

ECOHAB

The Ecology and Oceanography of Harmful Algal Blooms

A National Research Agenda

Preface

For many years, the United States has struggled to manage fisheries resources and marine ecosystems impacted by an expanding array of toxic and harmful algae. Our understanding of the fundamental ecological, toxicological, and oceanographic issues underlying these phenomena, however, is woefully inadequate. In recognition of this shortcoming, the National Science Foundation (NSF) and the National Oceanic and Atmospheric Administration (NOAA) co-sponsored a workshop on the Ecology and Oceanography of Harmful Algal Blooms at the Snow Mountain Ranch Conference Center, CO from 23-28 August 1994. This research agenda is the result of those deliberations. The forty workshop participants (Appendix A) included academic and government scientists as well as program managers and officials representing the NSF, NOAA Coastal Ocean Program, Sea Grant, and the National Marine Fisheries Service. The scientists attending the workshop were selected from among nationally recognized leaders in fields spanning both the biological and physical sciences. The diverse composition of the group (Appendix A) reflects the interdisciplinary nature of this subject as well as the need to solicit recommendations from the oceanographic community at large.

Position papers were prepared and distributed prior to the workshop so all participants would be familiar with the issues and questions associated with HABs. Selected participants were asked to present summaries of issues reflecting their area of expertise and geographical focus. Specific theme topics fell under the general categories of: *Physiology, Biochemistry and Genetics*; *Food-web/Community Interactions*; *Nutrients and Eutrophication in the Coastal Environment*; *Physical/Biological Interactions*; and, *Emerging Technologies*. Following these presentations and extensive plenary discussions, participants were divided into working groups and charged with identifying research priorities, approaches and essential technologies in three theme areas: *The Organisms*, *Environmental Regulation of Blooms*, and *Food-Web/Community Interactions*. Alternating between working group sessions and plenary discussions, research issues and priorities were refined for each of these program elements.

This national research agenda is being widely circulated to individual scientists, agency personnel, government officials and science administrators. Feedback is welcome at all stages and on all aspects of this planning process.

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Donald M. Anderson, Workshop Chair

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Executive Summary

Over the last several decades, the United States has experienced an escalating and worrisome trend in the incidence of problems associated with harmful and toxic algae. Impacts of these phenomena include mass mortalities of wild and farmed fish and shellfish, human illness and death from contaminated shellfish or fish, death of marine mammals, seabirds, and other animals, and alteration of marine habitats or trophic structure through shading, overgrowth, or adverse effects on life history stages of fish and other marine organisms. Formerly only a few regions were affected by harmful algal blooms (HABs) in scattered locations, but now virtually every coastal state is threatened, in many cases over large geographic areas and by more than one harmful or toxic species.

It is still a matter of debate as to the causes behind this expansion, with possible explanations ranging from natural mechanisms of species dispersal to a host of human-related phenomena such as nutrient enrichment, climatic shifts, or transport of algal species via ship ballast water. Whatever the reasons, virtually all coastal regions of the U.S. are now subject to an unprecedented variety and frequency of HAB events. The U.S. is not alone in this respect, as nations throughout the world are faced with a bewildering array of toxic or harmful species and impacts and disturbing trends of increasing incidence.

HAB events are characterized by the proliferation and occasional dominance of particular species of toxic or harmful algae. As with most phytoplankton blooms, this proliferation results from a combination of physical, chemical, and biological mechanisms and interactions that are, for the most part, poorly understood. Some HABs are unique, however, due to their production of toxins and the manner in which they affect co-occurring organisms and alter food-web function. As identified in *Marine Biotoxins and Harmful Algae: A National Plan* (Anderson et al., 1993), focused research into these ecological and oceanographic mechanisms is urgently needed. To achieve these ends, a workshop was co-sponsored by NSF and NOAA to develop a national research agenda to guide activities in the specific area of HAB ecology and oceanography.

The resulting program, called ECOHAB (ECology and Oceanography of Harmful Algal Blooms) provides a scientific framework designed to increase our understanding of the fundamental processes underlying the impacts and population dynamics of HABs. This involves a recognition of the many factors at the organismal level that determine how HAB species respond to, and potentially alter their environment, the manner in which HAB species affect or are affected by food-web and community interactions, and how the distribution, abundance, and impact of HAB species are regulated by the environment.

In its simplest form, the goal of the ECOHAB program is:

To develop an understanding of the population dynamics and trophic impacts of harmful algal species which can be used as a basis for minimizing their adverse effects on the economy, public health, and marine ecosystems.

The objective of the ECOHAB program is:

To investigate fundamental physical, biological, and chemical oceanographic questions critical to scientifically based management of fisheries resources, public health, and ecosystem health in regions threatened by toxic and harmful algae.

ECOHAB is thus a scientific program that addresses important societal issues through advances in fundamental or basic research. The research priorities identified at the workshop fell naturally into three main themes that represent the individual program elements of ECOHAB. The rationales, goals, and specific objectives and activities of each program element are listed below.

Program Element # 1

The Organisms

Rationale: The negative impacts of HABs reflect not only the growth and metabolism of individual algal cells, but the ecological selection of those cells within a diverse phytoplankton assemblage. Studies at the organismal level are essential if we are to understand the population dynamics of HABs and their toxic and/or harmful effects.

Goal: To determine the physiological, biochemical, genetic, and behavioral features and mechanisms of harmful algal species that influence their bloom dynamics, general ecology, and negative impacts.

Specific objectives:

- Develop methods to rapidly and accurately identify, enumerate, and physically separate HAB species from mixed phytoplankton assemblages.

- Identify the life history stages of major HAB species, determine what factors control transitions between those stages, and establish the role of the stages in bloom dynamics.

- Characterize the physiological responses and tolerances of HAB species to differing environmental conditions.

- Develop methods to permit in situ measurements of species-specific rates of growth, photosynthesis, and nutrient uptake,

and to assess the physiological condition of cells at different times and locations.

- Characterize the nutritional requirements, uptake and nutrient assimilatory characteristics of HAB species.

- Determine the functional role of toxins and/or exudates produced by HAB species.

- Define the genetic basis of toxin production, elucidate toxin biosynthetic pathways, and determine how toxin accumulation in cells is regulated.

- Investigate the mechanisms and importance of motility and other behaviors of HAB species.

Program Element # 2

Environmental Regulation of Blooms

Rationale: Concurrent with escalating influences of human activities on coastal ecosystems, the environmental and economic impacts of HABs have increased over recent decades. It is therefore

imperative to know if present trends of human activities and HABs will lead to unacceptable consequences, and if the means can be developed to mitigate negative impacts. The key to this knowledge is an understanding of the ecology and oceanography of HABs. An important facet of this complex topic is environmental regulation, that is, the influence of environmental factors on the population dynamics of harmful algal species and their competitors.

Goals: 1) Determine and parameterize the environmental factors that govern the initiation, growth, maintenance, dissipation and impacts of HABs; and 2) Formulate principles that explain similarities between ecosystems during HABs and understand how those systems are unique with respect to the types of blooms that occur.

Specific objectives:

- Determine the extent to which HAB events reflect increases in growth rate versus physical transport, immigration, and accumulation. Determine whether there is a specific suite of physical factors with which known HABs are associated.
- Investigate physical and ecological processes that control the partitioning of nutrients within a system and the relationship between nutrient inputs and population dynamics of HAB species.
- Investigate whether there are specific physical, chemical, and biological regimes or processes that are associated with HAB events.
- Determine whether some ecosystems are more susceptible to HABs than others. If so, determine what makes them unique and whether they share characteristics that can be used to anticipate HAB events in other systems.
- Characterize HAB population dynamics, including the rate processes required in predictive models of bloom incidence.

Program Element # 3

**Food-Webs and
Community Interactions**

Rationale: The negative impacts of HABs are the result of complex interactions that begin at the phytoplankton community level and extend to upper trophic level compartments. Habitat physics, life cycles, community structures, growth and grazing processes all combine to regulate the dynamics of the HAB event. Therefore, studies on the impacts of trophic interactions in the selection and dynamics of HABs, and conversely, the impacts of HAB events on trophic structure, processes and interactions are essential if we are to understand the ecology and oceanography of harmful algal blooms .

Goals: 1) Determine the impacts of trophic interactions on selection for, and dynamics of, HABs; and 2) determine the impacts of HABs on trophic structure, processes and interactions.

Specific objectives:

- Determine the extent to which bloom formation results from a breakdown of grazing or from harmful species outcompeting other phytoplankton for limiting resources.
- Determine whether biological controls (e.g., grazers, allelopathy, pathogens) are the cause of bloom termination.
- Investigate how HAB effects on the food-web are controlled by toxin dynamics, food-web routing of toxins, and the differential susceptibility of species at higher trophic levels. Determine whether chronic,

sublethal impacts of HABs are more significant than acute (lethal) impacts.

- Determine if HAB impacts are controlled by the degree of temporal and spatial overlap between blooms and critical life cycle stages of target species.
- Determine whether high biomass (non-toxic) HABs adversely impact the food-web directly through reduced food quality, or indirectly through environmental effects.

Program Implementation

The diverse nature of HAB phenomena and the hydrodynamic and geographic variability associated with different outbreaks throughout the U.S. pose a significant constraint to the development of a coordinated research initiative. Where other multi-investigator oceanographic research programs can concentrate field activities on one specific area of the ocean (e.g., GLOBEC on Georges Bank), no single ECOHAB study site could be identified that would permit all of the major biological and physical features that underly HAB phenomena to be investigated. Given this diversity, the ECOHAB program will rely on comparisons among large-scale, regional field programs, on laboratory and mesocosm studies by individual investigators or small groups, and on theoretical studies using new and existing models to provide realistic and testable simulations of HAB dynamics in different oceanographic systems. An integration of physical, chemical, and biological components is essential to all of these approaches.

The ECOHAB Research Agenda outlines research priorities that are intended to guide agencies in the efficient allocation of resources targeted to HAB issues, and to help them formulate new, multi-disciplinary HAB initiatives. The rate and extent of future progress will depend upon how the recommendations in this report are received and implemented. State and federal agencies should use this document to identify topics that they can support, and individual scientists should shape their specific research programs to meet the perceived needs of the HAB community.

Despite the focus on ecology and oceanography and the exclusion of many other aspects of HAB phenomena, the scope of the issues to be addressed by ECOHAB exceed the resources of any one agency or program. ECOHAB will be successful only if a nationally coordinated interagency effort can be implemented to focus research personnel, facilities, and financial resources to the common goals outlined in this comprehensive national strategy. Nowhere else do the missions and goals of so many government agencies intersect and interact as in the coastal zone where HAB phenomena are prominent. Every effort must be made to keep the program flexible, efficient and responsive to the needs of the agencies that become partners in this endeavor. As soon as the participating agencies and programs are identified, a Steering Committee will be appointed to oversee program implementation. Where necessary, small working groups or sub-committees will be convened to address specific program needs. Once ECOHAB is underway and research programs begin to accumulate results, regional and national workshops will be convened to identify common mechanisms and processes underlying the diverse array of HAB phenomena and their impacts. One of the strengths of ECOHAB lies in this "comparative approach," but resources must be allocated to facilitate the scientific communication that is required for successful implementation.

Rationale and Benefits

The significant economic, public health and ecosystem impacts of HAB outbreaks are strong, practical motivations for a research program such as ECOHAB, made all the more pressing by the apparently escalating trend in their incidence. The direct benefits to society from a research program of this kind are many, and include management issues such as bloom detection and prediction, control or mitigation strategies, site selection criteria for aquaculture, and assessment of impacts from altered nutrient loading, dredging or other coastal zone activities. There are indirect benefits as well. For example, support of multidisciplinary field HAB programs can address specific, practical problems while providing new techniques and basic scientific information relevant to plankton ecology and oceanography in general. In

this respect, one compelling aspect of the ECOHAB program derives from the need to study individual HAB species, rather than mixed planktonic assemblages. New autecological techniques must be developed, such as remote detection of bloom populations using satellites, swimming robots or moored instruments. Methods are needed to "tag" target species with molecular probes and then enumerate cells or separate them from co-occurring organisms, and techniques must be developed to estimate in situ growth rates or cell physiology. These are but a few examples of the areas where new technologies developed to meet the objectives of ECOHAB can benefit all of oceanography.

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1. Introduction

1.1 The Nature of Harmful Algal Bloom Phenomena

Among the thousands of species of microscopic algae at the base of the marine food chain are a few dozen which produce potent toxins. These species make their presence known in many ways, ranging from massive "red tides" or blooms of cells that discolor the water, to dilute, inconspicuous concentrations of cells noticed only because of the harm caused by their highly potent toxins. Blooms of non-toxic micro- and macroalgae (seaweeds) also cause harm due either to indirect effects of biomass accumulation (such as anoxia or habitat alteration) or to physical features (such as spines which lodge in fish gill tissue). Impacts of HAB phenomena include mass mortalities of wild and farmed fish and shellfish, human illness and death from contaminated shellfish or fish, death of marine mammals, seabirds, and other animals, and alteration of marine habitats or trophic structure.

The term "red tide" has been used to describe some of these phenomena, since in certain cases, microalgal species increase in abundance until they dominate the planktonic community and discolor the water with their pigments. The term is misleading, however, since non-toxic species can also bloom and harmlessly discolor the water or conversely, adverse effects can occur when algal cell concentrations are low and the water is clear. Furthermore, blooms of benthic or planktonic macroalgae can have major ecological impacts such as the displacement of indigenous species, habitat destruction, oxygen depletion, and even alteration of biogeochemical cycles. The causes and effects of macroalgal blooms are thus similar in many ways to those associated with harmful microscopic phytoplankton species. The scientific community now employs the term "harmful algal bloom" (HAB) to describe this diverse array of bloom phenomena.

1.2 HAB Impacts

1.2.1 Public Health and Ecosystem Effects

One major category of public health impact from HABs occurs when toxic phytoplankton are filtered from the water by shellfish such as clams, mussels, oysters, or scallops, which then accumulate the algal toxins to levels that are potentially lethal to humans or other consumers (Shumway, 1990). These poisoning syndromes are named paralytic, diarrhetic, neurotoxic, and amnesic shellfish poisoning (PSP, DSP, NSP, and ASP). Except for ASP, an alarming new syndrome that results in permanent short-term memory loss in victims, all are caused by biotoxins synthesized by a group of marine algae called dinoflagellates. The ASP toxin is produced by diatoms, a group of phytoplankton that until recently was considered free of toxins and generally harmless (Bates et al., 1989). A fifth human illness, ciguatera fish poisoning (CFP), is caused by biotoxins produced by epibenthic dinoflagellates attached to surfaces in many coral reef communities (reviewed in Anderson and Lobel, 1987). Ciguatera toxins are transferred through the food chain from herbivorous reef fishes to larger carnivorous, commercially valuable finfish. In a similar manner, the viscera of commercially important fish such as herring,

mackerel, or sardines are known to accumulate PSP toxins, endangering human health following consumption of whole fish. Whales, porpoises, seabirds and other animals can be victims as well, receiving PSP toxins through the food chain via contaminated zooplankton or fish (Geraci et al., 1989; Anderson and White, 1992). All of these poisoning syndromes occur within the U.S. and its territories.

Another HAB impact occurs when marine fauna are killed by microalgal species that release toxins and other compounds into the water (Box 1.2.1), or that kill without toxins by physically damaging gills or by creating low oxygen conditions as bloom biomass decays. These impacts frequently occur at aquaculture sites where caged fish cannot escape the harmful blooms. Farmed fish mortalities from HABs have increased considerably in recent years, and are now a major concern to fish farmers and their insurance companies. Wild fish, however, may also be affected. The list of finfish, shellfish and wildlife affected by microalgal toxins is long and diverse (Table 1) and accentuates the magnitude and complexity of this one manifestation of HAB phenomena. It does not, however, adequately document the true scale of that impact.

We are only now beginning to recognize that there can be impacts from toxic blooms in virtually all compartments of the marine food-web due to adverse effects on viability, growth, fecundity, and recruitment. As reviewed by Smayda (1992), toxins can move through ecosystems in a manner analogous to the flow of carbon or energy, and the impacts can thus be far-reaching and significant (Box 3.3.1). In this expanded context, it is evident that our present knowledge base is inadequate even to define the scale and complexity of many HAB phenomena.

Blooms of macroalgae (seaweeds) can also be harmful, especially to seagrass and coral reef ecosystems and the food-webs dependent on those habitats. Nuisance seaweed species replace indigenous macroalgae in the benthos and microscopic phytoplankton in the water column. They thus modify benthic habitats, affect microbial and macrofaunal foodwebs, and alter key biogeochemical features of coastal ecosystems. Because seaweeds are generally benthic organisms and inhabit inshore coastal waters that mark the interface between land and sea, they are often the first primary producers to be impacted by nutrient inputs from land. Indeed, increased nutrient supply seems to be implicated in virtually all harmful seaweed blooms. A dramatic example of the impact of macroalgal blooms was seen in Bermuda, where the green macroalga *Cladophora prolifera* formed widespread blooms of drifting, filamentous balls that overgrew seagrasses and corals in response to N and P enrichment from groundwater (LaPointe and O'Connell 1989). This bloom, which many consider to be the most dramatic ecological change in the recent history of Bermuda's waters, led to a dramatic decline in benthic species diversity, including the commercially-valuable calico clam.

1.2.2 Economic Impacts

The range of the economic impacts from HAB outbreaks and the magnitude of those costs have expanded with increasing public awareness, coastal development, and the growth of mariculture. Shellfish quarantines, wild or farmed fish mortalities, and frightened consumers who avoid seafood (including products which are totally safe) are well-known impacts of major blooms of harmful algae (Ahmed, 1991). Adverse health effects and lost sales of fish and shellfish products are direct costs, but constrained development or investment decisions in coastal aquaculture due to the potential for outbreaks of toxic algae are examples of indirect or hidden costs. Lost marine recreational opportunities are also important indirect costs of harmful algal bloom incidents. Unfortunately, no national estimate of the combined economic costs of HAB phenomena is available. Estimates from isolated, individual events provide some indication of the scale of the problem:

- A single PSP outbreak cost the state of Maine an estimated \$7 million in 1980 (Shumway et al., 1988). PSP outbreaks are annual events in Maine, and several have been more severe than the 1980 event.
- An NSP outbreak in 1987-88 closed more than 400 km of North Carolina coastline for shellfishing during the peak harvesting season, causing economic losses estimated at \$25 million (Tester et al., 1991)

- Brown tide outbreaks in 1985 and several succeeding years devastated the New York state bay scallop industry. Economic losses for the fishery were estimated at \$2 million per year (Kahn and Rochel, 1988). The brown tide has recurred on Long Island most years since 1985, and continues to have major ecosystem and economic impacts. At this writing, legislation is under consideration to have parts of Long Island declared Disaster Areas as a result of a massive 1995 outbreak.
- In 1987, phytoplankton blooms of the diatom *Chaetoceros convolutus* were linked to the mortality of 250,000 Atlantic salmon valued at over \$500,000 (Rensel et al., 1989). In other years, blooms of the flagellate *Heterosigma carterae* have caused farmed-fish mortalities in British Columbia and Washington state costing the industry \$4-5 million per year (Horner et al., 1991).
- PSP was detected in the rich shellfish beds of Georges Bank in 1989, forcing the closure of those offshore resources. The Georges Bank surf clam fishery alone, closed now for five successive years, has an estimated annual value of \$3 million (New England Fisheries Development Association).
- In 1917, the shellfish industry in Alaska produced 5 million pounds of product. Today, except for aquaculture, the state's commercial shellfish industry is virtually non-existent as a direct result of persistent product contamination by PSP (Neve and Reichardt, 1984). The value of the sustainable, but presently unexploited, shellfish resource in Alaska is estimated to be \$50 million per year.
- The Gulf coast of Florida experiences frequent red tides, often accompanied by dead fish washed up on beaches, NSP -contaminated shellfish, and human respiratory problems due to toxins aerosolized by the surf. Habas and Gilbert (1974) estimated a loss of \$20 million per event, including losses to the tourist industry, hotel/motel suppliers, commercial fisheries, and local governments for the expense of beach cleanup.
- Domoic acid in razor clams and Dungeness crabs in Washington and Oregon in 1991 caused economic losses estimated at \$15-20 million (T. Nosh, pers. comm.). Losses included reduced tourist trade, unemployment, reduced or delayed sales, lower prices, and bankruptcy for some commercial processors. Commercial oyster growers experienced declines in both sales and prices during the peak holiday period, although the oysters never contained detectable levels of domoic acid. Some losses continue as razor clam seasons are shortened or closed due to the continued presence of domoic acid in some areas.
- The states of Maine, New Hampshire, Massachusetts, Rhode Island, Connecticut, Florida, California, Oregon, Washington, and Alaska maintain annual shellfish monitoring programs to detect algal toxins in shellfish. The total cost of these programs exceeds \$1 million per year.

1.3 Recent Trends

The nature of the HAB problem in the United States has changed considerably over the last two decades (Box 1.3.1). **Where formerly a few regions were affected in scattered locations, now virtually every coastal state is threatened, in many cases over large geographic areas and by more than one harmful or toxic microalgal species.** Few would argue that the number of toxic blooms, the economic losses from them, the types of resources affected, and the number of toxins and toxic species have all increased dramatically in recent years in the United States and around the world (Anderson, 1989; Smayda, 1990; Hallegraeff, 1993). Disagreement only arises with respect to the reasons for this expansion. Possible explanations include: a) species dispersal through currents, storms, or other natural mechanisms; b) nutrient enrichment of coastal waters by human activities, leading to a selection for, and proliferation of, harmful algae; c) increased aquaculture operations which can enrich surrounding waters and stimulate algal growth; d) introduction of fisheries resources (through aquaculture development) which then reveal the presence of indigenous harmful algae; e) transport and dispersal of exotic HAB species via ship ballast water or shellfish seeding activities; f) long-term climatic trends in temperature, wind speed, or insolation; g) increased scientific and regulatory scrutiny of coastal waters and fisheries products; and h) improved chemical analytical capabilities that lead to the discovery of new toxins and

toxic events.

The trends are equally disturbing for macroalgae. The development of dense canopies of macroalgae in the benthos of shallow water bodies is an increasingly common phenomenon along virtually all of the world's shorelines. Human activities, including deforestation, agriculture, and generation of domestic and industrial wastewaters are increasing the concentrations and fluxes of nitrogen and phosphorus in coastal waters that in turn enhance seaweed productivity leading to high biomass levels (see Box 1.3.2). As with microalgal blooms, these trends are difficult to document statistically due to a lack of long-term datasets, the number of species involved, and the lack of a simple measure of population size or harmful impact that can be tabulated for all outbreaks. Nevertheless, workers in the field are united in their opinion that the problems are worse and the trends disturbing.

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2. The ECOHAB Program

2.1 Why a National Program on HABs is Needed

If we accept that HAB problems are expanding and that they have multiple causes, both natural and human-assisted, what can be done about them in a practical sense? What information is needed to efficiently manage affected marine resources, protect public and ecosystem health, encourage and support aquaculture development, and contribute to policy decisions on coastal zone issues such as waste or sewage disposal, aquaculture development, or dredging? If human activities are making the HAB problem worse, how can that be verified, and what steps should be taken to minimize further impacts? These are important practical questions, and the apparent trends in HAB incidence make them even more pressing. The need for applied, practical research on HAB bloom phenomena is clear. However, the problems are complex, and will require a comprehensive research program that includes basic and fundamental studies of HAB species, their environment, and the organisms that interact with them or their toxins. The ECOHAB program has been designed to address these issues.

2.2 What is ECOHAB?

ECOHAB (ECOlogy and Oceanography of Harmful Algal Blooms) is a scientific program designed to increase our understanding of the fundamental processes underlying the impacts and population dynamics of HABs. This program addresses the many factors at the organismal level that determine how HAB species respond to, and potentially alter their environment, the manner in which HAB species affect or are affected by food-web and community interactions, and how the distribution, abundance, and impact of HAB species are regulated by the environment.

In its simplest form, the goal of the ECOHAB program is to develop an understanding of the population dynamics and trophic impacts of harmful algal species which can be used as a basis for minimizing adverse effects on the economy, public health, and marine ecosystems.

2.3 Evolution of the ECOHAB Initiative

The U.S. is not alone in its struggle with the expanding HAB problem. Nations throughout the world are faced with a diverse array of toxic or harmful species and impacts, and many of these countries are poorly prepared for the threat posed to their coastal economies and ecosystems. As a result, international agencies or organizations such as the Intergovernmental Oceanographic Commission (IOC) of UNESCO, the International Council for Exploration of the Seas (ICES), the Scientific Committee for Oceanic Research (SCOR), the European Union (EU) and the Asia Pacific Economic Cooperation Program (APEC) have all established programs or working groups focused specifically on HABs and their impacts.

The IOC's HAB program is assuming a leadership role in the international arena. A series of workshops

was convened beginning in 1987, leading to the creation of a science plan which separates the IOC HAB program into three divisions - scientific, educational and operational. The scientific program has three branches: ecology and oceanography; taxonomy and genetics; and toxicology and toxin chemistry.

From the outset, the IOC HAB program was intended to be a coalescence of national and international programs. On the international side, ICES established a working group on the Dynamics of Harmful Algal Blooms which has planned several major field programs or pilot studies targeting key HAB phenomena. However, due to the regional nature of many HAB problems, it is evident that these field studies will involve individual countries or groups of neighboring countries, and not large multi-national teams of investigators, as is often the case in international programs. At the national level, however, it became clear that many countries did not have a national program or plan to attack HAB issues. In the U.S., this was true despite an array of problems associated with harmful algae and a long history of HAB research. In order to rectify this lack of coordination of HAB problems in the U.S., a workshop was convened in 1992 at the National Marine Fisheries Service laboratory in Charleston, SC. That meeting produced the report "*Marine Biotoxins and Harmful Algae: A National Plan*" (Anderson et al., 1993), which identified numerous impediments to progress in the HAB field and made specific recommendations to address those impediments. In addition to identifying areas for future research, the National Plan structured proposed HAB activities in a framework that helped agencies identify their roles in the overall program.

A prominent gap in U.S. attempt to deal with HAB issues was immediately apparent - that dealing with the ecology and oceanography of the blooms. The National Plan identified this as an important area for research, but a detailed scientific agenda was lacking and no agencies were actively supporting such research. NSF and NOAA then co-sponsored a workshop at Snow Mountain Ranch Conference Center in Colorado, involving participants representing an array of scientific disciplines, geographic regions, and agencies. The goal was to integrate field, laboratory, and theoretical studies into a focused effort to understand the fundamental issues underlying HABs and their impacts. The resulting ECOHAB program addresses these national needs, but it also represents a U.S. component of the international HAB programs of IOC and ICES.

2.4 The ECOHAB Strategy

The objective of the ECOHAB program is to combine field, laboratory and modeling studies in a coordinated effort to characterize the physical, chemical and biological processes governing the growth, distribution and impacts of HAB species.

Three program elements have been identified: *The Organisms; Environmental Regulation of Blooms;* and *Food-web/Community Interactions*. One challenge has been to design a program that could accommodate the wide array of HAB species, their impacts, and oceanographic regimes without being so broad or diffuse that implementation would be impossible. Given this diversity, ECOHAB will rely on coordinated, multi-investigator programs as well as projects by individual investigators or small groups. The program will require at least three types of research, all of which will involve an integration of physical, chemical and biological components.

Laboratory or Mesocosm Studies. Carefully controlled studies of HAB species and their food chain interactions are needed, focusing on genetic, biochemical, behavioral and life history processes that are important factors in the dynamics and impacts of blooms. These experimental studies will range from the organismal to the ecosystem level.

Field Investigations. Multi-investigator, multi-disciplinary field studies of HAB species are needed to document the distribution and dynamics of key elements of HAB ecosystems, emphasizing the complex interactions between biotic and physical or chemical factors. Since no single field program could possibly address the wide array of HAB phenomena, a series of regional field studies is envisioned, in the expectation that this comparative approach will reveal differences and commonalities when both hydrographic regimes and ecosystems are compared. An underlying challenge in all of these studies will

be the need to obtain species-specific or autecological information from natural populations.

Theoretical Studies. Existing models will be applied, and new approaches developed, which incorporate field and laboratory measurements into realistic and testable simulations of HAB dynamics in different oceanographic systems. Models will include conceptual studies in idealized flows that examine how circulation patterns affect biological processes at the level of the individual, population, community and ecosystem, as well as site-specific models that address mechanistic interactions of the physics and biology within a particular oceanographic regime.

2.5 Rationale and Benefits

The significant economic, public health and ecosystem impacts of HAB outbreaks are strong, practical motivations for a research program such as ECOHAB, made all the more pressing by the apparently escalating trend in their incidence. The direct benefits to society from a program of this kind are many, and include management issues such as bloom detection and prediction, control or mitigation strategies, site selection criteria for aquaculture, and assessment of impacts from altered nutrient loading, dredging or other coastal zone developments. There are indirect benefits as well. For example, many of the mechanisms underlying bloom formation by harmful algal species are the same as those responsible for blooms of other phytoplankton in the ocean. Multidisciplinary field HAB programs address a practical problem while also providing basic scientific information relevant to plankton ecology and oceanography in general.

Another compelling aspect of the ECOHAB program stems from the need to study individual HAB species, rather than mixed planktonic assemblages. New autecological techniques must be developed, such as remote detection of bloom populations using swimming robots or moored instruments. Methods are needed to "tag" target species with molecular probes and then enumerate or separate them from co-occurring organisms, and techniques to estimate in situ growth rates or to determine the physiological status of a species must be developed. These are but a few examples of the areas where new technologies developed to meet the objectives of ECOHAB can benefit all of oceanography.

2.6 ECOHAB Implementation

To address the many scientific issues outlined in this report, federal agencies must break away from the parochial view that has often dominated HAB research in the past. A comprehensive understanding of the present status of coastal waters, and the manner in which those waters and their ecosystems will respond to changes, both natural and human-assisted, cannot be achieved without effective interagency cooperation, coordination and collaboration. Nowhere else do the missions and goals of so many government agencies intersect and overlap as in the coastal zone where HAB phenomena are prominent. **ECOHAB will be successful only if a nationally coordinated interagency effort can be implemented to direct research personnel, facilities, and financial resources to the common goals outlined in this comprehensive national strategy .**

Thus far, the planning of the ECOHAB program has involved the NSF Division of Ocean Sciences, and several agencies or programs within NOAA, including the National Marine Fisheries Service, Sea Grant, and the Coastal Ocean Program. As the science plan evolves, more state and federal agencies are expected to join the program. The research agenda outlined herein is intended to guide these agencies in the efficient allocation of resources targeted to HAB issues, and to help them formulate new, multi-investigator, multi-disciplinary HAB initiatives as well. Joint interagency announcements of opportunity for research support as well as interagency cooperation on the provision of needed resources and facilities are thus possible. However, an additional feature of the ECOHAB program will be a reliance on proposals submitted by individual investigators or small groups, in recognition of the diversity of causative organisms, impacts, and oceanographic systems associated with HABs.

A Steering Committee has not yet been established, as that awaits a final indication of the agencies that will participate in ECOHAB. Once those issues are resolved, a committee will be selected to oversee

implementation of ECOHAB at the national level. Where necessary, small working groups or sub-committees will be convened to address specific program needs. Once ECOHAB is underway and research programs begin to accumulate results, regional and national workshops will be convened to identify common mechanisms and processes underlying the diverse array of HAB phenomena and their impacts. One of the strengths of ECOHAB lies in this "comparative approach" but resources must be allocated to facilitate the requisite scientific communication.

ECOHAB

The Ecology and Oceanography of Harmful Algal Blooms

A National Research Agenda

3. ECOHAB Program Elements

Program Element #1

3.1 The Organisms

3.1.1 Introduction

***Rationale:* The negative impacts of HABs reflect not only the growth and metabolism of individual algal cells, but the ecological selection of those cells within a diverse phytoplankton assemblage. Studies at the organismal level are essential if we are to understand the population dynamics of HABs.**

The impact of harmful algal blooms (HABs) is a function of the growth and metabolism of individual algal cells ecologically selected from a diverse phytoplankton assemblage. Growth is a general term reflecting photosynthesis, nutrient uptake and assimilation, and numerous other metabolic processes within cells. The inherent growth characteristics of species are genetically determined, but the realization of growth potential is often controlled by external environmental factors.

There is considerable diversity among HAB species with respect to strategies for growth and bloom formation in natural systems. Some cause harm at relatively low cell concentrations (e.g., DSP can occur with only a few hundred *Dinophysis* cells per liter), but in other cases, population growth of HAB species results in a monospecific bloom at high concentrations (e.g., a red tide). There are numerous explanations for that type of growth and accumulation, and many are rooted in the unique physiology of the organisms involved. For example, it has long been argued that production of toxins or other exudates allows some species to outcompete co-occurring organisms (e.g., Pratt, 1966) or to deter grazing (Huntley, 1982; Huntley et al., 1986; Ives, 1987). Practical demonstrations of these mechanisms are few, however.

Another survival and growth strategy involves the benthic resting stages of many HAB species. These cysts or spores provide a recurrent "seed" source or inoculum for planktonic populations, and this characteristic may be a critical factor in determining not only the geographic distribution of species, but possibly their eventual abundance as well (Anderson and Wall, 1978; Anderson et al., 1983). Some HAB species are motile, and under certain environmental conditions their swimming behavior may result in formation of high-density patches (e.g., Kamykowski, 1974; Cullen and Horrigan, 1981; Franks, 1992). Diel vertical movement by motile cells in a stratified environment undoubtedly has functional significance, for example, maximizing encounter frequencies for sexual reproduction, minimizing grazing losses, and allowing cells to obtain nutrients at depth and light at the surface.

These diverse issues can be incorporated into the following goal for the Organisms program element of ECOHAB:

Goal: To determine the physiological, biochemical, genetic, and behavioral features and mechanisms of harmful algal species that influence their bloom dynamics, general ecology, and impacts.

3.1.2 Research Agenda

The following issues are considered high priorities in organismal research within ECOHAB:

Issue:

There is a need to rapidly and accurately identify, enumerate, and physically separate HAB species from mixed phytoplankton assemblages.

Positive identification and enumeration of specific algal species in discrete field samples collected over large temporal and spatial scales is a labor intensive, but necessary process for the characterization of HABs. A common problem in research and monitoring programs focused on HAB species occurs when the species of interest is only a minor component of the planktonic assemblage. Many potentially useful measurements are simply not feasible because of the co-occurrence of other organisms and detritus. Studies must thus rely on tedious microscope counts to enumerate the target species, and measurements of toxicity or other physiological parameters are generally not possible for just the species of interest. Another constraint arises from the difficulties in adequately identifying and distinguishing between species or strains which are morphologically similar. (see Box 3.1.2) Considerable time and effort are often required to identify a particular species when its distinguishing characteristics are difficult to discern under the light microscope. Such fine levels of discrimination are not generally feasible in monitoring programs or other studies which generate large numbers of samples for cell enumeration.

At present, the time lag between sample collection and the identification and counting of specific organisms severely limits our ability to follow the population dynamics of HAB species in real-time. As a consequence, we are limited in our ability to predict when potentially harmful organisms may develop in areas where they might pose a threat to public health or wildlife. Once species identification is automated and/or greatly accelerated, population data can be collected that is compatible with high-frequency measurements of chemical and physical oceanographic features.

We need to:

- 1. Collect, isolate, and maintain a wide range of HAB species in unialgal and/or axenic cultures.**
- 2. Characterize key species using standard microscopic methods (e.g, light, epifluorescence, and electron microscopy as appropriate).**
- 3. Characterize key species using molecular genetic methods (e.g., RFLP, RAPD, gene sequencing).**
- 4. Develop molecular probes and application strategies for use in field and laboratory settings, and make these tools broadly available.**
- 5. Detect and quantify toxins produced by HAB species using bioassays, HPLC, immunoassays, and receptor binding assays; refine those techniques for routine use on field and culture samples.**
- 6. Develop optical sensors able to distinguish taxon-specific features such as pigments.**

Approach and Technology. The problem of uncertain and slow identification can be addressed by cross-disciplinary investigations that utilize a spectrum of techniques to distinguish between species,

strains of single species, and toxic and non-toxic forms. Culture collections, especially those including multiple strains of key species, are essential to this effort. A variety of identification techniques should be supported, as future applications of rapid detection methods for HAB species will likely employ multiple probe types. (see Box 3.1.2) These new technologies should be actively pursued, but traditional systematic or morphological investigations using standard microscopy or biochemistry should also be supported. The traditional methods are well-established, but considerable effort is needed to develop the species-specific probes and the methods to use them in a rapid and precise manner. Results from this aspect of the program will greatly accelerate progress in several other ECOHAB activities, especially those involving large-scale field programs.

Probe technologies can also be used to detect the toxins (rather than the cells) in environmental samples (see Box 3.1.3). This type of application has the potential to provide rapid and accurate information on toxin levels and distribution that can be highly useful to resource managers.

Issue:

There is a need to identify the life history stages of HAB species, to determine what factors control transitions between those stages, and to establish the role of each stage in bloom dynamics.

Many marine phytoplankton species produce cysts or spores during their life histories, and these resting stages can have a significant impact on many aspects of HAB phenomena (Anderson and Wall, 1978; Anderson et al., 1983). Cyst or spore germination provides the inoculum for many blooms, and the transformation back to the resting state can remove substantial numbers of vegetative cells from the population and be a major factor in bloom decline. Cysts are important mechanisms for population dispersal, they permit a species to survive through adverse conditions, and since sexuality is typically required for their formation, they facilitate genetic recombination (Wall, 1971). They can even be important sources of toxin to shellfish and other benthic animals. Clearly, all investigations of the ecology and bloom dynamics of HAB organisms must be based on a thorough understanding of an organism's life history, as well as the factors that regulate the transitions between dormancy and a vegetative existence.

Unfortunately, the state of knowledge about resting stages and life histories is neither complete nor uniform for the many HAB species. For several (e.g., *Alexandrium* spp., *Heterosigma carterae*, *Pfiesteria piscicida*), the existence of resting cysts has been documented. For many others, however, no life history information is available. The prevalence of life-cycle stages among other HAB species is not well known, and factors triggering transitions are poorly defined. Recognizing and determining the role of these stages in bloom initiation, growth and termination is critical to our understanding of HAB phenomena.

We need to:

- 1. Develop culture techniques which simulate in situ growth conditions sufficiently well that life history transitions can be induced and characterized.**
- 2. Isolate and culture many HAB species, and support the maintenance of HAB culture collections. The importance of maintaining multiple isolates of individual species must be emphasized here, given the genetic diversity observed in regional populations of HAB "species."**
- 3. Develop molecular probes that can assist in the identification of life history stages in natural samples.**
- 4. Incorporate studies of resting cyst or spore distribution, abundance, and dynamics into field investigations of HAB bloom dynamics.**

Approach and Technology. A combination of laboratory and field studies is required to determine the complete life histories of HAB species and to elucidate the factors that regulate transitions between life stages. Here again, culture collections of multiple HAB species are necessary, and multiple studies are required given the diversity of species represented by HAB organisms. Some technique development effort is needed, such as in the design and application of probes to identify life history stages of a target organism and refinement of culture techniques to permit full-cycle life history transformations to occur in the laboratory. Otherwise, methodologies are in-hand for these studies.

Issue:

It is essential to understand the physiological responses of HAB species to differing environmental conditions.

The manner in which HAB species respond to a changing environment determines their survival and growth. These responses are governed by the physiological requirements and tolerances of each species for environmental variables such as nutrients, light, temperature, and salinity. All HAB species must be characterized with respect to these tolerances if we are to understand and predict their distribution and occurrence in natural waters. For example, an extraordinary bloom referred to as "the Texas brown tide" has persisted in the Laguna Madre for five years (Buskey and Stockwell, 1993). There are multiple potential explanations for this dominance, one of which is that the causative species out-competes other phytoplankton for essential resources. In addition to possible selection on the basis of temperature or salinity tolerances, the brown tide alga cannot use nitrate as a nitrogen source. This unique nutritional strategy may be fundamental to the success of the species in that system, but further experimental studies are clearly needed to document how this might be occurring.

The brown tide in Texas is but one example of the need for information on physiological responses of individual HAB species to their chemical and physical environments. The same can be said for virtually all important HAB species. Why are *Alexandrium* blooms in the southwestern Gulf of Maine tightly linked to a coastal current of low salinity water (Franks and Anderson, 1992a)? Do the cells grow faster in that water mass due to its unique macro- or micro-nutrients? How is *Gymnodinium breve*, a red tide dinoflagellate from the Gulf of Mexico, able to survive during transport for 1000 km or more around the Florida peninsula and up the southeastern coast of the U.S. to North Carolina via the Gulf Stream (Tester et al., 1991)?

Experimental approaches to organismal physiology must include the following:

- 1. Establish new clones of key HAB species representing their entire geographical range.**
- 2. For multiple toxic and non-toxic clones of HAB species, determine tolerance ranges and optima for growth and toxin production in response to a suite of environmental variables.**
- 3. Conduct classical steady-state analyses of nutrient requirements and uptake rates for key HAB species.**
- 4. Compare laboratory results with data from mesocosm and field investigations.**

Approach and Technology. Species-specific physiological data can be most easily derived from experiments with unialgal cultures under controlled conditions. However, clones of a single species typically exhibit marked variation in numerous characteristics, including growth and toxin production (Maranda et al., 1985; Bomber, et al., 1989; Cembella et al., 1987; Hayhome et al., 1989; Anderson et al., 1994). Since no single isolate can be considered to be representative of a regional population, growth studies are needed for multiple strains to define the extent of genetic variability and environmental plasticity. Several laboratories in the United States have initiated "syndrome-based" culture collections of harmful marine microalgae, and these collections should be supported and exploited in this respect. As discussed below, techniques for some of the above physiological studies are not uniformly accepted.

It may be necessary to convene a small working group to standardize approaches and protocols for these investigations.

Issue:

***In situ* measurements of the rates of photosynthesis, growth, and nutrient uptake are essential for understanding the dynamics of HABs, as are assessments of the physiological condition of cells at different times and locations.**

This issue epitomizes a unique and challenging feature of HAB studies that separates them from more traditional process-oriented oceanographic investigations. Many techniques are available to assess the biological rate processes and biomass of planktonic communities (e.g., ¹⁴C-fixation, chlorophyll), but there are few methods suitable for determining growth or uptake rates or physiological condition of an individual species when it occurs in a mixed population and does not dominate the phytoplankton assemblage. Considerable methods development is thus required to fully address this autecological characteristic of ECOHAB.

Effort is needed in the following areas:

1. Investigate the physiology, biochemistry, and molecular biology of specific processes to identify "diagnostic indicators" for physiological condition.

2. Develop methods to estimate in situ rates of growth, photosynthesis, and nutrient uptake for HAB species.

3. Calibrate these methods carefully, and then apply them aggressively to field populations.

Approach and Technology. The necessary studies can build on molecular and biochemical techniques developed to assess growth rate (Dortch et al., 1983; Chang and Carpenter, 1991; Lin et al., 1994) and a suite of physiological processes within cells such as nitrogen fixation (e.g., Currin et al., 1990), nutrient uptake or limitation (Berdalet and Estrada, 1994; see Box 3.1.3) and photosynthetic activity (Orellana and Perry, 1992). The objective of these studies will be to develop analytical methods and diagnostic indicators that can be applied to individual cells. The more traditional bulk analyses work on communities rather than species. For some HAB organisms, it will be necessary to couple the above methods with identification probes and flow cytometry or cell imaging techniques to measure species-specific characteristics.

Although recent technological advances are encouraging in these fields, there is a clear need for an initial methods development and calibration effort within the ECOHAB framework. The ultimate goal is to apply these techniques in ecological studies.

Issue:

It is essential to know the nutrient requirements, uptake, and assimilation characteristics of HAB species.

Nutrient limitation of phytoplankton growth is a fundamental factor that places a limit on the accumulation of biomass and may determine the outcome of competition among species in mixed assemblages. It is often suggested that increasing incidences of harmful algal blooms in coastal waters are related to changes in nutrient loading from human activities (e.g., Smayda, 1990), so verification of this linkage would have important societal implications. These nutrients can stimulate or enhance the impact of toxic or harmful species in several ways. At the simplest level, toxic phytoplankton may increase in abundance due to nutrient enrichment but remain as the same relative fraction of the total phytoplankton biomass (i.e. all phytoplankton species are affected equally by the enrichment). Alternatively, there may be a *selective* stimulation of HAB species by pollution. This view is based on

the nutrient ratio hypothesis (Smayda 1990) which argues that environmental selection of phytoplankton species is associated with the relative availability of specific nutrients, and that human activities have altered these nutrient supply ratios in ways that favor toxic or harmful forms. For example, diatoms, the vast majority of which are harmless, require silicon in their cell walls, whereas other phytoplankton do not. Since silicon is not abundant in sewage effluent but nitrogen and phosphorus are, the N:Si or P:Si ratios in coastal waters have increased through time over the last several decades. Diatom growth in these waters ceases when silicon supplies are depleted, but other phytoplankton classes (which include most of the known toxic species) can proliferate using the "excess" nitrogen and phosphorus.

This concept is controversial, but is not without supporting data. A 23-year time series off the German coast documents the general enrichment of coastal waters with nitrogen and phosphorus, as well as a four-fold increase in the N:Si and P:Si ratios (Radach et al., 1990). This was accompanied by a striking change in the composition of the phytoplankton community, as diatoms decreased and flagellates increased more than ten-fold. As coastal communities and countries struggle with pollution and eutrophication issues, the implications of these concepts are profound and clearly deserve further investigation.

A few measurements of nutrient uptake kinetics and cell nutrient quotas of HAB species (e.g., dinoflagellates and raphidophytes) suggest that they have high nutrient requirements, indicating that they would be able to proliferate only in high nutrient environments (e.g., Eppley et al., 1969; Caperon and Meyer, 1972). Relatively few HAB species have been investigated in this context, however. Nutrient uptake and growth rate kinetics and nutrient quotas for HAB species must thus be determined to predict their growth response relative to other species. In addition, some supposedly autotrophic phytoplankton species appear to utilize dissolved organic nutrients (Cembella et al., 1984; Taylor and Pollinger, 1987) while others rely on mixotrophy to supplement their carbon requirements (Sanders and Porter, 1988). If confirmed in HAB species, these nutritional strategies may confer a competitive advantage over other phytoplankton.

Nutrient studies within ECOHAB should focus on the following:

- 1. Determine nutrient uptake and growth kinetics for HAB species under a range of environmental conditions. Depending on the species, this information is needed for N, P, Si, Se and Fe at least, but other micronutrients may need to be considered.**
- 2. Assess the prevalence among HAB species of unique nutritional strategies such as osmotrophy and mixotrophy.**
- 3. Develop and optimize culture techniques for fastidious HAB species.**

Approach and Technology. The hypotheses that changing nutrient ratios can influence competition dynamics and that HAB species have nutrient requirements different from other phytoplankton species can be assessed using a combination of nutrient kinetics and manipulative experiments with cultures and natural populations. Several approaches have been used to derive nutrient kinetic parameters, and there is the need to standardize experimental protocols before embarking on comparative studies among HAB species. Moreover, there is no general agreement on the merits and limitations of using batch versus continuous culture methods for nutritional requirement studies. As a result, it may be necessary to convene an ECOHAB community working group to address standard experimental approaches.

Issue:

The functional role of toxins and/or exudates produced by HAB species is not known.

Toxin production is a wide-spread, but not universal, characteristic of HAB species. The functional roles suggested for toxins are: 1) as deterrents to grazers (Box 3.3.1); 2) as allelopathic compounds that restrict the growth of co-occurring algal species; and 3) as storage products. It may well be that toxins

are secondary metabolites with no physiological function. Field observations suggest that some fish and zooplankton avoid dense concentrations of HAB species (Huntley, 1982) and laboratory studies indicate that toxic species can be rejected by grazers (Sykes and Huntley, 1987; Ives, 1987). These studies are limited to a few species and are only a beginning. They certainly have not addressed the diversity of grazer-algal relationships necessary to evaluate the role of toxins in natural populations.

We need to:

- 1. Conduct laboratory and field studies to determine if there is differential grazing on toxic versus non-toxic species.**
- 2. Determine the effects of toxic algae on ecologically significant grazers.**
- 3. Evaluate the allelopathic activity of exudates and toxins of HAB species.**

Approach and Technology. This work will depend upon a supply of appropriate isolates, our ability to manipulate them in culture, and the availability of sensitive and reliable methods of toxin analysis. In general, techniques are available to pursue this important line of investigation, although advances in 3-dimensional video analysis of grazer behavior when presented with HAB species can provide new and relevant insights.

Issue:

It is essential that we define the genetic basis of toxin production, elucidate toxin biosynthetic pathways, and determine how toxin accumulation in cells is regulated.

Toxin production is a distinguishing characteristic of many HAB species. However, the prevalence of toxin synthesis among these organisms is continuously being re-evaluated. For some species the toxins are well described (e.g., saxitoxins, domoic acid, brevetoxins; Hall et al., 1990; Shimizu, 1993; Falconer, 1993), although non-toxic strains or sub-species have been documented for several such taxa (e.g., Yentsch et al., 1978; Smith et al., 1990). Other species such as *Heterosigma carterae* and *Pfiesteria piscicida* are known to produce toxins, but the actual compounds remain uncharacterized (e.g., Burkholder et al., 1992; 1995). Still, other species are considered likely to be toxigenic based on an association with events such as fish kills, but their toxicity has yet to be confirmed. Our lack of information about this basic trait for many HAB species limits our ability to ascertain the nature and extent of HAB impacts or to evaluate mechanisms underlying trends in HAB incidence.

While relatively few organisms have been examined, the available data suggest that the amounts and forms of toxin contained in a cell vary with its physiological status. For the saxitoxins, environmental factors such as nutrient concentration and temperature can influence the expression of individual toxin derivatives (Hall, 1982; Anderson et al., 1990a,b). This is an important ecological consideration, because various derivatives can differ markedly in their potencies (Oshima et al., 1989). Production of algal toxins can also be modulated by co-occurring bacteria (Bates et al., 1995), and in certain cases, bacteria themselves represent autonomous sources of phycotoxins (Kodama et al., 1988; Doucette and Trick, 1995).

Nutrients have a clear and significant influence on the production of toxins by some algae. In species from a variety of taxonomic groups producing different toxins, cellular toxin content varies dramatically during nutrient starvation in culture. For example, the abundance of saxitoxins in *Alexandrium* species can vary by more than an order of magnitude depending upon whether phosphorus or nitrogen is limiting (e.g., Hall, 1982; Boyer et al., 1987; Anderson et al., 1990). Likewise, domoic acid production in *Pseudo-nitzschia* species varies with silicate availability (Bates et al., 1991; Bates and Douglas, 1993), and *Chrysochromulina polylepis*, the chrysophyte responsible for massive fish and invertebrate mortalities in Sweden and Norway in 1987, has been shown to be more toxic when phosphorus is limiting (Edvardsen et al., 1990; Granéli et al., 1993).

These and other demonstrations of the effects of nutrient availability on toxicity have major implications with respect to our efforts to understand the manner in which HABs are influenced by, and impact their environment. There are many unknowns remaining, however, as studies to date have only demonstrated the nature of the linkage between nutrients and toxicity, and then only for a few species. Biochemical and cellular mechanisms remain to be elucidated, as does the extent to which the nutrient limitations that alter toxicity are actually occurring in natural waters. Without more detailed information about the physiology of toxin production for a wider range of HAB species, it is very difficult to assess the ecological role of toxins in population and community dynamics (see sections 3.2 and 3.3).

The biosynthetic pathways for the production of several toxins have been described to the extent that elementary "building blocks" have been identified (Shimizu et al., 1984; Douglas et al., 1992; Box 3.1.4), but in no case have complete pathways, including all intermediates involved, been elucidated. Isolation of the enzymes involved in toxin synthesis or interconversions is also at a very early stage of development (Sako et al., 1995). At the most basic level isolation of the genes and enzymes directing the production of algal toxins remains an important but elusive goal.

Organismal studies of toxin production and its genetic control will need to include the following:

- 1. Determine the prevalence of toxin production among HAB species in culture and the time-varying concentrations of toxin at different stages of growth.**
- 2. Determine the linkage between bacteria and toxin production.**
- 3. Isolate and purify poorly characterized or unknown toxins and determine their chemical structures.**
- 4. Elucidate toxin biosynthetic pathways and characterize the genetics and regulation of toxin production.**
- 5. Determine the nutrient assimilation and partitioning pathways which permit toxin synthesis, and determine the factors which influence toxin production at the cellular level.**

Approach and Technology. A number of well-established experimental approaches are available and appropriate to address patterns of toxin production, typically involving the growth of an HAB species under a suite of environmental conditions and monitoring the manner in which toxicity varies. The isolation of toxin genes/enzymes is a critical first-step toward identifying the actual mechanisms underlying environmentally-induced toxin variability. Additionally, studies at the molecular level will allow us to evaluate the intracellular trade-offs between toxin production and maintenance of "normal" cellular metabolism, ultimately leading to a clearer understanding of "why" it might be ecologically beneficial for toxigenic organisms to synthesize toxins. Technological strategies for implementing these studies will include: classical laboratory culture methods, application of toxin probes, assays and analyses; biosynthetic feeding/label-incorporation experiments; standard chromatographic techniques; and, methods incorporating mutagenesis and gene expression protocols.

Issue:

We must understand the importance of motility and other behaviors of HAB species.

Some motile HAB species exhibit directed swimming behavior such as vertical migration or orientation towards prey, as has been demonstrated for the ambush dinoflagellate, *Pfiesteria piscicida* which is capable of detecting and swimming towards its preferred food. Vertical migration is thought to be a response to light, salinity, nutrient gradients, and even gravity (e.g., Holmes et al., 1967; Eppley et al., 1968; Kamykowski, 1974; Cullen and Horrigan, 1981). This mechanism is fundamental to the population dynamics of many motile HAB species, as it can result in dense concentrations of cells that affect grazing losses, light harvesting, nutrient availability, and encounter frequencies for sexuality. The

aggregation of cells is also directly related to the scale of the adverse impact from the blooms. Non-motile algae are also capable of orienting themselves vertically by changing their relative buoyancy.

We need to:

- 1. Conduct vertical migration studies at a variety of scales, from tube cultures to mesocosms to natural populations.**
- 2. Characterize the influence of environmental variables such as salinity, light, and nutrients on these behaviors.**
- 3. Refine models of swimming behavior and examine how different strategies interact with physical features such as pycnoclines, fronts, or internal waves.**

Approach and Technology. A combination of laboratory and field investigations is necessary to address the significance of these behaviors. Much of the technology required for such studies exists, but new approaches such as fine-scale sampling techniques, (e.g., Donaghay et al., 1992) will be needed to measure biological, chemical and physical parameters with appropriate resolution. Rapid detection and enumeration techniques for HAB species will be required (see Box 3.1.2), and mesocosm strategies will be important as well.

3.1.3 Summary

The Organism program element of ECOHAB reflects the fundamental importance of physiological, genetic, and behavioral studies in an initiative designed to develop an understanding of the population dynamics and trophic impacts of harmful algal species. **We take it as a given that studies of HAB blooms require a thorough understanding of genetic variability and regulation, nutritional and environmental tolerances and responses, behavioral adaptations, life history transformations, toxin physiology and function, and numerous other processes and features that will vary among HAB species.** Given the diverse array of HAB species in the U.S. and the many different environments in which they occur, this program element will likely be dominated by small research programs conducted by individual investigators or small teams. Focused, multi-investigator proposals are also envisioned on specific issues where coordination and comparisons between organisms would be beneficial. In many cases, the technology exists for the studies that are proposed, but a focused methods development effort will greatly accelerate progress on numerous other ECOHAB elements. For example, rapid and automated detection and enumeration of HAB species using probe technologies would eliminate a major constraint to field programs namely the time required to process cell count samples collected at spatial and temporal frequencies similar to those for hydrographic and chemical parameters. Such techniques would also make cell counts and even the physical separation of HAB species from co-occurring organisms fast and accurate, permitting measurements that otherwise would not be possible. Prioritization within the research issues highlighted above was not attempted, as the list already reflects an effort by workshop participants to include only the most important and timely research topics.

3.2.1 Introduction

***Rationale:* Concurrent with escalating influences of human activities on coastal ecosystems, the environmental and economic impacts of HABs have increased in recent decades. It is therefore imperative to know if present trends of human activities and HABs will lead to unacceptable consequences, and if the means can be developed to mitigate impacts. The key to this knowledge is an understanding of the ecology and oceanography of harmful algal blooms. An important facet of this complex topic is environmental regulation, that is, the influence of environmental factors on the population dynamics of harmful algal species and their competitors.**

The geographic range, persistence, and intensity of HABs are determined by both physical and

biological factors. For example, the initiation of a bloom requires successful recruitment of a population into a water mass. This may result from excystment of resting cells during a restricted set of suitable conditions (e.g., *Alexandrium* in the Gulf of Maine; Anderson and Keafer, 1987), transport of cells from a source region where blooms are already established (e.g., *Gymnodinium catenatum* in northwest Spain; Fraga et al., 1988), or exploitation of unusual climatic or hydrographic conditions (e.g., *Pyrodinium bahamense* and ENSO events in the Indo-West Pacific; Maclean, 1989). Once a population has begun growing, its range and biomass are still affected by physical controls and nutrient supply. Physical controls include long distance transport of populations (e.g., Franks and Anderson, 1992a), accumulation of biomass in response to water flows and swimming behavior of organisms (Kamykowski, 1974; Cullen and Horrigan, 1981), and maintenance of suitable environmental conditions (including temperature and salinity, stratification, irradiance, and nutrient supply; Whitedge, 1993). Aspects of nutrient supply include not only the amount of macro- and micronutrients, but also their ratio and mechanism of supply. Thus, physical forcings, nutrient supply, and behavior of organisms all interact to determine the timing, location, and ultimate biomass achieved by the bloom, as well as its impacts. The first goal of the Environmental Regulation program element of ECOHAB is thus:

Goal: To determine and parameterize the environmental factors that govern the initiation, growth, maintenance, dissipation and impacts of HABs.

Physiological responses and life histories of HAB species are varied, as are local and regional physical environments where HABs occur. Thus there is considerable variability in the relationship of HABs to their environment. In spite of this complexity, however, it is usually possible to elucidate the patterns underlying recurrent blooms in an area. Generalizations to other regions is not usually appropriate, however. An understanding of the relationship between an HAB species and its physical and biological environment is critical to predicting environmental and economic impacts and to the formulation of mitigation strategies to minimize those effects. Since it is impractical to study all of the ecosystems in which HABs occur, a second goal is:

Goal: To formulate principles that explain similarities between ecosystems during HABs and to understand how these systems are unique with respect to the types of blooms that occur.

3.2.2 Research Agenda

The following section outlines specific issues defining high priority field, modeling and experimental studies required in the Environmental Regulation program element of ECOHAB.

Issue:

To what extent do HABs reflect increases in growth rate versus physical transport and immigration? Is there a specific suite of physical factors associated with many HABs?

Physical factors in the environment influence HAB population dynamics both directly by moving and aggregating cells, and indirectly by influencing the cells' physical and biological environment. Factors that can influence the population dynamics and physiology of phytoplankton include: nutrients, temperature; salinity; irradiance; stability of the water column; turbulent mixing; currents; vertical advection, dispersion or dilution; wind stress; bottom stress and bathymetry. Temperature, salinity and irradiance directly influence growth rate, physiology, and in some species, behavior (e.g., Watras et al., 1982; Tyler and Seliger 1981). High shear associated with turbulent mixing may alter growth, behavior, and in extreme cases induce mortality (e.g., Pollinger and Zemel, 1981; Thomas and Gibson 1990; Berdalet 1992). Water motions (a complex interaction of most of these factors) determine the losses and gains from advection as well as losses to dispersion (Kamykowski 1979, 1981). In addition, water motions determine in large part an alga's ability to exploit light and nutrients. Finally, stratification of the water column allows weakly swimming algae to interact with current shear and thereby drastically alter immigration rates. The success of individual HAB species is associated with different hierarchies

of these influences, and we expect these associations to vary among ecosystems.

There are a wide variety of flow regimes in which HABs occur, from well-mixed estuaries to upwelling regimes and highly stratified river plumes. Among these diverse settings, vertical and horizontal transport processes play important roles in regulating bloom development, although the physical mechanisms and rates may vary considerably between environments. Characterizing transport processes in these stratified and/or spatially non-homogeneous regimes represents a challenging basic research problem. **Our progress in understanding HABs thus requires advances in coastal physical oceanography.** Some of these unresolved physics problems that have an important bearing on HABs include:

Turbulence and Vertical Transport. While there are many turbulence closure models that parameterize the effects of stratification (e.g., Martin, 1985), such models may not apply across the range of conditions found in coastal environments. A particularly difficult problem is the mixing in stratified shear layers removed from boundaries, such as those found at the base of buoyant plumes. The rate of vertical mixing must be accurately quantified and related to measurable mean properties in order to interpret observations and develop models of HABs. Vertical transport processes associated with upwelling, frontal processes and secondary circulations may be comparable to (or more important than) vertical mixing. These motions facilitate vertical exchange, but they also play an important role in the aggregation of plankton.

Horizontal Dispersion Processes. As is the case with parameterizing vertical mixing, the rate of horizontal dispersion is difficult to quantify at the scales relevant to HABs. In estuarine environments, there have been a number of attempts to relate flushing rate to easily measurable physical parameters. There has been some success in this regard, but there remains considerable room for improvement in parameterization of flushing. In coastal settings, Okubo's (1971) mixing diagrams still provide the benchmark for estimation of small-scale horizontal exchange. Dispersion theory has advanced considerably with the contributions of Young et al (1982) with respect to shear dispersion and Zimmerman (1986) for chaotic dispersion. However, there are few observational studies that provide the requisite measurements of small-scale velocity variation required to turn these theoretical ideas into estimates of horizontal dispersion. Given recent progress in measurement of small-scale variations of velocity (e.g., Geyer and Signell, 1992; Prandle 1991), there is great potential to make substantial progress on this important research area, and one mechanism for such study would be through ECOHAB.

Buoyant Plumes. HABs are frequently observed in association with buoyant plumes (Therriault et al., 1985; Franks and Anderson, 1992a; Tester et al., 1991). While the bulk characteristics of buoyant plumes are well established, the details of the velocity and density structure and their variability are not adequately understood to explain the transport, aggregation and dispersion of algal cells within plumes. The strong vertical and horizontal shears occurring within plumes result in a complex advective regime that may concentrate cells within the front or disperse them, depending on the interaction between the relative motion of the organisms and the flow field. Secondary circulations associated with winds, planetary rotation and topography also contribute to the complexity of the flow within these environments. Numerical models simulating theoretical coastal buoyant plumes under surface wind stress have been formulated (e.g., Chao, 1987), but no one has yet incorporated behavioral or physiological models of HAB species into such physical models.

Estuarine Circulation. The classic paradigm of two-layer estuarine circulation is a gross oversimplification of time-dependent and three-dimensional motions in estuaries. Wind-driven motions provide a large perturbation that in many environments may dominate the horizontal exchange, and tidal dispersive effects often control small-scale transport and in some cases regulate the estuary-scale exchange. Complex, three-dimensional motions due to the interaction of stratification, tides and winds play an important role in horizontal dispersion and vertical exchange, and they provide critical controls on the spatial distribution of plankton cells. These processes and their interactions are certainly complex, but careful consideration of both physical and biological features can lead to important insights into the

population dynamics of phytoplankton in an area. The work of Seliger et al., (1970) and Tyler and Seliger (1978) are noteworthy in this regard.

Each of the physical processes described above is an active area of research in physical oceanography, but much of this work is taking place without reference to phytoplankton populations. With respect to the ECOHAB program, it would be unrealistic to strive for a complete understanding of how each these processes can drive ecosystem response. There are, however, tractable physical problems that can be addressed, and large advances in our understanding can be obtained with collaborations between HAB biologists, physicists, and modelers.

Priority activities should be to:

- 1. Describe and model the dynamics of HABs in relation to their physical environment.**
- 2. Determine how variations in population growth rate and biomass depend on small scale turbulence through its influence on nutrient uptake, grazing, cell division, accumulation, and bloom structure.**
- 3. Determine how the vertical distribution of HAB populations regulates bloom development and dissipation, and how vertical distribution relates to physical (e.g., horizontal and vertical advection, vertical mixing and stability) and biological processes (e.g., motility, buoyancy control).**

Approach and Technology. To determine the influence of environmental factors on the development of HABs, it is essential to describe their distributions in time and space, and this will require coordinated and multidisciplinary studies. Information on the distributions of physical and biological variables should be synoptic in space (local to regional scales) and highly resolved in time (hours to days) covering successive bloom periods (interannual variability). This level of coverage would be ideal, but it is expensive and probably unrealistic in the context of ECOHAB alone, given the many sites where HABs occur. Excellent results have been obtained, however, with focused field programs that involve significant physical components (e.g., Seliger et al., 1970; Tyler and Seliger, 1978, 1981; Franks and Anderson, 1992a). Future collaborative efforts between physical and biological oceanographers should thus be emphasized and encouraged.

There is considerable potential for the use of moored optical sensors in red tide/toxic algae research and monitoring (see Box 3.2.2). When properly designed and calibrated, these sensors measure radiometric quantities that should be particularly appropriate for long time-series observations in coastal systems. That is, unlike measurements of chlorophyll, floristics, and stimulated fluorescence, which are somewhat dependent on equipment and methods, records of irradiance and radiance should be completely comparable over many years, documenting changes associated with eutrophication or remediation, for example. If such observations can be made autonomously and interpreted reliably, they would be ideal for the detection of HABs in coastal waters, even in remote locations. Because some harmful species can exert profound negative effects on coastal resources without dominating the phytoplankton and changing the color of the water, there are limitations to the usefulness of optical instruments for detecting HAB phenomena. Nonetheless, continuous optical measurements in coastal waters would be extremely useful for describing bloom dynamics and long-term trends. Furthermore, with the development of species-identification technologies described in section 3.1.2, it should become feasible to use moorings to obtain long-term records of HAB species distributions and associated environmental variables.

A hierarchy of remote sensing platforms would provide frequent, synoptic, near-surface spatial information (see Box 3.2.3). Aircraft-mounted units are needed to provide high-resolution distributions on local to regional scales (e.g., Millie et al., 1992). Satellite sensors, such as SeaWiFS, will provide lower temporal and spatial resolution, but over regional to global scales. Calibration and deployment of these instruments during HAB events is essential to the development of a remote sensing capability for

such phytoplankton blooms.

Shipboard research programs are also essential to the elucidation of HAB dynamics, not only to obtain direct measurements of rates and standing stocks of key components, but also as a means of relating component variability to information from moorings and remote platforms. Field programs aimed at understanding the interactions between HAB species and their physical environment are typically hampered by the enumeration of HAB individuals in a diverse assemblage of phytoplankton, microzooplankton and detritus. Small, low cost profilers are needed that can be rapidly deployed in the vicinity of HABs to define their spatial structure and temporal evolution. These profilers can be deployed with self-contained CTD/fluorometer/transmissometer/optical backscatter packages for measurement of key physical parameters that may directly or indirectly control bloom development.

Analytical and numerical models are important tools for studying physical-biological interactions in the ocean. Models have been used for decades to understand how physical forcings influence the distribution and production of HAB species. New architectures for physical models which incorporate turbulence-closure formulations for the small-scale motions (e.g., Blumberg and Mellor, 1987) now provide important platforms for the investigation of physical-biological interactions over scales of meters to hundreds of kilometers. The incorporation of behavioral and physiological models of HAB species into these physical models is a necessary and important step in elucidating the couplings between nonlinear physical flows and time-dependent biological responses (see Box 3.1.6). The formulation of theoretical models investigating the nature of interactions between physical flows and organism behaviors must be encouraged.

Issue:

How do physical and ecological processes control the partitioning of nutrients within a system and the relationship between nutrient inputs and the population dynamics of HAB species?

The availability of nutrients (inorganic and organic) to individual organisms ultimately regulates the growth rate and net biomass of blooms. Physical forcings, such as vertical mixing, stratification or advection can be significant factors in determining the availability of those nutrients. It is also clear that the relationship between nutrient inputs and population dynamics is complex and reflects many other, interacting processes. One of the explanations given for the increased incidence of HAB outbreaks worldwide is that these events are a result of increased pollution and nutrient loading of coastal waters. Some argue that we are witnessing a fundamental change in the phytoplankton species composition of coastal marine ecosystems throughout the world due to the changes in nutrient supply ratios from human activities (Smayda, 1990). There is no doubt that this is true in certain areas where pollution has increased dramatically. It is perhaps real, but less evident in areas where coastal pollution is more gradual and unobtrusive. In Tolo Harbor, Hong Kong, human population within the watershed grew 6-fold between 1976 and 1986, during which time the number of red-tide events increased 8-fold (Lam and Ho, 1989). The underlying mechanism is presumed to be increased nutrient loading from pollution that accompanied human population growth. A similar pattern emerged from a long-term study of the Inland Sea of Japan, where visible red tides increased steadily from 44 per year in 1965 to over 300 a decade later, matching the pattern of increased nutrient loading from pollution (Murakawa, 1987). Japanese authorities instituted effluent controls in the mid-1970's, resulting in a 50% reduction in the number of red tides that has persisted to this day.

As coastal communities and countries struggle with pollution and eutrophication issues, the implications of the trends in Hong Kong, Japan, and other countries are profound. The public, the press, and regulatory officials are concerned about whether this is happening in the U.S. as well, and are asking for predictions and answers about HAB incidence that exceed our present capabilities. **Unfortunately, competitive outcomes in phytoplankton species selection and succession cannot yet be predicted, nor can the relative effects of natural versus anthropogenic factors be resolved.** A variety of important issues involving nutrients and the manner in which they are supplied to and utilized by HAB species must thus be addressed.

We need to:

- 1. Determine how changes in the magnitude and elemental ratios of nutrient inputs to coastal ecosystems can influence ecological responses, especially those that favor HABs.**
- 2. Determine whether the frequency and duration of harmful algal blooms are increasing in coastal waters relative to increases in phytoplankton production in general.**
- 3. Investigate the extent to which HABs are indicators of local (point-source) or regional (diffuse input) increases in nutrient loading.**
- 4. Investigate how climatic variability from local to global scales influences the development and dispersal of HABs.**
- 5. Learn whether HABs are indicators of environmental or habitat changes induced by nutrient over-enrichment or other anthropogenic effects (e.g., alterations in freshwater or contaminant inputs).**

Approach and Technology. The potential stimulatory influence of anthropogenic nutrient inputs on HAB incidence is certainly one of the more pressing unknowns we face, and will require a focused commitment of resources and effort greatly in excess of that previously devoted to the topic. Time-series analysis of existing data bases for phytoplankton communities and of variables such as major nutrients or pollutants are required. Where such data are lacking, long-term monitoring programs must be initiated in key regions where anthropogenic changes are anticipated. Moored instrument packages including optical sensors and other devices to resolve HAB classes or species within the plankton would be highly effective in this regard. Laboratory studies of the stimulatory effects of chemicals contained in effluents or terrestrial runoff are also needed, as are kinetic studies and other experiments that can quantify the nutritional requirements and uptake capabilities of HAB species (see section 3.1.2 for a more detailed list of approaches to nutrient issues).

Issue:

Are there specific physical, chemical, and biological regimes or processes that are associated with HAB events? Are some ecosystems more susceptible to HABs than others?

Issue:

Population dynamics, including the rate processes required in predictive models of harmful blooms, cannot be adequately described or predicted, although this information is of fundamental importance to effective resource management.

Information on bloom dynamics can be gained through laboratory and field studies that define nutrient uptake kinetics, growth rates, loss terms, and life cycle dynamics. While field conditions such as circulation, meteorology, and water chemistry have long been recognized as critical elements in blooms of some toxic species, neither the initial boundary conditions, nor the hydrographic regimes within which harmful blooms occur are clearly understood. The comparative ecosystem approach adopted by ECOHAB will permit common features to be identified, such as coastal currents, upwelling, and nutrient enhancement of biomass levels. Additional insights will be obtained through numerical modeling efforts. Despite the fundamental importance of predictive models for harmful algal blooms in different regions, no such models exist for U.S. problem species (see Box 3.2.4). The ultimate goal is to couple population dynamics with physical circulation models for a given hydrographic regime, and to refine physically/biologically coupled models using field bloom observations and toxicity patterns.

We need to:

1. Identify environmental and biological cues or characteristics that can be measured and used to predict the onset and magnitude of HABs for the purposes of research and management.

2. Determine biological rate processes and initiate studies of coastal hydrography and water circulation for development of physically/biologically coupled models at temporal and spatial scales appropriate to harmful algal blooms.

Approach and Technology: Here again, shipboard observations, field programs, satellite remote sensing and moored instrument arrays can all provide the level of detail required for the identification of the mechanisms underlying HAB out breaks. The key is to obtain data at appropriate time and space scales for the blooms under study, and this will require careful planning and considerable advance study so that programs are mounted in the proper place at the proper time.

Theoretical and heuristic models that can be used to guide the formulation and testing of hypotheses and to evaluate the causes and consequences of variability in nature should be developed as an integral part of these multidisciplinary field studies. Models are required to represent the broad range of environmental dependencies that contribute to HABs. Since HABs reflect physical and biological dynamics over a broad range of time and space scales, a hierarchy of models will be required. On small scales, models that examine the vertical experience of HAB populations over the diurnal cycle are needed to elucidate cell dynamics. On larger scales, models that examine bloom transport and dispersion are essential. Model dynamics and parameterization must be driven by field and laboratory data that are sufficiently detailed to allow independent testing and corroboration. Such robust models may then have predictive capability. Numerous data sets exist that can be used for testing hypotheses regarding HAB dynamics, but often such data have not been examined in depth nor have they been examined in terms of potential interactions among physical, chemical and biological variables as they relate to HABs. Retrospective analyses of historical data and information may provide important insights at a relatively low cost.

Often the main limitation of models is the paucity of data available to formulate, force and test them. As discussed above, field programs must be combined with coupled physical-biological models to gain the most from limited resources and to test hypotheses concerning bloom initiation, transport, and patterns of accumulation and dispersal. Much can be accomplished with limited data if it is obtained at the right places and the right times.

3.2.3 Summary

The Environmental Regulation program element of ECOHAB addresses factors that act on harmful algal populations and regulate their distribution, abundance, and impact. Despite the diverse array of HAB species and the many hydrographic regimes in which they occur, one common characteristic of such phenomena is that physical oceanographic forcings play a significant role in both bloom dynamics and the patterns of toxicity or adverse impacts. Furthermore, the interplay or coupling between physical variables and biological "behaviors", such as swimming, vertical migration, or physiological adaptation, holds the key for understanding many HAB phenomena. This physical/biological coupling can occur at both large and small scales, and includes processes of great interest to both physical and biological oceanographers.

Understanding the small- and large-scale physics underlying HAB phenomena is a clear priority, but this need should not be restrictive. Observational and modeling studies of physical processes need not be massive in scale, cost, or complexity to provide useful information. Significant insights on HAB dynamics have been obtained from field programs with modest but focused physical components (e.g., Seliger et al., 1970; Tyler and Seliger, 1978; Franks and Anderson, 1992a).

The potential stimulatory influence of anthropogenic nutrient inputs on HAB incidence is a key unknown and time-series analyses of existing data bases are required, as are laboratory studies of the stimulatory effects of chemicals contained in effluents or terrestrial runoff.

This program element will require investigations spanning the spectrum from large-scale field studies to mesocosm and laboratory experiments. Modeling has a major role to play as well. In many cases, the technology exists to address the questions that are asked, but development is needed to permit biological data to be obtained on time and space scales similar to those currently possible with physical and chemical measurements. This challenge is compounded by the need to focus on individual species rather than communities.

The issues highlighted in this program element are entirely complementary to those of the Organism element, and together they outline a direct path toward the goal of understanding HAB dynamics and impacts. Managers must recognize the urgent need for better information about how the environment, and especially how human alterations to the environment, can alter coastal ecosystems and lead to harmful blooms.

3.3.1 Introduction

***Rationale:* The negative impacts of HABs are the result of complex interactions that begin at the phytoplankton community level and extend to upper trophic level compartments. Habitat physics, life cycles, community structure, growth and grazing processes all combine to regulate the dynamics of the HAB event. Therefore, studies on the impacts of trophic interactions in the selection and dynamics of HABs, and conversely, the impacts of HAB events on trophic structure, processes and interactions are essential if we are to understand the ecology and oceanography of harmful algal blooms.**

Phytoplankton blooms develop through a sequence of stages termed initiation, growth, maintenance and decline. A key to understanding bloom dynamics is the identification of processes leading to transitions between these stages; that is, what factors in the biology of harmful algal species and their grazers lead to changes in growth and loss processes at different phases of the HAB cycle? Of the terms included in the population growth equation given in Box 3.2.1, we consider in this section only the trophic interactions. Specifically, it is imperative that we understand how competitive interactions between harmful algal species and other phytoplankton contribute to the formation of blooms. Likewise, we must evaluate how grazing controls, or fails to control, HAB development. These issues define the first goal of the Food-Web/Community Interactions program element:

***Goal:* Determine the impacts of trophic interactions on selection for, and dynamics of, HABs.**

Harmful algal blooms involve multiple interactions among predators, competitors and the harmful algal species within an ecosystem. Many routes have been demonstrated by which HABs can impact food-webs (Box 3.3.1), yet little is known about the nature, extent, and ramifications of many of those pathways. We must therefore determine the relative importance of each of these interactions over appropriate spatial and temporal scales. Implicit in this task is the elucidation of the pathways and fates of HAB toxins in the food-web. The mechanisms by which the timing and frequency of HABs (both toxic and high-biomass types) affect community and trophic structure also need to be identified. Progress in these areas is essential to realizing our second goal:

***Goal:* Determine the impacts of HABs on trophic structure, processes and interactions.**

3.3.2 Research Agenda.

The following section outlines specific issues defining high priority field and experimental studies required to establish how trophic interactions regulate HAB species' selection and population dynamics, and how HAB events, in turn, influence community/trophic structure and trophodynamics.

Issue:

It is essential to know the extent to which bloom formation results from a breakdown of grazing

or from harmful species outcompeting other phytoplankton for limiting resources.

Interspecific competition influences HAB dynamics. The presence of co-occurring phytoplankton species reduces the capacity of the environment to support one species' requirements from a common pool of limiting resources (e.g., nutrients). Species competition coefficients are modified continuously by changes in growth parameters such as temperature, light and nutrient availability, and are further altered by changes in grazing pressure, community structure and allelochemical effects. Enhanced growth or physical accumulation alone does not always explain HABs, as some taxa secrete allelopathic substances that inhibit or stimulate the growth of competing and co-occurring algal species (e.g., Pratt, 1966; Gentien and Arzul, 1990) or inhibit grazing (Smayda, 1992).

Grazing control of HABs depends upon both the local abundance of grazers and their ability to ingest the harmful algal species (Box 3.3.2). Low grazer abundance can be critical in the early phases of bloom development by providing times or regions where grazing losses are less than increases from cell division. Low grazer abundance can result from a variety of external biological factors (e.g., predation on grazers), or physical factors (e.g., spatial separation of HAB species from benthic grazers). Reductions in grazer abundance may also occur in direct response to an HAB event (e.g., avoidance or mortality induced by HABs; Fiedler, 1982; Huntley, 1982), or as a result of the effects of past HAB events on grazer populations. In cases where grazers are abundant, grazing control may still not be exerted because toxins or small size can reduce the chance that HAB species will be ingested. If harmful algal species are consumed, grazers may be unaffected, impaired or killed. The response of zooplankton and benthic grazers to toxic algal occurrence is often species-specific in terms of behavioral responses and toxin susceptibility.

Grazing control of HABs can also depend on the population density of the harmful alga, as, for example, when suppression of grazing occurs above a threshold concentration of the alga, as demonstrated for the Narragansett Bay brown tide in 1985 (Tracey, 1988). A threshold effect may also occur if the daily production of new HAB cells becomes large enough to saturate the ingestion response of the grazers and the ability of grazers to increase their populations. In that case, population growth can accelerate dramatically (Donaghay, 1988). A breakdown of grazing control has been implicated in the brown tides in Narragansett Bay (Tracey, 1988) and in Texas (Buskey and Stockwell, 1993) and removal/loss of the grazer population has been reported to precede or accompany bloom development (Montagna et al., 1993). There is, however, little information on how the nature of the grazer response influences the timing, magnitude and duration of HABs.

In order to address the issue of trophic influences on HAB formation we need to:

- 1. Determine the role of differential growth rates, nutrients and nutritional strategies in competitive interactions among phytoplankton species.**
- 2. Determine the nature and extent of allelopathic interactions.**
- 3. Determine the importance of spatial and temporal separation between harmful algal species and grazers, and the relative contribution of pelagic and benthic grazing.**
- 4. Determine the role of harmful algal species' behavior, toxicity and food quality in reducing or avoiding grazing controls, as well as the importance of density-dependent processes (e.g., grazing thresholds).**
- 5. Determine the effects of mixed (toxic/non-toxic) assemblages on grazing control (e.g., does breakdown of grazing only occur once harmful algae become a dominant component of the phytoplankton?).**

Approach and Technology. A combination of field, mesocosm and laboratory studies will be required to elucidate the nature and extent of species' interactions and grazing regulation in HAB phenomena.

Quantitative data for growth rates and grazing-related mortality rates of harmful algal species are needed, as are measurements of population recruitment rates for both HAB taxa and their grazers. *In situ* estimates of growth and grazing rates, obtained in the context of sampling programs that define the temporal and spatial variability of an HAB and its potential grazers, are essential for quantifying the role of grazers in controlling HAB dynamics. Mesocosm experiments are needed to determine the patterns and dynamics of interactions between HAB species and grazers, and the population growth characteristics of competitors in the presence and absence of harmful algal species. Laboratory investigations are required to determine interactions between HAB species and individual grazers, to measure growth and grazing rates on harmful algal species in the presence of "organics" secreted by HAB species, and to elucidate allelopathic impacts, mechanisms and pathways involved in HABs.

Development and/or improvement of the following technologies are required to implement the approaches described above: species-specific molecular probes for HAB identification; methods for *in situ* detection and quantification of HABs during all bloom phases; diagnostic indicators of grazer physiological status; methods for assessing grazer food quality; video techniques for measuring *in situ* grazing and avoidance behavior; high-resolution sampling of fine-scale HAB and grazer distributions; and suitably scaled microcosm and mesocosm experimental strategies and systems.

Issue:

Are biological controls (e.g., grazers, pathogens) the cause of bloom termination?

The role of biological mechanisms in contributing to bloom termination remains largely unknown. In some instances, HAB impacts on grazers are so severe that these organisms may be of little consequence in the termination of blooms (e.g., Bricelj and Kuenstner, 1989; see Box 3.3.1). The occurrence of viral particles in cells of a harmful algal species has also been observed (Sieburth et al., 1988; Milligan and Cosper, 1994), but the efficacy of this mechanism to control natural HABs remains to be demonstrated. In addition, it has been suggested that bacteria may play a role in regulating the population dynamics of HAB species (Doucette, 1995).

Assessing the involvement of biological controls in terminating HABs requires investigation of:

1. The effects of HABs on the grazer community in terms of functional groups and their concentrations.

2. Alternative biological mechanisms for HAB decline and termination (e.g., pathogens).

Approach and Technology. Several approaches, including field studies and mesocosm and laboratory experimentation, are needed to determine how specific biological mechanisms can contribute to the termination of HABs. The majority of tasks described earlier as essential to defining the influences of trophic factors on bloom formation are also required here. In addition, the impacts of pathogens such as viruses and bacteria on HABs need to be assessed in a quantitative fashion in the context of both field studies and mesocosm manipulations. Sampling schemes must take into account the appropriate temporal/spatial scales relevant to these interactions, as well as environmental factors potentially regulating the distribution and abundance of the pathogens. When possible, pathogenic organisms should be isolated and examined in the laboratory to provide a mechanistic understanding of their effects on HAB taxa.

Strategies for elucidating factors involved in the formation and termination of HABs overlap considerably. Most of the technologies required to investigate bloom termination are well established in the phytoplankton and zooplankton literature. To evaluate the role of pathogens in terminating blooms, several new techniques must be developed or improved: taxon-specific molecular probes and their application for identifying and quantifying pathogens *in situ*; sampling methods for concurrently resolving HAB and pathogen distributions over a range of temporal/spatial scales; techniques for measuring negative impacts of pathogens on HAB species; identifying the underlying mechanisms, and assessing the taxonomic specificity of these effects; and appropriately "contained" experimental

microcosm and mesocosm systems.

Issue:

It is essential that we learn the manner in which the effects of HABs on the food-web are controlled by toxin dynamics, routing pathways, and the differential susceptibility of species at higher trophic levels.

The toxins of HAB species may have evolved to release these species from grazing pressure. Similar anti-herbivore defenses are well-documented in terrestrial plants, but have received scant attention in marine systems. Many algal toxins (e.g., PSP and DSP toxins) are endotoxins that affect planktonic and benthic grazers after consumption. Susceptibility to ingested toxins and, thus, the ability to accumulate toxins, vary markedly within and among taxa (Twarog et al., 1972), as suggested by reports that finfish appear to be more sensitive to PSP toxins than crustaceans or molluscs (Robineau et al., 1991). **If the grazing species are not killed, accumulated toxins may be transferred to other components of the food-web and affect other organisms at higher trophic levels.** This is an area where our knowledge is rudimentary at best, as subtle, ecosystem-level effects are probably pervasive, affecting many different trophic levels, depending on the toxin involved. Recruitment rates or year-class sizes of important commercial fish species may well be directly affected by brief exposures of larval or juvenile stages to toxic algae.

Zooplankton impaired by ingesting harmful algal species may be more susceptible to predation, and thus may become an important vector for transferring toxins in the pelagic food-web. Alternatively, zooplankton killed outright may sediment and allow toxins to enter benthic food-webs. Zooplankton fecal pellets may also be important sources of toxin to benthic communities. Thus, zooplankton can act as vectors of HAB toxins resulting in events such as fish kills (White, 1981; Smayda, 1992). Herbivorous fish can also accumulate and transfer toxins, and even cause mass mortalities of the marine birds that consume them (Work et al., 1993). Mortality of marine mammals linked to trophic transfer of HAB toxins has also been reported (Geraci et al., 1989). During their food-web transfers, toxins may be bioaccumulated, excreted, degraded or structurally modified, as in the case of enzymatic bio-transformation of PSP toxins in some bivalve molluscs (Cembella et al., 1993).

In order to define the role of toxins in mediating the effects of HABs on food-webs, it is necessary to:

- 1. Identify target species and their life-history stages that are adversely affected by toxic algae, and those that act as vectors of toxin transmission through the food-web.**
- 2. Determine pathways, transfer rates and mechanisms for bioaccumulation, transformation, degradation and elimination of algal toxins.**
- 3. Characterize modes of action of various phycotoxins (e.g., neurotoxic, cytotoxic, hemolytic) on marine fauna and determine their differential susceptibility.**

Approach and Technology. Field studies as well as supporting laboratory and mesocosm studies using algal toxins as tracers are needed to describe changes in toxin concentrations and transformations of toxins from one trophic level to another. Predictive and heuristic models of food-web transfer of algal toxins should be developed. These models might be analogous to those formulated for anthropogenic contaminants (heavy metals, radionuclides, organic pesticides). A comparative, experimental approach is also needed to determine dose-dependent behavioral (e.g., swimming avoidance), physiological (e.g., grazing inhibition) and cellular (e.g., toxin inactivation, compartmentalization) responses of marine organisms to toxic algal species.

In order to carry out the research on toxins associated with HABs outlined above, rapid, standardized toxin assays, with detection based on both chemical structure and toxic activity, must be developed to

elucidate toxin pathways. Toxin probes (e.g., antibodies specific for individual forms of a toxin), employed in conjunction with these assays, may be required to quantify and localize toxin derivatives in target organisms.

Issue:

Are chronic, sublethal impacts of HABs more significant than acute (lethal) impacts in altering food-webs or causing trophic dysfunction?

Blooms of harmful algae may be recurrent in some areas (e.g., red tides of PSP-and NSP-producing dinoflagellates in the Northeast U.S. and Gulf of Mexico, respectively), episodic (e.g., 1987-88 *Gymnodinium breve* red tide in North Carolina estuaries) or, more rarely, persistent (e.g., brown tides in Laguna Madre, Texas). Chronic, sublethal effects of HABs on marine biota have been documented, as in the case of brown tide persistence being linked to gradual reductions in eelgrass and shoalgrass meadows (Dennison et al., 1989). More often, however, it is the effects of brief, but acute blooms that have received the most attention because of their immediate impacts on ecosystems or humans (e.g., Shumway, 1988; Bates et al., 1989; Smayda, 1992; Burkholder et al., 1992). Episodic HABs are often associated with acute, lethal effects on adult stages of commercially important species (Tracey, 1988; Summerson and Peterson, 1990; Burkholder et al., 1992; Taylor, 1993). Removal of parental stocks may cause recruitment failure of some natural populations with limited dispersal capabilities (Peterson and Summerson, 1992). However, it is more likely that HABs adversely affect recruitment success by exerting sublethal, chronic impacts on reproduction (e.g., reduced fecundity), growth and behavior (Bricelj et al., 1987; Buskey and Stockwell, 1993). These chronic effects, which may have long-term consequences for year-class strength and persistence that are critical in the recovery of natural populations to pre-bloom levels, have received little attention and merit serious consideration.

Characterizing the relative importance of chronic, sublethal versus acute, lethal impacts of HABs on food-webs and their components requires that we:

- 1. Determine lethal and sublethal effects of HABs on life-history stages of key species in the food web.**
- 2. Identify mechanisms of recruitment failure, or reduction in affected species.**
- 3. Investigate the extent, time frame and mechanisms of recovery of natural populations impacted by HABs.**
- 4. Characterize differential effects of episodic, recurrent and chronic HAB events on food-webs.**
- 5. Identify HAB-induced changes in ecosystem energy/nutrient pathways.**

Approach and Technology. Field and mesocosm studies, as well as laboratory experimentation, are essential for evaluating the impacts of chronic versus acute exposure to HABs on food-web structure and trophodynamics. This suite of approaches is needed to determine the species-specific effects of harmful algal species on egg, larval, juvenile and adult stages of target species. Comparisons of HAB impacts are needed both among different habitats and within the same system where HABs occur with different frequencies, duration and intensities. We must quantify the effects of recurrent, episodic and persistent HAB events on recolonization rates of species from representative trophic levels, which vary in their life-history strategy, generation time and susceptibility to HABs. We also need to investigate the impacts of HABs at aquaculture sites, where stocks are concentrated at high densities and are routinely monitored for growth, mortality and disease incidence.

Several technological advances/improvements are needed to augment those currently employed for acquiring the types of information cited above: remote sensing technology and in situ high frequency optical devices to obtain more rapid, efficient measurements of phytoplankton abundance and

composition; computerized motion analysis to study behavioral effects; toxin assays and probes, including applications development, for quantifying and localizing toxins in target species.

Issue:

Are HAB impacts are controlled by the degree of temporal and spatial overlap between blooms and critical life cycle stages of affected species.

The impacts of HABs on sensitive life cycle stages of affected species higher in the food-web depend upon their co-occurrence in time and space, which varies dramatically with the degree of vertical stratification and exchange with surrounding waters. In some well-mixed estuaries and lagoons, HABs may be sufficiently persistent and dispersed throughout the system so that all species are equally exposed, as in the case of brown tides in Laguna Madre, Texas (Buskey and Stockwell, 1993). Such even exposure greatly simplifies the task of relating observed changes to the effects of HAB populations. However, in stratified waters, reduced vertical mixing may allow weakly swimming plankton, including HABs and potentially affected groups (i.e., microzooplankton, macrozooplankton and fish larvae) to form highly concentrated layers surrounded by regions of low or undetectable concentrations of the organisms (Donaghay et al., 1992). In stratified systems exposed to current shear from winds and tides, the distributions of both HABs and target plankton may also vary dramatically in response to lateral advection of layers and interactions between organismal swimming behavior and current shear. Regardless of the prevalent physical system, however, it is highly unlikely that HAB impacts can be predicted from average concentrations of HAB and affected species.

In order to assess the extent to which temporal and spatial factors control the impact of HABs on other species, we need to:

1. Investigate the temporal and spatial coincidence of susceptible life-history stages of key species (e.g., grazers) with HABs.

2. Determine the physical processes and biophysical interactions that control bloom development and grazer responses.

Approach and Technology. Determining the degree to which HAB impacts are regulated by temporal and spatial factors requires evaluation of impacts on affected species from actual time-space abundance measurements. The typical approach has been to estimate responses based on statistically derived *average* dispersion and abundance measurements, often on an annual basis. Yearly, and probably even seasonal means, are, however, inadequate to assess impacts of HAB species, which often form short-term, high-impact blooms in areas that provide critical spawning/nursery habitat for higher trophic levels. Consequently, we need to determine the small-scale, temporal/spatial distribution patterns and abundances of HAB taxa.

Resolution of co-occurrence effects in stratified waters will require both field and mesocosm studies. Field programs must be aimed at quantifying temporal and spatial scales, as well as in situ concentrations of HAB and co-occurring, affected species. This will require application of high resolution sampling techniques, both to detect changes in abundance on sub-meter scales, and to link those changes to physical structure and processes in the system where the HAB occurs. Such field investigations must be complemented by mesocosm experiments designed to elucidate the underlying mechanisms that lead to observed patterns of co-occurrence or avoidance.

Accurate, fine-scale characterization of the temporal/spatial aspects of HAB interactions with affected species will depend on: adapting spectral optical and video sensors for deployment on vertical profilers or towed systems to detect HAB distributions in real-time; designing "smart" sampling systems triggered by these sensors to collect discrete samples for simultaneous identification of and experimentation on HAB and affected species; developing techniques for measuring in situ swimming behavior of motile HAB taxa and affected species; and improving methods for rapid quantification of

HAB toxin concentrations.

Issue:

Do high biomass (non-toxic) HABs adversely impact the food-web directly through reduced food quality, or indirectly through environmental effects?

Harmful effects of algal blooms may occur in the form of anoxic/hypoxic events. Such incidents result from increased sedimentation of organic matter coupled with enhanced microbial decomposition of phytoplankton on the bottom, and/or via transient increases in water column respiratory demands of the phytoplankton (Box 3.3.3; Falkowski et al., 1980). Mass mortalities of benthic fauna associated with these events are widespread and affect a broad range of taxa (e.g., Swanson and Sindermann, 1979), but their connection to HABs is often circumstantial or speculative. High microalgal biomass and resulting light attenuation are also known to cause marked declines in biomass and distribution of seagrasses. Both effects have been noted in eelgrass and shoalgrass communities exposed to picoplanktonic brown tides in New York and Texas waters. Reductions in irradiance levels may also induce shifts in macrophyte species' composition toward less desirable forms. Blooms of picoplanktonic microalgae show enhanced light-scattering properties and are thus particularly likely to cause these light-related effects, yet the prevalence and magnitude of the problem have not been adequately characterized. Because seagrasses provide an important nursery habitat for many commercially valuable shellfish and finfish species as well as their associated fauna, issues related to these habitats are of special concern.

Nutrient-mediated macroalgal blooms can also lead to the decline of seagrass as well as coral reef ecosystems (LaPointe and O'Connell, 1989). In addition, high biomass HABs may limit growth and recruitment of grazers if the dominant algal species is poorly predated upon due to its unpalatability, small size and indigestibility, or because of physical impairment of feeding (e.g., Bass et al., 1990). High algal densities *per se* may also interfere with food uptake and utilization by many suspension feeders. Sublethal effects of food quantity/quality, which remain poorly understood (Donaghay, 1985), are potentially important determinants of recruitment success in grazer populations.

Evaluating the direct and indirect effects of high biomass HABs on food-webs requires that we:

- 1. Determine the relative importance of oxygen depletion from HABs in the water column vs. surface sediments.**
- 2. Understand the extent and ecological consequences of light attenuation from HABs, including relative effects on phytoplankton, epiphytic algae, seaweeds, seagrasses and their supporting fauna.**
- 3. Investigate the effects of HABs on food quality available to consumers (e.g., via changes in size spectra, chemical composition).**
- 4. Define the mechanisms and threshold levels at which high algal biomass interferes with food capture and utilization by grazers.**
- 5. Understand the controlling mechanisms for restructuring marine communities during recovery.**

Approach and Technology. Elucidation of high biomass HAB impacts on food-webs will require a multi-faceted approach, including field studies, experimental mesocosm manipulations and laboratory investigations. Field work should be aimed at quantifying organic sources and rate processes within the water column and in sediments to allow development of an oxygen budget. This will involve continuous monitoring of dissolved oxygen and irradiance levels at spatial and temporal scales relevant to HABs. Assessment of macrophyte and faunal coverages, as well as seasonal successional patterns, before and after bloom events are likewise needed, and should incorporate synoptic aerial mapping of the

macrophyte communities. The effects of changes in food quantity and quality must be evaluated in field and mesocosm experiments to determine their impacts on ingestion, growth and reproduction for critical life stages of target species. Mesocosm studies are required to assess the consequences of removal or perturbation of target species on food-web structure and processes. Laboratory and mesocosm experiments will provide insights into the mechanisms and linkages between high biomass HAB events and associated food-web /habitat responses.

Implementing the variety of studies needed to characterize the effects of high biomass HABs on food-webs will rely on: remote sensing approaches to aid in determining the occurrence and distribution of HAB events and high biomass coverage; moored instrumentation for continuous monitoring of impacted vs. non-impacted sites; techniques for automated, simultaneous sampling of the diel changes in vertical structure of light, oxygen, phytoplankton (distinguishing HAB taxa from other phytoplankton) and vertical mixing; regional geographic information system data bases for high-biomass blooms; computerized predictive models for oxygen deficits from HABs, relating water-column dissolved oxygen, BOD and algal abundance with sediment BOD and algal abundance; improving available methods for assessing food quality, both biochemically and by comparison to other known quality foods.

3.3.3 Summary

As algal toxins move through marine food-webs, they can have a broad spectrum of effects on marine organisms in inshore, offshore, pelagic, and benthic habitats (Box 3.3.4). The scope of these effects, resulting from both chronic and acute exposure to the toxins, has become more evident in recent years, since a wide variety of animals are now known to accumulate biotoxins and act as intermediate vectors to consumers at higher trophic levels. Algal blooms can also have harmful effects not related to production of toxins, such as overgrowth and shading by seaweeds, oxygen depletion of the water column from high biomass blooms, fish mortalities from over-stimulation of gill mucus production, and mechanical interference with filter-feeding structures. The Food-Webs/Community Interactions program element of ECOHAB recognizes the diverse nature of these processes, and highlights key areas for focused investigation. What is needed is a recognition by managers and regulatory officials that harmful algal bloom impacts extend far beyond the obvious manifestations of poisonous shellfish and dead fish, and include subtle, sub-lethal effects that can alter or even destroy ecosystems through time. Identifying such impacts and determining their extent and magnitude is a significant challenge for ECOHAB scientists.

This program element also emphasizes research in the other direction the effects of grazers and other organisms on the harmful algal blooms, since in many cases, the bloom reflects the suppression or absence of grazing. This again is an area of obvious importance to the dynamics of HABs, but one which has received only rudimentary study thus far.

ECOHAB

The Ecology and Oceanography of Harmful Algal Blooms

A National Research Agenda

4. Regional HAB Phenomena in the United States

4. Regional HAB Phenomena in the United States

The following regional summaries present brief perspectives on specific HABs. Written by workshop participants familiar with each region, they review available information and point out deficiencies. These are provided to document the extensive geographic scale of HAB impacts and to illustrate the diversity of the phenomena involved. In some cases, the regional summary only reflects one manifestation of a particular phenomenon, and thus understates the extent of the HAB problem. For example, macroalgal blooms in Florida are described, but similar outbreaks occur in coastal waters throughout the U.S. Similarly, cyanobacterial blooms are highlighted on the U.S. east coast, but occur on both coasts as well as in rivers, lakes and ponds throughout the country.

It is obvious from these summaries that serious information gaps exist in all regions and for all organisms, but some deficiencies are similar across multiple regions. The summaries also highlight how information bases differ among regions, due to different physical regimes, causative organisms, and/or level of research. This is true even when the organisms are the same or similar (e.g., *Alexandrium* spp. along the Northeast and Pacific coasts).

HAB phenomena are truly diverse, and it is this diversity that must be accommodated in the ECOHAB science plan. Clearly, single investigator and multi-investigator, regional projects are required to address the many identified deficiencies. This approach can address the immediate information needs of each region, but ECOHAB will derive significant benefit from comparisons among regions and attempts to highlight common principles or mechanisms underlying many of these phenomena.

Northeast Region: PSP

The most significant HAB problem in the northeastern U.S. is PSP caused by several closely related species in the dinoflagellate genus *Alexandrium*. The affected resources are predominantly shellfish, but PSP toxins also affect higher levels of the food-web, including lobsters, fish, and marine mammals.

Paralytic Shellfish Poisoning. PSP is a recurring problem that has affected large areas of the region every year for over two decades. Prior to 1972, shellfish toxicity was known only in eastern Maine and Canadian waters to the north. That year, a massive bloom introduced *A. tamarense* to southern waters, and there have been shellfish harvesting quarantines along large sections of coastline every year since. A second expansion of the regional PSP problem occurred in 1989, when the rich shellfish beds of Georges Bank and Nantucket Shoals were found to be contaminated with PSP toxins. The size of the offshore area affected, the difficulty in monitoring an area so far from land, and the slow depuration of toxin from the affected shellfish have necessitated a permanent closure of the surf clam fishery on

Georges Bank for the past five years.

One key feature of the ecology and bloom dynamics of toxic *Alexandrium* species in the northeast is that they include a dormant cyst stage in their life histories. Cysts germinate in the spring to inoculate overlying waters with a "seed" population.

In the southwestern Gulf of Maine, *Alexandrium* cell distributions are associated with a coastal current or buoyant plume formed by the outflow from rivers in southern Maine (Franks and Anderson, 1992a). The southward propagation of this plume and the entrained *Alexandrium* cells creates an annual north-to-south sequence of PSP toxicity, beginning in late May or early June. The plume is influenced by freshwater flow, wind, and bathymetry, with predictable consequences for the location and timing of coastal PSP events. Extensive freshwater flow in early May creates a strong, fast plume, while low river flow may preclude the formation of a coastal plume. Upwelling-favorable winds oppose the propagation of the plume, forcing it offshore and arresting its north-to-south motion. This tends to halt the alongshore propagation of toxicity, leaving the southern regions toxin-free. In contrast, downwelling-favorable winds force the plume against the coast, and accelerate its alongshore propagation. Such conditions lead to widespread toxicity, and may account for the recent occurrence of PSP on Georges Bank (Franks and Anderson, 1992b). Understanding of the physical forcings that influence the location and dynamics of the coastal buoyant plume, and information concerning the abundance of *Alexandrium* cells within the plume have given us a limited predictive capability of the location and timing of toxic outbreaks over large (>100 km) scales (Franks and Anderson, 1992b).

Other potential HAB Problems. The general view of the harmful algal bloom problem in New England is that PSP is widespread, persistent and expanding, while outbreaks of other harmful species cause sporadic damage on a smaller scale. A realistic concern is that HAB species already present within the region are likely to cause problems in the future. For example, an outbreak of *Gymnodinium mikimotoi* caused extensive benthic mortalities in 1988 in Maine, a region of New England that is rapidly expanding its salmon farming industry. This fish-killing species causes recurrent and significant financial losses to the fish farming industries throughout the world. Likewise, *Pseudo-nitzschia pungens* f. *multiseries*, a diatom responsible for ASP, has been detected in Gulf of Maine waters, and its toxin (domoic acid) detected in scallops. Over the last several years, DSP has been detected in Canadian shellfish located just to the north of New England, where DSP is not yet a problem.

Some species, identified in other regions as problem algae, are regular components of the Gulf of Maine flora. The prymnesiophyte, *Phaeocystis pouchetii*, is a regular and sometimes dominant component of the spring bloom, but does not reach the high cell densities recorded in western Europe, where foam on beaches, fouling of fishermen's nets, and other negative impacts are common.

Economic Impacts. The economic impact of these outbreaks is significant, though difficult to estimate in total. Shellfish monitoring programs in each of the coastal New England states have minimized illnesses from PSP-contaminated shellfish and prevented any deaths. This extraordinary level of human health protection has come at a cost that has never been directly quantified, but must be in the range of millions of dollars per year, including the annual cost of the state and federal shellfish monitoring programs (nearshore and offshore), the value of unexploited resources such as surf clams and roe-on scallops, and the loss to shellfishermen and retailers from shorter-term quarantines, including "spinoff" effects on other perfectly safe fisheries products that are rejected by consumers during red tide outbreaks. Estimates of the losses to shellfishermen and other seafood-related industries are few, but a single PSP outbreak cost the state of Maine \$6 million (Shumway et al., 1988).

Significant unknowns in our understanding of *Alexandrium* blooms include:

- What are the geographic origins of newly germinated cells that initiate the *Alexandrium* populations in the coastal current and the physical/behavioral mechanisms by which they enter the buoyant plume?
- Is accumulation of cells at small-scale fronts necessary for their entrainment in the buoyant plume?

What other physical -biological interactions are important to bloom dynamics on small (<10 m) scales?

- What is the nutrient physiology of the cells, their requirements, uptake rates and nutrient status during the long-distance transport?
- Is the localization of elevated *Alexandrium* populations within the plume a result of physical entrainment, or does it reflect an increased growth rate in response to unique chemical properties of plume waters?
- Are nearshore cells in the coastal current responsible for PSP offshore on Georges Bank?
- What are the hydrodynamic forcings that regulate PSP outbreaks in other areas of the Gulf of Maine?
- What effect does zooplankton grazing have on the *Alexandrium* populations, and what are the ecosystem impacts of toxin transfer through the food-web?

Mid-Atlantic Coastal Region: Brown Tides

Blooms of the small (2-3 μm) chrysophyte *Aureococcus anophagefferens*, referred to as "brown tide" due to the resulting water color, have been confirmed in many locations along the northeast coast of the United States, especially in Narragansett Bay, RI, Barnegat Bay, NJ, and the Peconics-Gardiners Bay estuary and south shores of Long Island, NY (Cosper et al., 1989a). The figure on the next page shows the widespread distribution of this organism in the northeastern U.S., including many areas with no previous history of visible or destructive blooms (Anderson et al., 1993).

Brown tides are restricted to shallow, vertically well-mixed waters, and occur during late spring and summer at maximum concentrations of 3×10^9 cells l⁻¹; bloom duration ranges from one to four months. The first outbreak occurred concurrently in New York and Rhode Island in 1985, and blooms have recurred in New York bays in subsequent years, with varying intensity, duration and geographic spread. An immunofluorescent method is used for the reliable identification and quantification of *A. anophagefferens*.

Severe light attenuation in Long Island bays due to the brown tide caused a significant reduction in the depth penetration and leaf biomass of eelgrass (Dennison et al., 1989), which serves as an important nursery habitat for numerous fish and shellfish. Brown tides also caused severe mortalities, recruitment failure, and growth inhibition of commercially important, suspension-feeding bivalves, including blue mussels in RI (Tracey, 1988) and bay scallops in NY (Bricelj and Kuenstner, 1989). Economic losses from the brown tide for the New York State bay scallop fishery were estimated at \$2 million per year during early outbreaks.

Aureococcus adversely affects feeding of larval and adult bivalves, but only through direct cell contact. Although specific cell toxins have not yet been identified, the cell surface of this microalga contains a bioactive compound that interferes with ciliary beat and thus food capture of bivalves, a response mimicked by the common neurotransmitter, dopamine. Thus, impaired grazing by zooplankton and filter-feeding benthos are believed to contribute to bloom occurrence.

The physico-chemical conditions that contribute to the formation of *A. anophagefferens* blooms are still largely unknown. Low annual rainfall, and increased residence time of bay waters that lead to increased salinity (> 28 ppt) may favor the development of the brown tide, as does increased water temperature (Cosper et al., 1989b). Year-to-year persistence of *A. anophagefferens* in the Long Island region is partly attributed to its wide temperature tolerance and thus its ability to survive overwintering conditions. Mesocosm experiments show that this alga grows well at relatively low concentrations of dissolved inorganic nitrogen (DIN), and a negative correlation has been described between the abundance of

Aureococcus and mean DIN concentrations experienced during blooms. Therefore, macronutrient loading of bays does not appear to be the direct cause of brown tide, but micronutrients, including trace metals such as iron and selenium, and certain chelators, have been implicated as growth promoters in its formation. The iron requirement of *Aureococcus* and its ability to grow in the presence of organic nutrients (e.g., glutamic acid) are higher than for many other common phytoplankton species. Viral particles have been described and isolated from field-collected *Aureococcus* cells, and viral-lysis of algal cells has been attributed a potential role in bloom dissipation (Milligan and Cosper, 1994).

A number of questions need to be answered in order to more fully understand the physical and biological mechanisms controlling the population dynamics of *A. anophagefferens*, and the effects of brown tides on nearshore marine communities.

- What role does micronutrient availability, especially via groundwater, play in controlling bloom dynamics?
- What climatological-meteorological and/or hydrographic events are associated with the regional occurrence of the brown tide in the northeast?
- To what extent do biological mechanisms (e.g., grazing depression, competitive interactions with other phytoplankton, and viral lysis) contribute toward the formation of monospecific blooms and subsequent decline of the brown tide?
- Is microzooplankton grazing negatively impacted by brown tide? What are the toxins/metabolites that cause species-specific inhibition of suspension-feeding in planktonic and benthic organisms?
- What are the time- and concentration-dependent effects of brown tides on marine fauna, during various life history stages? What are the effects on submerged aquatic vegetation and its associated community (secondary as well as primary consumers)? What are the long-term impacts of recurrent brown tides on community trophic structure?

East Coast Region: Cyanobacterial Blooms

Harmful cyanobacterial blooms (HCBs) are indicative of excessive nutrient loading in oligohaline estuarine waters. These blooms represent economic and environmental threats nationally, and have occurred in several large estuarine systems (e.g., Chesapeake Bay, Albemarle-Pamlico Sound, and Florida Bay). Cyanobacterial blooms are also serious problems in freshwater systems. **Here we highlight the east coast of the U.S., but cyanobacterial blooms occur in virtually every state, given the existence of toxic species in both freshwater and marine environments.**

Bloom taxa include filamentous (*Anabaena*, *Aphanizomenon*) and aggregated coccoid (*Microcystis*) genera, which exhibit severe neuro-, cyto-, and hepato-toxicity to a variety of mammals (including man), birds, farm animals, fish, and invertebrates (including zooplankton). HCBs accumulate as buoyant surface-dwelling, high biomass blooms. They impart negative aesthetic values, and cause taste and odor problems. These blooms rapidly terminate or "crash" in response to sudden physical perturbations (e.g., rapid drop in temperature, sudden destratification and water column turnover, or reduced irradiance associated with poor weather). When crashes occur, excessive oxygen consumption as the biomass decays can lead to anoxia. This chain of events has been responsible for major estuarine fish and shellfish kills and loss of habitat for benthic infauna (Paerl, 1988a, 1990).

Conditions which favor harmful cyanobacterial bloom development and persistence include: 1) enhanced P and N loading; 2) increases in water retention time; 3) water column stability; 4) relatively high dissolved organic matter content; and, 5) for nitrogen-fixing genera, molar N:P input ratios < 15:1. Typically, blooms develop in oligohaline tributaries experiencing periods of excessive spring N and P

loading (via runoff, wastewater discharge, etc.), followed by decreased flushing, persistent vertical stratification, and surface water temperatures $>20^{\circ}\text{C}$ (Reynolds and Walsby, 1975). Buoyant noxious species have photoprotective pigments that allow them to survive at the water surface where they can remain for weeks to months (Paerl, 1988b). Grazing pressure by macrozooplankton has little impact on either initiating or controlling cyanobacterial blooms. Trophic interactions and ecosystem structure are often radically altered in response to such blooms (Porter and Orcutt, 1980; Fulton and Paerl, 1987). While physiological and molecular knowledge of individual HCB species is good, knowledge of growth, reproductive, and trophic dynamics on the ecosystem level is at best fragmentary.

Informational needs include:

- What trophic alterations (e.g., community changes and food transfer) are attributable to HCBs?
- What are the dynamics of akinete (cyst) dispersion, activation, and bloom initiation?
- To what extent can known and novel HCBs (e.g., *Synechococcus* spp. in Florida Bay; *Nodularia*, *Schizothrix*, and *Lyngbya* in reefs and intertidal environments) disperse into nutrient-enriched mesohaline/euhaline waters?
- What are the genetic and physiological potentials for such species dispersal?
- How does the ability to fix atmospheric nitrogen (N_2), facilitate expansion into N-limited estuaries or freshwater systems?

East Coast Region: Fish Kills

In 1991, an ichthyotoxic dinoflagellate with "ambush predator" behavior and a complex life cycle was discovered at a fish kill in the Pamlico River, a large estuary in the Southeast (Burkholder et al., 1992, 1995). The organism, *Pfiesteria piscicida* (Steidinger et al., submitted; 1995), represents a new family, genus, and species of armored dinoflagellates. Its cryptic or "phantom-like" behavior was observed several years earlier when it appeared as a contaminant of unknown origin in aquarium fish cultures (Burkholder et al., 1992; Smith et al., 1989).

Unknown substances freshly secreted by finfish and shellfish stimulate *P. piscicida* to transform from benthic cysts or amoebae, or non-toxic flagellated stages to toxic zoospores. Highly lipophilic exotoxin(s) are released to the water and travel as micelles that narcotize finfish, slough fish epidermis, and cause formation of open bleeding sores (see photo below), while also damaging osmoregulatory function (Noga et al., in press). In some species, (e.g., striped bass), extensive hemorrhaging also occurs. This dinoflagellate has proven lethal to every fish species tested, including more than 20 native and exotic species (Burkholder et al., in press). At sublethal densities, *Pfiesteria*-like dinoflagellates likely cause significant chronic impacts to fish populations, affecting recruitment, reproduction, and disease resistance. Clinical research recently demonstrated that *P. piscicida* is the causative agent of the disease known as ulcerative "mycosis" in Atlantic menhaden (Noga et al., in press). The Pamlico is known for high incidence of fish ulcerations and up to 98% of all fish sampled in this estuary have manifested large, open, bleeding sores during warmer months.

The dinoflagellates consume bits of epidermal tissue and blood cells from affected fish while also engulfing bacteria, phytoplankton, and other microfauna. In addition, they produce gametes that complete sexual fusion in the presence of dying fish. Upon fish death, toxic zoospores and planozygotes form non-toxic amoeboid stages that feed on the fish remains, or without abundant food resources, the toxic stages encyst. In the absence of live fish, gametes and toxic zoospores revert to non-toxic zoospores that remain highly active in phosphate-enriched waters, especially when flagellated algal prey are abundant (Burkholder and Glasgow, 1995). Surprisingly, most of the 19 known life cycle stages

are amoebae that range in length from 5-250 μm . Under certain conditions (e.g., cold temperatures) some amoeboid stages become ichthyotoxic.

In enclosed laboratory conditions, human exposure to aerosols from toxic cultures with live fish has been linked to a variety of short- and long-term symptoms, including narcosis, respiratory distress with asthma-like symptoms, severe stomach cramping, nausea, vomiting, and eye irritation. Other autonomic nervous system dysfunction such as high, localized perspiring and erratic heart beat may last for weeks. Central nervous system dysfunction, including sudden rages and other erratic behavior can last hours to days, and reversible cognitive impairment for weeks; chronic effects such as sustained asthma-like symptoms and suppressed immune system may last for months to years (Glasgow et al., in press).

The extent of *P. piscicida*'s involvement in fish kills likely has been underestimated because of difficulty in reaching many kills when toxic zoospores are still present. Most *Pfiesteria*-associated field kills have occurred in quiet, upper estuarine tributaries with poor flushing rates, where both fish secretions and toxins can accumulate and be more readily detected. During the past three years, *P. piscicida* has been implicated as the causative agent of ca. 50% of the major fish kills in large estuaries of the Albemarle-Pamlico system, the only region where rigorous sampling protocols have been established (Burkholder et al., in press).

About two-thirds of the *Pfiesteria*-caused fish kills in North Carolina have occurred in the phosphate-rich Pamlico, and laboratory bioassays have shown that some life cycle stages are stimulated by organic phosphate sources. Field surveys documented significantly higher abundance of zoospores at sewage outfall sites relative to unpolluted sites. *P. piscicida* is euryhaline and eurythermal, with optimal growth at 15 psu and 26 oC, but with toxic activity from 2-35 psu and 10-33 oC (Burkholder et al., in press). Some stages can remain active down to 5 oC. The wide salinity/temperature tolerance of *P. piscicida* suggests that this species and its close relatives are probably widespread, at least in warm temperate/subtropical regions, acting as significant but often undetected sources of fish mortality and disease. This species has been documented in sediments or water from the mid-Atlantic to the St. Johns estuary in Florida. Recently, a second, apparently more subtropical, *Pfiesteria*-like species was identified (Landsberg et al., 1995).

Critical questions that need to be answered include:

- What is the geographic range of *Pfiesteria* -like dinoflagellates? Do they occur only in warm temperate/subtropical areas or do they occur in colder regions as well?
- Can molecular probes be developed to facilitate detection of the various life cycle stages and/or the toxins they produce?
- What are the toxins? What is their chemical structure?
- How do organic and inorganic nutrients control life cycle stages and/or toxicity?
- What chronic effects does *Pfiesteria* and its relatives have on fish recruitment, disease resistance, and survival?
- What is the role of dinoflagellates in estuarine microbial food-webs in light of the discovery of multiple, benthic amoeboid stages in *Pfiesteria*? Might these also be found in the life cycles of other dinoflagellates?

Southeast and Gulf of Mexico Regions: NSP

The toxic dinoflagellate *Gymnodinium breve* has a distribution from the Gulf of Mexico

(Mexico-Florida) to the South Atlantic Bight. This fragile species produces neurotoxins and hemolytic substances that can cause mass mortalities of marine animals, neurotoxic shellfish poisoning (NSP), and human respiratory irritation. Blooms are usually seasonal, starting in late summer/fall and lasting 3-4 months; they impact fishing and tourist industries and alter population levels or recruitment potential of affected marine animals. These recurrent bloom events cause an economic loss of approximately \$18-24 million per episode (Steidinger and Vargo, 1988; Tester et al., 1991). Associated with this economic impact is an unquantifiable "halo" effect that results in reduced sales of all seafood products within the region of the bloom and even outside the region.

The most likely scenario for the development of *G. breve* blooms in the Gulf of Mexico and South Atlantic Bight is the following. The source of the blooms appears to be on the west Florida shelf in the eastern gulf where the Loop Current may entrain bloom patches and transport them into the South Atlantic Bight via Loop Current filaments/eddies and the Gulf Stream system (Steidinger and Vargo, 1988; Tester et al., 1991, 1993). Eddies can also transport entrained blooms to the western gulf. Blooms are initiated in association with Loop Current intrusions accompanied by upwelling on the west Florida shelf. These blooms develop on the leading edge of the Loop Current front where the boundary layer is ideal for near -monospecific growth of *G. breve*. Typically the cyanobacterium *Trichodesmium* precedes or co-occurs with *G. breve* at bloom initiation and its presence may condition the water mass and enhance *G. breve* growth as well as reduce grazing pressure. Bloom initiation is followed by population growth in excess of predation, natural mortality, and advective loss, then by sustained growth (maintenance), and finally by dissipation by advection or mixing of water masses. The physical integrity of the water mass appears to be the key factor controlling growth and maintenance of *G. breve* blooms. Offshore populations of *G. breve* can be transported shoreward with winds and inoculate inshore waters. Nutrient availability in the nearshore waters then contributes to the duration and intensity of blooms. Although *G. breve* is more concentrated in surface waters, it is distributed throughout the water column down to >50 m depths.

A conceptual framework for understanding *G. breve* blooms thus exists, but there are a number of questions that need to be answered:

- In the life cycle of *G. breve*, does sexual reproduction only occur in the zone of initiation, and are resting cells such as cysts or zygotes present in sediments or at pycnoclines?
- Are there "hot spots" within the zone of initiation on the west Florida shelf that retain resting stages?
- Can molecular probes be used to detect toxins in seawater or identify different strains of *G. breve*?
- Is zooplankton grazing inhibited at moderate to high *G. breve* cell concentrations when brevetoxins or other substances are released? Does *G. breve* regulate plankton community structure?
- Are there multiple hydrographic features that are requisite for bloom initiation that can be detected using moored instrument arrays and remote sensing?
- Does *Trichodesmium* condition the water prior to *G. breve* blooms?
- What are the roles of macro- and micronutrients in the initial growth phase of blooms and how does the situation change over time with bloom development?

Tropical Regions (Florida, Puerto Rico, U.S. Virgin Islands, Hawaii): Ciguatera

Ciguatera fish poisoning (CFP) is the most frequently reported non-bacterial illness associated with eating fish in the United States and its territories. The actual number of cases is, however, estimated to

be 2-5-fold higher, since there is no confirmatory laboratory test, and diagnosis depends on a patient's clinical presentation. Southern Florida, together with Puerto Rico and the Hawaiian islands, account for the majority of documented CFP incidents in the U.S. In the Virgin Islands, it is estimated that nearly 50% of the adults have been poisoned at least once. Many CFP intoxications have been reported from temperate "inland" locations in the U.S., resulting from the commercial distribution of sub-tropical and tropical fish species.

Gambierdiscus toxicus, an epibenthic dinoflagellate, is the organism primarily responsible for ciguatera fish poisoning (Yasumoto et al., 1977). *G. toxicus* produces ciguatoxin precursors and analogues that are biotransformed during food-web transfers into ciguatoxin, the causative neurotoxin (Lewis and Holmes, 1993). The ciguatera toxins are transported through herbivorous fish to carnivorous species, where they accumulate and persist over extended periods. Fish exposed to ciguatoxin exhibit impaired swimming behavior, and as a result may be subject to increased predation. Other toxic dinoflagellates, including species of *Prorocentrum*, *Ostreopsis* and *Coolia*, share the same epiphytic habitat and entry routes into the food chain as *G. toxicus*, but remain only circumstantially linked to CFP since their toxins are not known to occur in fish at levels that can affect humans.

G. toxicus does not form pelagic blooms of motile cells, but is most prolific in shallow waters (3-15 m) primarily as an epiphyte on red and brown macroalgae associated with coral reefs and protected embayments. Field and laboratory studies have established the temperature and salinity ranges of *G. toxicus* as 20-34 °C and 25-40 psu, respectively. Ciguatera endemic areas in both the Caribbean and Pacific are characterized by oceanic salinities and are primarily associated with island land masses; CFP is essentially absent along continental perimeters. In Florida, most cases of ciguatera are contracted in the summer, which is consistent with the elevated *G. toxicus* abundance observed during this period. By comparison, in the Virgin Islands neither the number of CFP incidents nor *G. toxicus* abundance exhibit notable fluctuations. Overall, the spatio-temporal variability of CFP in a local area corresponds largely to the patchiness of *G. toxicus* populations; however, it is difficult to explain the often rapid, localized changes in the concentration of this species based on a response to any one environmental factor (e.g., temperature, salinity, nutrients, etc.). The variable, localized occurrence of ciguatoxic dinoflagellates within a region may also be related to their rafting on drift algae, which is considered to be a primary means of dispersal.

Phenotypic variation in toxicity observed between clones from distinct geographical areas are stable in acclimated cultures and thus are indicative of genetic differences. For CFP cases occurring in the Caribbean and eastern Atlantic, gastrointestinal symptoms occur first, while the characteristic neurological manifestations of ciguatera develop later and may persist for weeks to months or even years, producing chronic disabilities. Conversely, in the Pacific, neurological symptoms are exhibited first, while gastrointestinal symptoms are minor or absent. These patterns in symptomology may reflect different geographic distributions of individual CFP toxin(s).

Presently, no coordinated, systematic monitoring programs exist for CFP in the U.S. and its territories. This poisoning syndrome has a significant impact on commercial and recreational fishing activities in the U.S. and throughout the world.

Questions for future research include:

- Are there environmental factors that promote *G. toxicus* blooms or cause increases in the toxicity of this dinoflagellate? and, if so, can they be incorporated into predictive indices of CFP events?
- Can human activities such as reef destruction or pollution increase the scale of the problem?
- What roles do toxic species of *Prorocentrum*, *Ostreopsis* and *Coolia* play in CFP?
- Where and how are ciguatoxin precursors and analogues biotransformed in herbivorous and/or carnivorous fish? How do ciguatera toxins affect food-web function?

- Are there genetic markers that define the toxin content and profile of individual dinoflagellate clones?

Southeast Region: Macroalgae

Macroalgae cause problems throughout the coastal waters of the U.S. This summary for southern Florida provides one example of the nature and scale of the problem.

Over the past several decades blooms of macroalgae (seaweeds) have been increasing along many of the world's developing coastlines in response to nutrient enrichment associated with coastal eutrophication. In southern Florida, a diverse group of opportunistic macroalgal species outcompete, overgrow, and replace seagrass and coral reef ecosystems that are adapted to stable, oligotrophic conditions. Moreover, once they are established, the macroalgal blooms may remain in an environment for years to decades until the nutrient supply decreases. This is in contrast to phytoplankton blooms that are usually relatively short-lived (days to weeks).

The negative effects of eutrophication include nuisance blooms of macroalgae and attached filamentous epiphytes that reduce light availability to seagrasses (Sand-Jensen, 1977; Twilley et al., 1985; Silberstein et al., 1986). This results in lower seagrass productivity, habitat loss from hypoxia/anoxia, and eventual die-off of sensitive species (LaPointe et al., 1994).

Nutrient enrichment of Florida Bay and the Florida Reef Tract results from multiple nutrient sources and supply mechanisms, including: 1) advection of phosphorus-rich water from the eastern Gulf of Mexico into Florida Bay; 2) nitrogen-rich inputs from land-based agricultural activities that enter coastal waters through the Everglades via groundwater discharge and surface runoff; and 3) nitrogen and phosphorus-rich domestic wastewater generated in the Florida Keys that enters coastal waters via groundwater discharge (septic tanks, cesspits, and injection wells) and surface water outfalls. This cumulative nutrient enrichment can cause high biomass algal blooms, which include the red algae *Laurencia intricata* and *Spyridia filamentosa*, the brown algae *Dictyota* sp. and *Sargassum filipendula*, and the green algae *Enteromorpha* sp., *Codium isthmocladum*, and *Halimeda* sp.

Macroalgal blooms in South Florida, as well as other factors, have contributed to the marked decline in extent and vigor of seagrass ecosystems that provide a vital nursery habitat for pink shrimp, spiny lobster, and finfish. These commercially valuable marine species support multi-million dollar recreational and commercial fisheries that have undergone drastic declines over the past decade. The Florida Reef Tract, the third largest coral reef in the world and the only coral reef system in North America, supports the largest recreational dive industry in the world. This valuable reef system is being overgrown by macroalgal species. The trend could lead to ecological collapse of the Florida Reef Tract, with subsequent economic losses in the tourist-related industries that support the most visited coral reef and largest marine sanctuary in the world.

Questions for future research include:

- What are the physiological and ecological mechanisms that regulate the ability of macroalgae to alter the patterns of nutrient storage and primary production by reducing the role of benthic macrophytes (seagrasses) and increasing the importance of pelagic phytoplankton communities?
- How does increased macroalgal biomass accelerate nutrient release from sediment pore waters underlying seagrass communities, and how does this lead ultimately to seagrass die-off?
- What are the mechanisms for benthic-pelagic coupling of nutrients and primary production? How does increased nutrient availability mediate a shift in primary production from reef corals to macroalgal HABS?

- What are the existing nutrient inputs and their relationship to the initiation, growth, and maintenance of macroalgal blooms on the Florida Reef Tract? How does nutrient enrichment affect the early life histories of bloom-forming macroalgae?

Gulf of Mexico Region (Texas): Brown Tide

For over 5 years, regions of the South Texas coast centered around the Laguna Madre have experienced a continuous, dense algal bloom referred to as the "brown tide." The nearly monospecific bloom has been caused by high densities ($1-5 \times 10^9$ cells/L) of a small (4-5 μm diameter) chrysophyte similar to *Aureococcus anophagefferens* that causes brown tides on the U.S. northeast coast. Brown tide blooms occur in shallow (1-2 m depth) embayments and lagoons that have minimal advective transport and/or dispersion. The onset of the bloom was preceded by a drought (that increased the salinity) and severe freezes during periods of extremely low tides (Whitledge, 1993). Declines in invertebrate populations and widespread fish kills were associated with these conditions. High ambient concentrations of nutrients, especially nitrogen in the form of ammonium, resulted from the decaying fish. Ammonium is important because the Texas brown tide species cannot utilize nitrate (DeYoe and Suttle, 1994). Although bloom initiation depended on the increased ammonium, its persistence was facilitated by severe declines in grazer populations and continued low rates of advection and physical dispersion (Buskey and Stockwell, 1993). However, generalizations about nutrient effects, flushing, and trophic antagonism are not sufficient to predict the occurrence, persistence, or long term effects of the brown tide.

The environmental and economic impact of the Texas brown tide stems from effects on several components of the food web. Zooplankton and larval fish do not eat the brown tide alga, but more importantly, after a threshold cell density is reached, their mortality increases. Eggs of important estuarine fish species (e.g., red and black drum, spotted seatrout) have reduced hatching and the young larvae rapidly die from lack of food. Large declines in the abundance of benthic filter feeders have also been observed. Exudate(s) from the brown tide organisms are thought to be responsible for these effects, but specific inhibitory compounds have not yet been identified. Another harmful effect of dense brown tides is a decline in the abundance of seagrasses due to light absorption by the microalgae. Severe long-term ecological changes thus result from the combination of loss of seagrass habitat and the reduced abundance of secondary consumers in the water and sediments. The economic losses to tourism and recreational fishing caused by the Texas brown tide are estimated to be several million dollars annually.

Important questions for future research include:

- To what extent do external nutrient sources and their elemental composition moderate brown tide blooms?
- What external or internal factors besides nutrient availability lead to the decline or dissipation of a brown tide bloom?
- To what extent do brown tide organisms modify environmental conditions so as to enhance their survival?
- What is the nature of the growth and feeding inhibition associated with brown tide blooms? Are toxins involved?

Pacific Coast Region (California and Oregon): PSP, ASP

Paralytic Shellfish Poisoning (PSP). Paralytic shellfish poisoning has a long history on the U.S. west

coast, having been reported by early European explorers and coastal Indian tribes. The dinoflagellate, *Alexandrium catenella* is apparently the primary PSP producer in open coastal environments of the California and Oregon coasts, but relatively little is known about its bloom dynamics due to a lack of field surveys focused on this species. What little is known has been gleaned from shellfish-toxin monitoring programs (Price et al., 1991). In California, blooms of *A. catenella* cause toxicity nearly every year. PSP toxins are usually highest during July and August with most toxic events occurring from May to October. PSP is known to accumulate in numerous benthic filter feeders, and there is considerable variability among species with respect to toxin retention (Price et al., 1991).

Hydrographic mechanisms underlying the PSP problems along the west coast are poorly understood. A good case can be made that PSP outbreaks in some areas of California occur following the relaxation of seasonal upwelling. This moves offshore waters and their established dinoflagellate populations rapidly to the coast, causing increases in toxicity far faster than can be attributed to in situ growth alone. A similar mechanism linking shellfish toxicity to changes in upwelling conditions has been reported for the northwest coast of Spain (Fraga et al., 1988), where hydrographic conditions resemble those along the northern California coast.

A number of questions underlying California and west coast PSP outbreaks remain to be resolved:

- Where are the source populations for the coastal blooms? If cysts are involved, where are the seedbeds located? Do blooms spread from one or a few points of origin or do isolated blooms develop simultaneously in several locations in response to similar hydrographic conditions? Do blooms originate in offshore waters, to be advected onshore with changes in meteorological conditions?
- What are the important meteorological or hydrographic forcings underlying toxicity in the different regions along the coast?
- If outbreaks are tied closely to transport of offshore waters and cells to coastal sites, can those events be detected and predicted using moored instruments and weather forecasts?
- What effect do recurrent blooms of toxic dinoflagellates have on west coast ecosystems, at all levels from zooplankton to fish and marine mammals?

Domoic acid-producing diatom blooms. Domoic acid poisoning (DAP), associated with ASP in humans, first became a concern along the west coast of North America in September, 1991 when more than 100 brown pelicans and cormorants were found dead or suffering from unusual neurological symptoms in Monterey Bay, CA (Fritz et al., 1992; Work et al., 1993). This event was attributed to a bloom of the pennate diatom, *Pseudo-nitzschia australis* (Buck et al., 1992; Garrison et al., 1992). At the peak of the 1991 bloom, domoic acid levels were $>10 \mu\text{g/L}$ and *P. australis* reached over 106 cells/L. Since the 1991 autumn bloom, domoic acid has been detected in both autumn and spring plankton assemblages in Monterey Bay, but with domoic acid concentrations usually $<1-5 \mu\text{g/L}$, and *P. australis* densities of 104 -105 cells/L. Blooms during the 1991-1994 period often have been comprised of two or three potentially toxic species (i.e., *P. australis*, *P. pungens* f. *multiseriis*, and *P. pseudodelicatissima*); however, *P. australis* is believed to be the main source of the toxin. Domoic acid production from locally-isolated clones has only been confirmed for *P. australis* (Garrison et al., 1992) and *P. pungens* f. *multiseriis* (Villac et al., 1993).

Monitoring studies in Monterey Bay suggest blooms of *P. australis* are most common and persist longer during the summer to autumn months (Buck et al., 1992; Walz et al., 1994). Hydrographic conditions during this period are characterized by warmer sea-surface temperatures, thermal stratification, and lower concentrations of organic nutrients. In contrast, *P. australis* blooms in southern California appear to be most common in the late spring to early summer months, and may be associated with upwelling pulses (Lange et al., 1994).

The 1991 domoic acid producing bloom in Monterey Bay was somewhat unusual because toxin was

transmitted through the pelagic food-web via Northern anchovies to seabirds. Anchovies are also consumed by marine mammals, several finfish (Morejohn et al., 1978), and are occasionally eaten by human consumers. Domoic acid has also been found in other grazing zooplankton (Buck et al., 1992; Haywood and Silver, 1994). With the exception of seabirds, nothing is known of the effects or impact of domoic acid on the pelagic food-web.

It is difficult to assess the costs associated with the domoic acid blooms. In California, much of the cost of the domoic acid blooms is associated with the monitoring program conducted by the California Department of Health Services (Langlois et al., 1993) and U.S. Food and Drug Administration (FDA). The California Department of Health Services presently monitors domoic acid in conjunction with its established PSP monitoring program, using intertidal mussels as "sentinel" organisms. This strategy may prove to be inadequate because mussel monitoring is apparently not able to detect domoic acid when it is present in planktonic assemblages in low concentration (Walz et al., 1994). Mussel, rock crab, and razor clam harvesting is a small sport fishing activity in California and their monetary losses from blooms are difficult to assess.

Domoic acid-producing blooms are a relatively new phenomena in U.S. waters. Unanswered questions about these blooms include:

- What are the sources of domoic acid in West Coast waters? How many species of *Pseudo-nitzschia* are toxic? Are there other sources?.
- How is domoic acid production related to bacteria?
- Is domoic acid production in natural populations triggered by nutrient stress?
- How is domoic acid transported in marine food-webs? Are there effects on consumers at all trophic levels?

Other Potential HAB Problems. Dinoflagellate species (e.g., *Dinophysis* spp.) associated with diarrhetic shellfish poisoning (DSP), noxious bloom-forming species such as *Phaeocystis pouchetii*, and setose diatom species (e.g., *Chaetoceros convolutus* and *C. concavicornis*), that damage gills of pen-raised finfish (see below) are found throughout the California Current region. Red-tides, apparently all caused by non-toxic species, are common during the summer months.

Questions related to potential HAB problems include:

- What are the effects of high-density, monospecific blooms of non-toxic "red tide" forming species on food-web structure?
- What are the occurrences and distributions of the potentially harmful species in California coastal waters?
- How are HAB species dynamics related to hydrographic events on short-term, seasonal, and interannual time scales?
- What is the importance of meso-scale features and short-term events on bloom dynamics?
- How do the life cycles of the HAB species influence their distribution and population cycles?

Pacific Coast Region (Washington): PSP, ASP, Finfish Mortalities

In the Pacific Northwest, public health and economic problems from HABs are related to paralytic shellfish poisoning (PSP), domoic acid poisoning (DAP), and mortalities of pen-reared salmonids; diarrhetic shellfish poisoning (DSP) is a potential but as yet unverified problem for the area.

Paralytic Shellfish Poisoning. Following the deaths of three people and mass mortalities of seabirds in 1942, the Washington coast from Dungeness Spit on the Strait of Juan de Fuca to the mouth of the Columbia River is closed each year from 1 April through 31 October for the harvest of bivalve molluscs. PSP was not a problem in Puget Sound until 1978, but since then, it has apparently spread southward with some closures now happening every year in central Puget Sound. The first closure in southern Puget Sound occurred in 1988 and in northern Hood Canal in 1991.

The causative organisms are members of the dinoflagellate genus *Alexandrium*. Species known from the area are *A. catenella*, *A. acatenella*, and *A. tamarense*. Two other potentially toxic species, *A. ostenfeldii* and *A. hiranoi* have been identified recently in British Columbia (Taylor and Horner, 1994).

Hydrographic mechanisms underlying the PSP problem in western Washington are poorly understood. There have been no sustained field programs, so bloom dynamics and physical forcings remain significant and important unknowns. PSP along the ocean coast and in coastal bays appears to be caused by blooms originating offshore. In Puget Sound, blooms originate in situ and toxicity may be widespread or very localized (Nishitani and Chew, 1988). A combination of physical factors and nutrient supply may explain why PSP has not been a problem in central and southern Hood Canal (Rensel, 1993). Some PSP outbreaks have been correlated with El Niño events (Erickson and Nishitani, 1985).

Economic impacts include the costs of shellfish and phytoplankton monitoring by state health officials, the closure of many beaches to the recreational harvest of shellfish during the summer months, and lost tourist trade. Commercial sales may be affected if the public thinks shellfish are contaminated. Recently, the harvest of non-traditional shellfish, such as predatory snails, has become a problem (Matter, 1994).

Important questions concerning PSP outbreaks in western Washington include:

- Where are the source populations for the blooms? Are cysts involved? If so, where are the seed beds?
- What meteorological or hydrographical forcings affect toxicity? Are outbreaks related to upwelling events or other transport of offshore waters and cells to coastal areas? Can those events be detected/predicted? What hydrographic conditions are necessary for blooms in inland waters?
- Do nutrients regulate/limit blooms in some areas?

Amnesic Shellfish Poisoning. Domoic acid was first found in razor clams on the Oregon/Washington coasts in late October 1991, and both commercial and recreational harvests of razor clams were halted. Other bivalves, including commercially grown oysters and mussels were tested and did not contain the toxin. However, domoic acid was also present in the viscera of Dungeness crabs and their commercial harvest was closed for a short time. Since 1991, the fall and spring recreational seasons for razor clams have been delayed, shortened, or not opened due to domoic acid. Furthermore, depuration of domoic acid from razor clams is apparently slow (Drum et al., 1993; Horner et al., 1993). In November 1994, domoic acid was found for the first time in mussels from southern Hood Canal.

The causative organisms have not been identified with certainty, but it has been assumed that species of the diatom genus *Pseudo-nitzschia* are to blame. Known toxin-producing species present in Washington waters include *P. australis*, *P. pungens* f. *multiseriata*, and *P. pseudodelicatissima*. Both *P. pungens* f. *pungens* and *P. pungens* f. *multiseriata* were present in the bloom in Hood Canal when domoic acid was found in mussels.

As with PSP, hydrographic conditions related to domoic acid occurrence are not known.

Pseudo-nitzschia spp. are rarely seen in samples collected in nearshore waters when razor clams are most toxic, but perhaps the cells originate offshore and are advected to the coast. There has been no offshore sampling since the 1991 incident. It is possible that a series of *Pseudo-nitzschia* blooms occurred, extending from California to Alaska, linked to unusually warm weather conditions associated with an ENSO event in 1991.

The 1991 domoic acid incident caused an estimated \$15 - \$20 million in damages to the Oregon/Washington coastal economy. Losses included health effects, lost and/or delayed sales, lower prices, lost jobs, bankruptcies, and lost recreational opportunities and tourist trade. No estimate is available for losses since 1991.

Unanswered questions with regard to domoic acid and ASP include:

- What are the causative organisms? Are *Pseudo-nitzschia* spp. the only ones involved or are other diatoms and/or macroalgae also culprits?
- What is the source of the organisms? Is there an offshore bloom that is advected to inshore localities? In Puget Sound, are there local seed populations in some areas?
- What is the life cycle of the *Pseudo-nitzschia* spp.?
- What environmental conditions are needed for domoic acid production by the cells?
- How do the razor clams and Dungeness crabs obtain domoic acid? How long does it take them to depurate domoic acid? Under what conditions?
- Has the Washington incident been one event with slow depuration or is there continual re-intoxication?

Finfish Mortalities. Catastrophic losses of cultured and wild fish sometimes occur due to species of phytoplankton that do not cause illnesses in humans. Blooms of the raphidophyte flagellate *Heterosigma carterae* (sometimes called *H. akashiwo* or, erroneously, *Olisthodiscus luteus*) have occurred in British Columbia every year since the early 1960s and fish kills have been reported most years since 1986; in Washington, fish kills occurred in pen-reared fish in 1989 and 1990, and wild fish in 1994. Losses to the fish growers are about \$4-5 million per year when blooms occur. The way *Heterosigma* kills is not known, but superoxide radicals may be involved because fish can be protected with the addition of superoxide dismutase (Yang et al., 1993). This organism is a vertical migrator, usually occurring in surface waters during the day and at depth during the night. Vertical stability of the water column is probably an important factor in maintaining blooms.

The harmful diatom species *Chaetoceros concavicornis*, *C. convolutus*, and perhaps *C. danicus* have long setae armed with short secondary spines and may kill at fairly low concentrations (<10⁴ cells/L). Chains of cells apparently become lodged between secondary lamellae in the fish gills and cause blood hypoxia as a result of mucus production. These diatoms may be restricted to near-surface waters or mixed throughout the water column depending on local hydrographic conditions. Most fish growers have their own phytoplankton monitors who sample at the pen sites on a daily basis from April through September. They also rely on reports from other phytoplankton monitoring programs. Economic losses are about \$0.5 million per event.

Unanswered questions here include:

- What environmental conditions cause blooms of *Heterosigma*?
- Does *Heterosigma* produce a toxin? If so, what is it? How does it kill the fish? What environmental conditions are needed for toxin production?

- Are fish killed by *Heterosigma* safe to eat?
- Are *Chaetoceros concavicornis* and *C. convolutus* the only *Chaetoceros* species that kill fish or can any species with secondary spines (e.g., *C. danicus*) or capilli (long, hairlike siliceous spines, e.g., *C. radicans*) on the setae kill fish?
- Can harmful *Chaetoceros* species and/or other harmful phytoplankton species influence the distribution and abundance of finfish in inland waters of Washington State?
- What environmental factors affect the timing and magnitude of harmful *Chaetoceros* blooms in inland waters?
- Are phytoplankton associated with summer mortality of finfish? If so, which species?

Other HAB problems. *Ceratium fusus* and *Gymnodinium sanguineum* have been linked to mortality of oyster larvae and adults (Cardwell et al., 1977, 1979) and spot prawns (Rensel and Prentice, 1980) in southern Puget Sound. There is no indication of a chemical toxin and mortality may be due to mechanical means or oxygen stress when blooms decay.

The major questions for these species are:

- How do they cause mortality?
- How often do they cause shellfish mortality? Could these dinoflagellates, or other phytoplankton species, be implicated in summer mortality of oysters?

Pacific Coast Region (Alaska): PSP, ASP, Bitter Crab Disease

Alaska, with 54% of the U.S. coastline, has a significant problem managing the impacts of HABs. Although some baseline information is available, virtually all studies have been either of short duration and/or restricted to small geographic areas. No studies have critically and specifically evaluated HABs on a broad geographic scale.

Paralytic Shellfish Poisoning. PSP is the most significant HAB problem in Alaska. Numerous beaches, bays, and coves in the southeast and east are periodically or perpetually plagued with high levels of saxitoxins in blue mussels, butter, little necks, and horse clams, geoduck, oysters, and cockles. Commercially valuable crabs are also affected. The causative species is apparently *Alexandrium catenella*, but other toxin-producing species may also be present. Toxic blooms have been reported in almost every month of the year, making it difficult to ascribe bloom conditions to any particular environmental or hydrographical condition. One frequently reported trend is that shellfish from headwaters of estuaries have more toxin than those collected near the mouths, perhaps suggesting that blooms originate or grow better near the headwaters. It is certainly possible that the rising tide or certain wind patterns may push toxic algae into shallow areas, but there is a growing perception that blooms originate offshore and move inland. Despite the prevalence of PSP, large areas of the coast remain relatively free of toxins.

In 1917, 5 million pounds of shellfish products were harvested from Alaskan waters, but today the state's commercial bivalve industry is virtually nonexistent. The destruction of the clam industry, estimated at 25-50 million pounds of bivalves per year, is in large part a result of product contamination by PSP (Neve and Reichardt, 1984). Other commercially valuable species, such as Dungeness crabs, are also affected by PSP, presumably from consumption of tainted bivalves. Other economically valuable crustaceans have not tested positive for PSP.

Only commercially harvested shellfish are presently tested for PSP on a routine basis. Recently, the Alaska Department of Conservation (DEC) instigated a multicomponent program to detect PSP and identify blooms. The program relies on local fish farmers trained to identify toxic dinoflagellates from their swimming patterns, satellite imagery to identify and track blooms, and a citizen monitoring program with an 800 number for reporting PSP illnesses, discolored water, fish kills, unusual behavior of seabirds or mammals, etc. This program needs to be coupled with physical, chemical, and biological oceanographic studies being conducted on the coast to provide insights into bloom formation, spread, and collapse.

Questions that need to be answered with regard to PSP in Alaska include:

- What algal species are involved?
- What are their seasonal and geographic distributions?
- What hydrographic and environmental factors contribute to blooms?
- What information is needed to guide the development of a shellfish industry in a region with extensive PSP problems?

Domoic Acid. Alaska does not have a severe problem with domoic acid, but low levels have been found in razor clams and *Pseudo-nitzschia pungens* and *P. australis* have been found in Alaskan waters. Whether they produce domoic acid is not known.

While domoic acid is not yet a problem in Alaska, some questions are still pertinent:

- Do blooms of *Pseudo-nitzschia* occur in Alaskan waters? Are any of the populations toxic?
- What are the seasonal and geographic distributions of *Pseudo-nitzschia* spp., and what controls their abundance and toxicity?

Other potential HAB Problems. *Phaeocystis* blooms occur occasionally in Alaska, and under conditions that are not well understood. This alga can be a major component of the spring bloom or form a second, smaller bloom later. It produces both acrylic acid and DMSP, but the ecological and environmental impacts of these compounds are not known. Southeastern Alaska shares with Washington State the presence of several potentially harmful diatoms, e.g., *Chaetoceros convolutus* and *C. concavicornis*, however no problems have been associated with these species, since fish farming is not yet a major industry..

A parasitic dinoflagellate, *Hematodinium* sp., has been of increasing concern since 1985 because it causes "bitter crab" disease. The parasite infects crabs during their molt (Love et al., 1993; Meyers et al., 1987). Once established, it is 100% lethal and the crab meat becomes unmarketable before the crabs die.

Questions with regard to *Phaeocystis* and *Hematodinium* include:

- How extensive are the blooms, and what hydrographic/environmental factors favor them?
- What are the economical/societal costs of *Phaeocystis* blooms?
- What oceanographic conditions favor growth and survival of the parasitic *Hematodinium* sp. during its life cycle when it is not within the host Tanner crab? What natural controls are there to *Hematodinium* abundance?

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The Ecology and Oceanography of Harmful Algal Blooms

A National Research Agenda

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