Ohman et al., 1991); tracing the ingestion of species may be an important, yet unexploited contribution of molecular tools to community ecology. Molecular markers can also be used to evaluate the effects of predation on microbial communities (Pernthaler et al., 1997; Suzuki, 1997).

Symbiosis

Symbiotic relationships span a wide range of interactions between host and symbiont, from loose associations to relationships that provide substantial mutual benefit. Molecular techniques that provide high resolution at the species level, as well as the ability to identify individual organisms on the basis of immunoassays or nucleic acid probe hybridization have greatly facilitated the investigation of symbioses (Hackstein, 1997). Previously unidentified symbiotic relationships that are uncovered with molecular techniques may have important implications for biodiversity (Hackstein, 1997). Molecular probes have been used to identify organisms in association with cells, or determine the specific localization of microorganisms within cells or tissues (Cary et al., 1993). Symbiotic organisms can often be identified (Distel & Wood, 1992; Polz et al., 1994), and the interactions between host and symbiont studied at the molecular level. Signals between hosts and symbionts and their effects on gene expression can be studied, providing a model of symbiotic interactions at the molecular level (Weis et al., 1998). Mechanisms of symbiont transfer from generation to generation can be explored (Cary & Giovannoni, 1993). Furthermore, the relationships between diversity of hosts and symbionts can be evaluated (Rowan, 1998).

Adaptation

Important determinants of the distribution of species are the physiological, biochemical and behavioral characteristics that allow an individual species to compete in its unique niche. Studies of how organisms are adapted to their environment, including extreme environments, are enhanced by the use of molecular tools that allow the direct examination of the molecular basis for adaptation and provide information on evolution as well. Ecologically important molecules can be identified and characterized, such as the antifreeze protein in fish (Wang et al., 1995). Molecules involved in damage or responses to environmental factors such as UV, can be assayed by molecular techniques (Lyons et al., 1998) and the effects of factors such as UV-stress have implications for competitive interactions (Miller et al., 1998). The identification and understanding of the expression of these molecules is fundamental to understanding adaptation and selection, which determine the distribution of organisms in time and space, and the outcome of competitive interactions.

Summary

The application of molecular tools was initiated with an exploratory, developmental phase that has blossomed and provided new insights into structure, function, diversity and ecology. Perhaps during this phase the traditional ecologist has been disappointed in the products of molecular biology, but the understanding that has been obtained now poises the ecologist to merge molecular approaches with more traditional experimental techniques to exploit the full potential of molecular level understanding. The molecular approach has perhaps made the most revolutionary impact on microbial ecology, which previously had been limited by the technological ability to identify, characterize and study natural populations. Perhaps the most profound insights are yet to come, when ecological information on rate processes and biomass are routinely collected with molecular information, and when molecular approaches are better integrated into experimental ecology to directly address ecological questions. A number of pioneering studies have shown the potential payoff of using molecular techniques and recombinant organisms in ecological experiments (Sobecky et al., 1996; Pernthaler et al., 1997; Gonzalez et al., 1999).

The next step will be to address questions regarding the specific physiological properties that constitute ecological success under certain nutrient conditions or that characterize populations that are nutrient-limited (controlled by bottom-up forces) or external factors (e.g. predation, top-down mechanisms). There may be molecular markers that define *r* vs. *K* strategists, or that characterize the populations at different stages in community succession. Molecular markers that provide indications of disturbance can be used to assess stresses that may be useful for predicting long-term impacts of environmental effects on biodiversity.

At this juncture, we have not yet seen the complete maturation of molecular ecology in the aquatic sciences, but the fusion of molecular approaches with the classical concerns of the ecologist are on the horizon.

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