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MiniReview

Microbial indicators of aquatic ecosystem change: current applications to eutrophication studies

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Abstract

Human encroachment on aquatic ecosystems is increasing at an unprecedented rate. The impacts of human pollution and habitat alteration are most evident and of greatest concern at the microbial level, where a bulk of production and nutrient cycling takes place. Aquatic ecosystems are additionally affected by natural perturbations, including droughts, storms, and floods, the frequency and extent of which may be increasing. Distinguishing and integrating the impacts of natural and human stressors is essential for understanding environmentally driven change of microbial diversity and function. Microbial bioindicators play a major role in detecting and characterizing these changes. Complementary use of analytical and molecular indicator tools shows great promise in helping us clarify the processes underlying microbial population, community, and ecosystem change in response to environmental perturbations. This is illustrated in phytoplankton (microalgal and cyanobacterial) and bacterial community changes in a range of US estuarine and coastal ecosystems experiencing increasing development in their water- and airsheds as well as climatic changes (e.g., increasing hurricane frequency). Microbial indicators can be adapted to a range of monitoring programs, including ferries, moored instrumentation, and remote sensing, in order to evaluate environmental controls on microbial community structure and function over ecosystem to global scales.

Keywords: Estuaries; Microbial indicator; Nutrient; Eutrophication; Pollutant; Climate; Ecological change

1. Introduction

More than 70% of the world's human population lives in watersheds that drain to the coast [1]. Further population growth will be centered in these regions, exerting unprecedented pressure on riverine, estuarine and coastal habitats receiving human pollutants [1,2]. Multiple negative ecological impacts on these fragile habitats (i.e., loss of biodiversity, increasing frequencies of harmful algal blooms, hypoxia, disease and declines in fisheries) have been documented [3–6], yet the mechanisms underlying water quality and habitat degradation remain poorly understood. It is now imperative that we develop and apply novel and effective ways to detect, manage and correct man-induced loss of aquatic ecosystem biodiversity, water quality, fisheries, and recreational resources.

Most evident are water quality and habitat changes attributable to nutrient over-enrichment, leading to excessive primary production or eutrophication [3–5]. Eutrophication has caused significant changes in coastal nutrient (C, N, P, Si) cycling, water quality, biodiversity, fisheries, and overall ecosystem health [3–7].

Natural perturbations such as droughts, storms and floods additionally impact aquatic ecosystems. Like human disturbances, these events are predicted to increase in the foreseeable future [8]. Being able to identify and distinguish climatic and anthropogenic impacts on ecosystem structure and function is critical to understanding and managing factors controlling water quality, habitat condition and biotic resources.

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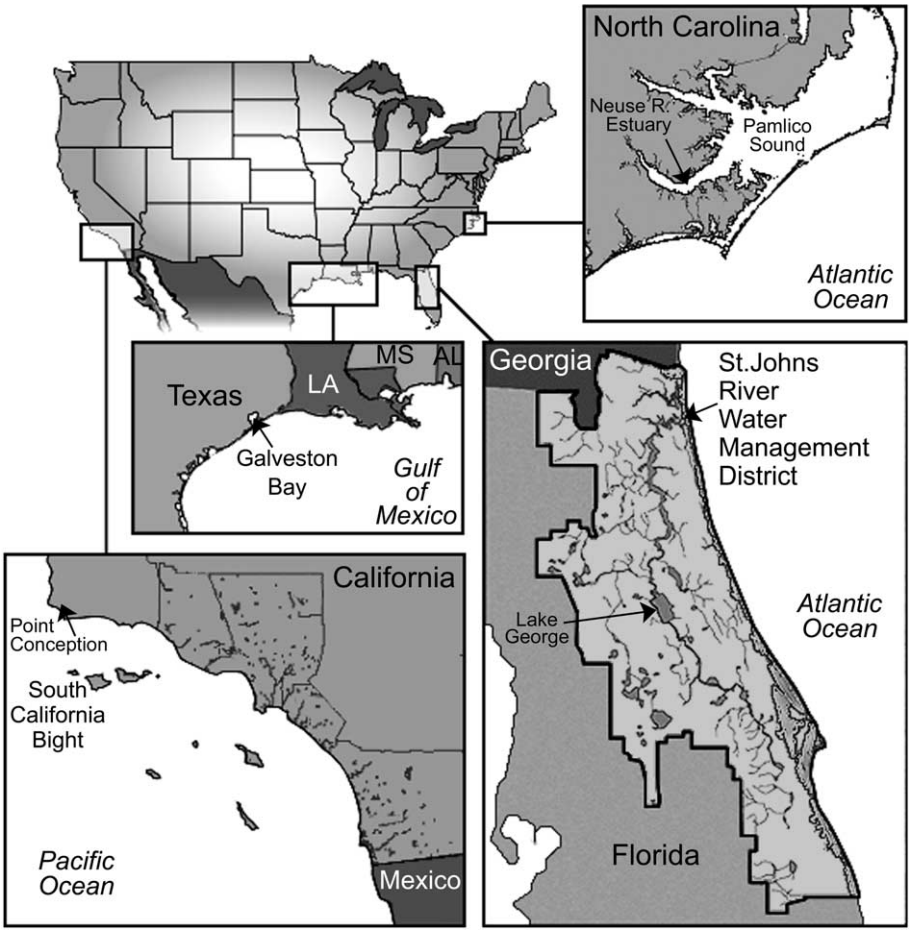

Fig. 1. Locations of some US estuarine and coastal systems in which microbial biochemical, chemotaxonomic and molecular indicator techniques have been applied. They include: (1) the Neuse River Estuary–Pamlico Sound, North Carolina, (2) the St. Johns River system, Florida, 3) Galveston Bay, Texas, and 4) the Southern California Bight, California–Mexico.

Among aquatic biota, microorganisms are generally highly sensitive to and profoundly affected by environmental perturbations. Microbes comprise the majority of aquatic biomass and are responsible for the bulk of productivity and nutrient cycling in aquatic systems. They have fast growth rates, and respond to low levels of pollutants as well as other physical, chemical, and biotic environmental changes. From detection and effect perspectives, they provide sensitive, meaningful, and quantifiable indications of ecological change.

Utilizing collective research results from geographically diverse US riverine, estuarine and coastal ecosystems (Fig. 1), we will explore recently developed microbial indicator tools for detecting and characterizing ecological change. Emphasis will be placed on the use of these tools for water quality and environmental health management over scales ranging from individual habitats to regions. We will use the word 'indicator' in a broad sense, including the detection of specific responses both in the entire microbial community and in individual organisms, and will consider responses to both known and unknown environmental perturbations.

2. Using microbial indicators to characterize ecological change in diverse aquatic ecosystems

2.1. *Eutrophication dynamics of the Neuse River Estuary–Pamlico Sound, North Carolina*

The Pamlico Sound (PS) system is the largest lagoon and second largest estuarine complex in the USA (Fig. 2). The Neuse River Estuary (NRE) is a key tributary of the PS. The NRE basin drains North Carolina's rapidly expanding agricultural, urban, and industrial Piedmont and coastal plain regions. Primary production in the PS and the NRE is N-limited throughout much of the year [9–11] and excessive N loading has been linked to eutrophication [10,11].

Symptoms of advanced eutrophication include nuisance algal blooms, dominated by cyanobacteria in the upstream oligohaline waters, and by dinoflagellates and cryptomonads in the downstream mesohaline zone. The resultant eutrophic conditions (>300 g C fixed m^{-2} yr^{1}) provide excess C that fuels periodic low dissolved oxygen (DO) bottom waters [10,11]. Hypoxia (DO <4 mg l^{-1}) and an-

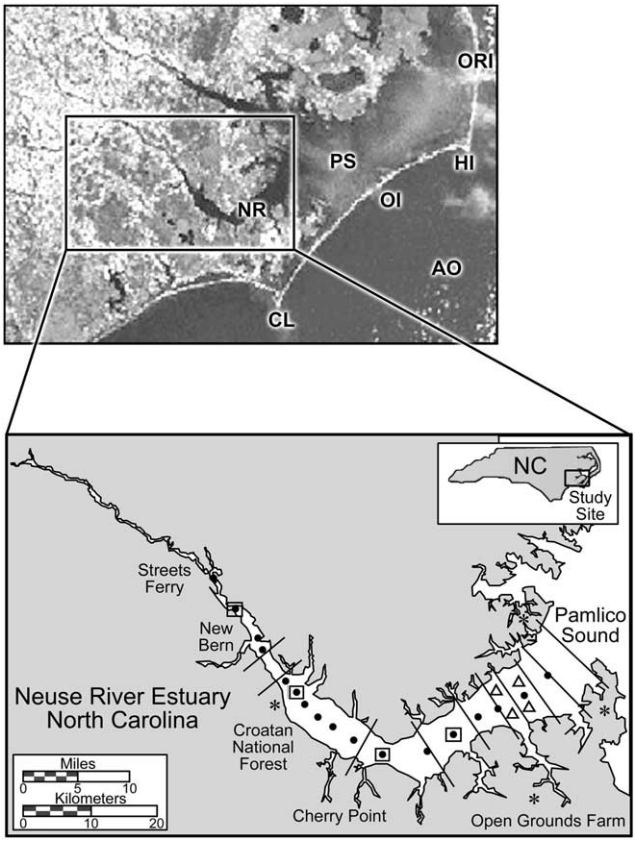

Fig. 2. Location of the Neuse River Estuary and Pamlico Sound, North Carolina. Shown are the Atlantic Ocean (AO), Oregon, Hatteras, and Ocracoke Inlets (ORI, HI, OI, respectively), Cape Lookout (CL), Pamlico Sound (PS), and the Pamlico and Neuse Rivers (NR). The NRE sampling sites for mid-river water quality (17 filled circles) and continuous in-stream monitoring (four open boxes) are shown. Triangles indicate sites for diel and other periodic studies during which additional samples are collected.

oxia (no detectable DO) are chief causative agents of fish kills [11] (also see: www.marine.unc.edu/neuse/modmon). Oscillating oxic–anoxic shifts dramatically alter microbial community structure and function [11].

This system has also been impacted by major climatic perturbations. During fall 1999, Hurricanes Dennis, Floyd, and Irene delivered up to 1 m of rainfall in a 6-week period, causing a 200–500-year flood in the PS watershed. Floodwaters turned the NRE and other tributaries of the PS completely fresh, and accounted for half the annual N load to this N-sensitive system (Fig. 3). Biogeochemical and ecological effects included hypoxic bottom waters, altered nutrient (N, P and C) cycling, a three-fold increase in algal biomass, shifts in microbial community structure and function, altered fish distributions, catches, and an increase in fish disease [12]. Predicted elevated hurricane activity may cause long-term biogeochemical and trophic changes in these systems [12].

To stem eutrophication, the North Carolina Legislature mandated a 30% reduction in external N loading to the NRE, to be in place in 2003. In addition, the US EPA has recommended an allowable total maximum daily load that also calls for a 30% N reduction. These management efforts will enable us to evaluate the impacts of an ecosystem-scale N reduction experiment on microbial community structure and function. Given expected increases of human population and changes in activities in the NRE and other coastal watersheds, it is likely that total amounts and types of N and other nutrients (e.g., P, Si) entering the ecosystem will change over time.

Such changes may alter microbial (bacterial, phytoplankton) community composition, phytoplankton–zooplankton interactions, with cascading impacts on invertebrate and fish consumers, nutrient cycling, and oxygen dynamics. If, for example, the growth of readily grazed phytoplankton (diatoms, chlorophytes) is favored, trophic transfer and nutrient cycling will largely take place in the water column. In contrast, if changes in nutrient input stoichiometry favor less palatable or toxic taxa (nuisance cyanobacteria and dinoflagellates), transfer will be poor and unconsumed phytoplankton biomass will accumulate in the sediments, exacerbating hypoxia and anoxia.

These ecosystem-scale research and management questions require rapid and accurate means of characterizing phytoplankton community compositional responses to shifts in nutrient inputs. Microscopic identification and

Table 1
HPLC-determined chemotaxonomic photopigments (chlorophylls and carotenoids) useful as markers for determining the relative abundance of major algal groups in phytoplankton communities

Algal Groups	Photopigments																	
	Chlorophyll a	divinyl-Chl a	Chlorophyll b	divinyl-Chl b	Chlorophyll c_1c_2	Chlorophyll c_3	Alloxanthin	Anheraxanthin	β-Carotene	Canthaxanthin	Diadinoxanthin	Diatoxanthin	Echinenone	but-Fucoxanthin	Fucoxanthin	hex-Fucoxanthin	Gyroxanthin	Lutein
Chlorophytes	■		■					▨	■									■
Chrysophytes (Chrysophyta)	■				■	■			▨		■	▨		■	■			
Cryptophytes	■				■		■		■									
Cyanobacteria (pelagic)	■							■	■									
Cyanobacteria (benthic)	■							■	■	■			■					
Diatoms	■				■				▨		■	▨			■			
Dinoflagellates	■				■				▨		■	▨						
Euglenophytes	■		■					▨	■		■	▨						
Eustigmatophytes	■								■									
Prymnesiophytes (Haptophyta)	■				■				▨		■	▨		■	■	■		
Pelagophytes	■				■				■		■	▨		■	■			
Prasinophytes	■		■						■									■
Prochlorophytes		■		■					■									
Raphidophytes (Chloromonads) (Chrysophyta)	■				■				■		■	▨			■			
Karenia brevis	■				■	■					■	▨		■	■	■		

The open squares indicate that the pigment does not occur in the algal group. The cross-hatched squares indicate that pigments occur in low concentrations for the algal group, while the black squares indicate that this is a major pigment for the algal group.

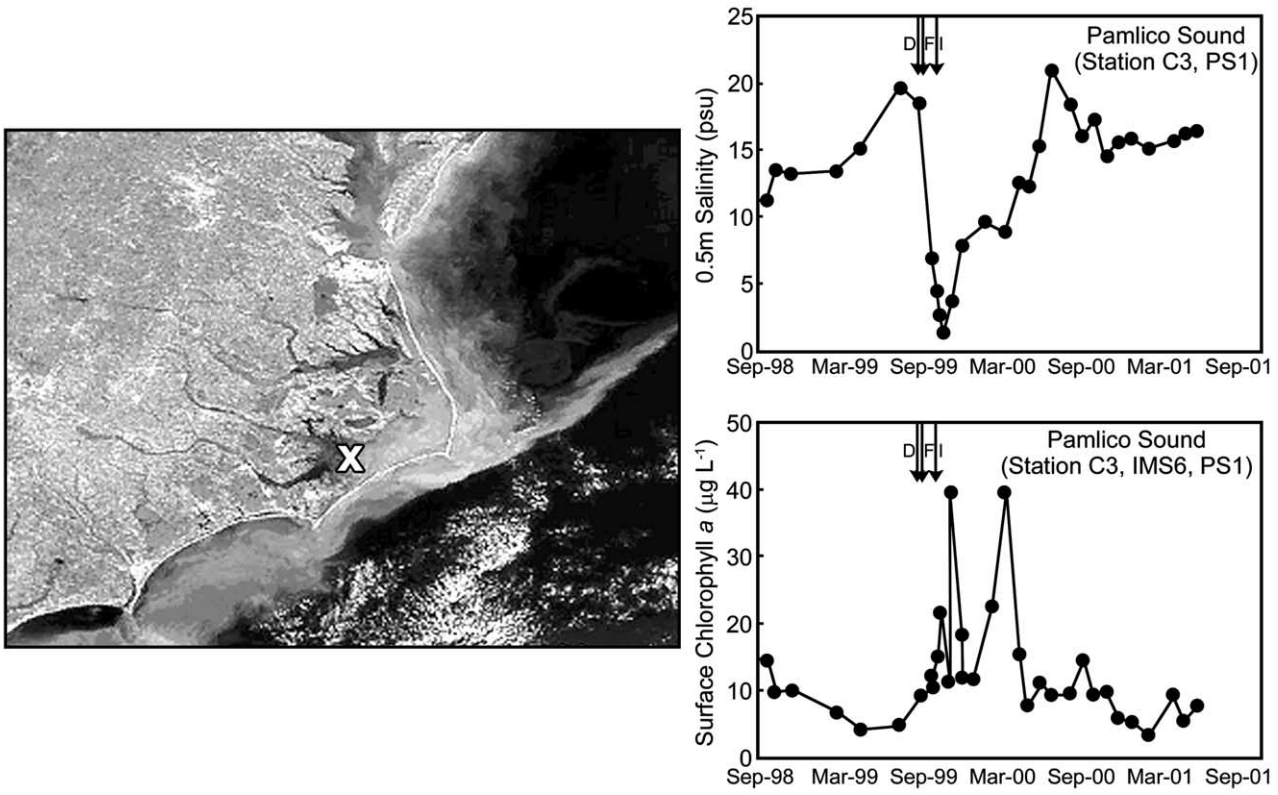

Fig. 3. Left: SeaWiFS satellite image of Hurricane Floyd's floodwaters inundating Pamlico Sound on September 23, 1999, one week after the hurricane's landfall. Upper right: Floodwater effects on salinity at a reference station (X) located in the western Pamlico Sound. Lower right: Impacts on phytoplankton biomass (as chlorophyll *a*) at station X. D = Hurricane Dennis, F = Hurricane Floyd, I = Hurricane Irene. Figure adapted from [12].

enumeration, while accurate and reliable in experienced hands, is time-consuming and impractical for coastal studies where there is a need for numerous rapid assessments. Alternatively, algal biomass can be quickly estimated from photopigment content. Chlorophyll *a* (Chl *a*), which is present in all microalgae and higher plants, has traditionally been used. However, Chl *a* cannot distinguish microalgal taxonomic groups, a taxonomic level useful for examining major compositional shifts in response to environmental change. High performance liquid chromatography (HPLC), coupled to photodiode array spectrophotometry (PDAS), can be used to characterize and quantify phytoplankton group composition based on diagnostic photopigments. These include specific chlorophylls (*a*, *b*, *c*), carotenoids and phycobilins [13–15] (Table 1). A statistical procedure, ChemTax [15], partitions Chl *a* (total microalgal biomass) into the major algal groups, to determine the relative and absolute contribution of each group. In the NRE, key photopigment markers include Chl *b* and lutein (chlorophytes), zeaxanthin, myxoxanthophyll and echinenone (cyanobacteria), fucoxanthin (diatoms), peridinin (dinoflagellates) and alloxanthin (cryptomonads) [16].

HPLC pigment analyses can be adapted to routine monitoring programs (cf. [16]). In addition, HPLC measurements can be used to calibrate remotely sensed (aircraft, satellite) phytoplankton distributions of the ecosystem and regional scale.

We have used HPLC–PDAS/ChemTax to characterize phytoplankton community compositional trends in the NRE since 1994 (Figs. 4 and 5). During this period, the NRE experienced the combined stresses of anthropogenic nutrient enrichment, droughts, and, since 1996, elevated tropical storm and hurricane activity. These distinct perturbations provide excellent research opportunities for examining the impacts of both anthropogenic and natural stressors on phytoplankton community structure at the taxonomic group level, a level pertinent to ecological (successional), biogeochemical (C, N and P flux), and food web (edibility, toxicity) changes. The effects of shifting hydrologic conditions on phytoplankton taxonomic groups are illustrated for a mid-river long-term monitoring location in the NRE (Fig. 4). Seasonal and/or hurricane-induced variations in river discharge and the resulting changes in flushing rates appear to have differentially affected taxonomic groups as a function of their contrasting growth characteristics. For instance, the contribution of chlorophytes, cryptophytes, and diatoms to the total Chl *a* pool coincided, and was therefore enhanced by periods of elevated river flow. It is hypothesized that these effects are due to the efficient growth rates and enhanced nutrient uptake rates of these groups. Cyanobacteria, however, demonstrated greater relative biomass when flushing was minimal and residence times were longer, specifically during the summer.

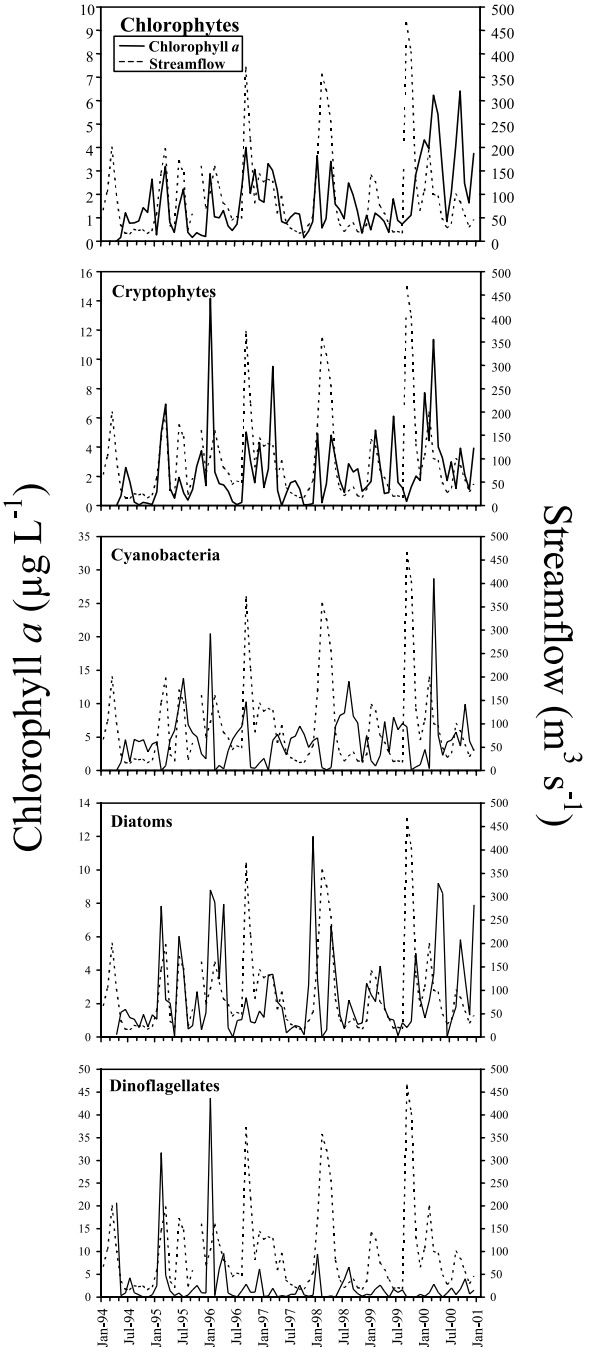

Fig. 4. Contribution of phytoplankton functional groups to total chlorophyll *a* during 1994 through 2000 as determined from ChemTax analyses of HPLC pigments. Values represent monthly means of bi-weekly surface samples collected from Station 120 in the NRE. Corresponding streamflow values (dotted line) were measured at the USGS stream gauge at Kinston (Station 2089500). Streamflow values represent monthly averages of daily mean streamflow.

Further evidence that changes in hydrologic conditions have significantly altered phytoplankton community structure is provided by the observed historical trends in dinoflagellate and chlorophyte abundance (Figs. 4 and 5). Both decreases in the occurrence of winter–spring dinoflagellate blooms and increases in the abundance of chlorophytes coincided with the increased frequency and magnitude of tropical storms and hurricanes since 1996. The relatively slow growth rates of dinoflagellates may have led to their reduced abundance during these high river discharge events. These results indicate that phytoplankton composition has been altered since 1994 in conjunction with hydrologic changes (Figs. 4 and 5). These changes in phytoplankton community structure could have potentially altered trophodynamics and nutrient cycling in the NRE during these years.

2.2. *Phytoplankton bloom dynamics and 'pink oysters' in Galveston Bay, Texas*

Galveston Bay (GB), Texas is the second largest estuary (1554 km^2) in the Gulf of Mexico. The bay is shallow ($\sim$2 m) and receives freshwater inputs from the Trinity (83%) and San Jacinto (8%) Rivers. Freshwater and nutrient inputs from the Trinity River extend well into Trinity Bay and GB, especially during periods of high river discharge in the spring [17]. Nitrate concentrations are inversely correlated with salinity and benthic regeneration of P leads to a PO_4^{-3} maximum in late summer [17,18]. Even though this system drains a highly industrialized region (Houston), heavy metal concentrations are not elevated over other Gulf estuaries [19]. As with many other North American estuaries, elevated nutrient loading is the main symptom of cultural eutrophication.

A bi-weekly sampling program collects data on water quality parameters, including nutrient concentrations and phytoplankton dynamics, at seven locations in GB and Trinity Bay (Fig. 6). The most common algal groups in GB were diatoms, cyanobacteria, chrysophytes, and cryptophytes [20,21]. Dinoflagellates, chlorophytes, and euglenoids were occasionally abundant, but on an annual basis were minor components of the phytoplankton community. Periodic blooms of large diatom species (*Rhizosolenia*, *Coscinodiscus*) have occurred during the summer. Nutrient addition bioassays have consistently shown that the phytoplankton community is N-limited [20,21]. In these bioassays, all major microalgal groups showed significant increases in biomass in response to the addition of NO_3^-. Either diatoms or cyanobacteria showed the largest response (in terms of biomass increase), depending on the time of year and the initial community composition. P or Si limitations have not been detected [20].

GB supports a large and commercially important fishery for the Eastern oyster (*Crassostrea virginica*), with annual harvests of near 400 metric tons [22]. Phytoplankton are a primary food source for oysters and the algal species that comprise the phytoplankton community vary in nutritional quality. Texas oystermen have recently expressed concern over the peculiar red/pink coloration of oysters ('pink oyster') from some commercial reefs in GB. Although the conspicuous color has no immediately apparent effect on oyster condition and is not known to pose a human health

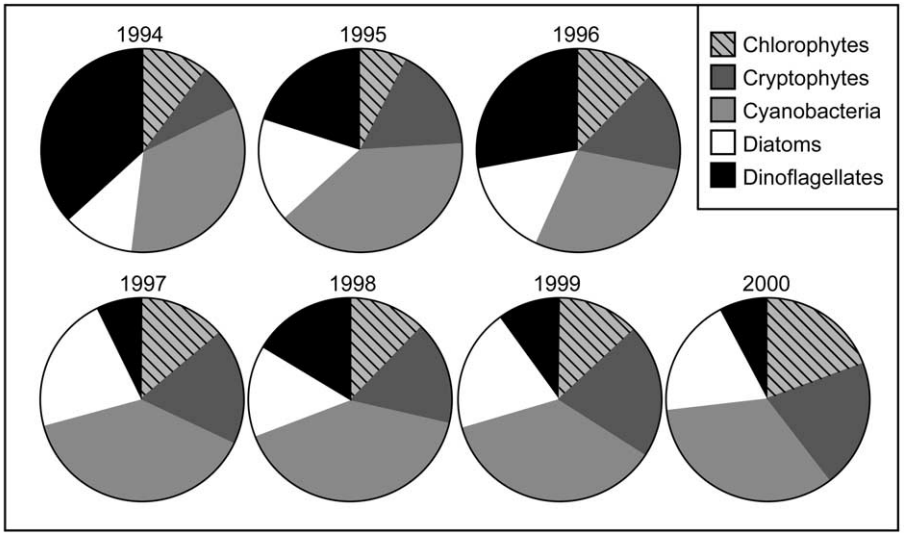

Fig. 5. Average phytoplankton composition per year at Station 120 in the NRE. Values represent surface phytoplankton assemblages.

hazard, the coloration adversely affects consumer acceptance of GB pink oysters. In addition, these oysters reportedly have an 'off-taste' that further detracts from their marketability. The magnitude and frequency of pink oyster events seem to be increasing in GB, suggesting that this is a growing problem.

Preliminary field and laboratory evidence suggests that this coloration may be caused by the phytoplankton upon which the oysters feed. Accessory photosynthetic pigments (carotenoids, phycobilins) from the algae may accumulate in the stomachs and livers of oysters following filter feeding, leading to the red-pink coloration.

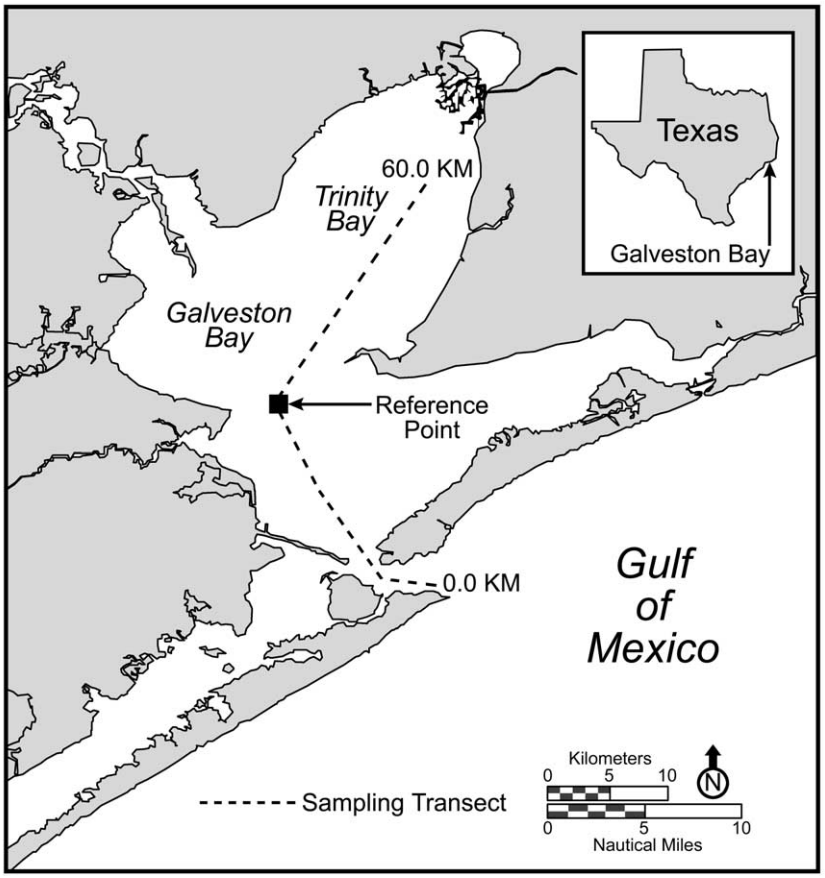

Fig. 6. Map of Galveston Bay, Texas. The dashed line down the center of the bay indicates the sampling transect (from 0.0 to 60.0 km) used to construct the spatio-temporal contour plots in Figs. 7 and 8. The Reference Point shows the location of several large commercial oyster reefs in the bay where pink oysters have been collected.

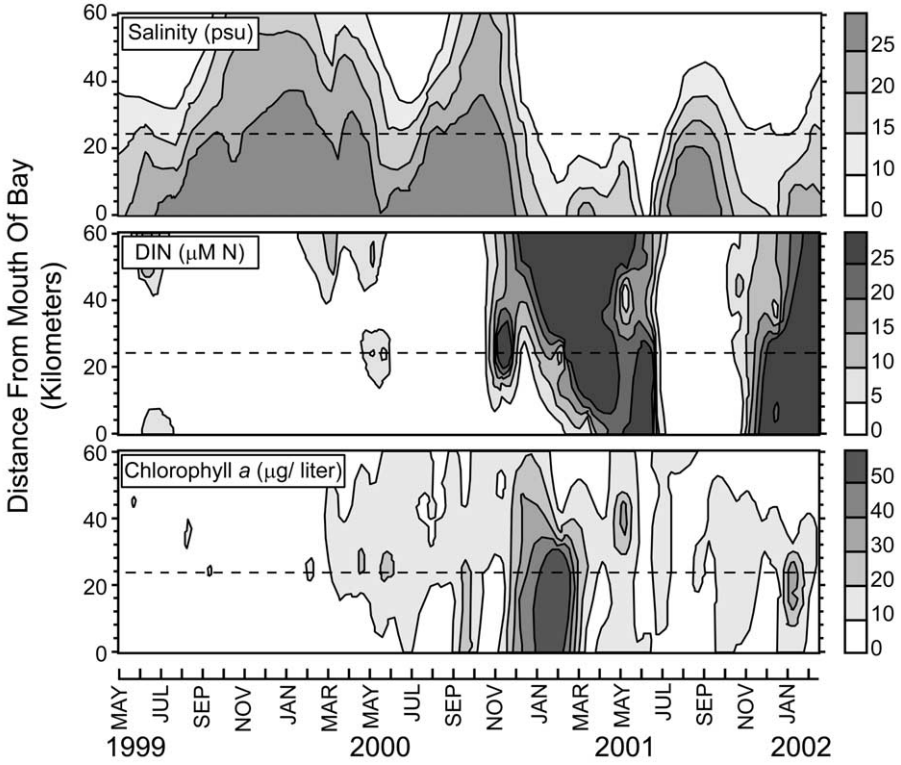

Fig. 7. Spatio-temporal contour plots of salinity, total dissolved inorganic nitrogen (DIN), and total Chl *a* (phytoplankton biomass) along the transect shown in Fig. 6. The horizontal dashed line indicates the location of the reference point shown in Fig. 6.

During the December 2000 pink oyster event, we obtained frozen samples of 'normal' and 'pink' oysters. The gut contents of both oyster types were analyzed by HPLC to determine if there were obvious differences in the phytoplankton groups present in the oyster guts. Although the analysis of gut pigments was qualitative, a comparison between the two oyster types revealed a higher concentration of peridinin in the guts of pink oysters relative to normal oysters. Peridinin is a bright red accessory pigment common in dinoflagellates [13–15]. Microscopic examinations of water samples during this period revealed that the most abundant dinoflagellate was *Prorocentrum minimum*. Therefore, the evidence suggested that the coloration could be due to this dinoflagellate. However, gut pigment analysis can be misleading because pigment degradation rates differ depending on the type of pigment and chemical conditions within the gut during digestion. Cryptophytes, which also have red accessory pigments (water-soluble phycoerythrin), were also present in high abundance during December 2000 and small amounts of alloxanthin (the indicator carotenoid pigment for cryptophytes) were detected in gut contents. Although the HPLC method used for these analyses cannot detect phycoerythrin, the presence of alloxanthin in the oyster guts does suggest that the oysters were grazing on the phycoerythrin-containing cryptophytes.

An examination of the water quality conditions and phytoplankton community composition over a 3-year period (1999–2001) offered useful insights into potential causal mechanisms for the occurrence and magnitude of pink oyster events. The salinity was relatively high in GB during the fall and winter 1999 (Fig. 7). A tropical storm in May 2000, high rainfall, and subsequent freshwater input from the Trinity River in late September 2000 resulted in lower salinities within the bay. Similarly, high rainfall in September–October 2001 lowered salinities. Riverine freshwater inputs resulted in elevated concentrations of dissolved inorganic nitrogen in excess of 25 μM N and fostered phytoplankton blooms with the bay (Fig. 7). The location of these blooms overlapped with the commercial oyster reefs in the central region of GB.

Phytoplankton community composition was determined using HPLC/ChemTax [15] (Fig. 8, Table 1). Cryptophytes and peridinin-containing dinoflagellates were the most abundant phytoplankton groups present when pink oysters were harvested. A comparison of the spatio-temporal distributions of cryptophytes and dinoflagellates suggests that cryptophytes were the primary contributor to the pink coloration of oysters. The timing of cryptophyte blooms and the occurrence of pink oysters seem to be more closely linked than for dinoflagellates and pink oysters. The dinoflagellate blooms may be linked to the cryptophyte blooms because the cryptophytes provide an abundant food source for the mixotrophic dinoflagellate *P. minimum* (the major dinoflagellate species in these blooms) [23]. Assuming a conservative oyster filtration rate of 1 ml water s^{-1}, and a cryptophyte biomass of 4 μg l^{-1} (the lower threshold concentrations in Fig. 8),

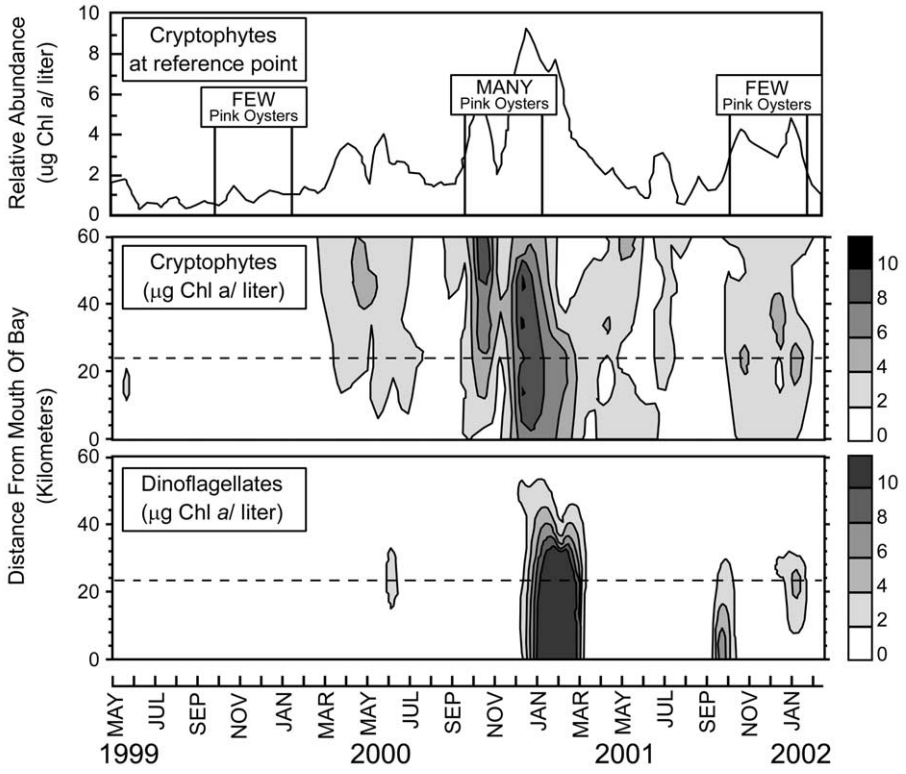

Fig. 8. Spatio-temporal contour plots of the relative abundance of cryptophytes and dinoflagellates along the transect shown in Fig. 6. The horizontal dashed line in the lower two panels indicates the location of the reference point shown in Fig. 6. The graph in the top panel illustrates the relative abundance of cryptophytes at the reference point in Fig. 6, the time period when pink oysters occur, and the prevalence of pink oysters during the 3 years.

an oyster would consume ca. 350 µg of cryptophyte biomass (in Chl *a* units) per day. This amount of material is more than sufficient to produce the pink coloration seen in the oysters. More intense cryptophyte blooms, such as those during the winter 2000–01, would likely produce more highly colored oysters and make the phenomenon more noticeable to harvesters and consumers. Thus, the field data are consistent with reported variability in pink oyster prevalence over the 3-year period.

These observations illustrate the applicability and underscore the importance of routine phytoplankton monitoring, supplemented by diagnostic microbial tools, for understanding the linkages between system-level 'driving' features (i.e., nutrient enrichment, phytoplankton blooms) and the 'health' of oysters, a key fisheries resource for GB and other estuaries of the Gulf of Mexico and Atlantic coasts.

2.3. *Eutrophication and cyanobacterial blooms in the St. Johns River, Florida*

The St. Johns River (SJR) is the largest river system in Florida (Fig. 1) and typifies US southeastern riverine–estuarine waters experiencing elevated nutrient loading from agricultural, urban, and industrial development in its watershed. The combination of reduced flushing and

Table 2
Phytoplankton community response to nutrient additions in a bioassay conducted on the St. Johns River Estuary

	Control	N	P	N+P
Primary productivity	48 ± 8	98 ± 14	115 ± 23	180 ± 26
Nitrogenase activity	123 ± 45	67 ± 35	543 ± 118	636 ± 130
C. raciborskii	5 921	13 645	8 980	13 903
C. raciborskii (0H)	1 030	2 317	97	515
C. raciborskii (1H)	2 703	9 912	1 738	3 347
C. raciborskii (2H)	2 188	1 416	7 145	10 041

The top rows show the response of phytoplankton primary productivity (^{14}C bicarbonate addition, µg C l^{-1} h^{-1}) and nitrogenase activity (acetylene reduction, nmol C_2H_4 l^{-1} h^{-1}) to additions of N, P and N+P. Values are means of five replicates and error is one S.D. The bottom rows show the response of *C. raciborskii* to nutrient additions in the bioassay in terms of total counts (trichomes ml^{-1}) and number of heterocysts per trichome. Trichomes were found with no, one, or two heterocysts (0H, 1H or 2H).

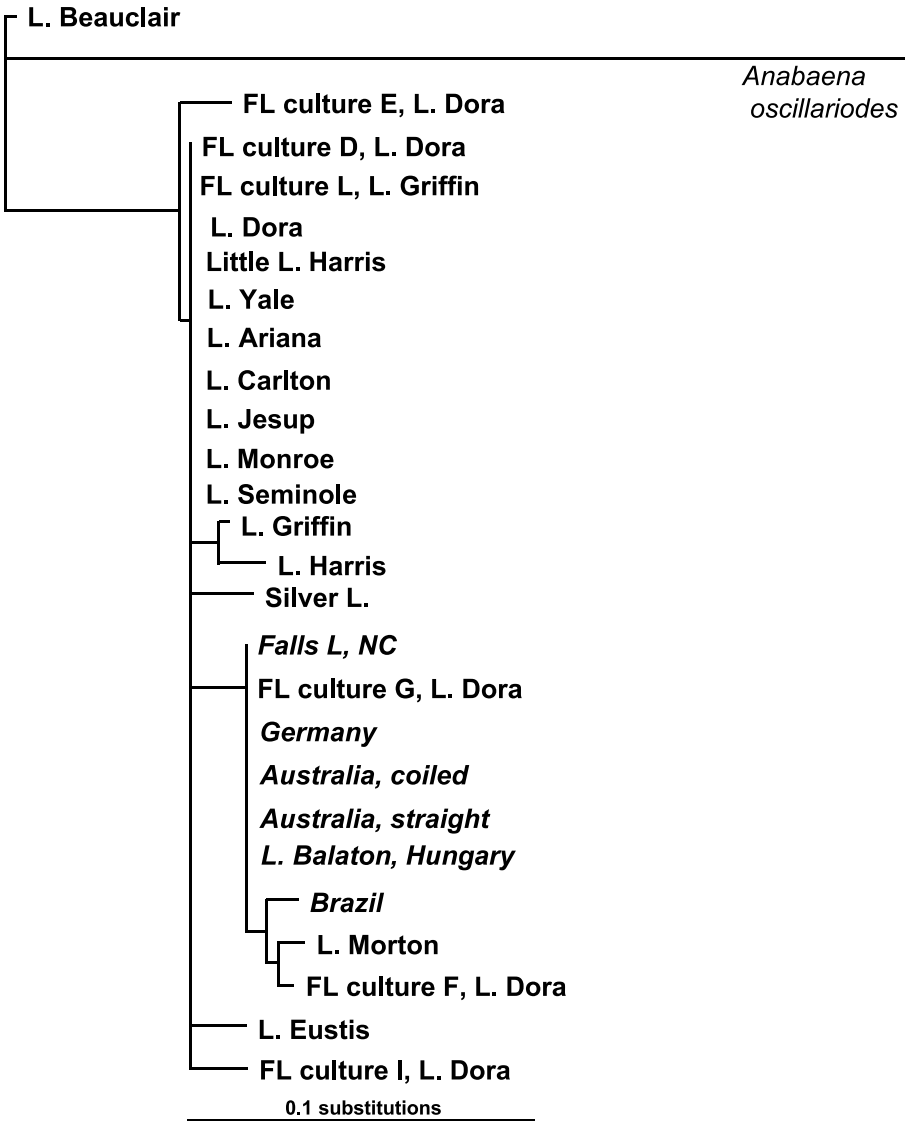

Fig. 9. Phylogenetic tree based on *C. raciborskii nifH* sequences for 14 Florida (FL) lakes. These sequences were compared to *C. raciborskii* cultures isolated from FL lakes as well as those from other parts of the world. *C. raciborskii* sequences from locations other than FL are noted in italics. Note that while most of the FL *C. raciborskii* sequences are nearly identical (as indicated by the lack of branching on the tree), there is genetic variability among some (i.e., Lakes Beauclair, Eustis and Morton). The phylogenetic tree was generated using the Dayhoff PAM matrix and neighbor-joining algorithm with PHYLIP software (University of Wisconsin Genetic Computer Group). *Anabaena oscillarioides* was used as the outgroup.

high nutrient inputs has created conditions suitable for expansion of opportunistic phytoplankton species, particularly nuisance cyanobacteria. N_2-fixing cyanobacterial bloom formers have proven particularly adept at taking advantage of these hydrologic and nutrient modifications and are indicators of eutrophication in these systems. Altering hydrologic and nutrient regimes result in changes in cyanobacterial dominance and bloom potentials, making these organisms useful indicators of water quality and environmental health. Our focus has been on *Cylindrospermopsis raciborskii*, a toxic cyanobacterium that is present in freshwater habitats throughout the world and has been implicated in a range of animal and human health issues [24]. This species is probably a relatively recent invader in the system, and during the past two decades it appears to have gained dominance over other non-nuisance cyanobacteria in the SJR and surrounding lakes. For this reason *C. raciborskii* is an attractive cyanobacterial indicator species for eutrophying waters.

In order to assess relationships between the native phytoplankton and changes in concentrations of growth-limiting nutrients, experimental nutrient manipulations were conducted in Lake George, a large lake in the headwaters of the St. Johns River (Fig. 1). Primary productivity at Lake George was stimulated most by the addition of both N and P, in comparison to the control in which no nutrients were added or N and P added individually (Table 2). However, N_2 fixation at Lake George was only stimulated by additions that included P (Table 2). Because P availability often limits the growth of N_2-fixing organ-

isms in freshwater lakes [25–27], reducing available levels of both N and P is necessary to prevent blooms of these cyanobacteria.

Cell counts of the heterocystous filamentous N_2 fixer *C. raciborskii* showed the highest increase in cells ml^{-1} in response to the additions of N and N+P, though the P addition still resulted in an increase in cell numbers over the control (Table 2). Additionally, the number of heterocysts per *Cylindrospermopsis* filament increased in the treatments that included P (Table 2), indicating that there was an increase in the potential for the community to fix N_2 when P was added. N_2 fixation may afford a competitive advantage in low N:P environments, allowing *C. raciborskii* to dominate the phytoplankton in estuaries experiencing these nutrient ratio addition scenarios.

In addition to microscopy, molecular techniques can be used to detect an indicator species in a mixed phytoplankton community, compare morphologically identical species and strains, and analyze the similarity of populations found at different locations. To analyze the local genetic variability among populations of *C. raciborskii* within this region, a survey of lakes within the St. Johns River Water Management District (www.sjrwmd.com) was conducted. The *nifH* gene (324-bp fragment), which is part of the gene cluster encoding the N_2 fixation enzyme nitrogenase [28], was chosen for this analysis because of its previously demonstrated utility for differentiating cyanobacterial populations and strains [29–31]. Polymerase chain reaction (PCR) primers designed to specifically amplify the *nifH* gene from *C. raciborskii* and not from other N_2 fixers [31] were used. We used these species-specific primers to look for presence of *C. raciborskii* in 16 Florida lakes that varied in size (<1 to >10 km in diameter), with and without visible cyanobacterial blooms. Of these, *C. raciborskii* was detected in all but two lakes. Within each lake, the percent similarity between *C. raciborskii nifH* genes sequenced was 97.7–100% (Fig. 9). Many lakes had genetically identical *C. raciborskii* strains, as is evidenced by the lack of branching among the *nifH* sequences from these lakes on the phylogenetic tree (Fig. 9). Most of the Florida *C. raciborskii* strains clustered separately from those originating in other parts of the world. The distinctiveness of *C. raciborskii nifH* sequences from Florida as well as the apparent higher genetic diversity among strains originating from Florida lakes in comparison to strains from Australia or Europe has also previously been noted [31]. This close clustering of most of the Florida *C. raciborskii* strains might suggest that these were a distinct population, but there are also a few *C. raciborskii nifH* sequences that have a high similarity to cultured isolates in other parts of the world (Brazil, Australia) and other parts of the USA (Falls Lake, North Carolina). The criterion for differentiating populations is not well defined, though one suggestion in another N_2-fixing cyanobacterial bloom-former, *Nodularia*, is that a sequence dissimilarity of more than 1% may indicate separate morphospecies [32]. Based on the analysis of just one gene, it is difficult to determine whether the *C. raciborskii* in these Florida lakes are indeed distinct populations or natural variations within a single population spread to different lakes. Further analysis is also necessary to identify the factors controlling the distribution of individual populations or strains. However, these data demonstrate the utility of using molecular methods of detection and characterization for target nuisance species such as *C. raciborskii*. Use of additional functional genes, such as ones regulating toxicity, will be useful in future studies.

2.4. Microbial indicators of water quality in the Southern California Bight

The Southern California Bight (SCB) is an open embayment that stretches from Point Conception, CA, USA to Cabo Colnett, Baja California, Mexico. The SCB is an important and unique ecological resource, world renowned for its recreational waters that attract more than 175 million visitors per year, and contains diverse habitats harboring a wide range of fish and invertebrate species. The SCB is also one of the most densely populated coastal regions in the USA, and the system is stressed due to the discharge of sewage effluents, non-point source contamination (stormwater and agricultural runoff), fishing and habitat modification, atmospheric deposition, and chemical contamination. Over 3 million US dollars are spent annually to monitor microbiological water quality in the SCB [33,34]. Currently implemented routine monitoring programs are designed primarily to protect the health of swimmers and surfers, and to provide important information about contamination from known discharges and effluents. However, the sampling sites of these programs tend to be known 'hot spots' or sites chosen because of their proximity to a known source of contamination. Site-specific routine monitoring of microbiological water quality does not lend itself to important larger-scale regional assessments, which are useful to determine overall deterioration of the resource. The Southern California Coastal Water Research Project (SCCWRP) has conducted several studies that provide an integrated assessment of the SCB by combining regional-scale monitoring program with analysis for a variety of biomarkers. The studies were conducted to examine environmental microbiology, water quality and coastal ecology (benthic toxicity and fish biology) [34,35].

One of the most important departures of the SCCWRP-conducted monitoring studies from other previously conducted studies was our regional assessment approach; we estimated the aerial extent of ecological change rather than assessing average conditions. To accomplish this, a rigorous sampling scheme was applied to the entire SCB (1000 km of coastline) to study fish biology, sediment toxicity, benthic health, and bacteriological and viral water quality. Using this approach for water quality assessment, it is

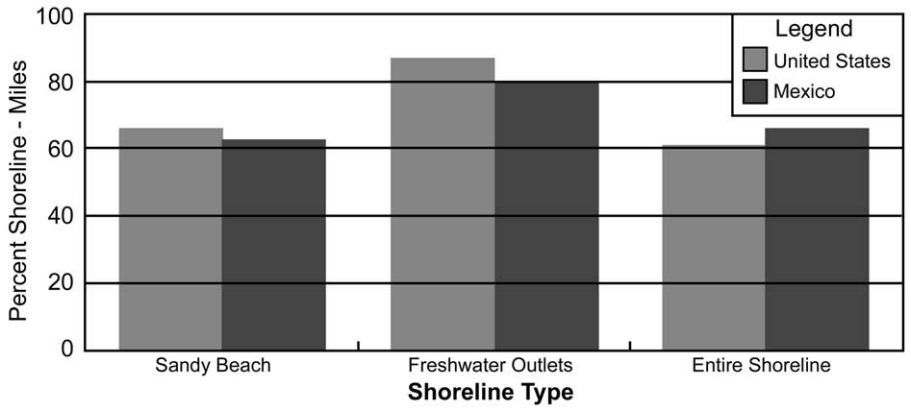

Fig. 10. Comparison of the percentage of shoreline-miles failing the State of California enterococci water quality standard (>104 MPN or cfu/100 ml) between the USA and Mexico after a large storm event (>2.5 cm precipitation over entire region).

possible to determine the area of the SCB that fails to meet water quality standards, important information for local policy decision-makers (see www.sccwrp.org). One advantage of estimating aerial extent is that it can be used as a tool to detect and delineate trends spatially. If conditions in the SCB (or other water bodies) change over time such that some areas improve and others worsen, the average condition might not change. By estimating the aerial extent of alteration, we are better able to assess ecosystem-scale change. Measuring multiple bacterial indicators permitted identification of the most responsive taxa, provided preliminary information necessary for risk assessment, and helped to develop efficient strategies for protecting the environment and human health.

For the microbiological water quality measurements, a stratified-random sampling scheme permitted an aerial assessment of the portion of the coastline (our metric is termed 'shoreline-miles') that either met or violated bacteriological water quality standards, currently used for management of beach closures [36]. Levels of bacterial indicators were used to provide aerial assessments of the extent of human perturbation of the coastline due to the presence of fecal contamination along the coast. During dry weather it was determined that 90–95% of the shoreline-miles exhibited 'good' microbiological water quality (met State of California water quality criteria) [37,38]. Violations of bacterial indicator thresholds were mostly present at sites where freshwater outlets (storm drains, rivers, and creeks) contributed fecal bacteria to the shoreline [37,38]. Furthermore, it was demonstrated using viral indicators of human fecal contamination, molecular methods, and a randomized sampling design that 50% of the freshwater outlets contained indicators of human fecal contamination [39]. During wet weather, however, over 50% of the coastline exhibited 'poor' water quality [40]. An example of the usefulness of this type of regional microbiological water quality assessment can be seen by comparing the magnitude of impact along the entire SCB, where we determined the percentage of shoreline-miles that exceeded State of California water quality standards for the bacterial indicator, enterococci. Using this regional assessment approach during a large storm event, we demonstrated that there was no significant difference between the percentage of shoreline-miles that exceeded standards along the shoreline of Mexico and the USA, regardless of the site type (sandy beach, freshwater outlet, or entire shoreline, Fig. 10 [40]). This result was interesting because previously conducted regional studies demonstrated a significant difference between the water quality of Mexico versus US beaches during dry weather [40]. Results of the regional study conducted during wet weather demonstrated that the entire shoreline was negatively impacted by the contaminated surface runoff from the freshwater outlets, regardless of whether the samples were from US or Mexican waters.

3. Advances in aquatic microbial detection and characterization studies: what does the near future look like?

Both the expansion of potentially harmful, coastal microbial populations and increased environmental impacts from anthropogenic activities necessitate improved protection of coastal and estuarine water bodies. To that end, the past few years have seen an impressive level of development and application of new technologies that will potentially enable real-time microbiological assessment of aquatic systems. There are a few advancements that hold promise for near real-time and quantitative detection of microbial indicators in environmental samples, such as DNA microarrays, PCR-based methods, and immunological methods. Development and application of these methods for the future will enhance our ability to conduct further regional assessments of the impacts of anthropogenic influence along our coastlines, while at the same time protecting the health of those using the waters for recreation.

Recently, there has been a rapid expansion of new technologies that will enable real-time assessment of aquatic systems. Among these is the promise of real-time, quantitative microbial indicators, especially in the field of water quality. Microarrays and quantitative PCR (Q-PCR) are two examples of new molecular approaches that show promise in aquatic ecology studies. DNA microarrays are slides to which complementary sequences of DNA are attached in an orderly arrangement. Hybridization of fluorescently labeled mRNAs or DNA from the environmental samples to microarrays indicates the presence of genes or cell types of interest, with the level of fluorescence indicating the quantity of the genes or cells. The absence of hybridization indicates that the cell, indicator, or pathogen of interest is not present. One of the advantages of microarrays is that researchers can probe for hundreds of different genes, as opposed to older techniques (such as PCR) that allowed the researcher to only detect one or a few genes at a time. Microarrays represent a potentially significant technology that can be used to detect multiple types of indicator organisms and/or pathogens from a single water sample. Their prior use has been primarily within the biotechnology sector, and they are not often used to assay environmental samples. However, recent work has proven fruitful in using DNA microarrays to detect indicator organisms, pathogens, and specific genes in aquatic environments. DNA microarrays can also provide the researcher with the ability to incorporate discrimination of cell activity via mRNA analysis. Current application of DNA microarrays includes the development of portable systems for near real-time detection of bio-indicators and pathogens [41].

Q-PCR is a novel primer-based molecular technique that combines the specificity of traditional PCR with the quantitative measurement of fluorescence for determining the presence of specific types of nucleic acid in environmental samples. Q-PCR employs dual-labeled oligonucleotide probes, e.g., Molecular Beacons™, which bear a 5′-fluorescent reporter dye and a 'dark' quencher group in the 3′-position. The probe has a hairpin loop structure designed to specifically hybridize to a unique target sequence. When the probe hybridizes to its target sequence, the hairpin structure is disrupted and the 5′-reporter is physically separated from the 3′-quencher, allowing fluorescence emission to be detected and measured quantitatively [42]. Brunk et al. [43] utilized Q-PCR to detect levels of *Bacteroides* sp. in environmental samples from the SCB in order to determine the presence of human fecal contamination.

Having these highly relevant diagnostic molecular techniques at our fingertips begs the exciting prospect and challenge of applying them to real-time, in situ monitoring. Most microbial indicator techniques being developed and tested can be readily incorporated in routine water quality monitoring programs, and can serve as stand-alone approaches, requiring no sophisticated sampling or preparatory steps prior to analyses. As such, these indicators can be piggybacked on existing monitoring protocols, including discreet sampling, platform-mounted or submersed, flow-through sampling.

In North Carolina's PS system some of these novel techniques are being adapted for ferry-based monitoring using Department of Transportation ferries as ships of opportunity for continuous water quality monitoring [44]. This program, 'FerryMon' (www.ferrymon.org), has been designed to: (1) assess and predict the relationships between human nutrient and other pollutant inputs, algal blooms and associated water quality changes, and ecosystem response, (2) provide critical information to long-term water quality and fishery management, and (3) develop FerryMon as a US national model for real-time assessment of coastal water quality. FerryMon is capable of collecting water samples for nucleic acid analysis, including *nifH* for examining the diversity of N_2-fixing microorganisms and other functional genes involved in critical N cycling, i.e., denitrification (*nosZ*) and nitrification (*amoA*). In addition, we are interested in detection of both enteroviruses and *Enterococcus* spp., two important indicators of human fecal contamination.

Aquatic microbial ecology is on the forefront of developing and applying a new generation of indicators of environmental stress and ecological change. Of particular promise is the 'arsenal' of molecular and chemotaxonomic identification, quantification and characterization (i.e., species-specific rate measurements, biomass-specific rates of production and nutrient transformations) techniques. Progress is being made in utilizing these techniques on the ecosystem scale, the scale at which human and climatic perturbations often coincide and interact. Complementary use of these techniques in dynamic estuarine and coastal environments offers great promise in ensuring their success as qualitative and quantitative indicators suitable for a wide variety of ecological applications. For example, using diagnostic photopigments together with nuisance taxa-specific molecular identification techniques ensures linkage to basic measurements such as phototrophic biomass (Chl *a*) made worldwide. This represents a significant conceptual and technical breakthrough for researchers, managers and decision-makers and their ability to delineate the ecological effects of human and climatic perturbations.

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