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Nutrients and Phytoplankton in Puget Sound

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NUTRIENTS AND PHYTOPLANKTON IN PUGET SOUND

Prepared by

Rensel Associates
2412 North 77th Street
Seattle, WA 98103

and

PTI Environmental Services
15375 SE 30th Place, Suite 250
Bellevue, WA 98007

Prepared for

U.S. Environmental Protection Agency
Region 10, Office of Coastal Waters
1200 Sixth Avenue
Seattle, WA 98101

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ANOVA	analysis of variance
ATP	adenosine triphosphate
CSO	combined sewer overflow
DIN	dissolved inorganic nitrogen (includes ammonium, nitrate, and nitrite)
DO	dissolved oxygen
DON	dissolved organic nitrogen
Ecology	Washington Department of Ecology
EDTA	ethylenediaminetetraacetic acid
EPA	U.S. Environmental Protection Agency
LOTT	Lacey-Olympia-Tumwater-Thurston sewage treatment plant
Metro	Municipality of Metropolitan Seattle
MGD	million gallons per day
NOAA	National Oceanic and Atmospheric Administration
NPDES	National Pollutant Discharge Elimination System
P-NMR	^{31}P nuclear magnetic resonance
PON	particulate organic nitrogen
PSP	paralytic shellfish poisoning
PSWQA	Puget Sound Water Quality Authority
SND	standard normal deviate
WASP4	Water Quality Analysis Program 4
WDF	Washington Department of Fisheries
WQI	water quality index

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EXECUTIVE SUMMARY

The purposes of this report are 1) to provide a general review of the state of knowledge regarding nutrient-phytoplankton relationships in Puget Sound and 2) to quantify the relative nutrient sensitivity of various areas in the sound. For the purposes of this report, "Puget Sound" refers to all of the inland marine and estuarine waters of Washington State, including Hood Canal and the Strait of Juan de Fuca, but excluding Grays Harbor, Willapa Bay, and the Columbia River estuary. Water quality problems associated with nutrient enrichment and phytoplankton blooms, such as depletion of dissolved oxygen, have been related to fish kills in poorly flushed embayments of Puget Sound. The geographic distribution and degree of shellfish toxicity related to phytoplankton-caused paralytic shellfish poisoning (PSP) has increased in Puget Sound since the mid-1970s. The occurrence of phytoplankton-related fish kills in mariculture facilities has also increased recently. This report summarizes the present state of knowledge about these conditions and identifies uncertainties about related ecological processes. Selected literature on known phytoplankton problems worldwide is reviewed to provide perspective about local conditions.

NUTRIENT-PHYTOPLANKTON RELATIONSHIPS

The quantitative aspects of nutrient-phytoplankton relationships in the sea, particularly nitrogen supply and phytoplankton productivity, have long been studied and debated. However, there is still no single, unequivocal method of determining when nutrients limit the production of phytoplankton in the marine environment. Rapid cycling among various organic and inorganic nutrient pools in water and sediment, as well as interspecific variation in nutrient requirements, confound attempts to identify nutrient limitation. Even when inorganic nutrient concentrations in the water are low or below detectable limits, rapid cycling of nutrients by phytoplankton and zooplankton may support relatively high rates of primary production. Phytoplankton require both macronutrients (e.g., phosphorus and nitrogen) and minor nutrients (e.g., trace metals and vitamins) in order to grow. The consensus of experts is that nitrogen is generally the primary macronutrient that controls or limits the growth of most phytoplankton in marine and estuarine environments, when other factors discussed below are not growth-limiting.

The most direct method of investigating nutrient limitation is to conduct nutrient-addition bioassays with indigenous species to determine whether the suspected limiting nutrient will promote algal growth for each specific geographic area of concern. A less expensive, alternative method of investigating potential nutrient limitation is to use the frequency of dissolved inorganic nitrogen (DIN) depletion in surface water. The frequency of DIN depletion is readily calculated because the concentration of DIN in Puget Sound surface waters has been measured frequently for many years at several locations. Areas with surface waters that frequently exhibit nutrient limitation are thought to be most susceptible to adverse effects of nutrient addition. In several such areas within Puget Sound, seasonally low dissolved oxygen concentrations in subsurface water may also occur that are harmful for some types of marine life.

Rates of macronutrient inputs to Puget Sound from the ocean have been estimated using available oceanographic data. Rates of macronutrient inputs to Puget Sound from anthropogenic and riverine sources can be estimated in some cases, although all of the data necessary to support accurate estimation of these rates have not been collected and analyzed to date. Rates of recycling of macronutrients within Puget Sound are known in only a general sense, and there is little information available on the dynamics of minor nutrients in Puget Sound. Most currently available evidence suggests that dissolved nutrients discharged into the main basin of Puget Sound, the Strait of Juan de Fuca, and the main channels of north Puget Sound will have little or no effect on the rate of phytoplankton production because of the naturally high background concentrations of nutrients. Phytoplankton production in these areas appears to be regulated primarily by the available sunlight and by other factors such as grazing by zooplankton, water temperature, water column stability, tidal- and wind-caused advection, mixing, and sinking of cells.

In poorly flushed bays and inlets of Puget Sound, dissolved nutrients (primarily nitrogen) in surface waters may be seasonally depleted as a result of optimum phytoplankton growing conditions and reduced flushing. Summer and fall blooms in restricted bays and inlets (e.g., southern Hood Canal and Budd Inlet) may be dominated by microflagellates and dinoflagellates that are functionally and morphologically distinct from diatoms that often dominate the phytoplankton in the main channels of Puget Sound. Dinoflagellate blooms typically occur under specific hydrographic conditions associated with calm weather. Many dinoflagellate species are able to migrate vertically to obtain nutrients from subsurface waters. Data from controlled experiments and field studies in tidally restricted waters elsewhere suggest that several nutrients other than nitrogen (e.g., trace metals and vitamins) or combinations of nutrients may occasionally regulate the growth of single species or mixed populations of dinoflagellates or microflagellates. Moreover, the ability of dinoflagellates to vertically migrate and thereby take advantage of subsurface reserves of nutrients suggests that their growth may not be nutrient-controlled in such areas, despite total exhaustion of dissolved macronutrient concentrations in surface water. Unfortunately, there is little empirical information (e.g., nutrient-addition bioassays) to evaluate potential effects of nutrient depletion in poorly flushed areas of Puget Sound.

Only some species of harmful phytoplankton and some coastal areas are prone to eutrophication effects from human sources. For example, the preponderance of opinion, from experts here and abroad, is that *initiation* of blooms of *Alexandrium* spp. that cause PSP is related more to seasonal temperature increases than to any other single factor. It does appear likely that some blooms may be *prolonged or intensified* by nutrients from human sources, although there is uncertainty regarding the molecular forms of nutrients required. Nitrogen, in the form of ammonium or nitrate, may prolong or intensify certain types of undesirable phytoplankton blooms in the poorly flushed areas of Puget Sound. Reduction of ammonium supply through advanced wastewater treatment processes may reduce the production of phytoplankton in some problem areas (e.g., inner Budd Inlet). As noted above, some uncertainty remains about the factors controlling general phytoplankton production in summer and fall within restricted inner inlets and bays, and about the factors controlling production by a few harmful species found throughout Puget Sound.

NUTRIENT SENSITIVITY RANKING

Areas of Puget Sound were identified as potentially nutrient-sensitive based on a ranking of the frequency of DIN depletion [defined for the purposes of this report as concentrations below 7 micromoles per liter (μM)] in surface waters and low dissolved oxygen concentrations at Washington Department of Ecology (Ecology) monitoring stations. The mean concentration of DIN of individual areas of Puget Sound was not used to assess their potential nutrient sensitivity because there is uncertainty regarding the appropriate concentrations of nitrogen (or other nutrients) that may limit the growth of locally dominant algal species. At the present time, the arbitrary use of any one selected concentration of nitrogen as a threshold of algal growth reduction would therefore be misleading and inaccurate. Although some limited information exists regarding nutrient requirements and growth limitation for some phytoplankton species commonly found in Puget Sound (e.g., *Gymnodinium splendens* and *Skeletonema costatum*), these data were not collected using strains from Puget Sound or under environmental conditions similar to Puget Sound, so there is doubt as to the applicability of the data (e.g., Carpenter and Guillard 1971).

Alternatively, it is stressed that the identification of potentially nutrient-sensitive areas based on ranking the frequency of nitrogen depletion is relative in nature, pending development of more sensitive methods that may include factors such as flushing rates and associated nutrient flux. As expected from these interrelated measures, there is a close, inverse correlation between mean DIN concentration during summer months and the frequency of DIN depletion during the phytoplankton growing season. The areas of Puget Sound judged to be relatively sensitive to anthropogenic nutrient inputs are listed in alphabetical order below:

Budd Inlet
Carr Inlet including Burley Lagoon
Case Inlet
Dabob Bay
Dyes Inlet
Eld Inlet
Hammersley Inlet near Oakland Bay
Henderson Inlet
Holmes Harbor
Hood Canal, central and southern
Liberty Bay
Penn Cove and Crescent Harbor
Port Orchard at Brownsville
Port Susan
Quartermaster Harbor
Saratoga Passage
Sequim Bay
Sinclair Inlet
Totten Inlet.

The areas of Puget Sound currently judged not likely to be sensitive to anthropogenic nutrient inputs in the near future are listed in alphabetical order below:

Bellingham Bay
Colvos Passage
Commencement Bay (except inner waterways)
Drayton Harbor (entrance only)
East Passage
Elliott Bay
Gordon Point to Nisqually Reach (excluding Oro Bay)
Main channel of central Puget Sound
Nisqually Reach to Dana Passage at Brisco Point
Pickering Passage
Port Angeles Harbor
Port Gardner
Port Townsend Harbor
Possession Sound
Rich Passage and Clam Bay
San Juan Channel
Skagit Bay (except Similk Bay)
Strait of Juan de Fuca
Tacoma Narrows (except Gig Harbor).

MANAGEMENT OF NUTRIENT-RELATED ALGAL PROBLEMS

An improved management strategy for preventing, controlling, and remediating nutrient-related algal problems is needed for potentially nutrient-sensitive areas of Puget Sound. Although controls on nonpoint sources to address eutrophication concerns may be coordinated within a watershed under existing programs (e.g., early-action watershed programs), nutrient-load allocation plans have not been developed for sensitive areas of Puget Sound. An inventory of riverine and anthropogenic nutrient inputs, which could be developed using available data for many potentially nutrient-sensitive areas of Puget Sound, would aid in determining priorities for further monitoring or mitigation. Regulatory decisions for siting of wastewater outfalls and for discharge permit limitations are made on a case-by-case basis. Available nutrient and phytoplankton models developed for Budd Inlet and south Puget Sound cannot be applied to most other areas without substantial modification (e.g., consideration of local hydrographic conditions and dominant phytoplankton species).

KNOWLEDGE GAPS AND RECOMMENDATIONS

Additional information about nutrient and phytoplankton dynamics is needed to improve understanding and management of potentially nutrient-sensitive areas of Puget Sound. Recommendations for further research and actions are summarized below:

- Studies of algal growth-limiting nutrients, phytoplankton species composition, nutrient flux, and distribution and dynamics of harmful species in potentially nutrient-sensitive areas, such as Budd Inlet, Lynch Cove (southern Hood Canal), and other areas of Puget Sound as identified in this report
- Investigation of the causes of fish kills, including analyses of key water quality and planktonic variables and analysis of fish tissues by pathologists
- Modification of the existing sound-wide monitoring program to include total nitrogen analysis during the fall and winter months, addition of chlorophyll *a* analysis for selected stations, routine monitoring of phytoplankton species

composition and abundance at a few sensitive areas and at least one main channel reference area, and analysis of long-term trends of oceanographic variables

- Preoperational and postoperational monitoring of new and improved municipal wastewater treatment plant discharges and receiving water quality in potentially nutrient-sensitive areas
- Standardization and trends analysis of PSP monitoring data collected by the Washington State Department of Health
- Empirical estimation of flushing and nutrient flux rates in potentially nutrient-sensitive areas.

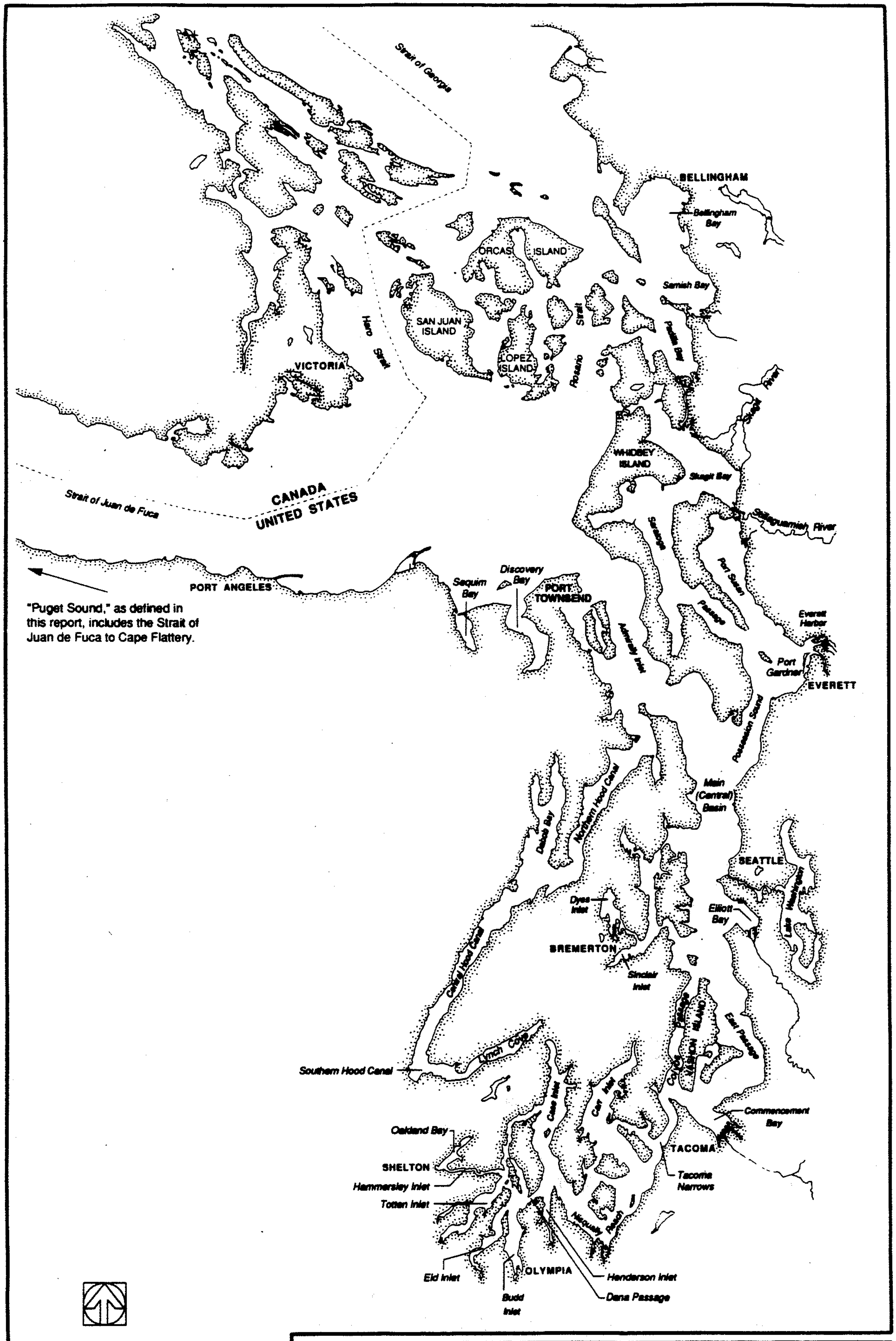
INTRODUCTION

With the rapidly increasing human population in the Puget Sound region, discharges of nutrients into Puget Sound will likely increase. Unless advanced wastewater treatment processes and other control measures are implemented, increased nutrient discharges could have adverse effects on algal communities in potentially nutrient-sensitive receiving waters. In turn, the increased production of algae may harm marine fish, shellfish, and other species, including humans, that use the resources of Puget Sound. When considering the gravity of this situation, it is important to compare the contribution of anthropogenic nutrient waste loads with the natural flux of nutrients in Puget Sound. Oceanic and natural riverine sources of nutrients far outweigh anthropogenic contributions in most of Puget Sound. However, there is concern for the restricted bays, inlets, and passages where tidal flushing is limited and where seasonally low dissolved oxygen (DO) or depleted dissolved inorganic nitrogen (DIN) concentrations in surface waters signal potential sensitivity to increases of nutrient loading.

For the purposes of this report, "Puget Sound" refers to all of the inland marine and estuarine waters of Washington State, including Hood Canal and the Strait of Juan de Fuca (Figure 1). The term "eutrophication" refers to an increase in the rate of supply of nutrients to a body of water, leading to increased algal productivity. Eutrophication and subsequent algal bloom decay may cause depletion of DO and associated problems (e.g., offensive odors and fish kills).

The incidence of harmful blooms of marine and estuarine phytoplankton may be increasing in coastal areas worldwide (Ayres et al. 1982; Tangen 1987; White 1987; Anderson 1989; Konovalova 1989; Smayda 1989a,b; Smayda and White 1990). In most areas, including Washington State, historical records of phytoplankton population dynamics are limited, with no systematic attempt to assess long-term trends. There is often little interest in harmful phytoplankton blooms until catastrophic events attract public attention or commercially valuable fisheries are harmed. Increased worldwide reports of phytoplankton blooms in recent years may be due to several factors: 1) more trained observers with better communication among governmental agencies, academic institutions, and marine-related businesses such as mariculture; 2) changes in short- or long-term weather patterns; 3) increased transport of harmful phytoplankton species through ship ballast; or 4) increased discharges of nutrients in industrial, agricultural, and domestic waste. Because harmful phytoplankton blooms may adversely affect fish and shellfish aquaculture, there is an increasing effort to monitor phytoplankton blooms in many parts of the world (Tangen 1987).

In estuarine and coastal marine waters, nutrient discharge has resulted in some clear cases of eutrophication and increased phytoplankton blooms (e.g., Underdal et al. 1989; Smayda and White 1990). However, the ecological processes leading to phytoplankton blooms are complex, and the connection between blooms and human activities is difficult to demonstrate definitively. On a worldwide basis, it is impossible to generalize about the role of nutrients in causing harmful algal blooms. Discharge of nutrients from municipal and agricultural sources is



"Puget Sound," as defined in this report, includes the Strait of Juan de Fuca to Cape Flattery.

Figure 1. Map of Puget Sound.

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frequently cited as contributing to specific harmful blooms, although the evidence is often circumstantial, suspect, or missing altogether. The lack of definitive evidence in most cases is not surprising because several interrelated factors may control phytoplankton production in coastal areas. Interrelationships among nutrient input and regeneration, light availability, zooplankton grazing, circulation, salinity, temperature, and density regimes require detailed study before determining which factors regulate phytoplankton production.

OBJECTIVES

The purposes of this report are to 1) provide a general review of the state of knowledge regarding nutrient-phytoplankton relationships in Puget Sound and 2) to quantify the relative nutrient sensitivity of various areas in the sound. The information contained herein could be used by environmental managers, scientists, planners, students, and interested citizens as background information for regulatory programs and research efforts. However, specific regulatory decisions may require site-specific studies [e.g., to determine the factor(s) controlling specific, harmful phytoplankton blooms] before controls on nutrient discharges from an anthropogenic source are imposed. Nevertheless, available information on nutrients and phytoplankton for Puget Sound allows generalizations to be made about existing conditions and possible trends. In particular, a list of potentially nutrient-sensitive areas, based on the frequency of DIN depletion, serves as a useful indicator of areas that should receive special attention regarding nutrient inputs. Given the rapid human population growth throughout most of the Puget Sound region, the introduction of additional nutrients into potentially nutrient-sensitive areas could cause significant problems in future years.

This report addresses the following issues:

- The extent of eutrophication problems in Puget Sound and relationships among nutrients, algal blooms, DO depletion, and fish and shellfish kills
- The relationship of nutrients to phytoplankton blooms responsible for paralytic shellfish poisoning (PSP)
- The relative importance of nitrogen, phosphorus, and micronutrients (e.g., trace metals and vitamins) in controlling phytoplankton growth in Puget Sound
- Macronutrient (i.e., nitrogen and phosphorus) sources to Puget Sound
- Possible effects of eutrophication on marine phytoplankton community structure
- Temporal trends in water quality conditions and occurrences of harmful phytoplankton blooms
- The relative sensitivity of various areas of Puget Sound to nutrient addition
- The use of selected simulation models and monitoring to assess eutrophication effects.

A key topic that is currently poorly understood is the coupling between phytoplankton production and nutrient supply (particularly nitrogen). The quantitative aspects of this linkage are currently widely debated; there is no single, unequivocal means to determine when nitrogen limits the production of phytoplankton (e.g., Dortch and Postel 1989). This report focuses on that uncertainty by examining what is known about the physiological requirements and behavior of phytoplankton species that inhabit areas of Puget Sound with limited flushing. These areas, rather than the open channels and more seaward basins, are more likely to experience nutrient-related problems.

REPORT ORGANIZATION

The next section of this report presents a review of selected literature on factors controlling phytoplankton production and nutrient dynamics throughout the world, including a few case studies dealing with nutrient discharges and phytoplankton blooms. The following sections address phytoplankton and their relationships to nutrients in Puget Sound, the relative sensitivity of various areas of Puget Sound to eutrophication, selected tools for managing nutrient problems, knowledge gaps, and recommendations for future action.

OVERVIEW OF PHYTOPLANKTON AND NUTRIENT DYNAMICS

The environmental factors responsible for phytoplankton blooms vary with habitat type, season, and species composition of the phytoplankton community. The term "bloom" usually refers to an exponential rate of growth in the phytoplankton resulting in a high phytoplankton biomass. Because harmful phytoplankton species may cause damage at relatively low concentrations, their occurrence may be referred to as a "harmful event" rather than a bloom. Within the broad category of harmful phytoplankton, there are toxic (toxin-producing) and noxious (harmful by nontoxic means) types of phytoplankton. The following sections provide background information on the major types of phytoplankton, factors potentially responsible for algal blooms, and selected case studies of eutrophication and related water quality problems.

TYPES OF PHYTOPLANKTON

Three major types of phytoplankton are prevalent in Puget Sound: diatoms, dinoflagellates, and microflagellates. However, each major type includes widely diverse kinds of cells. Aside from phytoplankton, other types of aquatic plants include macroalgae (e.g., seaweeds and kelp) and marine flowering plants (e.g., eelgrass).

Most "net" phytoplankton cells (i.e., greater than 20 micrometers in size and easily collected in phytoplankton sampling nets) are diatoms or dinoflagellates. Diatoms occur as either individual cells or chains of cells, with each cell enclosed in a silica shell. Some diatoms have spines that protrude from the main body, and nearly all diatoms are photosynthetic. Diatoms in calm waters may sink, although at times there may be physiological or structural adaptations that allow them to maintain buoyancy. Despite a well-mixed water column in some cases, diatoms have been known to maintain their vertical position within the euphotic zone (defined as the upper water layer to the depth of penetration of 1 percent of surface light; e.g., Ryther and Hulburt 1960). Diatoms are the most abundant phytoplankton group (measured by total cell carbon mass per volume of water) in central Puget Sound during the spring bloom and often throughout the summer months, at least for the few years for which adequate records are available (Anderson et al. 1984). Typically, diatoms are most abundant in coastal areas where a moderate amount of vertical mixing is followed by periods of vertical stratification within the euphotic zone. A spring bloom of *Skeletonema costatum* and other diatom species is typical throughout most of Puget Sound. In some years, a smaller bloom of diatoms occurs in the fall.

Of the approximately 2,000 living species of dinoflagellates, roughly one-half are photosynthetic (autotrophic) and the others are heterotrophic (Taylor 1987). All possess two flagella that enable them to move through the water. Some dinoflagellates are able to migrate vertically in the water column, obtaining nutrients at depth during the night and using sunlight near the surface during the day for photosynthesis. Estimates of migration speed average approximately 0.5–2.0 meters per hour (Darley 1982), although there is large interspecific variation of at least 0.03–6.5 meters per hour, as summarized by Levandowsky and Kaneta (1987). Dinoflagellates are found in all types of marine environments, but tend to be dominant in less turbulent areas that are vertically stratified and often seasonally depleted of nutrients in surface waters. Dinoflagellates may also dominate the phytoplankton community in relatively well-mixed areas (such as central Puget Sound) during periods of calm weather and solar heating. Vertical density stratification that is largely temperature-induced occurs in some of the restricted (i.e., shallow, poorly flushed) bays and inlets of Puget Sound during summer and some fall months, and this condition favors the growth of dinoflagellates.

Blooms of certain dinoflagellate species, a few species of protozoans, and microflagellate species may be responsible for the discoloration of waters commonly referred to as "red tide." Red tides are usually dominated by one or a few phytoplankton species and have been documented worldwide since biblical times. The term red tide may also refer to both harmful and benign blooms of phytoplankton, bacteria, ciliates, or even small zooplankton. About 20 of the more than 2,000 dinoflagellate species are known to cause most of the harmful red tides. A few dinoflagellate species may cause PSP in shellfish and other marine species, but only *Alexandrium catenellum* (formerly *Gonyaulax catenella* or *Protogonyaulax catenella*) is reported to cause PSP in Puget Sound. Because *Alexandrium tamarensis* (another species that causes PSP) occurs in neighboring British Columbia waters (Gaines and Taylor 1986), it may possibly occur in Puget Sound.

Red tides may also be caused by several harmless organisms. A common cause of visible red tides is *Mesodinium rubrum*, a ciliated protozoan that blooms in Puget Sound in the late fall to early spring and is not a health hazard to fish, shellfish, or humans (Horner et al. 1990). Other red tides in Puget Sound may be caused by the large heterotrophic dinoflagellate, *Noctiluca miliaris* (also known as *N. scintillans*). This organism may alter surface water coloration to a very noticeable orangish-red color and form windrows along beach areas, although *N. miliaris* is generally considered nontoxic to fish and other marine life. In other countries, however, high rates of ammonium release by this species have been related to mortality of natural and maricultural marine life (Suvapepun 1989). High concentrations of ammonium [to approximately 10 micromoles per liter (μM)] have been measured in the water during these events. To date, no known episodes of toxicity to marine life in Puget Sound have been caused by *N. miliaris*. However, this species is only one of several present in Puget Sound that have caused problems elsewhere in the world.

Microflagellates are not a taxonomically distinct group, but rather a collection of heterogeneous, small cells usually no greater than approximately 10 micrometers in size. Although they have been known to be numerically dominant during portions of the summer in central Puget Sound (Anderson et al. 1984), the Strait of Juan de Fuca (Chester et al. 1977, 1980), and Hood Canal (Rensel et al. 1989), little is known about their biology or behavior. These species, along with microbial and phage (virus) populations in marine waters, are currently the subject of many studies and may contribute significantly to the dynamics of marine food webs and nutrient cycling (Azam et al. 1983; Sherr 1988; Bergh et al. 1989; Bratak et al. 1990; Capriulo 1990). Many microflagellate species occur in Puget Sound, and one species, *Heterosigma akashiwo*, has been responsible for extensive losses of cultivated fish in Puget Sound and in diverse geographic locations around the world.

Other relatively minor types of phytoplankton (in terms of total cell carbon mass per volume of water) besides the three major types discussed above may occur in Puget Sound. One example is the picoplankton (Cyanophyceae), which are very small and not flagellated. Another example is the coccolithophorids, which commonly occur off the Washington coast, but are found infrequently in Puget Sound (Chester et al. 1980).

Published studies of phytoplankton species distribution and abundance in Puget Sound have been restricted to the main channels of the central and northern sound, including the San Juan Islands (Johnson 1932; Phifer 1933; Thompson and Phifer 1936; Chester et al. 1978; Anderson et al. 1984). Dexter et al. (1981) summarized information concerning life histories, major taxa, and spatial and temporal distribution of phytoplankton in Puget Sound. Although there is no published information about diatoms for the main channels of southern Puget Sound, limited information exists concerning diatoms (Tollefson 1959) and a few dinoflagellates (Cardwell et al. 1979) in certain inlets and passages of southern Puget Sound. The Washington Department of Fisheries (WDF) has collected some information about phytoplankton species occurrence in southern Puget Sound and some coastal areas, but little of that information has been published or is in a readily usable form (Westley, R., 12 December 1988, personal communication).

PHYTOPLANKTON SUCCESSION AND LONG-TERM TRENDS

In most temperate, coastal marine environments, there is a seasonal pattern of phytoplankton succession. Generally, this pattern begins with a community dominated by diatoms in the spring, shifting to a community dominated by dinoflagellates or microflagellates in the summer and early fall, and sometimes a return to diatom predominance in the late fall or early winter. Succession is tempered by, and in some cases appears to be directly caused by, seasonal water column stratification, eventual mixing in the water column, replacement of the surface layer by water rising from depth, and the effects of zooplankton grazing.

It is possible that elevated nutrient loading from human sources (e.g., sewage discharges and agricultural runoff) may alter the normal composition of the phytoplankton community in some areas of Puget Sound. Such perturbations have been demonstrated 1) in many freshwater lakes (Paerl 1988), including Lake Washington (Edmondson 1972); 2) in some shallow and restricted marine areas such as the Seto Inland Sea of Japan (Okaichi 1989); and 3) in coastal areas of Europe (Smayda 1989b). Different species may dominate at different concentrations or ratios of nutrients in laboratory studies (Takahashi and Fukazawa 1982) and field surveys (Sommer 1988; Smayda 1989b). Parsons and Harrison (1983) provide an extensive review of nutrient cycling and phytoplankton species competition under varying nutrient conditions. It has also been postulated that major interannual variations of the dominant species in Puget Sound may occur in response to interannual variations in the nutrient supply (Campbell et al. 1977). The degree of mixing and the nutrient supply to the surface layer may influence phytoplankton species competition and, hence, community succession. Because long-term data on phytoplankton species abundances in Puget Sound are unavailable, interannual variations in community structure and the possible long-term effects of changes in natural and anthropogenic sources of nutrients cannot be evaluated at present.

By the same token, interannual variations in hydrographic and weather conditions confound the interpretation of short-term monitoring studies for phytoplankton and nutrients in Puget Sound. Strong El Niño events in the early 1980s complicated attempts to characterize phytoplankton conditions during a 2-year survey of the main basin of Puget Sound (Anderson et al. 1984). Significant interannual variation in phytoplankton abundance was similarly noted in a 2-year study of the Strait of Juan de Fuca (Chester et al. 1980). These results suggest the need for longer time frames of monitoring programs to characterize the dominant types of phytoplankton in Puget Sound. Concurrent monitoring of insolation, nutrients, salinity, water temperature, and dominant zooplankton throughout the water column is needed to clarify nutrient-phytoplankton relationships, because all of these factors (and derivatives such as water column stability) have been shown to be important in determining species composition (Takahashi et al. 1973; Parsons et al. 1984b).

FACTORS CONTROLLING PHYTOPLANKTON ABUNDANCE

Blooms of phytoplankton are normal occurrences in most temperate and sub-arctic waters, including Puget Sound. Major blooms may occur in the spring as early as March or as late as May and may recur throughout the summer and fall. A bloom is possible only when appropriate physical and chemical factors and sufficient seed stock are present.

Four functional groups of phytoplankton have been identified based on growth dynamics and other characteristics: bloom diatoms (e.g., *Skeletonema* sp.); large, slow-growing diatoms (e.g., *Chaetoceros* spp.); microflagellates; and large dinoflagellates (Davis 1982). The dominance of one particular group at any one time or place may depend on habitat-specific combinations of light, temperature, and nutrient supply.

Marine environments may be divided into the following categories (defined in the following sections): open ocean, coastal/main channel, coastal/transitional, and coastal/restricted. In all four environments, light is a primary and sometimes dominant growth-controlling factor in temperate and higher latitudes, either on a seasonal or shorter-term basis. Other factors sometimes affecting phytoplankton production include nutrient supply, water temperature, self-shading, sinking of cells, zooplankton grazing, turbulence, and respiration requirements (Eppley 1972; Winter et al. 1975; White 1976; Anderson et al. 1984; Parsons et al. 1984b). The following discussion addresses these controls in the context of each type of marine environment.

Open Ocean Environment

The open ocean environment is defined as all offshore, oceanic waters of the world and may be categorized by latitude as tropical (20°N–20°S), subtropical (20–40°), temperate/subarctic (40–60°), and polar waters (60°+). In many tropical and subtropical oceanic areas, plankton productivity and nutrient supply are very low due to a strong, permanent pycnocline and thermocline (except in the tropical divergence zones found around the equator). In polar seas, annual phytoplankton productivity is relatively low, except near the retreating ice edge.

Phytoplankton productivity is generally greater in midlatitude temperate seas than in tropical and polar areas, but there are highly significant differences between the Atlantic and Pacific oceans. The north Atlantic Ocean has a classic spring bloom, partly in response to a shallowing and stabilization of the mixed (surface) layer. This spring bloom is followed by smaller summer and fall blooms and probably relatively little phytoplankton production in the winter. Phytoplankton production in the subarctic north Pacific (and possibly the temperate oceans of the southern hemisphere) is less variable throughout the year, due in part to the presence of a more stable pycnocline. The fact that there is relatively continuous phytoplankton production, and yet persistently high concentrations of macronutrients that appear to be underutilized by a relatively low standing stock of phytoplankton, is one of the great controversies in modern biological oceanography. Oceanic phytoplankton are considered important controls for the world's atmospheric concentration of carbon dioxide, known to be steadily increasing for several decades and thought to be leading to a greenhouse/global warming effect. Thus, it is important to understand what controls the production of phytoplankton in large expanses of the subarctic oceans, the subequatorial oceans, and the circumpolar southern oceans, all of which are characterized by relatively high concentrations of macronutrients within the euphotic zone throughout the year.

There are presently two major schools of thought regarding controls of phytoplankton populations in these oceanic regions. Although the factors are too complex to be discussed fully in this report, in essence one group of scientists believes that a lack of the micronutrient iron is the controlling factor, based on an ultraclean (no iron contamination) analytical technique, nutrient-addition bioassays, and related observations (Martin and Fitzwater 1988; Martin et al. 1989, 1990). The second group of scientists believes that grazing by microzooplankton, and not iron limitation, may be responsible for control of phytoplankton growth in these regions. They also believe that the nutrient-addition bioassays in which iron was introduced selectively enhanced the growth and survival of large diatom cells that are normally uncommon under typical conditions in the subject regions (Banse 1990, 1991; DeBarr et al. 1990). It may be possible that both groups are correct in some respects. There is evidence that the smaller phytoplankton cells are normally controlled by microzooplankton grazing throughout the year, while the less common, large diatoms are in fact limited by scarce supplies of iron (Frost, B., 9 April 1991, personal communication). Therefore, in terms of carbon biomass production, the effect of grazing, not nutrient supply, is the dominant controlling factor.

Coastal/Main Channel Environment

The coastal/main channel environment includes most of central and northern Puget Sound and the Strait of Juan de Fuca. In such areas, vertical density stratification is much greater and nutrient flux to the surface layer is generally less than in the coastal/transitional environment. Winter et al. (1975) found by mathematical modeling that primary productivity was relatively high in central Puget Sound compared with other temperate coastal waters, and the onset of spring blooms in Puget Sound occurred later in the spring than expected for a coastal area of this latitude. This high productivity was attributed to strong, persistent upwelling of nutrients and algal cells from depth, accomplished by tidal pumping of deeper water up into the surface layer, primarily at mixing and transitional zones. Winter et al. (1975) surmised that blooms in central Puget Sound were related to a specific combination of sunlight, weather, water stratification, and flushing characteristics of the upper water layer. Estuarine circulation (i.e., dense ocean water moving in at depth, and less dense surface waters moving out to sea) or occasional rapid advection of phytoplankton from an area because of sustained winds may also affect the abundance of phytoplankton in this environment.

Anderson et al. (1984) found that the time-averaged rate of change of sunlight (both intensity and duration) was the most important factor in determining the seasonal abundance and production of phytoplankton in central Puget Sound. Phytoplankton abundance reached a maximum around the time of the summer solstice and a minimum around the time of the winter solstice. Temporal correlations among nutrients, phytoplankton, and zooplankton occurred on a seasonal basis. The temporal variation and spatial heterogeneity among the variables was too great to detect correlations on lesser time scales of days and weeks.

Nutrient concentrations in the surface layer of the main basin of Puget Sound may fall below detection limits during the phytoplankton growing season for relatively short periods of hours or days. At these times, it is possible that the rate of nutrient supply limits phytoplankton production (see *Nitrogen Depletion and Algal Growth Limitation* below). This condition is much more prevalent in the Strait of Georgia than in the main channels of Puget Sound (see *Anthropogenic Effects on Nutrients and Phytoplankton in Estuarine and Coastal Waters: Case Studies).*

Coastal/Transitional Environment

Coastal/transitional environments are located between coastal/restricted environments (see below) and coastal/main channel environments (see above). This category of coastal environment includes areas of major sills in Puget Sound (e.g., Nisqually Reach and Tacoma Narrows), although a sill may be located between any of the four major groups of marine environments. Nutrient concentrations may be high in these areas because of vertical mixing, but phytoplankton production is typically low in the immediate area of the sills.

Conversely, the borders of the coastal/transitional environment may often have the greatest abundance of phytoplankton, as measured by chlorophyll *a* concentration (Pingree et al. 1978; Parsons et al. 1981; Jones et al. 1982). While these areas of Puget Sound have not been delineated, their occurrence may be fairly predictable if the hydrodynamics and physical and chemical conditions are known (e.g., Gowen 1984). Nutrient concentrations and the degree of vertical stratification in transitional surface waters often fluctuate tremendously, depending on seasonal, tidal, and weather effects. Biological "fronts" of enhanced phytoplankton productivity often occur in these areas, although they may shift location with each tidal cycle. This may present a problem if investigators use a limited number of fixed reference stations in attempts to determine primary or secondary production rates (Parsons et al. 1981). Coastal/transitional areas are generally less sensitive to nutrient addition from anthropogenic sources than are coastal/restricted waters, because flux rates of nutrients from natural sources are much higher and light limitation of phytoplankton production is more likely as cells are mixed in and out of the euphotic zone.

Coastal/Restricted Environment

The coastal/restricted environment includes semilandlocked, shallow, and relatively poorly flushed bays, inlets, and passages in the coastal environment. Nutrient flux in the coastal/restricted environment varies among areas and time scales depending on inputs of freshwater and materials from land and sediment sources, rates of tidal flushing, and vertical stratification of the water column. This environment is the focus of later portions of this report because of the likelihood of nutrient sensitivity. Although diatoms are common in the spring in the restricted areas, dinoflagellates are typically the dominant type of phytoplankton present in the summer and early fall when optimum conditions for their growth are common.

When vertical mixing is severely limited and adequate light is available, algal populations are often able to grow until dissolved nutrients become limiting in surface waters. At that point, further population growth is principally controlled by recycling of nutrients from living and decaying organisms because nutrient resupply from deep water is impeded by water column stratification. This assumes that all other factors such as grazing and advection of phytoplankton out of an area remain constant. The composition of phytoplankton populations under these conditions may shift to dinoflagellate or microflagellate species that are able to vertically migrate to obtain nutrients from subsurface waters. These mobile species may include several harmful and toxic species. For dinoflagellate populations, self-shading, tidal advection, grazing, and wind-caused vertical mixing may be more important controls than nutrient supply because subsurface nutrient reserves are available in all but the most shallow and isolated embayments of Puget Sound. In addition, the growth of at least one PSP-causing dinoflagellate is inhibited by turbulence in the water column (White 1976).

ANTHROPOGENIC EFFECTS ON NUTRIENTS AND PHYTOPLANKTON IN ESTUARINE AND COASTAL WATERS: CASE STUDIES

It is widely believed that anthropogenic sources of nutrients can adversely affect nutrient and phytoplankton relationships in estuarine and coastal marine waters, depending on the scale of nutrient input vs. flushing rates and other factors. Nevertheless, some harmful and benign species of phytoplankton are known to respond to anthropogenic sources of nutrients, while other species may not be obviously affected (Smayda and White 1990; Taylor 1990). The potentially unaffected species may include *Alexandrium catenellum*, the species responsible for PSP in the Pacific Northwest that is discussed later in this report, and the harmful species *Pyrodinium* and *Dinophysis* (Smayda and White 1990). Some have argued that increased nutrient discharge into coastal waters need not adversely affect the marine biosphere, but rather could be viewed as increased production at the base of the food web to support a larger population of higher organisms such as marine fish (e.g., Stockner et al. 1979). "High rates of nutrient addition are not undesirable per se, since the highest natural rates of primary production and fish production occur in naturally enriched areas such as upwellings" (Hecky and Kilham 1988). One problem with this position is that the composition of anthropogenic nutrient discharges may not be in balance with the ratios of nutrients required by benign types of phytoplankton, such as most diatoms, and may enhance the growth of harmful species, such as red tide dinoflagellates.

From advanced cases of coastal water eutrophication, there is strong evidence that shifts of species composition to less desirable or harmful species have already occurred. However, even in the often cited cases of Hong Kong Harbor, the Dutch Coast and Baltic Sea, the Seto Inland Sea, and the Aegean Sea near sewage outfalls, where experts agree that nutrients are implicated in causing the increased frequency of harmful blooms, the relative contributions of anthropogenic nutrient sources and natural sources are either unknown or, at best, poorly known. As noted in the introduction to this report, the frequency and intensity of harmful blooms appear to be increasing worldwide, but their cause is a subject that divides experts. Until mariculture of physiologically sensitive species such as the salmonids and some bivalves began increasing worldwide, there was no reliable long-term "bioassay" of coastal waters. "Caged or penned fish are much more vulnerable to harmful blooms than wild stock, since the latter can avoid the blooms and so the recent rise in fish farming is likely to lead to many more reports of these types of blooms" (Taylor 1990).

In this section, four case studies are presented in which increased phytoplankton activity or harmful blooms in estuarine and coastal marine waters have been reported. Even these fairly well-researched cases are sometimes inconclusive, ambiguous, or in conflict with each other. In general, studies of nutrient/phytoplankton relationships in semienclosed and poorly flushed shallow areas have been more definitive than have studies in more open, well-flushed coastal areas where sources and sinks of nutrients are not easily estimated. Accordingly, case studies from both types of areas are presented below.

The first case study concerns Chesapeake Bay in the eastern United States, where marked successes in reduction of estuarine algal biomass have been accomplished in some areas by phosphorus removal from wastewater. However, the more seaward areas of the bay have continued to decline in quality, probably because of the continued discharge of ammonium-nitrogen wastes. The second study, involving the southern Strait of Georgia, British Columbia, illustrates some of the controversies involved in relating phytoplankton blooms and eutrophication to anthropogenic sources of nutrients in an area immediately adjacent to Puget Sound. The third case study, involving the Seto Inland Sea of Japan, provides strong evidence that anthropogenic nutrient enrichment can be an important cause of repeated, harmful phytoplankton blooms in estuarine waters. The last case study focuses on the North Sea, the Dutch coast, and approaches to the Baltic Sea. Portions of these areas illustrate the long-term effects of agricultural, industrial, and municipal wastes that have dramatically and adversely altered nutrient loading to coastal waters and are thought to have caused an increase in blooms of the slime-forming harmful alga *Phaeocystis pouchetti* in some areas. Within the same general area, a catastrophic, toxic bloom of the normally benign microflagellate *Chrysochromulina polylepis* is cited to illustrate the profound effects of persistent, atypical weather patterns against a background of long-term and steadily increasing eutrophication.

Chesapeake Bay

There is general consensus that the water quality of Chesapeake Bay is rapidly deteriorating, with nutrient enrichment as the primary cause (Randall et al. 1987). However, some areas have achieved improved water quality, notably the upper Potomac estuary, where phosphorus removal from sewage treatment plant discharges apparently solved excess algal bloom problems (Jaworski

1990). Chesapeake Bay is an area strongly influenced by riverine sources, with salinity less than 16 ppt (unlike most of Puget Sound where salinities are closer to that of seawater). While wastewater treatment for phosphorus removal and a mass balance approach worked for the upper Potomac estuary, other upper estuarine areas of Chesapeake Bay such as the Patuxent River estuary alternate between nitrogen and phosphorus limitation, depending on the season and riverine discharge volume (D'Elia et al. 1986) (see *Nutrient Limitation of Phytoplankton Growth in Puget Sound* for background information on nitrogen vs. phosphorus limitation).

In the central basin of Chesapeake Bay, signs of eutrophication persist, including elevated concentrations of nitrogen and phosphorus and increased algal stocks compared with concentrations found in the late 1960s; increasing frequency of subpycnocline anoxia; high rates of ungrazed phytoplankton and microheterotroph production, with subsequent high rates of sedimentation; decline of several anadromous and marine fish and bay grass species; and occurrence of harmful dinoflagellates (Tyler and Seliger 1987; Boyston and Kemp 1985; McCloskey 1985; Sellner 1987; Tuttle et al. 1987). In 1986, a panel of experts called for nitrogen removal for wastewater treatment (Chesapeake Bay Program Science and Technical Advisory Committee 1986). Most of the phosphorus that enters the bay comes from point sources that are easily identified (e.g., wastewater from treatment plants), but much of the nitrogen comes from nonpoint sources, mainly runoff from agricultural sources (Macknis 1985). Accordingly, there have been several programs adopted to control agricultural and urban nonpoint sources (Jaksch et al. 1985).

Southern Strait of Georgia, British Columbia

The second case study involves the southern Strait of Georgia, British Columbia, where total phytoplankton production has reportedly increased since at least the mid-1960s (Stockner et al. 1979). Because this water body is contiguous with northern Puget Sound and in some ways is similar to the main basin of Puget Sound, albeit much larger, it is of special interest in the present discussion. Both areas have seasonally variable estuarine flow and periods of stratification in the spring and summer. However, the massive Fraser River discharge during late spring and summer has a dominant influence on stratification and other hydrographic features of the Strait of Georgia that is not duplicated in Puget Sound. It has been suggested that increases of phytoplankton stocks in the Strait of Georgia may be related to measured increases of nutrients from land sources carried primarily in the Fraser River plume (Stockner et al. 1979). Nitrogen and phosphorus waste loading into the strait doubled during the period from 1951 to 1977. Relocation of the city of Vancouver's marine sewage outfall into the river may have further distributed nutrients throughout the upper, euphotic layer of the southern Strait of Georgia. Through comparison of ^{14}C productivity and chlorophyll *a* data in the mid-1960s with data from the mid-1970s, as well as through other methods, Stockner et al. (1979) surmised that a significant increase in phytoplankton productivity had occurred in the southern Strait of Georgia near Vancouver. The increases in productivity were judged to have produced a positive impact on the aquatic food web and fisheries resources by providing an expanded base for the food chain.

Shortly after Stockner et al. (1979) published their report, Parsons et al. (1980) countered that there were other possible causes of the conditions and trends that Stockner et al. (1979) had observed. Parsons et al. (1980) questioned whether the Strait of Georgia was indeed becoming more eutrophic and cited potential problems in the Stockner et al. (1979) paper, including the selection of monitoring station locations near the Fraser River plume and throughout the strait, lack of a documented increase of nitrogen concentrations in the river, differences in calculating the relative contribution of nutrient-rich seawater to productivity in the Strait of Georgia, and other factors.

In a response to criticisms from Parsons et al. (1980), Stockner et al. (1980) agreed that the observed increase in phytoplankton production could have been the result of annual variation and/or cultural eutrophication. They also agreed that the contribution of the city of Vancouver's sewage effluent relative to upstream sources was small, but they disputed the validity of the riverine nitrogen concentration data for the Fraser River because the data did not account for episodic discharges and the known poor correlation between discharge volume and total nutrient load. Most importantly, they pointed out that phytoplankton growth depletes nitrogen levels to below detection limits from June to August in the surface waters of the Strait of Georgia (e.g., Takahashi et al. 1973), although an estimated 45 percent of the total annual phytoplankton production occurred during those months of so-called nitrogen limitation. Stockner et al. (1980) concluded that increasing human population in the greater Vancouver area will ultimately result in increased phytoplankton production in the Strait of Georgia as long as the receiving waters remain nutrient-limited throughout much of the phytoplankton growing season.

Subsequently, Harrison et al. (1983) provided a broad review of the biological oceanography of the Strait of Georgia and summarized the reasons for variation among the prior estimates of annual primary productivity. These reasons include differences in ^{14}C methodology, differences in frequency of distribution of vertical samples, choice of stations relative to the river and estuarine plume, methods of calculating primary productivity, and estimates of nitrogen inputs into the Fraser River from sewage. Harrison et al. (1991) traced the dynamics of the Fraser River plume (salinity 0–10 ppt) and its estuarine plume (salinity 10–15 ppt) to discover that high productivity around plume boundaries in July was due mainly to entrainment of nutrients from beneath, as the freshwater plume moved over the more dense, nutrient-rich seawater. The entrainment process leading to increased surface water concentrations of nutrients was thought to be more plausible than the previous suggestion of Stockner et al. (1979, 1980) that nutrients causing enhanced estuarine plume productivity were carried solely in the river water. In summary, the present preponderance of opinion is that there has been no statistically significant change in the nutrient status of the Strait of Georgia (Harrison, P.J., 11 December 1990, personal communication).

A direct comparison of the situation in the Strait of Georgia with Puget Sound's main basin is not warranted for a number of reasons. Foremost, DIN depletion is not common in the main channels of Puget Sound, and phytoplankton production is relatively great (Winter et al. 1975). Differences in dominant phytoplankton species could also have been related to interannual variation of hydrography in the Strait of Georgia. Phytoplankton studies in Puget Sound have documented significant interannual variation of numerical abundance (Chester et al. 1980; Anderson et al. 1984). However, inadequate data are available from both water bodies to judge whether dominant species assemblages have shifted dramatically on an annual or longer basis. This discussion underscores the need for an adequate database to judge whether environmental changes have actually taken place. The present Washington Department of Ecology (Ecology)

Puget Sound Ambient Monitoring Program, discussed later, provides much of the needed information to detect changes in inorganic nutrient concentrations, but there is a need for modification of the program if changes in phytoplankton dynamics and nutrient pools are to be detected. Major changes to the program have been proposed (Ecology 1990b) and some have been implemented (Janzen, C., 23 April 1991, personal communication).

Seto Inland Sea of Japan

One of the most studied areas of adverse nutrient-phytoplankton relationships in coastal waters is the Seto Inland Sea of Japan, where reports of red tide blooms increased greatly after 1968 (Okaichi 1989; Shirota 1989a). The sea is relatively large but hydrographically restricted, with a mean depth of only 30 meters. Damage to aquacultural products from harmful dinoflagellates and microflagellates peaked there in the mid-1970s. The recent reduction of damage is reportedly due to mitigative measures such as relocation of fish cages and timely harvesting of the fish, rather than to a reduction in the severity of phytoplankton blooms. More sensitive fish, such as yellowtail, are no longer reared in areas where harmful phytoplankton blooms frequently occur. Instead, the more sensitive fish have been replaced by fishes such as herring, which have lower commercial value in Japan but are less sensitive to the blooms. Significantly absent in the reports is mention of mortality to wild fish and marine invertebrates. It may be that some of the species of fish being cultured in the Seto Inland Sea would not naturally inhabit the area because of the extreme natural conditions of water temperature, which may average as high as 25°C in the summer.

The microflagellate *Chattonella antiqua* apparently causes most of the fish kills in the Seto Inland Sea, although a closely related species, *Heterosigma akashiwo*, and the dinoflagellate *Gymnodinium nagasakiense*, are reported to be involved in the fish kills at times. *H. akashiwo* is of particular interest because it also occurs in Puget Sound. *C. antiqua* is reported to be a vertical migrator, with a speed of approximately 1 meter per hour. At certain times and locations in the Seto Inland Sea, inorganic phosphate has been considered a major limiting nutrient for these species, although at other times, one or more other nutrients including nitrate, ammonium, iron, and vitamin B_{12} have been reduced to concentrations thought to be growth-limiting (Watanabe et al. 1982, 1988). Physiological assessments of *H. akashiwo* conducted by Watanabe et al. (1988) showed that at least phosphate was sequestered by the cells at depth during the night. The growth of *C. antiqua* has been modeled by an index that considers both DIN and phosphorus concentrations (Nakamura et al. 1989a). Subsequent studies of *C. antiqua* using stratified microcosms and ^{31}P nuclear magnetic resonance (P-NMR) spectroscopy have verified that the uptake of nitrogen and phosphate is synchronized to the time the cells are below the nutricline (Watanabe et al. 1990). The ability to vertically migrate was judged to be essential for *C. antiqua* to compete with coastal diatoms. These reports, along with others in the Japanese and European literature, support the notion that microflagellate nutrient requirements are often complex and site- and species-specific.

While many experts concluded that nutrient discharge is obviously linked to phytoplankton problems in the Seto Inland Sea (Smayda and White 1990), the particular nutrient (or nutrients) of highest concern remains unidentified. To date, phosphate has been the primary focus of mitigation programs, which may be reasonable given the monsoon climate with intense precipitation and runoff in early summer and fall that probably results in an excess of nitrogen entering the sea from riverine flow (see *Phosphorus Depletion and Algal Growth Limitation*). Direct mitigative measures implemented by the Japanese have included installation of wastewater treatment facilities for the surrounding large population centers, reduction of the use of raw feed (i.e., unprocessed fish wastes) for caged fish, and elimination of phosphates in detergents. As a result, measurable decreases in total nitrogen and total phosphorus concentrations in certain areas of the Seto Inland Sea have been documented. From 1970 to 1980, the estimated oxygen demand from land sources was reduced by 50 percent.

The number of red tide blooms in the Seto Inland Sea peaked in 1976, and by the mid-1980s, was reduced to about 150 blooms per year (compared with the maximum number of 299 reported earlier). However, Shirota (1989a) reported that the number of blooms lasting between 6 and 31 days has not significantly declined over the period from 1973 to 1987. Because fishery damage was reduced by mitigative action, and prolonged blooms have not been reduced, there is some question about the efficacy of the mitigative measures aimed at nutrient reduction that have been implemented to date. In part, the apparent lack of reduction in phytoplankton problems may be due to inaccuracies in reporting the spatial and temporal extent of harmful blooms. Also, nutrient supply in the Seto Inland Sea may have remained high because of leaching from enriched, muddy sediments. This source of nutrients has reportedly been very significant in some nearshore areas (Shirota 1989a,b).

The Baltic and North Seas

A primary example of long-term eutrophication of coastal waters is the Baltic Sea, a semienclosed estuary in northern Europe constituting the largest brackish water area in the world. Nitrogen and phosphorus loading to the Baltic Sea has increased by factors of 4 and 8 times, respectively, in this century (Larsson et al. 1985). Elevated nitrogen loading is a function of increased use and runoff of agricultural fertilizers, livestock waste, and precipitation carrying nitrate from fossil fuel combustion. Phosphorus increases are primarily from municipal and industrial sources carried in rivers (Wulff and Rahm 1988). Subsurface anoxia or hypoxia, nearly total loss of biota below the pycnocline, and high bacterial productivity in the aphotic layer are strong indicators of eutrophication in the Baltic Sea. Although no significant shift in species composition has been detected in the pelagic community (Wulff et al. 1986), several authors [see review in Smayda (1989b)] report increasing rates of primary productivity from some areas of the Baltic Sea.

The hydrography of the Baltic Sea is not similar to any major regions of Puget Sound, but adjacent coastal areas of the North Sea are similar in some respects. In recent years, it is apparent that parts of the North Sea have received accelerated inputs of nitrogen and phosphorus from agricultural, industrial, and domestic wastes carried primarily in the major rivers of western Europe. In some areas, the nutrient loading has reportedly caused dramatic reductions in the abundances of diatom species and increases in the abundances of flagellate species, occurrences of novel harmful phytoplankton species, and geographic spreading of other harmful species, although the extent and causes of the changes are controversial (Smayda 1989a,b).

Shifts in phytoplankton species composition in the North Sea may be due to massive loading of nitrogen and phosphorus from human sources that stimulates diatom growth and demand for silicate, which is required for cell (frustule) growth. Concurrently, the seasonal riverine and near-coastal supply of silicate, most of which is from nonanthropogenic sources, is nearly exhausted in some areas (Gieskes and Schaub 1990). One example of this trend is the steadily increasing epidemic of the slime-forming flagellate *Phaeocystis pouchetii*, which has occurred in Dutch coastal waters since 1973 (Cadee 1986). This species secretes nearly two-thirds of its assimilated carbon as a slime that is most unpalatable to fish, has interfered with fisheries (Estep and MacIntyre 1989), and is an aesthetic problem on some beaches. However, blooms of *P. pouchetii* along other North Sea coastlines in Britain that predate major problems along the Dutch coast have inexplicably disappeared in recent years (Taylor, F.J.R., 30 April 1991, personal communication). It is possible that the same species may be present in Puget Sound, although there has not been enough examination of local waters to be sure (see Table 1 in the following section, *Algal Problems in Puget Sound*).

An exceptionally damaging bloom of the microflagellate *Chrysochromulina polylepis* occurred in May and June of 1988 along approaches to the Baltic Sea and the Norwegian coast of the North Sea. This species had not caused any previous toxicity problems and was considered a normal part of the phytoplankton community, as it probably is in Puget Sound (Taylor, F.J.R., 30 April 1991, personal communication). However, in 1988, *C. polylepis* caused extreme mortalities of fish, shellfish, and marine mammals over a surprisingly large area (Underdal et al. 1989).

The bloom of *Chrysochromulina polylepis* originated in the Kattegat, near southern Sweden, at the entry to the Baltic Sea. It moved with the prevailing currents to the west and south coasts of Norway. Cell numbers increased to several tens of millions per liter. Production of a group of toxins by the *C. polylepis* bloom reportedly caused mortality to many forms of sea life (Estep and MacIntyre 1989). Maximum cell numbers were often found in a very narrow band near the bottom of the pycnocline (i.e., near the dividing layer between nutrient-rich, saline, deep waters and the more diluted surface water) (Barth and Nielsen 1989; Haumann 1989). The alga produced slime that was first thought to cause clogging of fish gills. Later it was noted that permeability changes in the cell membrane of the gills had taken place, affecting the fishes' osmoregulation (Reiersen and Iversen 1989). No evidence of a toxin was found in fish flesh after death.

Although the precise cause (or causes) of the North Sea bloom remains the subject of study and speculation, hydrographic conditions were probably the most important factor (Rosenberg et al. 1988). Coastal currents transport nutrients from the coasts of Holland, Germany, and Denmark and from the Baltic Sea to the bloom area. Reduction of nutrient content in runoff from agricultural and urban areas throughout those parts of northern Europe has been proposed as a mitigative measure (Reiersen and Iversen 1989). However, Barth and Nielsen (1989) also surmised that weather factors that influenced hydrographic conditions were probably more important than nutrient dynamics in the propagation of the bloom. The bloom was preceded by a relatively mild winter with a large amount of precipitation. In the late spring, a vertically stable and warm upper water column was exposed to long periods of sunshine and low wind activity (Barth and Nielsen 1989). The effect of nutrients from land sources as a primary cause of the bloom was discounted, in part because most of the *Chrysochromulina polylepis* cells were located at the pycnocline and could draw from abundant natural sources of nutrients in the deep water. Because *C. polylepis* may migrate vertically, the evidence strongly suggests that *C. polylepis* was positioned at depth to take advantage of the naturally nutrient-rich waters below. The effect of increased runoff in creating a stable water column may have been more important than nutrient inputs associated with the runoff in favoring the bloom of *C. polylepis*. A similar view is held by Lindahl and Dahl (1990), who also stress that the unusual toxic properties of the alga may have enhanced its competitiveness against other algae during this harmful event.

In summary, available evidence suggests that nutrients from natural or human sources certainly may have contributed to, but were not the primary cause of, a massive bloom during 1988 in the North Sea. Most authorities concur that unusual weather and hydrographic conditions seem to have been very important factors. Other massive losses of marine life have occurred near the North Sea because of unusual weather patterns, although the exact causes may vary from case to case. For example, a massive fish kill in one semirestricted bay on the Brittany (French) coast may have been caused by extremes of precipitation followed by calm weather and neap tides, leading to hyperstratification, phytoplankton blooms/decomposition, and anoxic water in an area subject to long-term increases in nutrient discharge (Merceron 1990).

Research goals suggested by Barth and Nielsen (1989) after the *Chrysochromulina polylepis* bloom point to the need for developing a quantitative understanding of how physical and biological processes regulate phytoplankton production in the North Sea. This understanding would require the use of mathematical models, as well as field and laboratory work. Using such a comprehensive approach, future events such as the *C. polylepis* bloom of 1988 may be categorized either as normal, infrequent occurrences or as events pointing to trends of increasing algal problems.

Summary

Several case studies presented here illustrate that each phytoplankton bloom or body of water is unique, requiring site-specific investigations to understand nutrient-phytoplankton relationships. In some cases, data are limited and disagreement exists regarding the precise causes of blooms. Nevertheless, massive nutrient inputs from human sources have apparently played a significant role in the increased frequency and spread of some harmful phytoplankton blooms.

ALGAL PROBLEMS IN PUGET SOUND

Eutrophication is often considered a primary cause of harmful phytoplankton blooms, DO depletion, fish kills, and other water quality problems. Occurrences of such problems in Puget Sound are discussed in the following sections. Table 1 is a list of harmful species of phytoplankton known to be present in Puget Sound. The list is most likely incomplete, as many of the potentially harmful species are small and may have been overlooked in past surveys. Some of the phytoplankton species present in Puget Sound and adjacent waters are known to be harmful at other locations in the world, but to date have not caused major problems locally. One form of a relatively common diatom, *Nitzschia pungens*, has been responsible for shellfish toxins that affected human consumers on the east coast of Canada (Bates et al. 1988, 1989). *N. pungens* has produced domoic acid that results in a very serious condition known as amnesiac shellfish poisoning in humans. While at least one form of this species is common in Puget Sound, it is not known whether the Puget Sound form of this species is the one that has caused problems elsewhere. In Europe, *Dinophysis acuminata* and *D. fortii* cause diarrheic shellfish poisoning (Kat 1985). These species have also been reported in Puget Sound, but apparently have not caused significant problems to date. In the next section, special emphasis is placed on the evaluation of relationships between nutrient dynamics and potential blooms of *Alexandrium catenellum*, the phytoplankton species responsible for PSP in Puget Sound.

PARALYTIC SHELLFISH POISONING AND NUTRIENT DYNAMICS

Probably the most troublesome species of harmful phytoplankton in Puget Sound is the dinoflagellate responsible for PSP, *Alexandrium catenellum*. Its PSP toxins may be accumulated in some animals or kill them outright, depending on the dose and the susceptibility of the species. If accumulated, the toxins may kill a predator higher in the food chain. A dose of PSP toxin lethal to humans (approximately 500-1,000 μg) can be obtained from ingesting a single filter-feeding mollusc (e.g., a clam or oyster) when the ambient concentrations of *A. catenellum* in the water are as low as 10,000 cells per liter (Gaines and Taylor 1986). For comparison, normal spring blooms may contain over 10 million cells per liter of all species, and some red tides may have over 250 million cells per liter of water of a single species.

Causes of Paralytic Shellfish Poisoning Phytoplankton Blooms

Optimum growth conditions for *Alexandrium catenellum* likely include a stable water column (little wind or strong currents), adequate light and nutrients (especially phosphorus and nitrogen), a seed population, and 13-17°C water temperatures in the surface layer (Nishitani and Chew 1984; Nishitani et al. 1985, 1988). Anomalous weather conditions during El Niño events are thought to have promoted *A. catenellum* populations along parts of the northeast Pacific Rim because of increased temperature and stability of the upper water column at those times

TABLE 1. HARMFUL PHYTOPLANKTON AND AFFECTED ANIMAL SPECIES FROM PUGET SOUND AND ADJACENT WATERS

Phytoplankton Species	Affected Species	Reference
Alexandrium catenellum *(Protogonyaulax catenella)*	Humans, marine fish, birds, and invertebrates	Nishitani et al. 1988; Nishitani and Chew 1984; Erickson 1988; White 1980
Ceratium fusus	Shellfish larvae, spot shrimp, and coho salmon in net-pens	Cardwell et al. 1979; Rensel and Prentice 1979; Fraser 1976
Chaetoceros convolutus *Chaetoceros concavicornis* (Subgenus *Phaeoceros*)	Net-pen salmon, possibly wild salmon	Gaines and Taylor 1986[a]; Bell et al. 1974; Schiewe et al. 1988; Farrington 1988[a]; Rensel et al. 1989
Chrysochromulina polylepis	Fish, invertebrates, and marine mammals	Taylor, F.J.R., 30 April 1991, personal communication[b]
Dinophysis fortii *Dinophysis acuminata* *Prorocentrum micans*	Human (diarrheic shellfish poisoning)[a]	Gaines and Taylor 1986[a]
Gymnodinium splendens *Prorocentrum gracile*	Shellfish larvae	Cummins et al. 1976
Heterosigma akashiwo	Net-pen salmon	Gaines and Taylor 1986[a]
Nitzschia pungens	Humans via shellfish consumption[b]	Bates et al. 1988, 1989
Noctiluca miliaris *(N. scintillans)*	Fish (NH_3 production may be toxic)[a]	Adnan 1989; Horner et al. 1990
Phaeocystis pouchetii	Fish (forms gelatinous masses that may be toxic)[a,b]	MacKienzie et al. 1983; Gaines and Taylor 1986; Smayda 1989b
Silicoflagellates *Dictyocha speculum*	Fish (reported to cause problems in Denmark)[b]	Thomsen and Moestrup 1985; Gaines and Taylor 1986

[a] No reported harmful cases in Puget Sound although species is present. References may include citations from other areas.

[b] Reports from British Columbia or Alaska.

Note: Species listed in alphabetical (not taxonomic) order.

(Erickson and Nishitani 1985). Leading phytoplankton experts recently concluded that there are two major types of blooms: those in which nutrient additions to coastal waters are obviously implicated (e.g., Seto Inland Sea and Tolo Harbor, Hong Kong) and those not obviously associated with nutrient enrichment. A key word here is "obviously," indicating some uncertainty. The experts include blooms of the genus *Alexandrium* in the latter category (Smayda and White 1990).

Nishitani et al. (1988) specify conditions that must exist concurrently for nutrients from anthropogenic sources to stimulate the growth of *Alexandrium catenellum*:

1. An initial seed population of *A. catenellum* in the receiving water
2. Growth-limiting concentrations of nitrogen or phosphorus in the upper water column
3. Water temperatures greater than or equal to 13°C in the surface layer
4. Nutrients discharged into the uppermost 10 meters of the water column
5. Persistence of the water parcel which receives the added nutrients for sufficient time for growth to occur (i.e., several days to perhaps 3 weeks, depending on the initial density of *A. catenellum* in the receiving water)
6. Nutrients present in forms available to *A. catenellum*.

It should be noted that the present state of knowledge is poor regarding the second and sixth conditions; that is, the nutrients that limit the growth of *A. catenellum* at various times and places and how they interact are unknown.

Norris and Chew (1975) found laboratory growth rates of *Alexandrium catenellum* to vary from one to three divisions per day and final cell yield proportional to nitrate or ammonium concentrations from 10 to 100 μM. The cells were eurythermal and euryhaline and grew best in the range of 11 to 23°C at a salinity of 29 ppt and in the range of 14 to 20°C at a salinity of 21 ppt. Growth of the cultures was best at relatively low light intensities. This finding is in apparent contradiction with previous field observations by Sribhibhadh (1963) and Dupuy (1968), who, working in Sequim Bay, found that abundance of *A. catenellum* was positively related to the amount of sunlight, increasing surface water temperature, and the decline in diatom abundance. The apparent contradiction may be explained by the vertical migration of *A. catenellum* to appropriate light levels that may occur *in situ*. Gaines and Taylor (1985) found a slight correlation between water temperature and PSP-caused shellfish toxicity based on over four decades of field data from coastal areas of British Columbia.

Nishitani et al. (1988) presented field data that at times suggested a positive correlation between dissolved phosphate concentration and growth rate of *Alexandrium catenellum*. At other times, their field data suggested that DIN was more likely to be the growth-limiting factor. Nishitani et al. (1988) recommended that the best way to adequately address the question of limiting nutrients is to conduct controlled laboratory studies.

Cyst Formation and Germination

Alexandrium catenellum has also been of interest to researchers studying the conditions that favor cyst formation and germination by this species. Cysts represent a dormant stage in the dinoflagellate's life cycle. Limited supply of DIN or dissolved phosphate in the water column resulted in cyst formation of a related species, *Alexandrium tamarensis*, on the east coast of the United States (Anderson and Wall 1978) and in British Columbia (Turpin et al. 1978). Water temperature was a related factor in cyst formation, and no cysts were formed at some temperatures that permitted growth of the cells. Glibert et al. (1988) suggested that the cell's sexual reproduction, which leads to cyst formation, is a result of nitrogen limitation. These studies support the hypothesis that some sort of environmental stress leads to cyst formation.

Temperature, and in some cases, genetic controls, are factors thought to control cyst germination. It is unlikely that changes in nutrient concentration are responsible for cyst germination in Puget Sound, because nearly all of the deeper waters of the sound contain relatively high levels of nutrients throughout the year. However, in shallow waters of Puget Sound, both water temperature and light intensity vary significantly through the seasons.

Studies of *Alexandrium tamarensis* conducted on the east coast of the United States have shown that some genotypes may be adapted to germination offshore in deep water where environmental conditions are relatively constant. A biological clock regulating cyst germination that is insensitive to environmental cues has been detected in *A. tamarensis* populations that occur in offshore Atlantic Ocean waters (Anderson and Keafer 1987). However, it is unknown whether populations of *A. tamarensis* in shallow water have similar mechanisms.

Increases of water temperature in late spring have been related to the seasonal appearance of large numbers of the harmful microflagellate *Heterosigma akashiwo* in British Columbia over two decades (Taylor, F.J.R., 22 February 1990, personal communication). Concurrent appearance of this species in northern Puget Sound and throughout portions of the inland passage of British Columbia in the summer of 1990 suggests conditions were favorable for cyst germination over a wide area.

Cell Toxicity

Possible relationships between ambient nutrient concentrations and the toxicity of individual cells of *Alexandrium tamarensis* have been investigated by Boyer et al. (1987). They found that the toxin *content* of *A. tamarensis* cells in phosphorus-limited cultures increased three- to fourfold with age of the culture, compared with nitrogen-limited or control cultures, where toxin content decreased with age of the culture. Toxin *composition* (i.e., the mixture of toxins) remained constant, suggesting the possibility of a genetic control on toxin production that is insensitive to changing environmental factors. Because the toxin is nearly one-third nitrogen, the toxin may act as a nitrogen storage compound. The ecological advantage, if any, of this form of nitrogen storage to the phytoplankton is unknown. In addition to nutrient supply, many other factors may affect toxin content of *Alexandrium catenellum* (see Anderson et al. 1990 for a review).

Spatial and Temporal Trends

PSP has been a recurring phenomenon in the Pacific Northwest since it was first documented in 1793 by Captain George Vancouver (Quayle 1969). Prior to 1978, however, PSP toxicity in Washington State was restricted to the outer coast, the Strait of Juan de Fuca, and immediately adjacent waters. No cases of PSP had been reported in Puget Sound south of Port Townsend or in the Whidbey basin (i.e., Saratoga Passage and Skagit Bay), but there had been limited observations of *Alexandrium catenellum* in Puget Sound prior to that time. A very large bloom of *A. catenellum* occurred in the Whidbey basin in 1978, which apparently increased the population of cells and possibly cysts in central and southern Puget Sound. Sampling of phytoplankton and shellfish was subsequently initiated and showed either low levels of toxin or *A. catenellum* cells in all major branches of southern Puget Sound. A major bloom in 1988 caused several people who consumed commercially harvested oysters from southern Puget Sound to become ill. Until 1988, there had been no PSP illness from consumption of commercially harvested shellfish in Washington State since a monitoring program began in 1957. PSP illnesses from sport-harvested shellfish have been limited to cases in 1978 and 1979, the latter from shellfish harvested in a closed area (Nishitani and Chew 1988).

The proportion of *Alexandrium catenellum* blooms in Puget Sound that originate offshore (Pacific Ocean and Strait of Juan de Fuca) vs. inshore (bays and inlets) is unknown. It is probable that many blooms of *A. catenellum* originate from seed stock within Puget Sound (e.g., the breeder bay concept for *A. catenellum*; see Nishitani and Chew 1984). The presence of PSP toxin during most years in shellfish from some outer coastal beaches indicates the possibility of other *A. catenellum* blooms originating in the ocean. Blooms from different source areas may combine concurrently or sequentially, as found by Anderson and Morel (1979) on the east coast of the United States. In coastal waters, harmful blooms may originate offshore, then drift inshore where they are noticed (Yentsch 1982; Parker et al. 1982). For example, Steidinger and Haddad (1981) used aerial remote-sensing equipment to detect the movement of thermal fronts that were associated with the onshore movement of a harmful bloom of the fish-killing dinoflagellate, *Ptychodiscus brevis*, near the Florida coast.

Nishitani et al. (1988) reported increases in the frequency and duration of PSP-causing blooms in Washington, Oregon, and California. Although the geographic extent of such blooms has increased in Washington State, there has been no published study of the duration of these blooms or attempts to correlate weather or water quality conditions with their occurrence. It is interesting to note that the major bloom of 1978, which apparently seeded large portions of Puget Sound, occurred early in the 1977-84 warm/dry period as identified in the decadal analysis discussed in the section *Nutrient Limitation of Phytoplankton Growth in Puget Sound, Decadal Variation*. Unusually high riverine flow during the otherwise generally warm/dry period, which caused intense water column stratification, may have been another contributing factor to the major bloom of 1978 (Nishitani et al. 1988). Finally, it should be noted that preliminary studies in British Columbia have not detected a progressive increase in PSP toxicity of shellfish over approximately the past 40 years (Gaines and Taylor 1985).

To date, closure of shellfish harvesting beds due to PSP has occurred in all waters of Puget Sound except Hood Canal. In the waters of south Puget Sound inside Dana Passage, PSP toxicity is rare and has not reached closure levels. The reason for this is unknown, but it may be related to the prolonged seasonal depletion of DIN in surface and subsurface waters in both areas (see *Nitrogen Depletion and Algal Growth Limitation, Coastal/Restricted Waters Environment*). There are valuable shellfish beds in these areas and the risk to the resource appears great. A shellfish monitoring program is conducted by the shellfish section of the Washington State Department of Health and cooperating county health departments. Information from tissue toxicity monitoring programs allows the commercial harvesting of oysters, mussels, and scallops to occur in areas prone to the PSP problem. A standardized laboratory assay that employs isolation and injection of the PSP toxin into mice is used to assess shellfish tissue toxicity. Historical data from such assays may be useful for detecting the timing of the onset of past PSP-causing blooms, which may in turn be useful for predicting future PSP-causing blooms. However, the unpredictably long and variable retention times of toxin in shellfish tissue may negate the use of such assay data for determining the length of time *Alexandrium catenellum* cells were present in the water column.

FISH KILLS IN AMBIENT WATERS

Reported fish kills in marine and estuarine waters of western Washington are relatively rare, but their actual extent is unknown. Some limited information is available from Ecology, WDF, and the Municipality of Metropolitan Seattle (Metro). WDF is the agency responsible for investigating fish kills caused by natural phenomena or for estimating the extent of loss from other kills. Ecology is responsible for assessing fish kills resulting from spills and discharges. There is no single database for collecting and recording information about all fish kills in Washington State, regardless of cause, and historical information within all agencies is not compiled in a detailed fashion. Therefore, because the cause (or causes) of at least some fish kills in Washington State is unknown (U.S. EPA 1976), it is unclear in those cases which agency is responsible for conducting fish kill investigations and compiling fish kill information.

In freshwater and estuarine areas, many fish kills are caused by adverse environmental factors such as low DO concentrations or elevated water temperatures. Salmonids are particularly sensitive to low ambient DO concentrations, and subchronic physiological effects have been noted in several studies of anadromous salmon at DO concentrations below 6.7 mg/L (SD = 2.57), equivalent to a pO_2 of 111 mm mercury (Davis 1975). When ambient DO concentrations fall below the level required for basal metabolism, fish lose their self-righting ability and die from internal hypoxia. The exact level at which death occurs is somewhat variable depending on the species, size and age of the fish, physiological condition, prior low DO conditioning, exposure time, and water temperature. Salmonids in marine waters may be severely affected if they are subjected to prolonged exposure to DO concentrations less than 4.0 mg/L (Davis 1975), especially at high water temperatures, and when fish health may be compromised by pollution, disease, or other factors. Strong avoidance of DO concentrations of 1.5, 3.0, and 4.5 mg/L has been noted in laboratory studies of Chinook salmon at normal summer water temperatures, but not at 4.5 mg/L during cooler fall temperatures (Whitmore et al. 1960).

A primary cause of low DO concentrations in Puget Sound is the rapid intrusion of oxygen-poor oceanic waters that results from upwelling along the Washington coast (Collias et al. 1974). This intrusion is particularly evident in subsurface waters of the Strait of Juan de Fuca

(Herlinveaux and Tully 1961) and expedites the partial replacement of Puget Sound basin deep waters and the normal estuarine flow pattern (i.e., landward at depth and seaward at surface). Although often noted in the summer and fall when inflowing seawater is most dense, rapid intrusion into Puget Sound deep waters may occur at any time of the year (Cannon 1975).

Other probable causes of low DO concentrations in restricted portions of Puget Sound are the respiration of algal stocks after an abrupt change from sunny to cloudy weather or the decay of algal stocks after a bloom (both macrophytes and phytoplankton). Vertical density stratification of the water column because of temperature and/or salinity differences tends to maintain low DO concentrations below the surface layer. The turnover of poorly flushed areas such as southern Hood Canal in the fall may result in fish kills when the subsurface water moves toward the surface (see below, *Lynch Cove*). Respiration of vertically migrating dinoflagellates during blooms may also contribute to low DO concentrations in some areas (Smayda et al. 1979), although the contribution of this source has not been documented or quantified in Washington State. In other cases, even the respiration of large schools of wild fish may result in measurable decreases in DO concentrations (Oviatt et al. 1972), although wild fish probably attempt to avoid areas of inappropriately low DO concentrations (Whitmore et al. 1960; Heath 1987). Other causes of low DO concentrations in marine waters could include sediment oxygen demand from areas subject to high rates of nutrient input and sedimentation, discharge of waste materials or runoff with a high biochemical oxygen demand, and, indirectly, other sources of nutrients (discussed below; see *Sources of Nitrogen and Phosphorus*).

High water temperatures and fish diseases are other factors that have reportedly caused fish kills in all aquatic environments (Tracy and Bernhardt 1972). Toxic chemicals from natural or anthropogenic sources have also been implicated in a number of urban and rural fish kills [LeVander, L., 17 March 1988, personal communication; Ecology information from Budd Inlet cited below; various unpublished Metro staff memos (e.g., Farlow, R., 14 and 28 January 1983, personal communication) primarily regarding the Duwamish River waterway]. Exposure to high concentrations of many toxic chemicals causes damage to the gills of fish and internal hypoxia or anemia. Lower doses of many chemicals may cause alteration of other physiological processes (Heath 1987).

While some of the reported cases of fish kills have been attributed to low DO concentrations, the possibility of phytoplankton being a direct cause of wild fish mortality in Puget Sound has never been investigated adequately. There is mounting evidence that *Alexandrium catenellum*, the dinoflagellate species responsible for PSP in Puget Sound, can be responsible for kills of wild fish, birds, and marine mammals through accumulation of toxins through the food chain (White 1980; Erickson 1988). No documented cases have been reported from Washington State, although occasional kills of marine mammals, sea birds, other wildlife, and fishes in other areas, summarized by Nishitani et al. (1988), may have been related to PSP toxin spreading through the food web. Pathology investigations that could implicate other causes (such as phytoplankton) may be impeded because of the time lapse between reports of fish kills and subsequent investigations. Pathologists require specimens that have just died or are moribund to avoid rapid change of fish tissue and blood after death. Even under these conditions, it is difficult to identify phytoplankton on and in gill tissue (Harrell, L.W., 2 June 1989, personal communication) because proteolytic enzymes act rapidly after fish death.

Some fish kills may be caused by certain diatom species that interfere with gas exchange or possibly cause bleeding from the gills. Less than 10^5 cells per liter of *Chaetoceros convolutus* has been found to cause acute mortality of Pacific salmon in laboratory bioassays [Farrington 1988; Rensel, unpublished (a)]. At such relatively low concentrations, these diatom species may produce no noticeable change in water transparency or oxygen content (Rensel et al. 1989), and thus their detection as the cause of fish kills can be difficult. Concentrations as low as 10^4 cells per liter of a closely related species, *Chaetoceros concavicornis*, have been found to induce a rapid increase in the production of mucus on the gills and a severe hypoxia combined with elevated carbon dioxide content in the blood of Atlantic salmon (Rensel, in press). Hood Canal, and in particular Dabob Bay, is an area where annual blooms of these harmful species may be expected (Rensel et al. 1989), particularly in September or October (Frost, B., 9 April 1991, personal communication), although the geographic extent of the blooms and possible relationship to frequent kills of wild fish in southern Hood Canal is not known.

During the fall of 1990, dead adult chinook salmon were found in north Hood Canal, although DO concentrations were not abnormally low in that area at the time (Logan, R., 14 May 1991, personal communication). These losses may have been caused by the harmful diatom, *Chaetoceros concavicornis*, that bloomed profusely throughout the month of October 1990 in Dabob Bay and was also present in waters of north Hood Canal. This fish kill may not have been directly related to other losses of fish and shellfish in south Hood Canal during the first 2 weeks of October 1990. The south Hood Canal mortalities were reportedly associated with low DO concentrations (Messman, unpublished).

There were several reports of wild fish kills and wild fish acting in a distressed manner during a widespread bloom of *Heterosigma akashiwo* in Puget Sound and the Strait of Juan de Fuca during the summer of 1990 (for a discussion of possible effects of this species on fish reared in net-pens, see *Fish Kills in Mariculture Facilities*). Field investigations and aerial surveys failed to validate the reports (Allen, R., 12 July 1990, personal communication); however, there was a lag of at least a day between the reports and the surveys.

In the 1970s, the U.S. Environmental Protection Agency (EPA) published annual summaries of fish kill reports from fresh and marine waters (e.g., U.S. EPA 1976). Throughout EPA Region 10 (Washington, Oregon, Idaho, and Alaska), the fish kills were attributed to unknown causes (33 percent), municipal water system and sewage discharge problems (20 percent), agricultural effluent (17 percent), industrial effluent (13 percent), transportation system problems (3 percent), and other operations (14 percent). The causative agents of the fish kills (e.g., low DO concentrations or toxic chemicals) were not reported.

More recently, Ecology began publishing semiannual summaries of statewide water quality assessments [305(b) reports] that cover all aspects of water quality, although with no special reference to fish kills. A recent summary report (Ecology 1988) suggests that bacterial problems, heavy metal wastes, and EPA priority pollutant organic compounds are more important in adversely affecting estuarine water quality than are organic enrichment or low DO concentrations. The report indicates that 25.1 square miles of estuaries in western Washington have minor to moderate nutrient problems to the extent that they are unable to fully support normal uses. However, there is considerable qualitative judgment involved in designating problem areas and causes of water quality impairment (Rashin, E., 3 March 1990, personal communication). For example, Lynch Cove and southern Hood Canal are generally considered nutrient problem areas (Tetra Tech 1988) and together represent an area larger than 25 square miles.

Lynch Cove

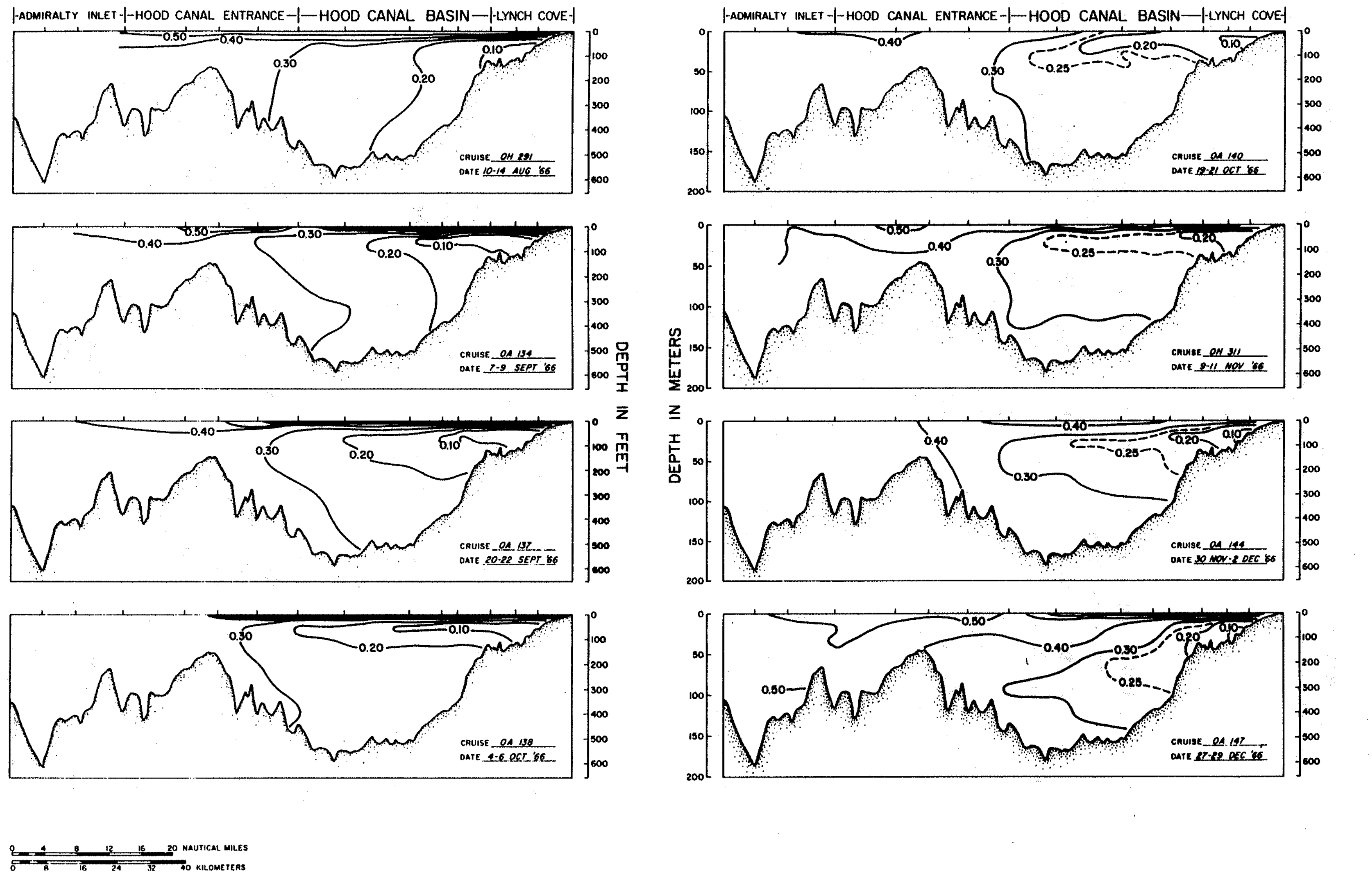
Occasional fish kills near Hoodsport, in central Hood Canal, have been related to the seasonal occurrence of low DO concentrations in Lynch Cove of southern Hood Canal (Westley, R., 12 December 1988, personal communication; Ecology 1991). In Figure 2, a longitudinal section plot of depth vs. DO concentration shows low DO water initiating in shallow, subsurface water of Lynch Cove (Collias et al. 1974). A "tongue" of low DO water displaced from depth moves northward in the canal during the late summer and fall, occasionally surfacing in other portions of southern or central Hood Canal. The problem may be exacerbated in the late summer and fall by the upwelling of water with naturally low DO concentrations.

Algal productivity and decay in the shallow, poorly flushed Lynch Cove area has been implicated as the cause of low DO concentrations through examination of surrogate measures of productivity (i.e., low Secchi disk transparency values and elevated DO saturation values in surface water). Barlow (1958) measured concentrations of chlorophyll *a* greater than 40 mg/m^3 in subsurface waters of Lynch Cove during May. Average values in Puget Sound surface waters are typically less than 10 mg/m^3, except during blooms, when peak values of 25 mg/m^3 or more have been noted.

Budd Inlet

The inner harbor of Budd Inlet is another area that has reportedly been subject to fish kills. Migrating juvenile salmon released from Percival Cove in Capitol Lake and returning adult salmon have died in this area during summer months. The causes may include DO depletion resulting from sewage discharges, algal decay after blooms, inflows of low DO water from Capitol Lake, hydrogen sulfide released from Capitol Lake, or other unknown causes (Yake 1981; Bernhardt and Yake 1983). The relative contributions of these sources to low DO concentrations are uncertain.

The mortality of juvenile salmonids in southern Budd Inlet during June 1981 was caused primarily by the accidental spillage of brewery wastes. The spill aggravated already marginal water quality conditions in the inner inlet (Singleton, L., 25 April 1991, personal communication). The fish kill was also possibly related to hatchery release practices in effect at that time (Peck, L., 13 December 1989, personal communication). In the early 1980s, hatchery fish were often removed from the lake by lowering the water-elevation control gates, which entrained the fish along with water containing low DO and high hydrogen sulfide concentrations from a deep area of the lake near the gates. The young salmon, already subjected to stress during adaptation to seawater, apparently succumbed to the adverse water quality conditions at these times. In recent years, there have been no known losses of hatchery salmon in Budd Inlet, which may be due to an alteration of the release practices and the installation of a siphon into the deep water



Source: Collias et al. (1974)

Figure 2. Longitudinal profiles of dissolved oxygen concentration (mg-at/L) in Hood Canal.

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of Capitol Lake. Fish are now allowed to freely migrate out of the lake rather than be flushed out in large numbers.

FISH KILLS IN MARICULTURE FACILITIES

Phytoplankton have been associated with occasional kills of fish reared in marine net-pens in Washington State. The purpose of this section is to discuss the possible effects of nutrients from marine fish farms and the species of phytoplankton involved in these fish kills.

Results from studies in Puget Sound (Pease 1977; Weston 1986; Rensel 1990) indicate that nutrients from marine fish farms in Washington State are not responsible for blooms of phytoplankton (harmful or otherwise) in Puget Sound. Nutrients from these fish farms could affect phytoplankton blooms if large fish farms were located in poorly flushed inlets (Gowen and Bradbury 1987; WDF 1990). Under present guidelines and requirements, fish farms are either located in well-flushed areas or are limited in size in areas with moderate flushing (SAIC 1986; WDF 1990). Commercial net-pen culture is not allowed in poorly flushed areas. Recommendations for siting and monitoring marine net-pens, used as lease requirements by the Washington Department of Natural Resources, are considered the most stringent in the world (SAIC 1986; WDF 1990).

Many kills of cultured fish in Puget Sound have been associated with blooms of certain diatoms of the genus *Chaetoceros* (Rensel et al. 1989). Occurrences of *C. convolutus* or *C. concavicornis* have been related to fish kills in the state hatchery at Hoodsport, in net-pens no longer present in northern Hood Canal, in Port Angeles Harbor, at Cypress Island (Rensel et al. 1989), and at Squaxin Island (Fraser 1976). The possible mechanisms of fish mortality caused by these diatom species were discussed in the previous section.

Heterosigma akashiwo, a microflagellate, was responsible for millions of dollars in losses of fish cultured in net-pens in Puget Sound in 1989 and 1990. The species has been present for at least the past 25 years in neighboring British Columbia (Taylor, F.J.R., 22 February 1990, personal communication) and was noted once at Clam Bay near Manchester in 1976 (Horner, R., 22 February 1990, personal communication). *H. akashiwo* blooms in Puget Sound have been geographically widespread and apparently unrelated to the operation of the relatively few fish farms (Banse et al. 1990) that are mostly located in main channels and areas replete with nutrients (SAIC 1986). Haigh and Taylor (1990) suggest that shallow marine embayments of British Columbia serve as the major source of annual *H. akashiwo* blooms that are later advected into main channels. In Japan, Nishimura (1982) found that water samples from fish farms and yellowtail tuna feces extract had no effect on the growth of *Chattonella antiqua*, a species closely related to *H. akashiwo*. A possible growth-enhancing effect was noted on an unrelated dinoflagellate species, *Gymnodinium nagasakiense*, from the addition of mackerel meat extract, but addition of inorganic nitrogen, inorganic phosphorus, and vitamins produced a similar effect. Raw, unprocessed fish as a food source is not allowed for use in Puget Sound net pens. *Gymnodinium nagasakiense* may be synonymous with *Gyrodinium aureolum* (Shumway 1990), but neither is known to occur in Puget Sound. The cause of fish mortality associated with *H. akashiwo* is unknown, although a closely related species in Japan, *Chattonella marina*, produces a neurotoxin and has hemolytic and hemagglutination characteristics (Onoue et al. 1990).

Other types of mortality in cultured fish have been attributed to phytoplankton, but the algal species and mechanisms involved are unknown. Numerous attempts to grow fish in south Port Townsend Harbor have led to abandonment of one net-pen site. Chronic mortality of Atlantic salmon at that site has been associated with a liver disease thought possibly to be caused by an algal toxin (Kent 1988). A nearby pulp mill was suspected of releasing a toxicant, but fish bioassays of discharge water failed to implicate that source as the cause. It is possible that net-pen fish at that location are affected by the presence of a fouling algae on the nets, a possibility presently being investigated.

In other cases of Atlantic salmon mortality, a physiological imbalance, possibly carried over from their residence in fresh water, may predispose the seawater-adapted smolts to extensive mortality when a phytoplankton bloom or some other environmental challenge occurs (Dickhoff, W., 11 November 1989, personal communication). Because net-pen culture is not conducted in restricted inlets and bays of Puget Sound (as it was to a very limited extent during the 1970s; compare with Rensel and Prentice 1979), mortality of fish due to naturally occurring dinoflagellate blooms is not likely to be a problem.

SHELLFISH MORTALITY DUE TO PHYTOPLANKTON

Cardwell et al. (1977, 1979) and Cardwell and Woelke (1979) mapped water quality conditions throughout marine waters of western Washington and used bioassays with oyster larvae to characterize toxicity related to dinoflagellate blooms. The armored dinoflagellate, *Ceratium fusus*, and the naked dinoflagellate, *Gymnodinium splendens*, were associated with larval oyster mortality. Because of its pointed shape, *C. fusus* apparently causes physical damage and mortality. Circumstantial evidence indicates that *G. splendens* is toxic to oyster larvae, although no details of the presumed toxin are known. Open-water areas and well-mixed passages exhibited little or no mortality of oyster larvae. The highest mortality occurred in sheltered and restricted waters, such as Budd Inlet in southern Puget Sound. Maximum abundance of dinoflagellates was recorded in restricted waters at a depth of approximately 10 meters during daytime surveys.

Periodically, oyster hatcheries in Puget Sound have reported poor survival of larvae, potentially attributable to dinoflagellate blooms. Cummins et al. (1976) reported problems with shellfish larvae apparently caused by *Prorocentrum gracile* and *Gymnodinium splendens* from Liberty Bay, Washington. Similar problems have been noted at times at hatcheries in central Puget Sound at Clam Bay and in Quilcene Bay on Hood Canal (Waterstrat, P., and Donaldson, J., 22 September 1988, personal communication).

Hood Canal spot prawns, *Pandalus platyceros*, reared in surface waters of Henderson Inlet suffered mortality during dense blooms of *Ceratium fusus* (Rensel and Prentice 1979). However, prawns reared in cages on the bottom of the inlet in water with much lower concentrations of phytoplankton had excellent survival rates. These results confirm diving observations (Rensel 1976) and hydrographic measurements (Pease 1977), indicating that blooms of *C. fusus* could have been restricted to warmer surface waters. Prawns reared in surface waters of central Puget Sound suffered chronic mortality sporadically through the summer during periods of rapidly decreasing Secchi disk transparency. Because adult spot prawns normally inhabit deep water, phytoplankton in the surface waters may have caused problems with respiration or other physiological processes.

MACROALGAL PROBLEMS

Thom et al. (1984, 1988) and Thom and Albright (1990) concluded that nutrient enrichment and associated abundant macroalgal growth and decay in a shallow, protected area of central Puget Sound's nearshore environment resulted in offensive odors and potentially toxic gases. The affected area, Seahurst Bight, is typically calm during the algal growing season (a period of sufficient light and favorable water temperatures). The nearshore waters had late spring and summer periods of DIN depletion below 10 μM (0.14 mg/L-N), and at times DIN was not detectable. If other factors are not controlling growth, seaweed growth is generally nitrogen-limited when concentrations of DIN fall below about 10 μM for sustained periods (a much higher level than for most phytoplankton) (Lobban et al. 1985). In these studies, seaweed biomass was found to decrease when dissolved nitrate was less than 8 μM, dissolved inorganic phosphate was less than 1.6 μM, and the atomic ratio of DIN to orthophosphate was less than 5. There was no seasonal trend in ammonium concentrations, although very high concentrations of ammonium in and below the seaweed mat indicated remineralization was occurring.

Review of the scant literature on nearshore eutrophication in Puget Sound suggests that macroalgal blooms in nearshore areas due to eutrophication are not a serious problem at this time. Reports of strong odors from decaying seaweed in other areas of Puget Sound are not uncommon (e.g., Fauntleroy Cove in recent years and False Bay, San Juan Island, since at least 1955) (Duxbury, A.C., 8 May 1990, personal communication), but there is presently no means to quantify adverse conditions or temporal trends. Annual trends of increased seaweed biomass in the shallow, nearshore areas of Puget Sound could be the first indications of adverse effects related to nutrient enrichment and eutrophication, although no routine monitoring is being conducted at this time.

NUTRIENT LIMITATION OF PHYTOPLANKTON GROWTH IN PUGET SOUND

Macronutrients that are required for phytoplankton growth include various forms of nitrogen and phosphorus, as well as carbon, hydrogen, oxygen, and sulfur (Raymont 1980). In addition, diatoms require silicon for construction of their frustule. Liebig's law of the least limiting nutrient (Liebig 1843) indicates that (monoculture) crop yield is essentially determined by the availability of the nutrient in shortest supply relative to an organism's requirements. In marine systems, nitrogen, and occasionally phosphorus, silicon, micronutrients (e.g., metals and vitamins), or some combination of any of these nutrients may limit the growth of mixed-species algal populations when other factors provide good growing conditions. The cycling of nutrients, their status in limiting phytoplankton growth in Puget Sound, and their sources are discussed in the following sections.

FORMS AND CYCLING OF NUTRIENTS

Nitrogen in the form of DIN is most commonly accepted as the primary limiting nutrient in marine waters (Dugdale 1967; Ryther and Dunstan 1971). DIN refers to the total combination of ammonium (NH_4^+), nitrate (NO_3^-), and nitrite (NO_2^-) ions. Ammonia (NH_3) can be hydrolyzed to ammonium ion in water and is grouped with ammonium for this discussion. At common pH and temperature levels in Puget Sound, only about 2 percent of the total NH_4^+ and NH_3 is in the un-ionized (NH_3) form that is toxic to fish and other forms of aquatic life. Of the inorganic forms of nitrogen, ammonium is preferentially taken up by some phytoplankton at concentrations down to very low levels (less than 0.5 μM), and it may be used directly for the synthesis of organic compounds by the cell (McCarthy et al. 1977). However, in most well-oxygenated and unpolluted environments, the ambient concentration of ammonium is low, as it is rapidly used by many species of phytoplankton or converted by microbial action to nitrate through nitrification (Harris 1986). Because of the naturally low concentrations of ammonium in most marine environments (except near sewage treatment plant discharges and river plumes), most phytoplankton must use nitrate, and, under some circumstances, nitrite (also typically in short supply), as the primary sources of nitrogen. Nitrogen is primarily used in the cell's structural components, not in the energy cycle of the cell (Parsons et al. 1984b).

Organic forms of nitrogen include particulate organic nitrogen (PON) and dissolved organic nitrogen (DON). Some forms of DON, such as urea and dissolved amino acids, have been shown to be moderately used for algal growth (Paul 1983; Flynn and Butler 1986), but their ambient concentrations are typically low (Sharp 1983). Phytoplankton may eventually use nitrogen from PON and some forms of DON after remineralization to inorganic forms through microbial action. Organic nitrogen is cycled at intermediate rates, midway between slow-cycling organic carbon and faster-cycling organic phosphorus (Sharp 1983).

Phosphorus is essential to many cellular reactions, including the generation of adenosine triphosphate (ATP), which is needed for cellular energy metabolism. Orthophosphate is the form of phosphorus most commonly measured in marine waters. It is also the form most readily available for plant uptake and use. Phosphorus is also present in dissolved organic and particulate forms, the former contributing to the growth of phytoplankton in some cases (Provasoli and McLaughlin 1963). Massive increases of phosphorus and nitrogen loading to some coastal areas of northern Europe (i.e., Baltic Sea, Kattegat, Dutch coastal waters, and areas of the North Sea) have resulted in reduced relative availability of silicon and a concurrent shift from diatoms to dinoflagellates and microflagellates as the dominant species [see Smayda (1989b) for a review].

Various combinations of micronutrients such as iron, copper, zinc, cobalt, selenium, manganese, molybdenum, boron, sodium, vitamin B_{12}, biotin, and thiamine are also important for the growth of phytoplankton. However, micronutrients are generally considered to be growth-limiting less often than nitrogen for most species in marine environments. Evidence exists, however, that the growth of some harmful species may be limited by micronutrients rather than nitrogen or phosphorus under some conditions (see *Nutrient Limitation: Micronutrients and Species Variation*).

The ambient concentration of a nutrient such as nitrogen may be a poor indicator of its supply rate for the production of phytoplankton. The total amount of DIN, PON, and DON and their turnover rates (i.e., the rates of exchange between dissolved and particulate forms) among those nutrient pools are the rate-limiting factors (Smith 1984; Harris 1986). However, little information concerning supply rates of DIN and DON is available for understanding nutrient dynamics in Puget Sound and applying this understanding to the control of nutrient inputs. Therefore, the concentration of dissolved inorganic nutrients in surface waters may be used as an approximate indicator of the nutrient status of an area. For example, DIN concentrations in surface and near surface waters may be very low or unmeasurable during periods of intense algal production. At these times, grazing by zooplankton, their excretion, and associated microbial organisms act to quickly cycle organic matter back to the phytoplankton as DIN, which may result in virtually no nutrient limitation of algal growth. Hecky and Kilham (1988) note that

> . . . there are numerous phytoplankton species and communities that have such high affinities for N [nitrogen] and P [phosphorus] that nutrient limitation, if it occurs, occurs at concentrations not analytically detectable. Consequently, dissolved nutrient data cannot be interpreted in terms of growth limitation given the present analytical capabilities.

Nevertheless, dissolved nutrient concentrations often vary inversely with phytoplankton production in marine waters (Copping 1982), at least on a broad time scale of seasons.

While it is likely that the rate of DIN supply limits the growth of most species of phytoplankton in many of Puget Sound's restricted bays and inlets during summer months, the concept that a single nutrient has to be *the* limiting nutrient must be seriously questioned when considering mixed assemblages of phytoplankton. There is good evidence that an assemblage of coexisting or competing phytoplankton species may be limited by the availability of several nutrients, with individual species limited by different nutrients (Levinton 1982; Parsons et al. 1984b).

In a local context, inner inlets of southern Puget Sound are apparently dominated by several species of dinoflagellates in summer and fall, including *Ceratium fusus* and *Gymnodinium splendens*. There is no evidence to suggest that these species have the same nutritional requirements, although it is important to reiterate that despite the knowledge gap, DIN is most probably the limiting nutrient in shortest supply for these species. For blooms of certain harmful species, in particular *Heterosigma akashiwo* and the related species *Chattonella antiqua* (not present in Puget Sound), there is much stronger evidence of a key role for micronutrients, especially iron or vitamin B_{12} (Yamochi 1989; Nishijima and Hata 1989).

Finally, nutrient limitation of natural phytoplankton communities may be demonstrated through various indirect and direct methods. The most common indirect method is to measure the ambient concentration of a given nutrient which allows inference regarding its supply and uptake rates. Shortcomings of this method were discussed above. Direct methods include using radioisotope-labeled nutrients or stable isotopes and measuring the response in cell growth or uptake (Dugdale 1967). Other direct methods involve nutrient-addition bioassays, discussed in more detail in the following section, *Nitrogen Depletion and Algal Growth Limitation*. Because marine phytoplankton communities are typically composed of a mixture of species, there may be some doubt regarding the applicability of experimental results to the natural milieu because differential responses, including cell death or dominance, are common for different species in the bioassay culture (Tranter and Newell 1963; Venrick et al. 1977). This may be ameliorated by experimental protocols that track dominant species dynamics (e.g., cell counts at frequent intervals) or through the use of unialgal cultures of species that are often numerically dominant during local blooms.

LIMITATION OF ALGAL GROWTH BY NITROGEN OR PHOSPHORUS

Much of the discussion that follows revolves around the relative availability of nitrogen (N) vs. phosphorus (P) in aquatic systems and the effects on algal populations. This discussion hinges on the ratio of N to P concentrations measured in the water column, termed the N:P ratio, that is sometimes useful as an indicator of which macronutrient is potentially limiting to phytoplankton growth. The N:P ratio used in this discussion is based on gram-atomic weights (i.e., atomic ratios). First, the physical processes that affect nutrient budgets are considered.

Smith (1984) reviewed the pertinent N vs. P limitation literature and concluded that "phosphorus versus nitrogen limitation is a function of the relative rates of water exchange and internal biochemical processes acting to adjust the ratio of ecosystem N:P availability." Oceanic deep waters supplied to many estuaries are balanced in terms of the supply of N and P for the physiological requirements of algae (Hecky and Kilham 1988, referring to the Redfield ratio, discussed below). As discussed later in more detail, riverine and anthropogenic sources of nutrients are often not balanced with respect to these requirements, the former providing excess N, the latter generally providing a relative abundance of P (except existing wastewater treatment plant discharges; see *Sources of Nitrogen and Phosphorus*).

The physical processes of tidal exchange and estuarine flow are often paramount in controlling nutrient budgets of fast flushing estuaries. In these areas, "nutrient budgets are dominated by advective input and output" and "nutrient concentrations are likely to be nearly conservative with respect to nonreactive tracers such as salinity and details of internal flux are not easily deciphered" (Smith 1984). In slower flushing systems, detection and modeling of nutrient throughputs is more feasible.

Redfield (1958) first observed that the atomic ratio of DIN to orthophosphate of oceanic seawater was typically 15 to 1, a ratio similar to the average ratio of the same nutrients within phytoplankton cells (16 to 1). Ryther and Dunstan (1971) cited oceanic and coastal distributions of DIN to orthophosphate (N:P) and nutrient-addition bioassays as evidence that nitrogen, not phosphorus, was often the limiting macronutrient for phytoplankton. Although they acknowledged the variability of the N:P ratio over space and time, they noted that ratios less than 15 to 1 are most common in coastal marine surface waters. The variability caused by complex interactions of physical transport, biological modification, and terrigenous inputs results in a situation where a "normal" N:P ratio must be determined empirically for each area of interest.

N:P ratios are potentially useful as indicators of nutrient supply and phytoplankton activity in restricted waters with minimal tidal exchange. The N:P ratio, coupled with a knowledge of the actual concentration of the lesser abundant nutrient, may serve as a potential indicator of nutrient limitation, but not as an absolute measure. In areas of more active physical transport, abiotic factors such as riverine flow, tidal mixing, and upwelling may significantly alter the ratio. Many nutrient budgets are controlled by advective inputs and outputs of water via tides and wind-driven circulation (Jaworski 1981; Monoget et al. 1981). Nitrification, denitrification, or both may be significant processes that alter the forms of nitrogen in some systems. The rate of exchange between pools of dissolved and particulate forms also affects nutrient availability. Simply considering low N:P ratios as evidence of nitrogen limitation or high ratios as indicative of phosphorus limitation is unwarranted (Smith 1984). The supply of nitrogen, phosphorus, or both may be adequate for phytoplankton growth in these cases, despite an unusually low or high N:P ratio. Actual algal growth limitation could be demonstrated through nutrient-addition bioassays, knowledge of the nutrient requirements of the dominant species, and other means previously discussed in *Forms and Cycling of Nutrients*, but these involve complex measurements that have not been conducted often enough or over a broad enough geographic range to be useful here.

A few authors (e.g., Shirota 1989a,b) have determined N:P ratios of seawater using total nitrogen (from combustion, not total Kjeldahl nitrogen) and total phosphorus as indicators of nutrient/algal conditions. As an indicator of available plant nutrients in the Baltic Sea, Wulff and Rahm (1988) stated that the *total* N:P ratio is a poor indicator because of the presence of a large reserve of organic, humic nitrogen. The Japanese have used total nitrogen and total phosphorus to track eutrophication trends in the Seto Inland Sea (Shirota 1989a,b). In Puget Sound, a body of water less influenced by large humic inputs than the Baltic Sea, the *total* N:P ratio cannot be used presently because of the absence of total nitrogen data from most areas.

NITROGEN DEPLETION AND ALGAL GROWTH LIMITATION

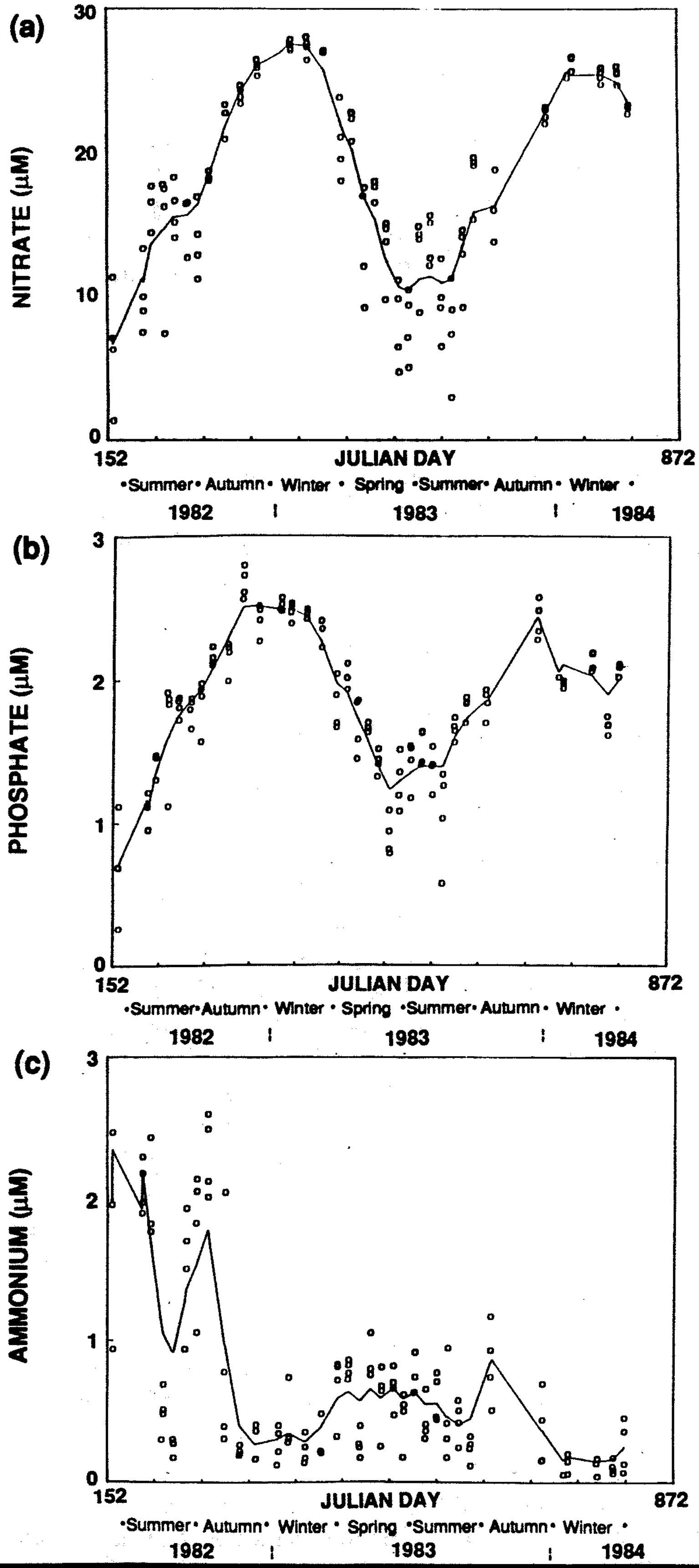
The threshold concentration at which nitrogen may limit phytoplankton growth is species-specific and influenced by light intensity and temperature (Parsons et al. 1984b). Different marine habitats have characteristic physical, chemical, and biological properties. The following discussion of nitrogen limitation in Puget Sound addresses the coastal/main channel (e.g., the main channels of Puget Sound and the Strait of Juan de Fuca) and the coastal/restricted environments (e.g., bays and inlets) separately.

Coastal/Main Channel Environment

In most main channel waters of Puget Sound, DIN supply is rarely low enough to limit phytoplankton growth. This assessment applies to waters of the main basin of Puget Sound, Nisqually Reach in southern Puget Sound, the Strait of Juan de Fuca, the waters around the San Juan Islands, and portions of the Strait of Georgia (Winter et al. 1975; Shim 1976; Campbell et al. 1977; Anderson et al. 1984; SAIC 1986). Nevertheless, DIN depletion in the surface waters in these areas may occur at least briefly. For example, during fair-weather spring surveys, Munson (1969) found periods of at least 72 hours when nitrate was not present at measurable concentrations in surface waters of the main basin of Puget Sound. In the southern Strait of Georgia, nutrient depletion is often more prolonged (Takahashi et al. 1973), in part because of the strong influence of fresh water from the Fraser River that increases vertical stratification.

Extensive water column studies were conducted over 2 years in central Puget Sound by Anderson et al. (1984). The annual cycle of nitrate, phosphate, and ammonium at stations in the central basin of Puget Sound is shown in Figures 3a, 3b, and 3c. Only rarely did nitrate or phosphate concentrations decline below about 5 or 1 μM, respectively. Ammonium was spatially and temporally more variable, with lowest concentrations in the winter, but generally in excess of 0.2 μM. Ehlers (1983) found a low N:P ratio of 3.3 and near-depleted concentrations of nitrate in samples of surface waters from central Puget Sound during 2 of 12 sampling cruises over the months of April through August. These conditions suggest that nitrogen limitation of phytoplankton growth could be possible during such periods of depletion. Additions of varying amounts of nitrogen and fixed amounts of phosphorus to water samples in Ehlers' (1983) study generally resulted in increased phytoplankton biomass. However, results were inconclusive when ambient nitrogen content was at its lowest level, which is the period of most interest to this discussion.

Welch and Messner (1984) conducted 7- to 10-day nutrient-addition bioassays using monospecific cultures of *Skeletonema costatum* added to filtered Puget Sound water. Growth rates of *S. costatum* measured by the ^{14}C technique and chlorophyll *a* yields were not consistently or significantly reduced below expected rates at low nitrogen concentrations. The addition of ammonium produced no effect on the growth rate of *S. costatum* for any of the sampling dates, including periods of very low DIN. The authors stated that the growth rate was probably saturated at very low nitrogen concentrations (i.e., growth may not normally be limited until a very low concentration of DIN is reached), below that assessed in their study.



Source: Modified from Anderson et al. (1984)

Figure 3. The annual cycle of nitrate (a), phosphate (b), and ammonium (c) from the euphotic zone of offshore stations in central Puget Sound.

Uptake constants or rates of nitrogen (nitrate or ammonium) uptake are sometimes used as an approximation to describe growth rate (Eppley and Thomas 1969) and will vary significantly with water temperature. An earlier study of *Skeletonema* in California found that the half-saturation uptake constant for nitrate was below detection at 8°C and 0.5 μM at 18°C (Eppley et al. 1969). For ammonium, the half-saturation uptake constant was greater, ranging from 0.8 to 3.6 μM at 18°C. Although Welch and Messner (1984) assumed their growth rate results for ammonium addition were inconsistently lower than those of Eppley et al. (1969), the reduction may have been caused by their use of lower water temperatures (12 vs. 18°C) and the confounding interactions of differing light exposures and intensities that existed between the two studies. Thus, the results of Eppley et al. (1969) could corroborate why no growth stimulation was found in the nutrient-addition bioassays of Welch and Messner (1984), because background levels of nitrate and perhaps ammonium were always relatively high in their Puget Sound study area compared with the physiological requirements of the bioassay species.

Many short-term ^{14}C bioassay results may be complicated by the ability of at least some phytoplankton species to take up and store nutrients prior to the bioassays ("luxury uptake"). Such storage could support initial growth during the bioassay despite low concentrations of DIN in the bioassay water (Dortch et al. 1984, 1985), although this may be sorted out in longer-term bioassays by selecting the log growth phase to calculate growth rate. Healy (1979) and Lean and Pick (1981) demonstrated a short-term growth lag in bioassays of a few algal species following nutrient addition. Although nutrients were rapidly taken up by the cells, the rate of net photosynthesis (as measured by ^{14}C fixation) declined below its preaddition rate for several hours, which reflects the carbon-rich (carbohydrate and lipid) cell composition of most nitrogen-starved cells. Healy (1979) suggested that dark respiration and ATP content may be more useful measures in short-term enrichment experiments than carbon fixation or uptake rates. Some prior nutrient-enrichment research has depended on cell conditioning methods such as preincubation at appropriate DIN concentrations in an attempt to avoid the lag problem (e.g., Eppley et al. 1969).

In summary, the main channels of Puget Sound are known to be replete with DIN at most times. Surface waters in these areas may be depleted of measurable concentrations of DIN for short periods, although conclusive proof of phytoplankton growth limitation at these times has not been demonstrated in the few nutrient-addition bioassays conducted to date.

Coastal/Restricted Waters Environment

Previous analyses of surface water data from Puget Sound (Yake 1981; SAIC 1986) have indicated several areas where DIN depletion to low or undetectable concentrations is common during the phytoplankton growing season. The observed depletion is an indicator of low DIN supply rates that may limit the growth of phytoplankton for varying periods in these environments. At other times, DIN supply for phytoplankton growth may be much greater, despite the apparent lack of measurable DIN. This apparent paradox is due to the potentially rapid cycling of nitrogen among particulate, dissolved inorganic, and dissolved organic forms previously discussed (see *Forms and Cycling of Nutrients*). To further understand this phenomenon, it is necessary to further discuss the role of dinoflagellates, as well as their growth rates, nutrition, and ability to vertically migrate.

Dinoflagellates are slower growing than diatoms, usually doubling at less than 0.5 times per day at temperatures common in Puget Sound. Growth rates of other types of phytoplankton are generally either between those of diatoms and dinoflagellates or less than those of the dinoflagellates (Banse 1982). Dinoflagellates are subject to nitrogen limitation (as measured by half-saturation constants of growth or nutrient uptake) at higher concentrations than are diatoms, but there is considerable interspecific variation (Bowie et al. 1985). Dinoflagellates have relatively high rates of dark respiration relative to the rate of growth or net photosynthesis (Raven and Beardall 1981). They also show only slight reduction in respiration when growing under low light conditions and they have characteristically high rates of photosynthesis and growth at low light intensities. Among diatoms, uptake rates of nitrate (and therefore growth rates) tend to be greater for eutrophic (i.e., coastal) than oligotrophic (i.e., oceanic) species, with half-saturation constants greater than 1.0 μM for the former and less than 0.2 μM for the latter (MacIssacs and Dugdale 1969).

Beyond the above generalizations, however, it is imprudent to attempt to generalize about growth rates within groups like dinoflagellates and diatoms, and most authors are careful to list the species or study area and taxonomic group when stating the average phytoplankton growth rate. One reference (Bowie et al. 1985) summarized maximum growth rates and half-saturation constants from a few studies by general taxon. The results show the great divergence within and among taxonomic groups, often spanning at least an order of magnitude. Caution is required when using these data because specific growth rates appear as exponents in models of phytoplankton growth and therefore even small differences, over a period of time, would lead to very large differences in the standing stock of a particular species (Parsons et al. 1984b).

The ability of dinoflagellates to vertically migrate to subsurface depths where increased reserves of nutrients may be available results in an apparent paradox regarding nitrogen limitation of their growth. Holligan (1985) states that dinoflagellate growth is ultimately controlled by physical factors such as advection and the depth, stability, and steepness of the pycnocline. Although of prime importance in some cases, chemical (nutrients) and biological (grazing) factors are generally of only secondary importance (Raven and Beardall 1981). Although DIN concentrations and supply rates in surface waters of restricted water bodies may be extremely low, blooms of dinoflagellates may occur by using available subsurface reserves. It thus becomes important to know whether subsurface reserves of DIN regularly exist in areas of interest.

Data from bays, inlets, and main channels at Ecology's south Puget Sound monitoring stations are presented in Table 2 to illustrate the differences between surface and subsurface (10-meter depth) DIN concentrations during the summer. The table is also useful to show the potential importance of differing DIN concentrations for specific species of dinoflagellates. Subsurface waters in most areas shown in Table 2, especially the inlets, have greater mean DIN concentrations compared with surface concentrations. Oakland Bay is an exception, but the sampling location there is located near a narrow, well-mixed channel (Hammersley Inlet) and several large creeks. The greater relative abundance of subsurface DIN in most south sound inlets may be the primary source of nitrogen for many autotrophic dinoflagellate populations during calm weather periods of the summer and fall. Cardwell et al. (1977, 1979) found maximum cell counts of certain harmful dinoflagellates in subsurface waters (10-30 feet and deeper) in some Puget Sound inlets in conjunction with increased toxicity to shellfish larvae in

TABLE 2. COMPARISON OF SURFACE AND 10-METER DEPTH DISSOLVED INORGANIC NITROGEN CONCENTRATIONS FOR SOUTH PUGET SOUND AREAS[a]

	Mean DIN[b] Concentration, June to August[c] (μM)	
Area	Surface	10-Meter Depth
Budd Inlet (central)	2.7 (4.15)	7.3 (5.82)
Carr Inlet	3.5 (6.39)	12 (4.72)
Case Inlet	3.7 (3.43)	8.6 (2.92)
Dana Passage (limited data)	6.4 (3.71)	10 (2.19)
Eld Inlet	3.0 (3.21)	5.7 (5.12)
Hammersley Inlet/Oakland Bay	4.2 (5.24)	3.7 (3.50)
Nisqually Reach	12 (2.31)	16 (4.58)
Pickering Passage	5.2 (2.66)	6.2 (3.71)
Totten Inlet	2.2 (2.55)	4.0 (3.69)

[a] Data are from the Washington Department of Ecology's routine monitoring stations in southern Puget Sound, June 1981 to July 1985.

[b] DIN - dissolved inorganic nitrogen.

[c] Standard deviations are shown in parentheses. (Concentrations below detection limits were considered to be zero.)

bioassays. The dinoflagellates may either migrate to depth at night to obtain nutrients or perhaps stay near the nutricline (layer of nutrient concentration discontinuity) continually. The nutricline will generally be shallower than 10 meters in these restricted bays and inlets (e.g., central Budd Inlet; URS 1986a) and may coincide with the thermocline depth.

There is only a weak correlation ($r = 0.56$) between the concentrations of DIN at the surface and the concentrations of DIN at subsurface depths for the areas shown in Table 2, indicating that the DIN concentration in surface water is a poor indicator of the DIN concentration in subsurface water. It is reasonable to assume that those bays and inlets with greater subsurface DIN concentrations (i.e., Carr Inlet, Budd Inlet, and Case Inlet) also have the ability to support a greater production of dinoflagellates than the other sheltered waters, if other conditions necessary for dinoflagellate growth are similar.

A final point relating to Table 2 regards the distribution of PSP-causing *Alexandrium catenellum*. As previously discussed, only Carr Inlet and its approaches in south Puget Sound have had significant concentrations of PSP toxin in shellfish as a result of the dinoflagellate *A. catenellum*. Of all the areas in south Puget Sound with seasonally stratified waters (i.e., Budd Inlet, Carr Inlet, Case Inlet, Eld Inlet, Oakland Bay, Totten Inlet, and probably Henderson Inlet), Carr Inlet has by far the highest concentration of subsurface (10 meters) DIN during the summer (mean = 12.4 µM vs. 8.6 µM for the next highest area, Case Inlet). Although the threshold DIN concentration for growth limitation of *A. catenellum* is not known, it is probably less than 10 µM DIN (Norris and Chew 1975). If the threshold of DIN concentration for growth limitation is similar to or less than the mean value for many dinoflagellates summarized by Bowie et al. (1985) of approximately 8 µM, then south Puget Sound areas other than Carr Inlet may not have enough DIN in summer months to support growth and reproduction of *A. catenellum* at the surface or 10-meter depths. Other dinoflagellates that are apparently more common throughout south Puget Sound (e.g., *Ceratium fusus* and *Gymnodinium splendens*) may be adapted to lower DIN concentrations. For example, the nitrate uptake half-saturation constant for *G. splendens* was found to be 1.0 µM at 18°C (Thomas and Dodson 1974). If Thomas and Dodson's (1974) data are applicable to *G. splendens* in Puget Sound, the results could explain why this species occurs in the bays and inlets with very low mean DIN concentrations that to date have not harbored large populations of *A. catenellum*.

The foregoing analysis may have an important bearing on the control of nutrient inputs to potentially nutrient-sensitive areas in Puget Sound. For example, from Table 2 it follows that Totten Inlet, Hammersley Inlet/Oakland Bay, and Eld Inlet are potentially more nutrient-sensitive because both surface and subsurface waters have relatively low concentrations of DIN during the summer. Any significant increase in DIN loading during the summer could result in more algal biomass, probably in the form of dinoflagellates. As previously discussed, cyst germination and blooms of *Alexandrium catenellum* are likely initiated by changes of water temperature, not nutrient dynamics. However, the growth and survival of these harmful species could be exacerbated by an increase of nitrogen discharge into potentially nutrient-sensitive areas of south Puget Sound (and similar potentially nutrient-sensitive areas in other parts of Puget Sound such as Hood Canal). Increased nitrogen discharge in these areas may lead to the further spread of PSP toxicity and the closure of shellfish beds heretofore unaffected. This analysis points to the need for increased understanding of nutrient dynamics for specific harmful species in Puget Sound, which is discussed later in *Knowledge Gaps and Recommendations*.

PHOSPHORUS DEPLETION AND ALGAL GROWTH LIMITATION

Phosphorus limitation of algal growth has generally been considered common in freshwater lakes and rivers that are oligotrophic or mesotrophic. However, eutrophic lakes and rivers may have algal growth limited seasonally by the supply of nitrogen (Welch 1980). Estuarine and some coastal marine areas may be variable with regard to nitrogen or phosphorus limitation of algal growth, depending on the extent of riverine vs. oceanic water inputs and the proportion of nutrients that each carries (Hecky and Kilham 1988). In many cases, riverine waters have relatively greater amounts of nitrogen than phosphorus when compared with oceanic water, but variation among rivers is possible. As a result of excess nitrogen carried in most rivers, algae in many estuaries may be growth-limited by phosphorus in the inner, riverine-influenced areas, shifting to growth limitation by nitrogen at the outer, seaward areas (Sakshaug and Olsen 1986; Harrison et al. 1990). Despite high N:P ratios in some areas, many have argued that phosphorus limitation in estuarine and coastal systems is improbable because of the rapid turnover (food web) cycling of phosphorus (e.g., Kubiak 1983). Increasing salinity has been correlated with the shift from phosphorus to nitrogen limitation (Caraco et al. 1987; Graneli 1987), but the connection is coincidental, not causal.

Through nutrient-addition bioassays, Harrison (1990) determined that large areas of the south China coast were phosphorus-limited because of the influence of several large rivers. As cited by the same author, the upper Chesapeake Bay, Hudson River, Apalachicola Bay in Florida, the Mediterranean Sea, some estuaries in Australia, and certain brackish areas in Norway are other likely areas of phosphorus limitation.

In many estuarine and coastal marine areas, inflows of nitrate-rich fresh water provide adequate concentrations of DIN, and orthophosphate concentrations may be well above detection limits. At such times, neither nutrient is likely limiting to phytoplankton growth, and the growth may be either uncontrolled for short periods (i.e., the exponential growth phase) or controlled by zooplankton grazing, lack of light, advection out of the area, vertical mixing into subsurface waters, or lack of some other nutrient substrate besides nitrogen or phosphorus. For reasons discussed in the next section, it is improbable that phosphorus limitation of algal growth is a large-scale and prolonged phenomenon in Puget Sound. However, it is possible that phosphorus limitation of algal growth occurs for at least short periods, in limited areas of Puget Sound when riverine discharge is great and algal growth conditions are concurrently optimum.

NITRATE-TO-PHOSPHATE RATIOS IN PUGET SOUND

General background information on the interpretation of N:P ratios was presented above. The following analysis was conducted to investigate, using existing Puget Sound data, water quality conditions that accompany various categories of nitrate-to-phosphate ratio observations and to draw inferences concerning which nutrient (nitrogen or phosphorus) is more likely to limit phytoplankton production in Puget Sound.

Data Analysis Methods

A computer database, previously assembled for a study of spatial and temporal trends in water quality of Puget Sound (Tetra Tech 1988), was used in the present study for examining Puget Sound nutrient conditions. The data set includes measurements by various state and local agencies and the University of Washington. For this report, the data set was screened by selecting those data that had both nitrate and orthophosphate measurements from the surface or 10-meter depths. Only Ecology and a few WDF data fit these criteria. All data were from the months of April through November except for the WDF data, which were annual data from Oakland Bay only. Metro data were not used because of incompatible methods of nutrient monitoring [as discussed by Tetra Tech (1988)]. None of the available University of Washington data in this database had both nitrate and orthophosphate concentrations from routine measurements over a period of several years.

The number of occasions when either nitrate or dissolved orthophosphate concentrations were very low or when their ratios were extreme was used to investigate possible algal growth limitation. Because of the lack of ammonium data, the use of DIN-to-orthophosphate (N:P) ratios was not possible. Instead, nitrate-to-orthophosphate (herein simply "nitrate" and "phosphate") ratios ($NO_3^-:PO_4^{3-}$) were calculated. By not including ammonium, the frequency of DIN depletion may have been overestimated using this ratio. Conversely, the frequency of phosphorus depletion may have been underestimated using this ratio. Use of the approximate ratio of $NO_3^-:PO_4^{3-}$ may be warranted, however, because nitrate is the predominant form of DIN in many habitats of Puget Sound. For example, all available Ecology surface water data from Carr Inlet indicate an average of 4.9 times as much nitrate as ammonium from April to October, while the average ratio of nitrate to ammonium is 8.1 for Admiralty Inlet. This general trend does not hold in some areas, such as the West Bay of Budd Inlet near a municipal wastewater treatment plant outfall [Station BUD002 (URS 1986a)], where ammonium concentrations are high. In addition, although surface waters of central Budd Inlet have much higher nitrate than ammonium concentrations during spring and fall, nitrate content declines to less than that of ammonium during the summer (URS 1986a).

Nitrate-to-phosphate ($NO_3^-:PO_4^{3-}$) ratio data were grouped into four major categories:

1. Both nitrate and phosphate not detectable
2. $NO_3^-:PO_4^{3-}$ ratio less than 5, with two subgroups:
    a. Only nitrate not detectable
    b. All other observations of $NO_3^-:PO_4^{3-}$ less than 5
3. $NO_3^-:PO_4^{3-}$ ratio between 5 and 20
4. $NO_3^-:PO_4^{3-}$ ratio greater than 20, with two subgroups:
    a. Only phosphate not detectable
    b. All other observations of $NO_3^-:PO_4^{3-}$ greater than 20.

Analysis of the difference between water quality variables among groups and, specifically, surrogate indicators of phytoplankton production was the basis for determining which group or groups were likely associated with growth-limiting conditions.

The total number of data points in the entire data set was 12,709. Of these, there were 2,892 data points with concurrent measurements of nitrate and phosphate from surface or 10-meter depths (Table 3). These observations were made during the period from 1964 to 1986, but most were from the late 1970s and 1980s, precluding long-term trends analysis. Approximately 91 percent of the observations were from embayments, restricted passages, and Hood Canal. The remainder were from Nisqually Reach and the Tacoma Narrows. Detection limits for nitrate and phosphate in the database were 0.7 and 0.3 μM, respectively. Concentrations reported at less than the detection limits were treated as the detection limit concentrations in accordance with Tetra Tech (1988). Statistical tests included a one-way analysis of variance (ANOVA) followed by a *post hoc* test, the Tukey-Kramer honestly significant difference multiple comparison test for unequal sample sizes.

Individual area data are shown in Appendix B and summarized by type of environment in Table 3. The NO_3^-:PO_4^{3-} ratios were grouped in the above manner to bracket the mean N:P value of 15:1 found in seawater by Redfield (1958). However, because values of the ratio in the euphotic zone of Puget Sound or other coastal areas are typically less than 15 (Ryther and Dunstan 1971), and because the database used here lacked ammonium measurements, the bracketing included a large range of observations with ratios between 5 and 20 to conservatively isolate those observations at both ends of the NO_3^-:PO_4^{3-} range. The distribution included a NO_3^-:PO_4^{3-} ratio break point of 5 because it is more common to find ratios of about 10 in Puget Sound even during periods of vertical mixing and input from deep or oceanic water sources. In addition, Thom and Albright (1990) found a decrease of macroalgal biomass when atomic N:P ratios declined below 5.

Results

ANOVA and multiple comparison statistical tests indicated significant ($p \leq 0.05$) differences in mean water quality values among groups in spite of large standard deviations (Table 4). In part, this is a result of the large number of observations in each test that influence standard error and other values used to calculate test results (Conquest, L., 7 November 1990, personal communication). In the context of this report, statistically significant differences do not necessarily indicate significant biological differences.

The grand average of all NO_3^-:PO_4^{3-} ratios in the Puget Sound data set was 10.2. The average in bays, inlets, and passages was 10.1. In main channels, the average ratio was only slightly different (i.e., 11.7). If concentrations of ammonium had been available in the database, the mean ratio values would have been slightly higher, about 1 to 3 units of the N:P ratio, given the normal scarcity of ammonium in Puget Sound surface waters (assuming nitrate-to-ammonium ratios of 4 to 8 or greater, as discussed above). Given these caveats, the results of this analysis agree with those of Ryther and Dunstan (1971), who first brought widespread attention to the fact that N:P ratios in surface waters of coastal areas are typically less than the Redfield ratio of 15:1 for oceanic seawater.

TABLE 3. SUMMARY OF NITRATE-TO-PHOSPHATE RATIO DATA[a]

	Number of Data Points	Frequency (%)
Embayments, Restricted Passages, and Hood Canal		
Mean $NO_3^-:PO_4^{3-}$ ratio = 10.1		
1. NO_3^- and PO_4^{3-} less than detection limits	161	6
2. $NO_3^-:PO_4^{3-}$ ratio less than 5		
a. NO_3^- (only) less than detection limit	409	16
b. All other $NO_3^-:PO_4^{3-}$ less than 5	483	18
3. $NO_3^-:PO_4^{3-}$ ratio between 5 and 20	1,251	48
4. $NO_3^-:PO_4^{3-}$ ratio greater than 20		
a. PO_4^{3-} less than detection limit	182	7
b. All other $NO_3^-:PO_4^{3-}$ greater than 20	138	5
Total	2,624	100
Main Channels (Nisqually Reach and Tacoma Narrows)		
Mean $NO_3^-:PO_4^{3-}$ ratio = 11.7		
1. NO_3^- and PO_4^{3-} less than detection limits	0	0
2. $NO_3^-:PO_4^{3-}$ ratio less than 5		
a. NO_3^- (only) less than detection limit	2	1
b. All other $NO_3^-:PO_4^{3-}$ less than 5	12	5
3. $NO_3^-:PO_4^{3-}$ ratio between 5 and 20	237	88
4. $NO_3^-:PO_4^{3-}$ ratio greater than 20		
a. PO_4^{3-} less than detection limit	5	2
b. All other $NO_3^-:PO_4^{3-}$ greater than 20	12	4
Total	268	100
All Data Combined		
Mean $NO_3^-:PO_4^{3-}$ ratio = 10.2	2,892	--

[a] April to November, 1964 to 1986, except 16 of the data points from Oakland Bay that were from December to March.

TABLE 4. WATER QUALITY COMPARISON AMONG DIFFERING NITRATE-TO-PHOSPHATE RATIO GROUPS[a]

Group	NO_3^-:PO_4^{3-} Ratio[b]	Month[c]	Water Temperature (°C)	Salinity (ppt)	Secchi Disk Depth (meters)	Dissolved Oxygen (mg/L)	Dissolved Oxygen Saturation (%)	Nitrate (µM)	Phosphate (µM)	NO_3^-:PO_4^{3-} Ratio
1	0	$6.5^{2,3}$ (1.53)	$14.3^{3,4}$ (3.04)	24.0^{*} (4.30)	4.2^{3} (2.10)	10.2^{*} (1.89)	115.9^{*} (21.44)	0.7^{*} N/A	0.3^{*} N/A	N/A
2	<5	7.2^{1} (1.69)	$14.0^{3,4}$ (2.28)	27.0^{*} (2.64)	4.1^{3} (1.99)	9.5^{*} (1.97)	111.1^{*} (22.99)	2.7^{*} (2.93)	1.3^{*} (0.67)	1.8^{*} (1.44)
3	5-20	$7.5^{1,4}$ (2.53)	$11.2^{1,2}$ (2.02)	27.8^{*} (3.04)	4.9^{*} (2.5)	$8.5^{1,2}$ (1.72)	$93.8^{1,2}$ (18.61)	16.6^{*} (6.37)	1.6^{*} (0.58)	10.5^{*} (34.69)
4	>20	6.9^{3} (2.64)	$11.1^{1,2}$ (2.55)	26.1^{*} (4.69)	3.4^{*} (1.78)	$8.7^{1,2}$ (1.90)	$94.0^{1,2}$ (20.44)	15.7^{*} (9.94)	0.5^{*} (0.30)	34.6^{*} (33.93)

[a] Standard deviations appear in parentheses. Superscript * indicates results are significantly different from all other groups at $p<0.05$. Superscript numbers refer to specific groups of statistical difference at $p<0.05$. For example, for the column labeled "Month," Group 1 was significantly different from Groups 2 and 3. Group 2 was significantly different from Group 1, etc. Mean NO_3^-:PO_4^{3-} ratio is based on compilation of a ratio for each individual data point.

[b] Group 1 (i.e., NO_3^-:PO_4^{3-} = 0) includes data points with both NO_3^- and PO_4^{3-} at or below detection limit; see text for more details on definition of other groups.

[c] Month refers to the mean period of measurement (e.g., 6.5 = mid-June).

An analysis of potential differences between data that were pooled from 0- and 10-meter depths in the Table 4 analyses is given in Appendix C. Trends of water quality variables at each depth are similar to those noted for the pooled data. Even salinity values for the separate depth data showed the trend, with lowest values at the surface for Groups 1 and 4 (Appendix C, Table C-1). It is important to note that many of the water quality variables are interrelated. For example, the oxygen-carrying capacity of seawater varies with water temperature and salinity.

Both Nitrate and Phosphate at or Below Detection Limits—In the event that both nitrate and phosphate concentrations were at or below detection limits, this condition suggests that one or both of the nutrients could have been limiting to phytoplankton growth at the time of measurement. Although encompassing only 6 percent of the total data points, data in this group (Group 1) included the highest mean DO saturation (116 percent, suggestive of elevated rates of algal production), the highest mean water temperature (14.3°C), and the lowest mean salinity (24 ppt) (Table 4). The mean period of measurement for all data points in this group occurred slightly earlier (mid-June) than for other nutrient ratio groups. This period coincided with the summer solstice, typically a period when phytoplankton production in central Puget Sound reaches an annual maximum (Anderson et al. 1984).

Nitrate-to-Phosphate Ratios Less Than 5—This condition suggests the possibility of, but does not demonstrate (as previously discussed), nitrogen limitation of algal growth. The mean $NO_3^-:PO_4^{3-}$ ratio in this group (Group 2) was 1.8. All but 25 data points in this group had nitrate concentrations less than 10 μM, and the mean nitrate concentration was 2.7 μM. There may have been significant differences between the two subgroups of this group (nitrate less than the detection limit; all other low ratio data points), but the relatively high detection limits do not allow an objective evaluation of potential differences.

In embayments, restricted passages, and Hood Canal, 34 percent of the data points were in this group, while only 6 percent of the data points from the main channels fit this group (Table 3). The associated hydrographic data suggest that low $NO_3^-:PO_4^{3-}$ ratio water was warmer by an average of approximately 3°C, and DO saturation was elevated 17 percent over that of water with higher $NO_3^-:PO_4^{3-}$ ratios (Table 4).

Nitrate-to-Phosphate Ratios From 5-20—This group of data (Group 3) was judged to be "normal" (i.e., neither nitrate nor phosphate was in short supply). No concentrations of nitrate less than 3.5 μM occurred in this group.

Normal $NO_3^-:PO_4^{3-}$ ratios were noted for 48 percent of the embayment data and 88 percent of the main channel data. Hydrographic data for this group exhibited the highest salinity and Secchi disk depths, as well as the latest average measurement date, although the latter parameter was not significantly different from that of the low $NO_3^-:PO_4^{3-}$ ratio group (Table 4). Water temperature and DO saturation for the normal group were significantly less than those for the low $NO_3^-:PO_4^{3-}$ ratio group, but not significantly different from those of the high ratio group.

Nitrate-to-Phosphate Ratios Greater Than 20—This condition suggests the possibility of, but does not demonstrate, phosphate limitation. The mean $NO_3^-:PO_4^{3-}$ ratio for this group (Group 4) was 34.6. Included were all measurements of undetected phosphate accompanied by detectable levels of nitrate, which accounted for approximately 55 percent of the data in this group. This group did not include undetected phosphate when nitrate was also undetectable (i.e., Group 1). $NO_3^-:PO_4^{3-}$ ratios greater than 20 were relatively infrequent in both embayments (12 percent) and main channels (6 percent).

Mean Secchi disk depth for this group was significantly less than that for the other groups. This group had significantly cooler water temperatures (2.9°C lower) and significantly lower DO saturation (17.1 percent lower) than did the low $NO_3^-:PO_4^{3-}$ ratio group (Group 2), but the values of these variables were not significantly different from those of the normal ratio group (Group 3). The DO saturation data, in particular, suggest that net phytoplankton oxygen production may have been less for Group 4 compared with observations from the low $NO_3^-:PO_4^{3-}$ ratio group (Group 2).

Some high $NO_3^-:PO_4^{3-}$ ratio observations in Puget Sound are probably related to the influence of riverine flow, which may carry relatively more nitrogen than phosphorus. Slightly reduced salinity was noted for this group compared with the normal (Group 3) and low (Group 2) $NO_3^-:PO_4^{3-}$ groups. The highest concentrations of nitrate in Puget Sound's main basin are often during February, coincident with high levels of runoff (Duxbury, A.C., 8 May 1990, personal communication). Winter nutrient measurements were not available in the historical database used in this report. More pronounced differences of salinity among the $NO_3^-:PO_4^{3-}$ ratio groups might be apparent if winter nutrient measurements were available. The lower mean Secchi disk depth (3.4 meters) measured for this group may be a function of suspended particulates carried in the riverine flow.

Because Groups 2 and 4 were each composed of two subsets that potentially would represent different water quality conditions, the data from each subset were examined to see whether the overall trends apparent among the four major groups were also consistent among each pair of subsets. The data within each pair of subsets were found to follow the trends noted above among the major groups.

Appendix B includes a summary of observations by station within each area. The frequencies of occurrence of either low $NO_3^-:PO_4^{3-}$ ratios or undetectable concentrations of both nutrients (Groups 1 and 2) are listed for various stations in Table 5. The four highest frequencies of low $NO_3^-:PO_4^{3-}$ ratios (Groups 1 and 2) occurred in four inlets of south Puget Sound, although Nisqually Reach, also in south Puget Sound, had very infrequent low $NO_3^-:PO_4^{3-}$ ratios. Three Hood Canal stations also had relatively frequent low $NO_3^-:PO_4^{3-}$ ratios. Inlets and bays adjacent to central Puget Sound had less frequent occurrences of low $NO_3^-:PO_4^{3-}$ ratios.

Extreme ratios of $NO_3^-:PO_4^{3-}$ may result from selective removal of nutrients by the plankton or differing ratios in source water. Analysis of $NO_3^-:PO_4^{3-}$ ratios and associated water quality data from surface and 10-meter depths in Puget Sound suggests that low ratios (less than 5:1) or undetectable concentrations of both nutrients are associated with water quality conditions more conducive to, or reflective of, increased phytoplankton production. The use of ratios must

TABLE 5. FREQUENCY OF LOW NITRATE-TO-PHOSPHATE RATIOS FOR 0- AND 10-METER DEPTHS AT ECOLOGY MONITORING STATIONS

Area	Number of Data Points	Percent Frequency of $NO_3^-:PO_4^{3-}$ <5 and Both Not Detectable
South Puget Sound		
Inner Budd Inlet (BUD003)[a]	45	56
Central Budd Inlet (BUD005)	160	61
Eld Inlet (ELD001)	126	69
Totten Inlet (TOT001)	133	74
Hammersley Inlet (OAK004)	101	70
Case Inlet (CSE001)	120	46
Carr Inlet (CRR001)	132	31
Nisqually Reach (NSQ001)	129	8
Main Basin Bays and Inlets[b]		
Sinclair Inlet (SIN001)	149	42
Dyes Inlet (DYE001)	148	35
Inner Commencement Bay (CMB006)	172	7
Commencement Bay (CMB003)	128	10
Tacoma Narrows	127	1
Whidbey Basin		
Port Gardner (PSS008)	84	14
Hood Canal		
South Hood Canal (HCB004)	147	58
Central Hood Canal (HCB003)	136	60
Dabob Bay (HCB002)	118	57
North Puget Sound		
Bellingham Bay (BLL008)	139	37

[a] Budd Inlet data may have been affected by ammonium from sewage treatment plant discharge.

[b] No data available from this data set for the main channels of central Puget Sound. Generally, outer Commencement Bay is representative of conditions for the main channel of that area.

be coupled with examination of nutrient concentration data to infer possible algal growth limitation. One surrogate indicator of phytoplankton productivity, DO saturation, was significantly greater in both the undetectable (Group 1) and low NO_3^-:PO_4^{3-} ratio (Group 2) groups, suggesting that low DIN concentrations are associated with high phytoplankton productivity. The fact that the high NO_3^-:PO_4^{3-} ratio group (Group 4) had a lower DO saturation suggests that low PO_4^{3-} concentrations are not associated with high phytoplankton productivity. These two facts suggest that DIN, and not dissolved orthophosphate, was relatively more scarce and therefore potentially limiting to algal growth during times of high phytoplankton productivity. While this analysis does not rule out phosphate limitation of phytoplankton growth in Puget Sound at limited times and places, it does show that nitrate limitation is much more likely. Because many routine sampling locations for bays, inlets, and passages are located in the middle portions of these areas, the data may not adequately reflect conditions nearer riverine inputs.

NUTRIENT LIMITATION: MICRONUTRIENTS AND SPECIES VARIATION

All algae require nitrogen and phosphorus for growth and metabolism (Raymont 1980). With few exceptions, all red tide dinoflagellates and microflagellates can use nitrate, nitrite, ammonium, urea, and uric acid as nitrogen sources (Iwasaki 1979). In addition, certain trace nutrients are necessary for growth of most phytoplankton. Depending on the species, these nutrients may include metals such as iron, magnesium, manganese, copper, and zinc, or vitamins such as thiamine, biotin, and vitamin B_{12}.

Organic Trace Nutrients

Organic compounds, humic acids, and other materials both from land and sea sources have been reported as capable of promoting phytoplankton growth. Some of these substances may act as metal chelators, promoting the availability of various trace metals. Metals are of special importance to many dinoflagellates and microflagellates. This is evidenced by the common use of laboratory media containing ethylenediaminetetraacetic acid (EDTA), a metal chelator, for culturing microflagellates and other types of phytoplankton.

Organic trace substances, especially vitamins, may be very important in regulating the dynamics of phytoplankton populations, especially certain harmful species. Almost all marine phytoplankton require vitamin B_{12} for growth and, in some cases, thiamine or biotin as well (Provasoli and Carlucci 1974). Vitamins are both required by and may be released from phytoplankton, depending on the stage of cell growth and species (Carlucci and Bowes 1970a,b). Both bacteria and phytoplankton play important roles in supplying and removing vitamins from the water (Swift 1980). Vitamins required for algal growth are generally thought to be in adequate supply in most coastal and oceanic areas. However, Droop (1968) and others have noted that the concentration of vitamin B_{12} may be extremely low in some circumstances. Investigators in Japan have found vitamin B_{12} to be a major growth-limiting factor for phytoplankton in the Seto Inland Sea at times (Nishijima and Hata 1989; Iwasaki 1989), but not always during prime phytoplankton growing seasons (Nakamura et al. 1989b). In that relatively shallow and enclosed sea, balanced supply and removal rates of vitamin B_{12} by bacteria and phytoplankton were documented, with contributions from rivers and sediments and degradation by light far less important in the total vitamin budget. It has been suggested that the balance could be upset by the introduction of large amounts of polluted river water or organic wastes, leading to a bloom of harmful phytoplankton that have a requirement for a higher than normal background concentration of vitamin B_{12} (Nishijima and Hata 1989).

Vitamin B_{12} dynamics were studied in marine waters of the Pacific Northwest during an investigation in the southern Strait of Georgia and the eastern Strait of Juan de Fuca (Cattell 1973). The distribution of this vitamin was found to be more complex than in other study areas outside the Pacific Northwest because of the influence of the Fraser River and its particulate matter-vitamin B_{12} interactions. This is not surprising because bacteria that may produce the vitamin are often most abundant in association with particulate matter. Unlike inorganic macronutrient concentrations that declined in surface waters during the summer, vitamin B_{12} concentrations peaked during early summer, coincident with the peak Fraser River discharge. At other times during the phytoplankton growing season, the concentration of vitamin B_{12} followed the seasonal pattern of macronutrient concentrations, demonstrative of its role in phytoplankton growth processes.

Assuming a similar relationship holds in Puget Sound, vitamin B_{12} concentrations probably follow the pattern of macronutrient concentrations, except seasonally in areas affected by major rivers. It is unknown whether vitamin concentrations in Puget Sound decline to the point where phytoplankton growth is limited. If this decline does occur, it would be most likely in the water column of restricted bays and inlets, which are also subject to seasonal stratification and macronutrient depletion. However, in restricted nearshore areas in Japan, it has been shown that bottom sediments may be relatively rich in vitamin B_{12} and some trace metals. Takahashi and Fukazawa (1982) have speculated that there may be some connection between low dissolved oxygen concentrations and elevated ammonium concentrations in bottom waters and the release of trace metals that could stimulate *Heterosigma* blooms. Haigh and Taylor (1990) have theorized that vitamin B_{12} concentrations in shallow marine waters of British Columbia are elevated, and therefore these areas may serve as the major source of annual *Heterosigma* blooms that are later advected into main channels.

In Scotland, the harmful dinoflagellate *Gyrodinium aureolum*, when exposed to very high concentrations of biotin (100 μg/L), was found to be toxic to Atlantic salmon; cells grown with lower concentrations of biotin [similar to those likely to be found in Puget Sound, 10 μg/L (Cattell 1973)] had no adverse effect on the fish (Turner et al. 1987). Biotin is an essential vitamin used in fish feeds, and the researchers concluded that under extremely poor flushing conditions in the vicinity of fish cages, accumulation of biotin could occur and possibly lead to localized toxicity of *G. aureolum*. *G. aureolum* is not known to occur in Puget Sound, but has been noted on the coast of Maine and may be synonymous with *Gymnodinium nagasakiense* (Shumway 1990).

Trace Metals

Trace metals are also important to phytoplankton as cofactors for growth and in oxidase systems. Trace metal availability and utilization by cells is affected by chelators, some of which are produced by the phytoplankton. The possibility of iron limitation in the open ocean was previously discussed (*Open Ocean Environment*) and is discussed relative to *Heterosigma* blooms below.

Species-Specific Nutritional Requirements

The available evidence suggests a great variety of nutritional requirements among phytoplankton taxa. Because diatoms are phylogenetically distant from the dinoflagellates and microflagellates, there is no *a priori* reason to expect that nutrient requirements will be similar. Most red tide microflagellates can use forms of organic phosphate as well as the inorganic forms used by other phytoplankton; for these microflagellates, nitrate is sometimes preferred over ammonium as a nitrogen source (Iwasaki 1979). Although it has been widely held that all phytoplankton prefer ammonium over nitrate, after extensively reviewing the literature, Dortch (1990) concluded that "the reduction of nitrate uptake in the presence of ammonium is rarely so severe that it completely inhibits nitrate uptake, and that it is a highly variable phenomenon" [see Flynn (1991) for a recent model of nitrogen uptake]. In addition, the total yield of microflagellate production may be increased by increasing the supply of nutrient salts, but vitamins (especially vitamin B_{12}) and sometimes trace metals could play a significant role in red tide events (see references below). Research discussed below indicates that certain harmful species have varying nutrient requirements and little is known about the nutrient requirements of other harmful species. The literature is voluminous regarding this topic and, by necessity, this discussion only briefly summarizes what is known about nutrient requirements of a few harmful species.

One microflagellate species of local importance, *H. akashiwo*, is reported to be growth-limited in waters near other countries by one or more nutrients, including phosphate, nitrate, ammonium, manganese, iron, and vitamin B_{12} (Takahashi and Fukazawa 1982; Watanabe et al. 1982, 1988, 1989; Yamochi 1989). *Heterosigma* causes massive fish kills in temperate coastal areas of both hemispheres and is perhaps the most detrimental species for marine fish culturists. There may be varying strains of *Heterosigma*, with varying physiological requirements that could account for the variety of responses (Watanabe et al. 1982). Iwasaki (1989) reviewed Takahashi and Fukazawa's (1982) often-cited study of mixed-species, nutrient-addition bioassays and concluded:

> Each flagellate showed characteristic growth responses under different nutrient conditions. *H. akashiwo* maintained a high rate of growth even under poor macro-nutrients, and a further growth enhancement was obtained by moderate concentrations of ammonia, manganese, and vitamin B_{12}.

Takahashi and Fukazawa (1982) concluded that "ammonium was not the primary controlling factor for forming a red tide of *Heterosigma*" in their study area.

Studies of a closely related species, *Chattonella antiqua*, in a restricted area of the Seto Inland Sea of Japan, indicate that vitamin B_{12} associated with polluted river water and organic wastes was responsible for initiating some red tide outbreaks, particularly after a period of heavy rain and in shallow, nearshore areas (Nishijima and Hata 1989). *C. antiqua* was found to require 10-20 ng/L of vitamin B_{12} for maximum population growth, a level found nearshore but somewhat higher than in offshore waters of the Seto Inland Sea. By comparison, vitamin B_{12} values for the southern Strait of Georgia and the eastern Strait of Juan de Fuca did not exceed approximately 10 ng/L during a 17-month period of monthly surveys (Cattell 1973). However, during that study there were no nearshore stations where *H. akashiwo* would be likely to germinate and bloom, and all samples were from stations located in relatively deep water (greater than 100 meters). See the previous section, *Fish Kills in Mariculture Facilities*, for further discussion of this species.

Another locally important species, reported throughout restricted waters of Puget Sound, is *Gymnodinium splendens* (Cardwell et al. 1977, 1979). Takahashi and Fukazawa (1982), in nutrient-addition bioassays discussed above, found that the growth of *Gymnodinium* sp. was controlled equally by macronutrients (nitrogen and phosphorus) and certain micronutrients (iron and vitamin B_{12}). Addition of nitrate alone did not increase its growth rate under laboratory-controlled conditions, and ammonia only increased its growth moderately. Concurrent addition of forms of nitrogen and phosphorus had a stimulating effect on its growth, but some micronutrients, alone and in combination, also had significant growth-promoting effects. In comparison, growth of the common diatoms *Skeletonema costatum* and *Thalassiosira* sp. appeared to be enhanced through the addition of only combinations of macronutrients (i.e., various forms of nitrogen and phosphorus).

In other studies of the genus *Gymnodinium*, ammonium was found to be less than half as effective as nitrate in promoting the growth of *Gymnodinium simplex* (Thomas 1966), contrary to the consensus opinion that ammonium is always preferentially used by phytoplankton over nitrate. Iwasaki (1979) noted that *Gymnodinium nelsoni* cannot use inorganic nitrogen at all, preferring instead yeast extracts. There is no laboratory information showing the relationship of these results to *Gymnodinium splendens*, the Puget Sound species. However, Eppley et al. (1969) reports half-saturation constants for *G. splendens* at 18°C to be 3.8 μM for nitrate and 1.1 μM for ammonium, but the effect of varying concentrations of nutrients was not assessed. A field study in a small, very shallow lagoon on the southern end of Vancouver Island concentrated on a red tide bloom caused by *Gymnodinium sanguineum*, another name for *G. splendens* (Robinson and Brown 1983). Correlative data strongly suggest that nitrate runoff regulated the bloom until the seasonal decrease of sunlight became limiting. Micronutrients were not assessed. The results of these studies suggest that dinoflagellates of the genus *Gymnodinium* are not necessarily limited only by forms of nitrogen and that interspecific differences may be great.

The purpose of this discussion has been to demonstrate the uncertainty that exists regarding the types of nutrients that may control dinoflagellate and microflagellate growth. It is clear that generalizations among species are inappropriate unless empirical evidence is available.

SOURCES OF NITROGEN AND PHOSPHORUS

Three primary sources of nitrogen and phosphorus exist in coastal waters, including Puget Sound: 1) upwelling of nutrient-rich water, 2) input from land sources, and 3) recycling of nutrients within surface waters and sediments (Harris 1986). Nutrient-laden seawater from the Pacific Ocean provides a continuous supply of macronutrients to all of Puget Sound except for the surface waters of certain restricted passages and embayments. During periods of fair weather or reduced tidal action, the oceanic nutrient supply may be restricted to subsurface waters in these restricted areas. Factors such as increased river discharge, lack of wind, and neap tidal cycles act to enhance stratification and retard vertical mixing of nutrients to the surface.

In Puget Sound, the relative contribution of nitrogen from human sources is known only in a very general sense. Anthropogenic sources of nutrients include wastewater discharges (e.g., sewage treatment plants and pulp and paper mills), septic system drainage, urban stormwater, and nonpoint runoff from fertilized agricultural, residential, and forest lands. The amount of dissolved nutrients discharged into the main basin of Puget Sound from municipal sources has been considered small compared with the amount of nutrients from upwelling of deep water and oceanic sources (Duxbury 1975; Collias and Lincoln 1977). A similar situation exists in the main channels of other Puget Sound basins, but sheltered bays and inlets such as West Bay in inner Budd Inlet near Olympia may have significant inputs of macronutrients from anthropogenic sources (URS 1986a). The relative contributions of a few anthropogenic and riverine sources of nitrogen to Puget Sound are compared with the oceanic input in Figure 4. Atmospheric deposition of nitrate from nitrogen oxide emissions has greatly increased in the eastern and northern midwest United States and in northern Europe, but is not yet a major contributor of nitrogen to Pacific Northwest waters (Smith et al. 1987; Wulff and Rahm 1988).

No attempts have been made to provide a mass balance estimate of macronutrient flux through Puget Sound. Complex nutrient cycling processes and unquantified physical, chemical, and biological sources and sinks impose a formidable barrier to providing such an assessment. However, some basic data relevant to a mass flux estimate are available. Physical transport and efflux/reflux coefficient data have recently been improved (Cokelet et al. 1990a,b,c). Riverine nutrient and discharge data are available from the U.S. Geological Survey, Ecology, Metro, and others. Because sampling of these nutrient sources is regular and periodic, and not episodic as would be needed to assess the effects of peak flows, macronutrient data may not be representative of the total annual loading. Monthly vertical profiles of macronutrients in the main basin of Puget Sound (Collias and Lincoln 1977) show major inputs of nitrogen and, to a lesser degree, phosphorus, related to riverine sources during the winter months. In general, riverine sources to Puget Sound carry proportionately much more nitrogen, in the form of nitrate and ammonium, than phosphate. For example, N:P atomic ratios of large and small rivers entering Puget Sound are typically greater than 30:1. The largest contributing river, the Skagit, contributes over 7,200 kg of nitrate and ammonium daily compared with less than 430 kg of orthophosphate (Weston 1986), which converts to an N:P atomic ratio of 37.6. Smaller rivers and creeks may have similarly high N:P ratios [e.g., Stillaguamish River (N:P = 68.9) and Chambers Creek (N:P = 173.6)].

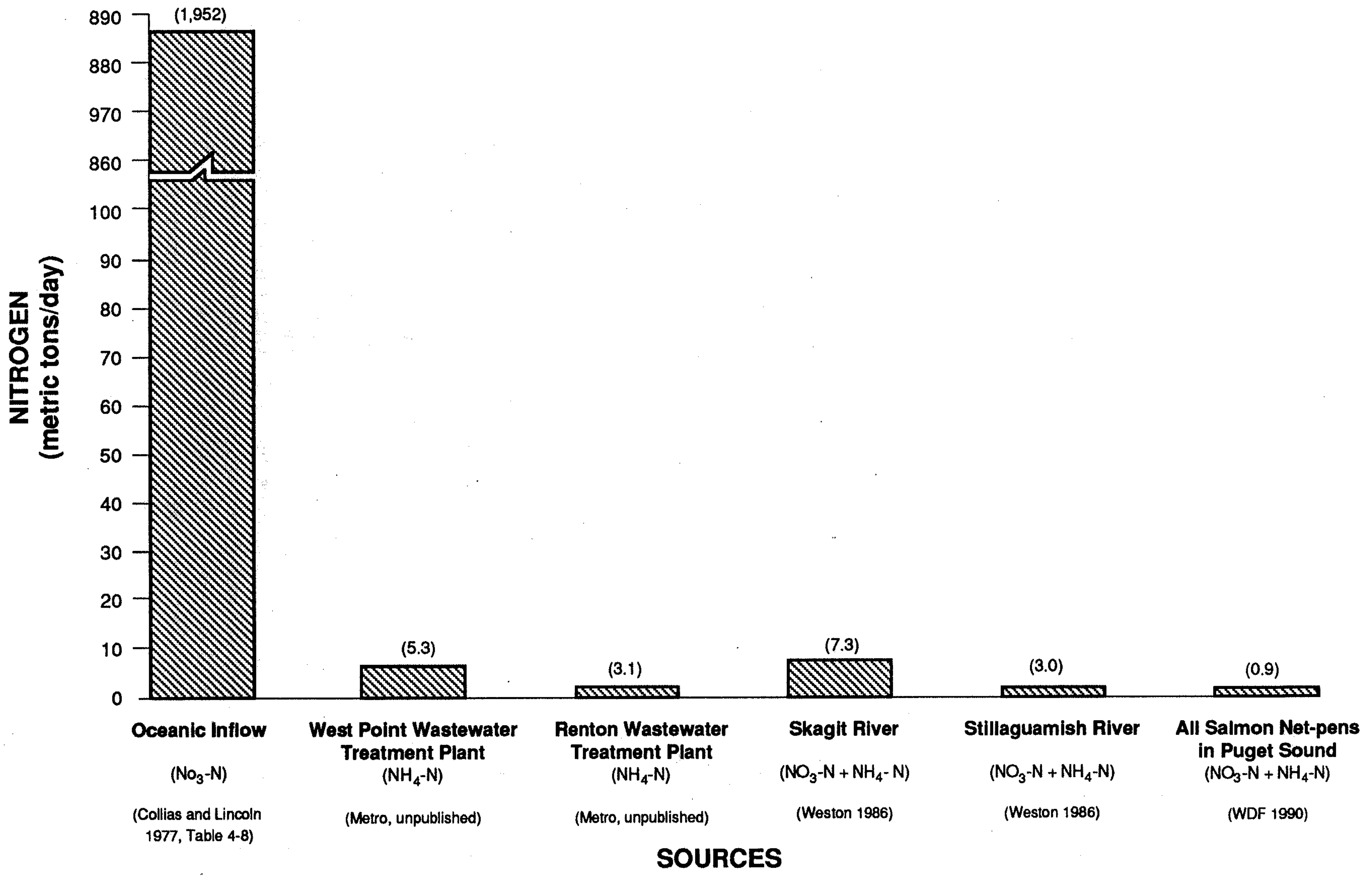


Figure 4. Annual nitrogen loading from oceanic and riverine sources compared with examples of other representative inputs.

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Studies have been conducted to assess the fate of dissolved nutrients from the Metro discharge at West Point, the largest discharger of treated sewage in Puget Sound (Collias and Lincoln 1977). Due to a lack of historical nitrogen data for the Metro effluent, no estimate of its contribution of nitrogen to Puget Sound was possible at the time of their studies. Field measurements indicated a measurable increase in the concentration of ammonium in the discharge area to a maximum distance of 1.5 km from the outfall. The average daily discharge of orthophosphate from all central Puget Sound municipal wastewater plant discharges was calculated to be approximately 1 percent of the average daily flux of orthophosphate into or out of the main basin of Puget Sound. The authors found approximately a 10-percent increase in ambient orthophosphate concentrations in the main basin by comparing the period from 1932 to 1963 (prior to the initiation of the West Point discharge) with the period from 1974 to 1975. Because this increase was less than 1 standard deviation greater than the earlier period's mean, it was not considered significant. This procedure, however, did not allow for possible interannual variation because only 2 years were assessed in the 1970s.

Metro presently monitors nutrient discharge from its two largest systems, West Point [about 100 million gallons per day (MGD)] and Renton (about 50 MGD) (Huber, K., 29 January 1990, personal communication). Combined, the two plants annually discharge approximately 3,066 metric tons of ammonium (Metro, unpublished). Three smaller wastewater treatment plants operated by Metro (producing an average total discharge of 12 MGD) do not have nutrient monitoring. Metro's contribution of ammonium is large compared with that from the Skagit River, the largest river flowing into Puget Sound, which annually discharges approximately 0.3 million kg of ammonium and 2.3 million kg of nitrate (Weston 1986). While the West Point discharge enters the seaward-flowing surface layer, the Renton discharge is to deeper water, entering the southward-flowing deep layer that is consistently rich in nutrients from oceanic sources. In terms of total water volume and quantity of ammonium, discharge from the West Point plant was essentially the same in 1989 as it was in 1975, but the quantity of discharged orthophosphate declined at least 30 percent. This assessment is based on a comparison of data from Collias and Lincoln (1977) with unpublished Metro data (1989). To perform the comparison, it was necessary to use total phosphorus data for 1989, which resulted in overconservative estimates of the quantity of orthophosphate. The decline of discharged orthophosphate may not have occurred in a linear fashion because the West Point plant abruptly ceased processing sludge from the Renton plant in 1988.

The N:P atomic ratio of effluent from the West Point treatment plant averages 13.2 [calculated from unpublished Metro data (1989)]. Other treatment plants may be similar [e.g., the Lacey-Olympia-Tumwater-Thurston (LOTT) sewage treatment plant discharges effluent with an N:P atomic ratio of about 8.0; Metro's Renton treatment plant's ratio is 11.8 (from calculations based on data given in Weston 1986)]. These ratios approximate the elemental nitrogen and phosphorus ratio (and therefore nutritional requirements) of phytoplankton (i.e., the Redfield ratio, 15:1, previously discussed in *Limitation of Algal Growth by Nitrogen or Phosphorus*), although silicate, required by the generally benign diatom species, is not discharged in elevated concentrations to match the nitrogen and phosphorus found in existing treatment plant effluent.

Studies of urban runoff have typically focused on priority pollutants, and not on nutrients. In addition, most associated nutrient data are for particulate matter, not dissolved nutrients (e.g., Galvin and Moore 1982; Pitt and Bissonnette 1984). Over 10 years of monthly dissolved nutrient data are available for various streams and lakes in Metro's database (Saunders et al. 1985). These data could be used to estimate urban nonpoint source nutrient loading and trends. Past studies of combined sewer overflow (CSO) discharges did not include monitoring of nitrogen content. Untreated CSO discharges presently total approximately 2.4 billion gallons/ year to fresh water and estuarine waters in the Metro service area (Huber, K., 29 January 1990, personal communication). However, the nutrient content of these waters is unknown. A CSO control plan, if fully implemented, may substantially mitigate the problem of such uncontrolled discharges (Metro 1988).

In summary, anthropogenic sources of nitrogen and phosphorus account for a small portion of the total macronutrient load into the main channels of Puget Sound. Most of the nutrients in the sound originate from upwelling of deep water and oceanic sources. However, in sheltered bays and inlets, municipal sewage treatment plants, industrial wastewater discharges, and nonpoint sources may contribute significant amounts of macronutrients. Available data are not adequate to generate a mass balance estimate of nutrient flux through Puget Sound.

NUTRIENT TRENDS IN PUGET SOUND

Duxbury (1975) sought to determine trends in nutrients and other factors that could influence, or be affected by, phytoplankton in Puget Sound. He compared records of orthophosphate and dissolved oxygen concentrations from a depth of 10 meters in the main basin of Puget Sound during the 1930s with those from the period after 1950. Duxbury (1975) reasoned that hydrographic information from the 10-meter depth would be the best available data to use for this comparison because the depth would be deep enough to eliminate variability due to nonhomogeneity and rapid motion of surface waters, while being shallow enough to retain some of the effects of the annual cycles of phytoplankton. He concluded from plots of the data that there was a slight, but real trend of increasing orthophosphate concentrations from 1937 to 1952, followed by a decline from 1952 to 1970, which cooccurred with an inverse trend in DO saturation. Through further analyses of salinity, precipitation, and runoff, Duxbury (1975) attributed the changes in orthophosphate to interannual variability in the strength of upwelling or influx of seawater, not to anthropogenic effects. However, the database used in this study appeared to have large temporal gaps, which limited the usefulness of the data for detailed statistical analyses.

SPATIAL AND TEMPORAL TRENDS STUDY

Recently, EPA Region 10 sponsored a study of water quality trends in 11 embayments or passages and 2 main basin areas in Puget Sound (Tetra Tech 1988). An analysis of recent (from 1973 to 1986) and long-term trends (since 1932) was conducted for several physical and chemical variables extracted from Ecology's database and other data sources. Observations of nitrate, phosphate, Secchi disk depth, and DO saturation were included. For all of the areas of Puget Sound considered, there was no assessment of long-term trends for any form of nitrogen. Only after improvements in analytical methods were made could routine measurement of that nutrient be performed from the late 1960s and early 1970s.

In recent years, nitrate concentrations decreased at 4 of the 13 sampling stations (Tetra Tech 1988). No trend was observed at seven stations and a subsurface increase was noted for one station. Since 1973, the concentration of nitrate has decreased in Port Gardner (10 meters), Carr Inlet (surface waters), and, possibly, central and southern Hood Canal (10 and 30 meters). The reduction noted in central and southern Hood Canal may be related to subsurface phytoplankton activity. The decrease of nitrate concentrations in Carr Inlet was accompanied by reduced Secchi disk depths and elevated DO saturation, suggesting the changes may have been caused by increased phytoplankton abundance and production.

Long-term phosphate concentrations have decreased since the 1950s in seven of nine areas of Puget Sound, both urban and rural, where data were assessed (Tetra Tech 1988). A more recent trend of significant increases in phosphate concentrations was demonstrated at stations near urban centers in 6 of the 13 study areas (Figure 5). The lack of change in rural areas suggests that the urban area changes may have been due to anthropogenic influences. The possible effect of this trend on phytoplankton populations is unknown.

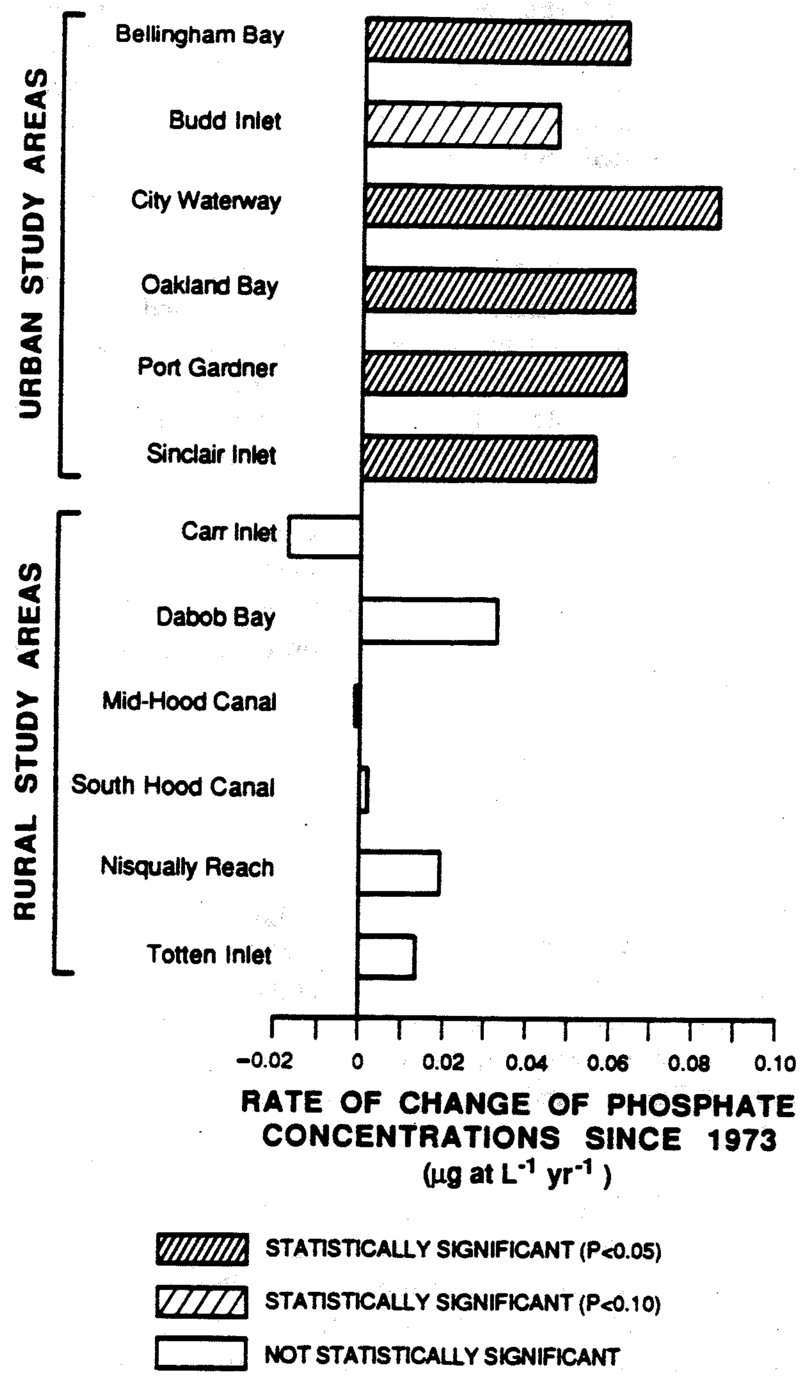
The Point Jefferson station was included as representative of conditions in the main basin of Puget Sound. However, neither nitrate nor dissolved phosphate trends could be determined on either a short- or long-term basis because of the incompatible data sets for this station. Secchi disk transparency increased and DO saturation decreased at the Point Jefferson station in recent years, suggesting a decrease in phytoplankton abundance and production.

DECADAL VARIATION

In evaluating nutrient conditions in Puget Sound, it is important to consider long-term trends caused by climatic shifts or cycles. Variation in Puget Sound flushing rates, temperature, salinity, vertical mixing, stratification, and nutrient sources or sinks may be influenced by regional or broader weather patterns. Phytoplankton populations may respond to these influences by shifts in abundance, species assemblages, or distribution. The purpose of this section is to review recent information on regional climate patterns and their physical, chemical, and biological influences on Puget Sound, with special attention to surrogate factors or indicators of phytoplankton production.

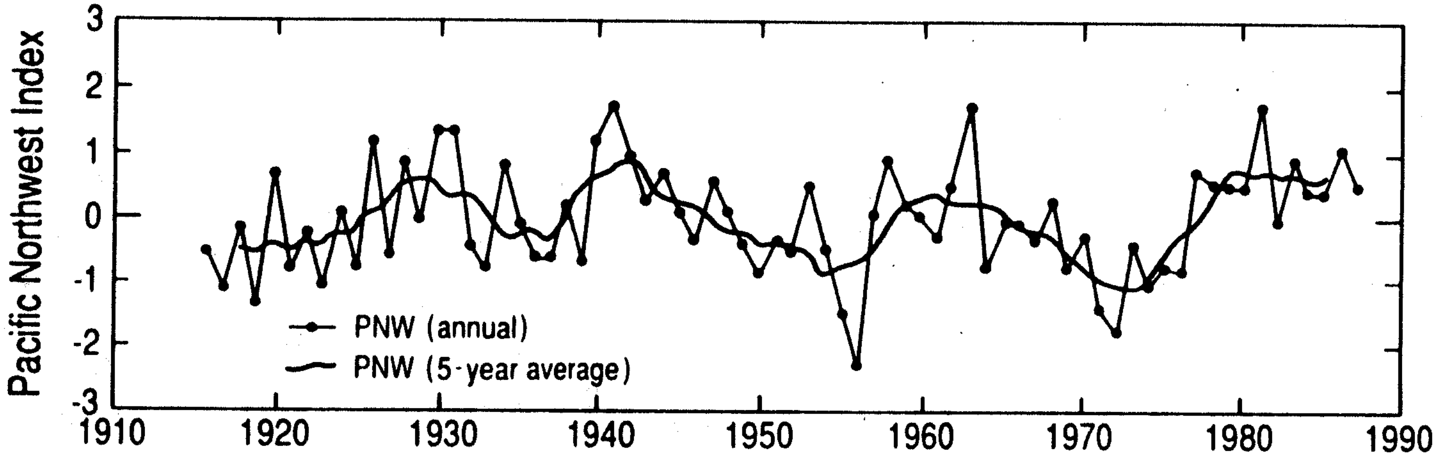
Ebbesmeyer et al. (1989) recently demonstrated a dynamic linkage between Aleutian low pressure systems in the north Pacific Ocean and circulation in Puget Sound. As the position of the Aleutian low pressure cell shifts, fluctuations in many Puget Sound environmental variables were found to covary at approximately 10-year (decadal) periods. Their study was initiated when review of current meter data from the central basin of Puget Sound suggested decadal shifts in circulation patterns. Subsequently, other factors such as storm winds, coastal temperature, Puget Sound temperature, Puget Sound lowland winds, Cascade Mountain Range snow depth, precipitation, and runoff were found to vary concurrently.

Figure 6 illustrates the decadal variations using the Pacific Northwest Index derived by Ebbesmeyer et al. (1989). The Pacific Northwest Index is the average of the standard normal deviates (SNDs) of three variables: coastal water temperature at Neah Bay, Puget Sound water temperature at 150-meter depths, and snow depth at the Paradise ranger station at Mount



Source: Modified from Tetra Tech (1988)

Figure 5. Rates of change of phosphate concentrations during algal bloom seasons in urban and rural areas of Puget Sound.



Source: Ebbesmeyer et al. (1989)

Figure 6. Time series (1916-87) of the Pacific Northwest (PNW) index.

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Rainier. This figure presents annual values as well as the 5-year running average. The running average accounts for about 40 percent of the variance of the annual values. The variation suggested two regimes: a warm/dry period alternating with a cold/wet interval with four to five complete oscillations evident since 1916. The nearly complete cycle of the decadal oscillation from the mid-1960s through the mid-1980s can be seen in Figure 6.

As a further application of the approach of Ebbesmeyer et al. (1989), environmental data including surrogate indicators of phytoplankton production in Puget Sound from recent decades were analyzed for this report. To examine decadal variation in other variables, SNDs from annual values of 19 variables were computed for the period from 1968 to 1984. An average of the SNDs was calculated during two subintervals: 1) the cold/wet regime during 1968-75 and 2) the warm/dry regime during 1977-84. Table 6 lists the difference of the SNDs averaged during the two periods, ranked from the highest to lowest difference.

Table 6 also contains data covering a range of oceanographic disciplines (from the meteorology of the Pacific Ocean and the Pacific Northwest to hydrology and marine biology of Puget Sound). During the years between 1968 and 1975, the SND averaged −0.56 with a standard deviation of 0.206. During the years between 1977 and 1984, the SND averaged +0.56 with a standard deviation of 0.153. The change in the mean SND between the two intervals equals 1.12, which is equivalent to more than 5 standard deviations, a major shift in the environment of the Pacific Northwest.

Figure 7 shows the SND averaged over the 19 variables by year during the entire 1968-84 interval. These and 21 additional variables discussed elsewhere (Ebbesmeyer et al. 1989) show a strong step-like change around the pivotal year of 1976, indicated by the dashed line in Figure 7. Data presented in Figure 7 and Table 6 suggest that major changes in the physical and biological environment of Puget Sound took place during these intervals. A comparison of nutrient concentrations from central Puget Sound during this interval was not possible because of the lack of consistent data for any form of nitrogen or phosphorus. However, analyses of main basin DO saturation, temperature, and Secchi disk transparency data were performed for this report. Results of these analyses show a strong signal that matches the decadal trend of the other variables (Table 6).

In the main basin of Puget Sound, DO saturation of the surface layer increased during the cold/wet period of 1968-75. The available water temperature data for the main basin of Puget Sound, from a depth of 150 meters, indicated lower temperatures during the 1968-75 period. Although surface water temperature data were unavailable for the main basin, it is likely that surface water in the main basin was also colder during this period because surface water temperature at Neah Bay was colder and solar radiation was lower (Table 6). Secchi disk transparency values increased during the same period. Because solar radiation was reduced, surface waters were likely cooler, and Secchi disk transparency increased during this period, the increase in DO saturation was probably not related to phytoplankton activity or solar heating. The following decadal period, a warm/dry period from 1977 to 1984, was marked by decreased DO saturation, increased salinity and water temperatures, and reduced Secchi disk transparency. Similar trends of increased deep water temperature and decreased DO saturation and increased salinity at the surface were noted by Tetra Tech (1988) for the years 1973 to 1986, which overlap the time periods of decadal variation used here. The Tetra Tech analysis, however, was

TABLE 6. STANDARD NORMAL DEVIATES OF PHYSICAL, CHEMICAL, AND BIOLOGICAL PARAMETERS FOR THE PERIODS 1968-75 AND 1977-84

	Average Standard Normal Deviate			
Parameter	1968-75	1977-84	Difference	Source
Main basin temperature, T_b	−0.83	+0.88	1.71	(1)
Coastal temperature, T_c	−0.61	+0.96	1.57	(2)
Benthic abundance	−0.91	+0.61	1.52	(3)
Secchi disk[a]	−0.86	+0.49	1.35	(4)
Salmon diversion	−0.60	+0.75	1.35	(5)
Percentage south winds	−0.82	+0.51	1.31	(6)
Chlorophyll *a*, N. Pacific	−0.64	+0.67	1.31	(7)
Dungeness crab production	−0.74	+0.51	1.25	(8)
Main basin dissolved oxygen saturation[a]	−0.59	+0.55	1.14	(9)
Pacific N. American index	−0.50	+0.55	1.05	(10)
Main basin salinity	−0.45	+0.57	1.02	(11)
Cedar Lake precipitation[a]	−0.55	+0.44	0.99	(12)
500 millibar winds[a]	−0.55	+0.41	0.96	(13)
Skagit River discharge[a]	−0.42	+0.49	0.91	(14)
Zonal sea level pressure gradient	−0.46	+0.45	0.91	(15)
Mt. Rainier snow depth[a]	−0.42	+0.46	0.88	(16)
University of Washington solar radiation	−0.34	+0.52	0.86	(17)
Downwelling index 42° north[a]	−0.31	+0.45	0.76	(18)
Fraser River discharge	−0.12	+0.41	0.53	(19)
Mean	−0.56	+0.56	1.12	
Standard deviation	0.206	0.153	0.303	

[a] The sign of the standard normal deviate has been reversed to facilitate ranking.

Sources:

(1) Temperature in main basin at 150-meter depth in the vicinity of Point Jefferson (Ebbesmeyer et al. 1989)

(2) Coastal sea surface temperature at Neah Bay (Ebbesmeyer et al. 1989)

(3) Abundance of *Macoma carlottensis* at 200-meter depth at Station 2 in the main basin off West Point (Nichols 1988)

(4) Metro stations offshore of Point Jefferson: KSHK01, JSPH01, JSNK01, JSPRO01, KSBP01; May, June, and July, 1968 to 1984

(5) Fraction of Fraser River sockeye salmon returning around the northern end of Vancouver Island (Hamilton 1987)

(6) Percent frequency of south winds at weather station in Tacoma, Washington, 26th and Pearl Street

TABLE 6. (Continued)

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(7) Integrated chlorophyll *a* in the central part of the north Pacific Ocean (Venrick et al. 1987)

(8) Puget Sound commercial Dungeness crab production (Bumgartner 1990)

(9) Surface water dissolved oxygen for Metro stations offshore of Point Jefferson: Stations KSHK01, JKPH01, JSNK01, JSPR01, KSBP01; May to July, 1968 to 1984

(10) Pacific North American Index of Wallace and Gutzler (1981) (McLain, D.R., 1989, personal communication)

(11) Surface salinity at Metro stations offshore of Point Jefferson: Stations KSHK01, JSPH01, JSNK01, JSPR01, KSBP01; May to July, 1968 to 1984

(12) Annual total precipitation at Cedar Lake, Washington (Seattle Water Department and Seattle City Light)

(13) Annual mean wind speed at 500 millibars, Medford, Oregon (U.S. Weather Service Station)

(14) Annual (January to December) average discharge at Concrete, Washington

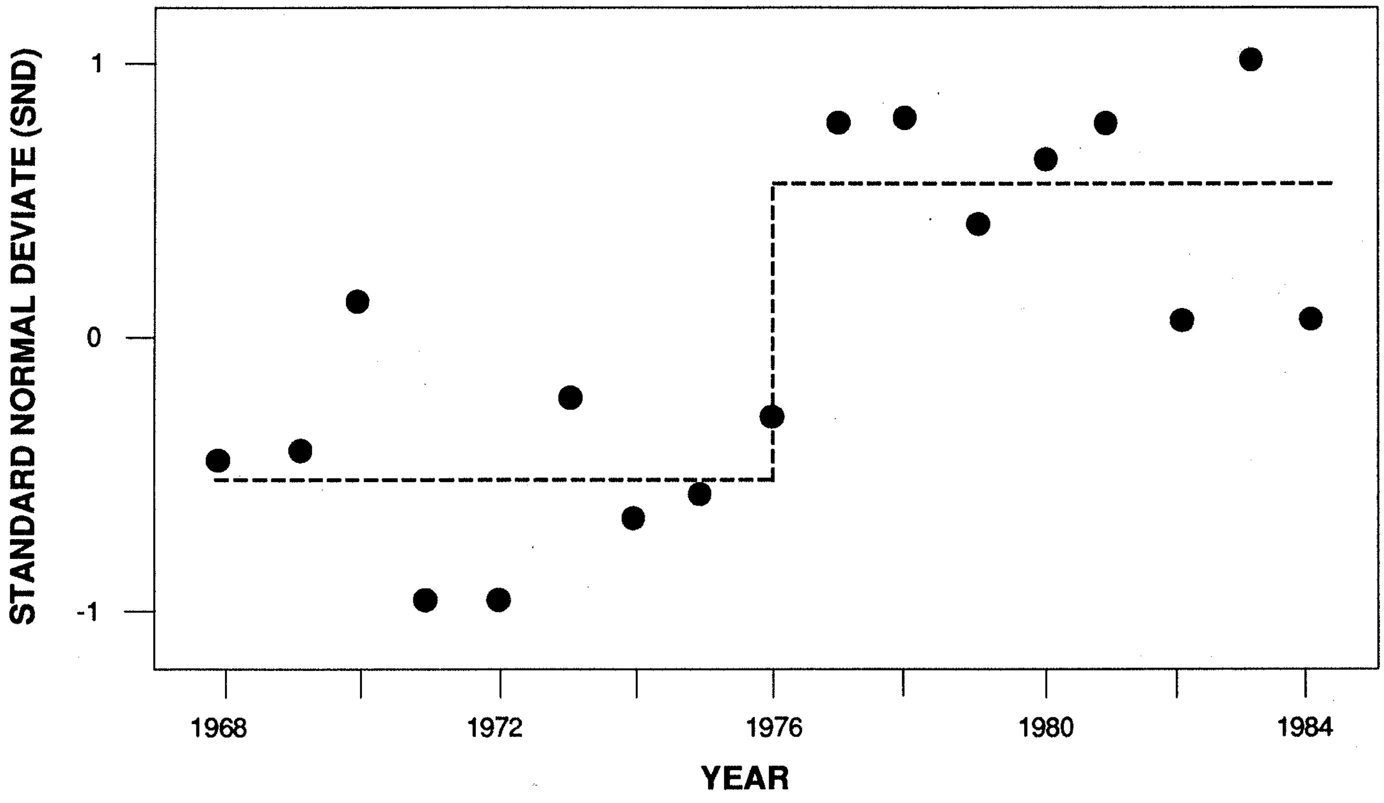
(15) Sea level pressure gradient along 50° north (Seckel 1988)

(16) Snow depth on 15 March at Mt. Rainier (Albright, M., 1989, personal communication)

(17) Daily solar radiation averaged during May and June at the University of Washington and Lake Washington (Albright, M., 1989, personal communication)

(18) Updwelling index for winter (Norton et al. 1985)

(19) Annual (January to December) average discharge at Hope, British Columbia (Environment Canada 1989).



Source: In part from Ebbesmeyer et al. (1989)

Figure 7. Mean standard normal deviate for 19 physical, chemical, and biological variables in the Puget Sound region.

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performed without knowledge of the decadal cycles discussed here. The increased DO saturation at the surface during the cold/wet period could be related to the influence of cold, relatively oxygen-rich riverine water flowing into Puget Sound. The riverine water, although colder, is less dense and persists at the surface prior to being mixed into the water column. Although not as pronounced as the increase in DO saturation during spring, the riverine effect on surface water DO saturation typically peaks in January or February (Collias et al. 1974; Collias and Lincoln 1977).

If macronutrient concentrations in surface waters of the main basin were persistently low or exhausted during periods of phytoplankton growth, increased productivity of phytoplankton stocks would be anticipated as increased discharge of nutrient-laden riverine water mixed with surface waters of the sound and stimulated phytoplankton growth during the wet/cold period. Because macronutrient concentrations are normally high in the main basin, it is likely that the macronutrients in the riverine water do not increase phytoplankton production as they do in areas where nutrient depletion of coastal waters is more common (e.g., Dutch coastal areas that experience blooms during wet periods due to riverine nutrient loading; see Gieskes and Schaub 1990). Conversely, increased phytoplankton productivity would be expected to occur seasonally in nutrient-depleted bays and inlets during the wet/cold periods, although insufficient hydrographic or nutrient data are presently available to test this hypothesis.

Overall, these results suggest that Puget Sound's hydrography varies on a frequency approaching a decadal basis. Surrogate indicators of phytoplankton abundance also vary, but interactions of riverine and ocean inputs may be responsible for the perceived signals. It is important that future trends analysis of Puget Sound nutrients and water quality consider the possibility of decadal and other time-scale variation that may occur in an abrupt, step-like function. Such step variations (Figure 7) would not be detectable by previously conducted forms of trend analyses that rely on gradual shifts in water quality variables over time (e.g., linear regression and nonparametric ANOVA of randomly selected time periods) (Tetra Tech 1988). Once an adequate time series of nutrient and phytoplankton data are available (especially chlorophyll *a* and abundance indices of dominant species), it is likely that decadal or other time-scale variation would be more easily detected in the semiconfined bays and inlets of Puget Sound that do not receive significant nutrient supply from anthropogenic sources.

NUTRIENT SENSITIVITY RANKING SYSTEM

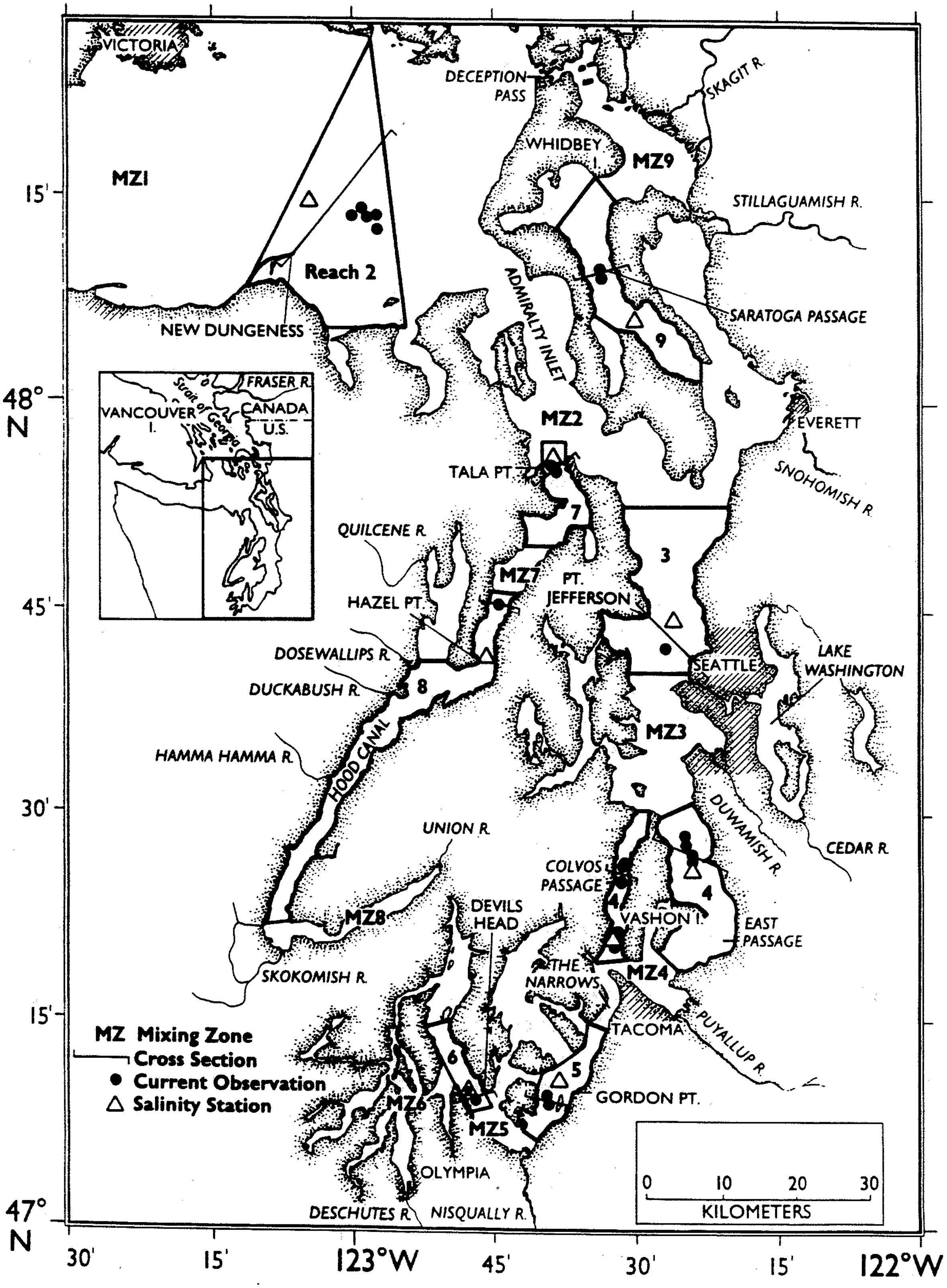
This section provides an assessment of the relative sensitivity of algae stocks in various bays, inlets, passages, and basins of Puget Sound to additional inputs of nutrients, herein defined as their "nutrient sensitivity." Estimates of nitrogen depletion in surface waters were used to quantify the relative sensitivity of the various areas. This ranking is provided to identify locations that may require site-specific studies prior to outfall siting decisions or upgrading of wastewater treatment facilities. The water quality index (WQI) developed by EPA and used in the past by Ecology (e.g., Bernhardt and Yake 1983) was not included here because it includes some factors not directly related to nutrients and phytoplankton. Physical transport information is presented below because of the relationship of the degree of flushing to nutrient sensitivity, but physical transport data were not sufficient for use in the ranking of areas.

PHYSICAL TRANSPORT: MAJOR REACHES OF PUGET SOUND

The rate of water exchange with neighboring waters and the associated flux of nutrients is a primary determinant of nutrient dynamics in each area of Puget Sound. The availability of physical transport data (flow volumes per unit time measured at several places across a channel) is mostly limited to main channels and sill areas in Puget Sound. Comprehensive physical data for many bays and inlets (e.g., estimates of reflux and efflux rates, vertical mixing rates, stratification data, and circulation patterns) are not available. Although current meter records are available for many areas of Puget Sound (Cox et al. 1984), cross channel arrays are required to accurately estimate transport volume. If comprehensive and accurate records were available, there are relatively simple models for using the data, along with nutrient concentration data, to provide first-order estimates of nutrient sensitivity (see *Alternate Ranking Method* and *Knowledge Gaps and Recommendations, Flushing Estimates and Modeling*).

Water transport coefficients have been calculated for the nine major reaches of Puget Sound (Figure 8) (Cokelet et al. 1990a,b,c). These reaches were ranked from highest to lowest flushing potential based on the water volume flowing in and out of each area (Table 7). Transport calculations take into account river runoff, current meter records, salinity measurements, water transport coefficients, and recycling. A relative ranking code was assigned to each reach, ranging from 1 (designating the highest transport) to 7 (which denotes the lowest transport). The maximum transport within Puget Sound proper (i.e., Admiralty Inlet and southward) occurs in Colvos Passage and East Passage, with a transport of 31 percent of that in the western Strait of Juan de Fuca. Three reaches have relatively small transports: 1) Dana Passage in southern Puget Sound, 2) southern Hood Canal, and 3) Saratoga Passage in the Whidbey Basin.

The last two reaches listed above and areas landward (west) of Dana Passage are considered likely to have nutrient-related problems. Recent studies of Budd Inlet and the region inside of



Source: Cokelet et al. (1990c)

Figure 8. The reaches and mixing zones of the Strait of Juan de Fuca/ Puget Sound system (Reach 1, not shown, is at the entrance to the Strait of Juan de Fuca).

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TABLE 7. VOLUME TRANSPORT FOR SUBREGIONS AND RELATIVE RANKING

Reach	Area	Transport[a] (m^3/second)		Relative Ranking of Transport Flow
Pillar Point	Strait of Juan de Fuca	104,000	(8,000)	1
New Dungeness	Strait of Juan de Fuca	41,000	(10,000)	2
Colvos and East passages	Central Puget Sound	32,000	(2,000)	3
Gordon Point	North of Nisqually Reach	15,000	(2,000)	4
Point Jefferson	Central Puget Sound	13,000	(2,000)	5
Devils Head	East of Nisqually Reach	5,000	(500)	6
Inner Southern Puget Sound	South Puget Sound[b]	3,000	(500)	7
Southern Hood Canal	Inner Hood Canal	3,000	(300)	7
Dabob Bay	Northern Hood Canal	N/A[c]		7
Saratoga Passage	Whidbey Basin	3,000	(300)	7

[a] Numbers in parentheses indicate standard error.

[b] Refers to marine waters westward of Dana Passage.

[c] Not available, see text.

Source: Cokelet et al. (1990c); URS (1986b)

Dana Passage (primarily consisting of Budd Inlet, Eld Inlet, Totten Inlet, Hammersley Inlet, and Oakland Bay) have predicted that region to be sensitive to small incremental additions of nitrogen (URS 1986a,b). Southern Hood Canal is also considered sensitive to nutrient addition because of its low transport ranking. The average transport of water through Saratoga Passage is as low as that of southern Hood Canal despite the high seasonal runoff from the Skagit and Snohomish rivers. Dabob Bay has been added to this group because historical data have shown occurrences of stagnant water behind its sill for up to 2 years (Ebbesmeyer 1973). All of these areas are known to have waters with seasonally low DO concentrations at depth.

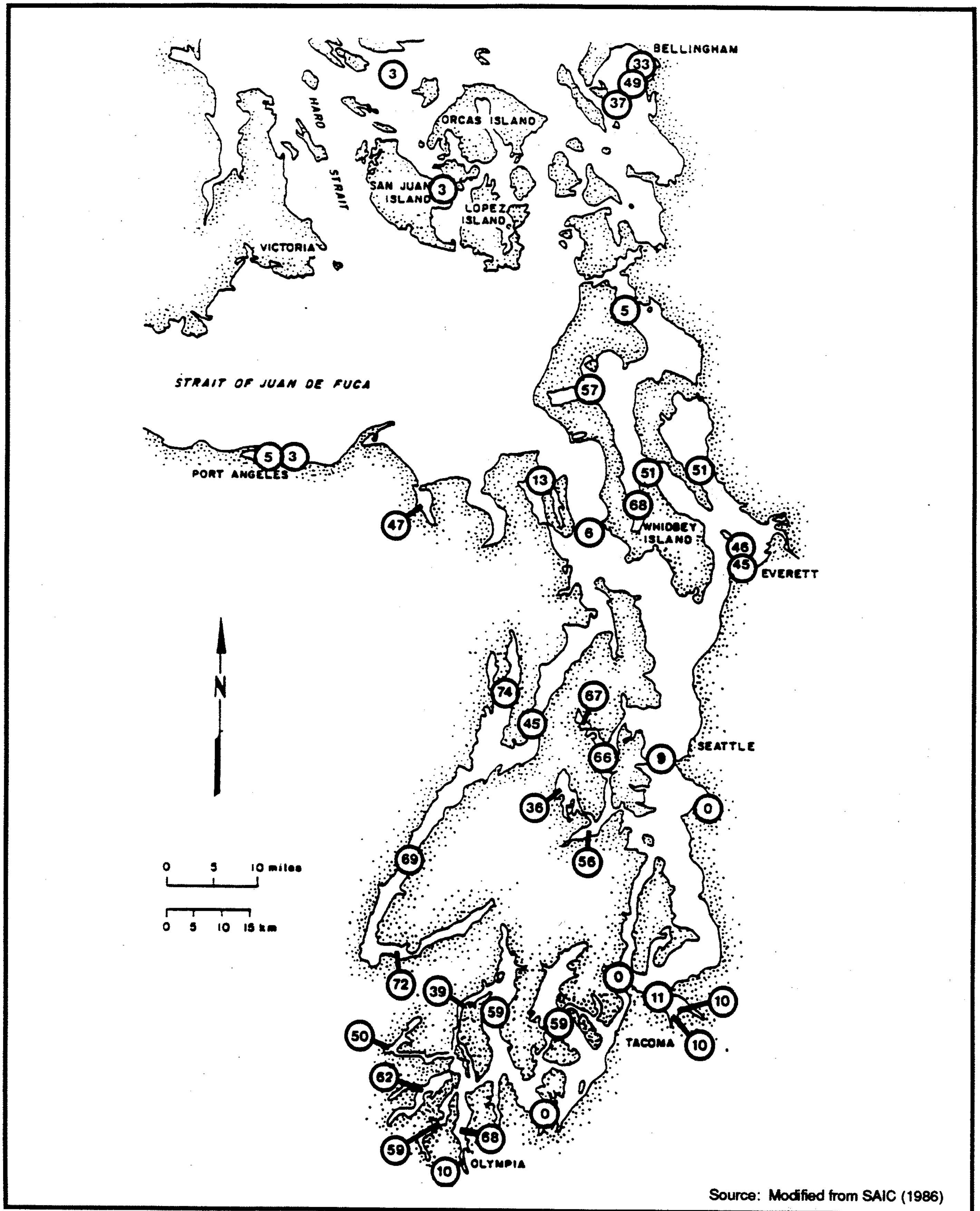
Although the largest transport within Puget Sound proper occurs in Colvos Passage and East Passage around Vashon Island, about 50 percent of the transport northward through Colvos Passage is fluxed southward via East Passage. Further south at the Tacoma Narrows, tidal flow across a major sill forces deep water toward the surface and enriches the surface waters with nutrients. Some of this water returns northward via Colvos Passage and some continues southward to Nisqually Reach and other areas of south Puget Sound. West of Nisqually Reach, most of the transport is through Dana Passage, a major sill in south Puget Sound. Although volume transport is relatively low through Dana Passage compared with other reaches of Puget Sound, velocity and probably vertical mixing are high there because of the narrow profile of the passage compared with other low transport areas with wide or deep, slowly moving waters (e.g., Saratoga Passage or south Hood Canal).

The preceding information suggests that several areas of Puget Sound could be sensitive to the addition of nutrients based only on transport considerations. Unfortunately, data are lacking for calculation of transport and efflux/reflux at the entrances to many potentially sensitive inlets and passages. Other factors must also be considered, such as the relative flushing rate during the phytoplankton growing season and the proximity of a body of water to well-mixed channels. Accordingly, the transport data presented here are useful, but these data are not used to assess nutrient sensitivity. Recommendations for determination of flushing rates for shallow embayments are provided in a following section (see *Knowledge Gaps and Recommendations*).

NITROGEN DEPLETION AND LOW DISSOLVED OXYGEN

The frequency of DIN depletion [defined for the purpose of this report as the sum of nitrate, nitrite, and ammonium (= DIN) concentrations less than 7 μM (0.1 mg/L N)] in surface waters of Puget Sound and approaches was estimated by SAIC (1986) from Ecology data (Figure 9). The word "depletion" is used here meaning both greatly reduced or undetectable concentrations of DIN. Measurements from April to November (roughly the bulk of the algal growing season) during the period from 1981 to 1985 were available and were ranked in Table 8. The original data used by SAIC (1986) were obtained and other results from the same Ecology database for stations at West Point, Dana Passage, and the entrance to Drayton Harbor were added.

The 7-μM DIN concentration represents a very conservative measure of nitrogen depletion in surface waters and the reader should not assume that this value has any special biological significance with regard to algal growth limitation. It was merely a convenient starting point to calculate the percent-frequency depletion of DIN. Most phytoplankton are probably not growth-limited by DIN at a concentration of 7 μM. Although the frequency of DIN depletion



Source: Modified from SAIC (1986)

Figure 9. Percent frequency of nitrogen (DIN) depletion less than 7.0 μM (0.1 mg/L-N) for surface waters at Washington Department of Ecology routine monitoring stations in Puget Sound from April to November 1981-85.

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TABLE 8. FREQUENCY OF DISSOLVED INORGANIC NITROGEN DEPLETION AND LOW DISSOLVED OXYGEN CONCENTRATIONS DURING APRIL TO NOVEMBER[a]

	DIN[b] <7 µM (0.1 mg/L N) at surface		DO[c] <5 mg/L % Observations	
Area	% Observations	Rank	10 meter	30 meter
Southern Basin				
Budd Inlet/Port Dock (BUD002)[d]	10	30	18	--[e]
Central Budd Inlet (BD005)	68	4	0	--
Eld Inlet (ELD001)	59	9	0	--
Totten Inlet (TOT001)	62	8	0	--
Hammersley Inlet (OAK004)	50	16	0	--
Pickering Passage (PCK001)	39	23	0	--
Dana Passage (DNA001)[f]	25	27	0	0
Case Inlet (CSE001)	59	9	0	0
Carr Inlet (CRR001)	59	9	0	0
Nisqually Reach (NSQ001)	0	38	0	--
Main Basin				
Liberty Bay (POD006)	67	6	0	--
Sinclair Inlet (SIN001)	56	13	0	--
Port Orchard/Brownsville (POD005)	66	7	0	--
Dyes Inlet (DYE001)	36	25	0	--
Inner Commencement Bay (CMB006)	10	30	0	--
Commencement Bay (CMB003)	11	29	0	0
Tacoma Narrows (NRR001)	0	38	0	0
Elliott Bay (ELB005)	0	38	0	--
Admiralty Inlet (ADM001)	6	33	0	0
West Point (PSB003)[d]	9	32	0	0
Whidbey Basin				
Holmes Harbor (HLM001)	68	4	0	15
Penn Cove & Crescent Harbor (PNN001)	57	12	6	0

TABLE 8. (Continued)

Area	DIN[b] <7 μM (0.1 mg/L N) at surface		DO[c] <5 mg/L % Observations	
	% Observations	Rank	10 meter	30 meter
Port Susan (SUZ001)	51	14	0	--
Port Gardner (PSS008)	46	19	0	0
Saratoga Passage (SAR003)	51	14	0	0[g]
Skagit Bay (SKG001)	5	34	0	--
Possession Sound (PSS019)	46	19	0	0
Strait of Juan de Fuca				
Sequim Bay (JDF005)	47	18	0	--
Port Angeles Harbor (PAH003)	5	34	0	--
Hood Canal				
Southern Hood Canal (HCB004)	72	2	41	79
Central Hood Canal (HCB003)	69	3	3	36
Dabob Bay (HCB002)	74	1	0	24
Northern Hood Canal (HCB006)	45	22	3	--
Northern Puget Sound				
Drayton Harbor (DRA001)	46	19	0	0
Bellingham Bay, Point Francis (BBL009)	37	24	0	--
Bellingham Bay, Post Point (BBL008)	49	17	0	0
Inner Bellingham Bay (BBL006)	33	26	5	--
San Juan Channel (SJI001)	3	36	0	--
Haro Strait/Skipjack Island (HRO001)	3	36	0	0
Port Townsend Harbor (PTH005)	13	28	0	--

[a] Source: Ecology data, modified from SAIC (1986).

[b] DIN - dissolved inorganic nitrogen.

[c] DO - dissolved oxygen.

[d] Nitrogen depletion directly affected by sewage treatment plant discharge.

[e] Dash indicates station not deep enough.

[f] Data from limited number of observations.

[g] Low DO conditions occur in northern Saratoga Passage (Collias et al. 1974).

provides only limited information regarding nitrogen supply rates (see above, *Nutrient Limitation of Phytoplankton Growth in Puget Sound* and *Forms and Cycling of Nutrients*), it is the best widely available indicator of potential nutrient sensitivity for Puget Sound.

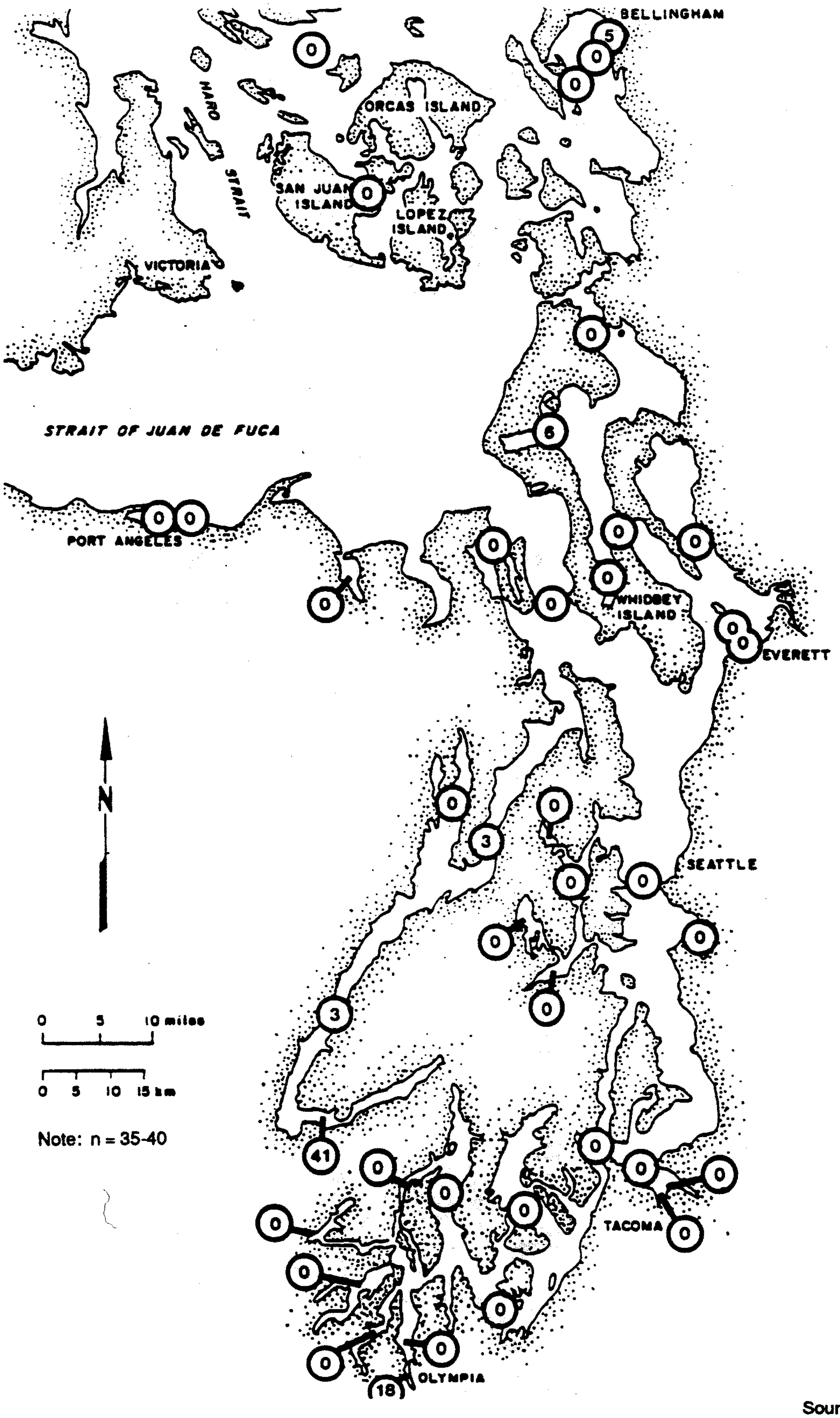
The frequency of low DO concentrations (less than 5 mg/L) in subsurface waters (Figures 10, 11, and 12; Table 8) from the same literature source was also available as a surrogate measure of potential nutrient sensitivity. However, subsurface DO concentrations less than 5 mg/L tend to be localized within a specific bay, inlet, or passage, and may not be detected by the use of a monitoring station at a fixed location. For example, the monitoring station in Saratoga Passage is in the middle of the passage, but the low DO concentrations are restricted to the north end of the passage (Collias et al. 1974).

The mean concentration of DIN in surface water of individual areas of Puget Sound was not used to assess their potential nutrient sensitivity because there is uncertainty regarding the appropriate concentrations of nitrogen (or other nutrients) that may limit the growth of locally dominant algal species when nutrients, and not other factors, are growth-limiting. At the present time, the arbitrary use of any one selected concentration of nitrogen as a threshold of algal growth reduction could therefore be inaccurate and misleading. Although some limited information exists regarding nutrient requirements and growth limitation for some of the common phytoplankton species in Puget Sound (e.g., *Gymnodinium splendens* and *Skeletonema costatum*), none of the data were collected from Puget Sound clones or under Puget Sound conditions, so there is doubt as to the applicability of the data (e.g., Carpenter and Guillard 1971).

Alternatively, it is stressed that the ranking of potential nutrient sensitivity using the frequency of nitrogen depletion is relative in nature, pending development of more sensitive methods that may include factors such as flushing rates and associated nutrient flux (see *Other Models and Approaches*). As expected from interrelated measures, there is a close, inverse correlation between mean DIN concentration during summer months and the frequency of DIN depletion during the phytoplankton growing season (April to November) [Table 9, correlation coefficient between DIN depletion and DIN concentration (r) = –0.91, 38 df]. The mean concentration of DIN during the summer (June through August) was selected for the comparison in Table 9 to more closely approximate the period of most pronounced DIN depletion. The mean concentration of DIN at the surface in summer exceeds 1 μM at all Puget Sound sampling stations. The half-saturation constants of many coastal diatom species have been found to be in the range of less than 1 to about 1.5 μM for DIN uptake, while 1 μM is in the lower range of half-saturation constants for DIN uptake by many dinoflagellate species (MacIssacs and Dugdale 1969; Eppley et al. 1969; Carpenter and Guilliard 1971; Bowie et al. 1985).

RANKING OF AREAS BY POTENTIAL NUTRIENT SENSITIVITY

To provide a relative ranking of potential nutrient sensitivity, information on existing phytoplankton biomass, nutrient availability, or adverse algal bloom effects was needed for areas throughout Puget Sound. The best widely available indicator at present is the frequency of DIN depletion of surface waters from Ecology's Puget Sound database. Subsurface DO concentration is also a widely available measure, but areas with frequently low DO concentration also had



Source: SAIC (1986)

Figure 10. Percent frequency of observations with less than 5 mg/L dissolved oxygen at a depth of 10 meters

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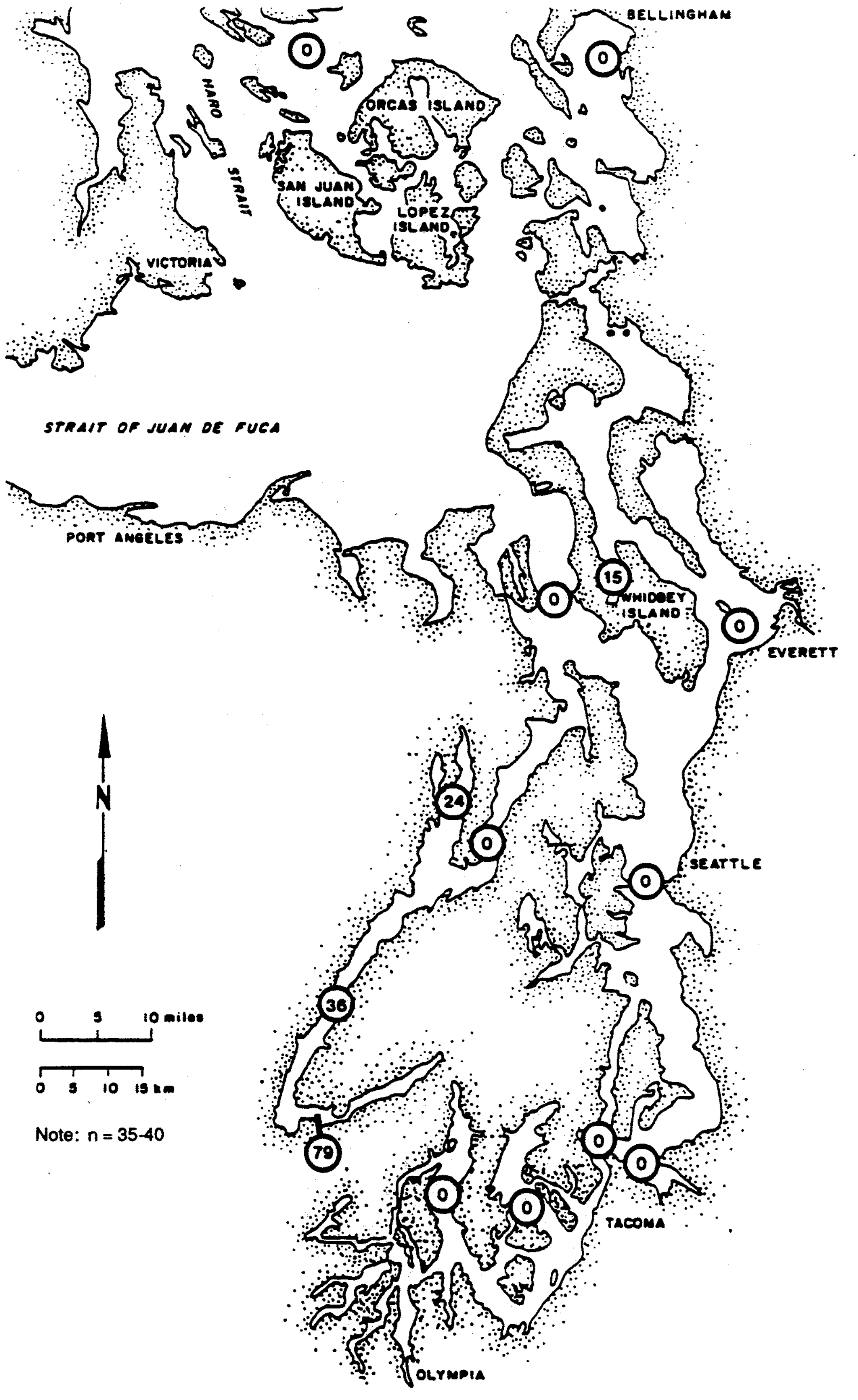

Source: SAIC (1986)

Figure 11. Percent frequency of observations with less than 5 mg/L dissolved oxygen at a depth of 30 meters.

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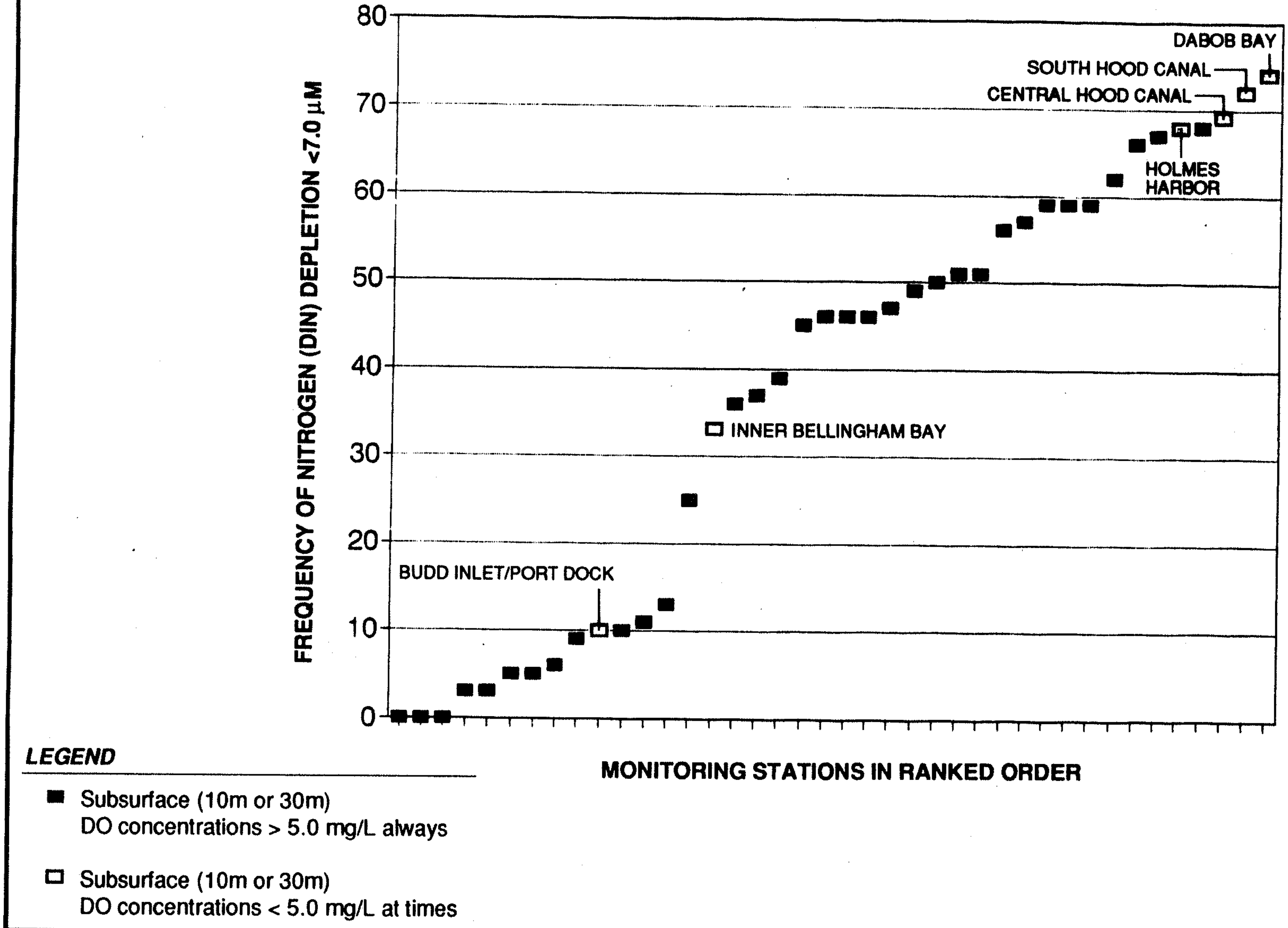

Figure 12. Percent frequency distribution of nitrogen (DIN) depletion less than 7.0 µM (0.1 mg/L-N) for surface waters at Washington Department of Ecology routine monitoring stations in Puget Sound.

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TABLE 9. COMPARISON OF DISSOLVED INORGANIC NITROGEN DEPLETION FREQUENCY VS. MEAN DISSOLVED INORGANIC NITROGEN CONCENTRATION FOR SURFACE WATERS AT ECOLOGY MONITORING STATIONS, 1981-85

Area	Percent Frequency of DIN <7 μM (April-November)	Mean DIN Concentration (μM) (June-August)
✓ Dabob Bay[a]	74	1.01
✓ Southern Hood Canal[a]	72	1.43
✓ Central Hood Canal[a]	69	1.91
Central Budd Inlet[a]	68	2.73
Holmes Harbor[a]	68	1.22
Liberty Bay[a]	67	2.86
Port Orchard at Brownsville[a]	66	3.32
✓ Totten Inlet[a]	62	2.46
✓ Case Inlet[a]	59	3.66
✓ Carr Inlet[a]	59	3.48
✓ Eld Inlet[a]	59	3.03
Penn Cove and Crescent Harbor[a]	57	4.16
Sinclair Inlet[a]	56	4.29
Port Susan[b]	51	2.40
Saratoga Passage[b]	51	4.23
Hammersley Inlet[b]	50	4.16
Bellingham Bay at Post Point	49	3.03
Sequim Bay[c]	47	3.82
Possession Sound	46	5.51
Port Gardner	46	6.09
Drayton Harbor	46	6.18
✓ North Hood Canal	45	5.00
✓ Pickering Passage	39	5.17
Bellingham Bay-Point Francis	37	6.09
Dyes Inlet[c]	36	6.07
Inner Bellingham Bay	33	6.39
Dana Passage	25	6.43
Port Townsend Harbor	13	10.76
Commencement Bay	11	12.06
Inner Commencement Bay	10	14.20
Budd Inlet at Port Dock[c]	10	14.96
West Point	9	11.68
Admiralty Inlet	6	15.49

TABLE 9. (Continued)

Area	Percent Frequency of DIN <7 μM (April-November)	Mean DIN Concentration (μM) (June-August)
Skagit Bay	5	16.31
Port Angeles Harbor	5	19.21
San Juan Channel	3	19.16
Haro Strait	3	15.55
Nisqually Reach	0	12.06
Tacoma Narrows	0	16.94
Elliott Bay	0	27.44

[a] Characterized as potentially nutrient-sensitive only because of DIN depletion; mean concentration = 2.7 μM, SD = 1.02.

[b] Characterized as potentially nutrient-sensitive because of DIN depletion and other reasons discussed in the text.

[c] Characterized as potentially nutrient-sensitive for reasons other than DIN depletion discussed in the text.

Mean DIN concentration of all potentially nutrient-sensitive areas: 3.7 μM, SD = 2.84.
Mean DIN concentration of all nonnutrient-sensitive areas: 11.5 μM, SD = 6.15.
Grand mean DIN concentration for all areas: 7.8 μM, SD = 6.23.

frequent DIN depletion (Figure 12 and Table 8, with two exceptions explained below). Accordingly, the frequency of DIN depletion was the primary measure used to categorize areas with respect to their potential nutrient sensitivity in this report.

Other types of information were used when DIN depletion or DO concentration data were limited or unavailable, and for areas with DIN depletion considered to be borderline (see below). Possible improvements to this system or the use of other methods are discussed after the ranking. Within categories, areas are listed in alphabetical order. Areas of Puget Sound are based on McLellan (1954); it should be noted that there are no well-defined biological or nutrient status boundaries between areas. This is reflected in Figure 9, which indicates the frequency of DIN depletion at specific sampling stations within areas, but does not show specific boundaries.

Potentially Nutrient-Sensitive Areas

The distribution of DIN depletion frequency during the April to November phytoplankton growing season was plotted (Figure 12) and interpretation suggests three groupings of data ranging from low to high. Areas with frequencies of DIN depletion greater than 55 percent were combined to form a potentially nutrient-sensitive category. Because the separation between the medium and high groupings was relatively small, other indicators [e.g., the frequency of low DO occurrence, probable physical transport based on bathymetry, and frequency of PSP toxin in shellfish from algal blooms (e.g., McCallum 1991)] were used to assess the potential nutrient sensitivity of areas near the 55-percent breakpoint, as explained below.

Nutrient loading to the following areas is of chief concern (additional literature citations and comments are listed for areas without adequate data):

Budd Inlet
Carr Inlet, including Burley Lagoon
Case Inlet
Dabob Bay
Dyes Inlet
Eld Inlet
Hammersley Inlet near Oakland Bay
Henderson Inlet (Rensel 1976; Pease 1977; Cardwell et al. 1979; Rensel and Prentice 1979)
Holmes Harbor
Hood Canal, central and southern areas including Lynch Cove
Liberty Bay
Penn Cove and Crescent Harbor
Port Orchard at Brownsville
Port Susan
Quartermaster Harbor (inner portions) (Nishitani et al. 1988)
Saratoga Passage (particularly the northern end)
Sequim Bay
Sinclair Inlet
Totten Inlet.

Within the potentially nutrient-sensitive category, three of the four areas of Hood Canal had low DO concentrations and the highest frequency of DIN depletion. Other potentially nutrient-sensitive areas were distributed throughout the various regions. Inner Budd Inlet, although only showing a 10-percent frequency of DIN depletion, was included in this group. The low DIN depletion frequency in that area is related to the discharge from a sewage treatment plant.

Sequim Bay (47-percent DIN depletion frequency) was included because of the repeated occurrence of PSP in the bay. Hammersley Inlet near Oakland Bay (50-percent DIN depletion frequency) was included because of its proximity to stratified, poorly flushed areas of Oakland Bay. Saratoga Passage and Port Susan (each 51-percent DIN depletion frequency) were included in the potentially nutrient-sensitive group because of weak currents and the occurrence of low DO concentrations for the former, and shallow bathymetry of the latter. The sampling location in Port Susan is probably not representative of the shallow inner areas. Dyes Inlet (36-percent DIN depletion frequency) was included in the potentially nutrient-sensitive category because of weak currents, shallow bathymetry, and isolation from the main channels of central Puget Sound.

Additional nutrient inputs to the potentially nutrient-sensitive areas need to be thoroughly evaluated because of:

- The potential to exacerbate harmful or other types of phytoplankton or macroalgal growth that may cause harmful events
- The potential to load the bottom sediments and water column with oxygen-demanding plant matter following blooms, such that fish kills may occur
- The need to preserve the aesthetic qualities of the water, such as transparency and lack of odor.

Reduction or management of existing anthropogenic sources of nutrients may be desirable in some of these areas, but without site-specific studies, the data reviewed herein are not sufficient to allow this determination.

Areas Unlikely to be Nutrient-Sensitive

This category includes all areas with frequencies of DIN depletion less than 55 percent, with the exception of a few areas just discussed. Based on the data from the historical sampling stations and the current state of knowledge, these areas are unlikely to be nutrient-sensitive in the near future. Also included are main basin areas known to be replete with nitrogen at most times. Shallow or isolated portions of some of the following areas may be potentially nutrient-sensitive (e.g., shallow waters of Seahurst Bight), but generally there are inadequate data to define such subareas at this time.

Based solely on nutrient data, the areas unlikely to be nutrient-sensitive include the following (additional literature citations and comments are listed for areas without adequate data):

Bellingham Bay
Commencement Bay (except inner waterways)
Drayton Harbor (entrance only)
Elliott Bay
Nisqually Reach to Dana Passage at Brisco Point
Pickering Passage
Port Angeles Harbor
Port Gardner
Port Townsend Harbor
Possession Sound
Rich Passage and Clam Bay (Lincoln and Collias 1975)
San Juan Channel
Skagit Bay (except Similk Bay) (Stober and Salo 1973; Collias et al. 1973)
Tacoma Narrows (except Gig Harbor).

Based on physical transport data and proximity to monitoring stations with low frequencies of DIN depletion, the following are also included:

Colvos Passage
East Passage
Gordon Point to Nisqually Reach (excluding Oro Bay)
Main channel of central Puget Sound
Strait of Juan de Fuca.

Routine Ecology monitoring is not conducted in several of these areas, but special studies (e.g., Winter et al. 1975; Collias and Lincoln 1977; Chester et al. 1980; Anderson et al. 1984; and others cited in this report) have demonstrated the infrequency of nitrogen depletion in these areas.

Inner Bellingham Bay (33-percent DIN depletion frequency) was included in the category considered unlikely to be nutrient-sensitive, even though low DO concentrations were observed in 5 percent of the data points. These low concentrations were apparently not related to algal problems. Instead, the low DO concentrations in that area appeared to be related to the influx of low DO seawater during August and September, accompanied by very high nitrate concentrations. Possession Sound (46-percent DIN depletion frequency) is probably a transition zone, with alternating periods of nutrient sensitivity and insensitivity, but is included in this category because of its marginally lower DIN depletion frequency.

Areas with Limited Nutrient Data

Nutrient data for the following areas are limited, but tentative categorization is possible based on the literature and rationale presented below for each area. Some of these areas are likely to be transition zones, which were previously discussed. DIN concentrations may fluctuate dramatically, depending on tidal stage and amplitude, wind and weather conditions, and specific sampling location.

- **Bellingham Channel**—Not nutrient-sensitive, based on proximity to the Strait of Juan de Fuca, unpublished 1990 data of Postel and Rensel, and unpublished 1988 and 1989 data of Rensel.

- **Discovery Bay, northern end**—A transition zone between the Strait of Juan de Fuca and the remainder of Discovery Bay; not likely sensitive to small inputs of nitrogen, based on hydrographic studies, nutrient and phytoplankton monitoring, and numerical modeling conducted for this area (Rensel 1987, 1988; Ebbesmeyer et al. 1989; Kiefer and Atkinson 1989).
- **Discovery Bay, central and southern end**—Likely to be nutrient-sensitive based on shallow bathymetry and previous nutrient studies (Rensel 1987, 1988).
- **Peale Passage**—Probably not nutrient-sensitive, given the condition of source waters at either end of the passage and limited hydrographic/phytoplankton studies (Rensel 1990). Long-term data are not available.
- **Port Madison**—Probably not nutrient-sensitive, given its proximity to the main basin and strong tidal flow and mixing at Agate Passage.
- **Quilcene Bay**—Probably nutrient-sensitive, at least in the inner areas, given its proximity to Dabob Bay and central Hood Canal.
- **Samish, Padilla, and Fidalgo bays**—Probably nutrient-sensitive, given their shallow depths and weak currents. Some information is available in theses and unpublished reports from the University of Washington and Western Washington University.

Areas with Nutrient Status Unknown

Nutrient or phytoplankton data for the following areas are not available to assess their potential nutrient sensitivity (additional literature citations and comments are listed for areas with other types of available data):

West of Dana Passage from Brisco Point to Pickering Passage [some physical transport data available from URS (1986b) and LOTT (1989)]
Gig Harbor
Hale Passage at Fox Island (probably a boundary zone between Tacoma Narrows and Carr Inlet)
Kilisut Harbor
Port Gamble Bay (some unpublished hydrographic data available from Port Gamble Klallam Tribe)
Port Ludlow [physical data available from Ebbesmeyer (1986)]
Port Townsend Bay, southern end
Oro Bay, Anderson Island
Small or shallow bays of San Juan and adjacent islands (some information available from theses, unpublished reports, and other literature).

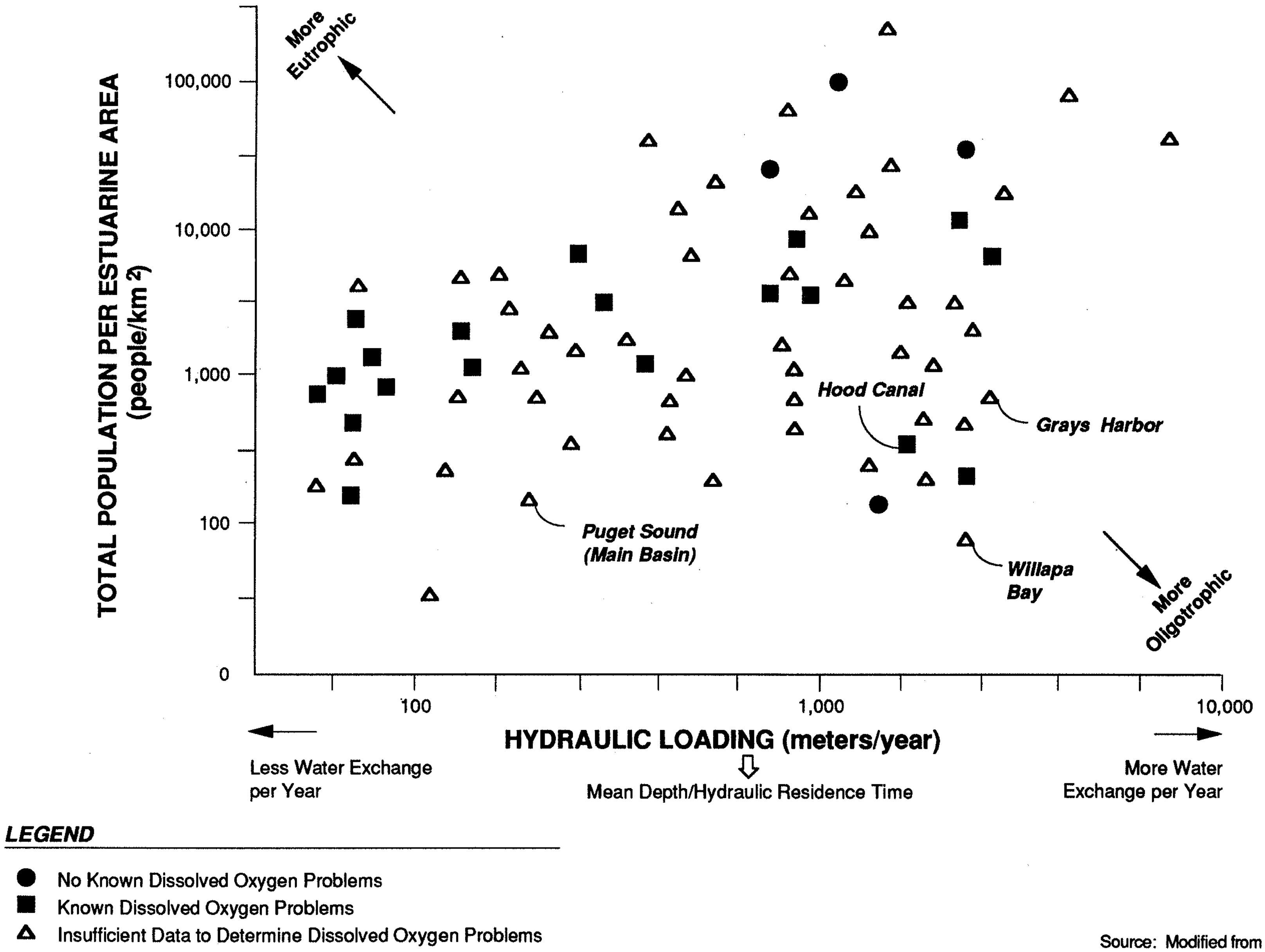
ALTERNATIVE RANKING METHOD

A different method of estimating potential nutrient sensitivity in specific areas of Puget Sound has been used in the past. The method estimated the flux of nitrogen through areas and used an exponential decay/tidal prism model (SAIC 1986; see below, *Management of Nutrients in Puget Sound, Nutrient and Phytoplankton Models, Tidal Prism Model)*. This method was not employed here because it does not account for the flux of nitrogen-rich deep water through an area, only the flux of nitrogen through the surface layers (Duxbury, A.C., 15 December 1987, personal communication). Accordingly, the method underestimates the true flux of nitrogen in most areas with deep profiles. Moreover, the area divisions presently available (McLellan 1954) are too large and reflux/efflux estimates are not known in most cases. Should these problems be resolved, the method could provide a useful measure of potential nutrient sensitivity (see *Knowledge Gaps and Recommendations, Flushing Estimates*). Other methods or indices could be constructed, in part using data from future Ecology databases, if a consistent measure of primary productivity were available throughout Puget Sound. Existing data may give a good approximation of the standing stock of phytoplankton (i.e., from chlorophyll *a* measures), but because of grazing by zooplankton, mixing, and advective processes, standing stock is a poor indicator of phytoplankton production and the algal stock's degree of dependency on nutrients.

COMPARISON OF PUGET SOUND WITH OTHER ESTUARIES

A recent nationwide survey of estuarine susceptibility to nutrient and toxic pollution used physical, hydrologic, and population data available in the National Oceanic and Atmospheric Administration's (NOAA) National Estuarine Inventory and the United States census database (Biggs et al. 1989). Surrogate indicators of anthropogenic nutrient and toxic pollutant discharge rates were used, such as total population size and subpopulation of agricultural and chemical/metal workers. Surrogate measures were used because there are no nationwide, systematic data that include actual nutrient concentration or nutrient supply data. These surrogate measures were compared with estimated flushing rates, degree of stratification, and other variables to classify pollution sensitivity.

Puget Sound (referring to the main channels of the central and northern basins) was judged to be among the least susceptible to change from anthropogenic nutrient input and pollution from heavy industries (Figure 13). However, a significant increase in nutrient loading, as a result of population growth, could shift the status toward eutrophy (Figure 13). The survey identified estuaries with low DO problems, but did not use this information in estimating their relative susceptibility to pollution. For example, Hood Canal was categorized among the least susceptible estuaries for general population, heavy industry, and agricultural effects because of low human populations relative to estuarine surface area, moderate hydraulic loading (defined as flushing in this case), and other factors. The results of the present study conflict with this categorization of Hood Canal as being among the least susceptible estuaries. This demonstrates the need to examine DO concentrations as an important indicator of potential nutrient sensitivity. It also indicates that flushing rate estimates used in the nationwide survey may be inappropriate or incorrect for Hood Canal.



Source: Modified from Biggs et al. (1989)

Figure 13. Surrogate point source nutrient loading diagram for major United States estuaries.

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MANAGEMENT OF NUTRIENTS IN PUGET SOUND

Management strategies to prevent, control, and remediate nutrient-related problems in Puget Sound have been developed by EPA, Ecology, the Puget Sound Water Quality Authority (PSWQA), Metro, and other agencies (e.g., U.S. EPA 1987, 1988a; PSWQA 1989, 1990). However, most current management strategies do not address nutrient discharges directly. Selected management tools relevant to nutrient-related problems are discussed in this section. These management tools include regulatory policies and programs based on eutrophication concerns, selected models for evaluating nutrient loading from wastewater discharges, and monitoring programs.

REGULATORY POLICIES AND PROGRAMS

Regulatory policies and programs for preventing and controlling pollutant discharges to Puget Sound generally do not provide direct guidance or limits on nutrient discharges. Point sources of nutrients from human activities are indirectly regulated by Ecology through permit limits on surrogate factors (e.g., biochemical oxygen demand) under the National Pollutant Discharge Elimination System (NPDES). Historically, wastewater discharges from pulp and paper mills released large amounts of sulfides, organic acids, sugars, and lignins into some inner bays of Puget Sound, causing major fish kills and other water quality problems associated with DO depletion (Strickland 1983). These problems have largely been corrected through improved treatment of industrial wastes by the imposition of limits on biochemical oxygen demand and suspended solids loadings in NPDES permits. The requirement for advanced wastewater treatment with nutrient removal at the LOTT sewage treatment plant represents the most significant regulatory action that directly addresses concerns about nutrient waste discharges to Puget Sound (PSWQA 1990). The regulation of nutrient discharges from nonpoint sources is addressed indirectly through a variety of programs involving local conservation districts, county and state health departments, land use regulations, the state Dairy Waste Management Plan, early action watershed plans, and other elements of the 1991 Puget Sound Water Quality Management Plan (PSWQA 1990). The lack of a sound-wide policy or strategy for managing nutrient releases to Puget Sound is reflected by the inclusion of a section on nutrient effects in the unfinished agenda of the 1991 Puget Sound Water Quality Management Plan (PSWQA 1990).

Discharges of organic or inorganic matter that cause, or tend to cause, water pollution are unlawful based on RCW 90.48.080. Siting and discharge limits are generally set on a case-by-case basis. Plume modeling is typically used to locate outfalls so that contact with shoreline areas and other sensitive areas (e.g., shellfish beds) is minimized. At present, Ecology does not have an official outfall location policy with regard to nutrient discharges. It is probable that Ecology would not have the legal authority to enforce an outfall location policy for water quality protection as long as water quality standards were not violated and the State Environmental Policy Act (i.e., environmental impact statement) process was followed (Smith, D., 30 May

1990, personal communication). However, because of the sensitivity of Hood Canal to nutrient inputs, Ecology has an official policy (Ecology 1987) that severely restricts wastewater treatment plant discharges to that water body.

Although discharges of pollutants are required by law not to exceed levels that would violate water quality standards, specific standards for nutrient limitation have been difficult to set because of the complexity of determining nutrient concentrations that would result in adverse effects. Determining specific discharge limits for nitrogen and phosphorus requires site-specific modeling, which has only been conducted in a few areas in Washington State (e.g., Budd Inlet and the Spokane River). Developing nutrient limits in an estuarine system is more complex than in a freshwater system and requires a much higher level of effort.

NUTRIENT AND PHYTOPLANKTON MODELS

Determining regulatory criteria for locations and amounts of nutrient discharged into Puget Sound requires an understanding of specific receiving water dynamics and the ability to predict the effects of nutrient addition. Predictions can be made for a selected area by using mathematical models that simulate nutrient and phytoplankton dynamics in nature coupled with field data to support calibration and verification of the model. Models have been used to simulate water quality conditions in various areas of Puget Sound, including Budd Inlet (URS 1986a) and the general south Puget Sound area (URS 1986b; Kiefer and Atkinson 1989). The following sections summarize selected models (i.e., tidal prism model and Budd Inlet model) that have been developed for management of wastewater discharges to Puget Sound. Although a comprehensive review of other models that could be applied to management of nutrients and phytoplankton in the sound was beyond the scope of this project, other models and criteria for model selection are discussed briefly in the final two sections below.

Tidal Prism Model

The approach of the tidal prism model is to simulate the residence time of water in an embayment using a simple exponential decay equation and a reflux value as follows:

$$t = \frac{\ln (Vt/Vl) \times (Vt/Vp) \times 0.52}{(1 - R)}$$

where:

t = Residence time (days)

Vt = Total volume of embayment at mean high water

Vl = Volume of original water remaining

Vp = Volume difference in the embayment between mean high water and mean low water

R = Decimal percent of water refluxed back into the embayment

0.52 = Number of days per tidal cycle.

Assuming conservative constituents and total mixing within a nonstratified embayment, residence times may be coupled with the rate of constituent input to predict constituent concentrations over time. The tidal prism model has been used to simulate the effect of salmon net-pen cultures on nutrient flux through subareas of Puget Sound (SAIC 1986). Dr. Alyn Duxbury suggested in 1983 (Duxbury, A.C., 15 December 1987, personal communication) that Ecology use the tidal prism approach as a method for estimating flushing rates in nonstratified estuarine systems. Flushing rates estimated by the University of Washington (1971), Duxbury et al. (1972), and URS (1986b) using this model were compared with flushing rates estimated by the University of Washington (1971) and Duxbury et al. (1972) using net seaward transports calculated from actual runoff and salinity measurements. This latter approach, based on a water and salt budget, assumes that because the volumes of embayments do not change, the net seaward movement must be equal to the runoff plus precipitation. Thus, the net seaward movement of less saline water and the net landward movement of deeper marine water can be calculated using measurements of fresh and salt water entering, within, and outside the embayment of study. The flushing rate estimated for Budd Inlet using the tidal prism model (1.4 days) was lower than that estimated using the water and salt balance approach (2.4 days). This difference may be attributed to the fact that the tidal prism estimate was calculated using a reflux value of 0, resulting in the fastest possible flushing by tidal action. When refluxing is taken into account using the water and salt balance approach, the flushing rate is slower.

The following is a summary of the strengths and weaknesses associated with using the tidal prism model as a regulatory tool:

Strengths:

- The simplicity of the model makes it quick and easy to set up and use. Using a spreadsheet or database management system to generate and store results makes it accessible to anyone with basic computer skills.
- The model is completely general and may be applied to any embayment assuming reflux and volume values are known and assuming that a nonstratified system holds.

Weaknesses:

- Because the basic model does not incorporate any terms for chemical or biological interaction, it lacks realism in estimating water quality variables for nonconservative components, limiting its applicability as a regulatory tool (Singleton, L., personal communication). Modification of the model to include simulation of biological and chemical dynamics could greatly increase its usefulness for evaluating potential nutrient sensitivity of Puget Sound areas. Attempts to incorporate nutrient flux into the model (SAIC 1986) have, however, been criticized (Duxbury, A.C., 15 December 1987, personal communication; see *Nutrient Sensitivity Ranking, Alternative Ranking Method*).
- Reflux rates are not well known for most areas of Puget Sound, which means that some fieldwork would be needed before the model could be calibrated for a given area.

- Estimating the volume of the embayments to be modeled is not a trivial task, adding to the difficulty of applying the model (see McLellan 1954).

Budd Inlet Model

In 1984 and 1985, URS (1986a) performed field studies to calibrate a dynamic circulation and water quality model for Budd Inlet in Puget Sound. The model used by URS (1986a), hereinafter referred to as the Budd Inlet model, is a modification of a laterally averaged, two-dimensional, finite difference model developed by J.E. Edinger Associates. Modification of the basic Edinger hydrodynamic and transport model for use in Budd Inlet studies involved addition of a water quality module that incorporates biochemical processes.

The Budd Inlet model was calibrated using primary productivity and water quality data from studies conducted by URS in Budd Inlet in September 1984 and May 1985. Three main variables, the Michaelis-Menten constant, the maximum phytoplankton growth rate, and the carbon-to-chlorophyll *a* ratio, were manipulated to calibrate the phytoplankton component of the model. Because site-specific values for these variables would have required elaborate field and laboratory studies beyond the scope of the project, they were determined by estimating the maximum growth rate (from literature sources) and varying the other two factors until the output plots coincided with the observed field estimate of ^{14}C growth rate from one day and station during the May bloom (providing that all factors fit in the expected ranges of values from the literature). Estimates of the light-saturated growth rates used for diatoms in the Budd Inlet model appear to be appropriate (e.g., Eppley 1972), but the growth rates used for dinoflagellates as stated in the URS report (1986a) are too high (2.5-3.0 day^{-1}, when approximately 0.2-0.8 day^{-1} would be more appropriate). However, according to Boatman (3 March 1990, personal communication), and the values shown in the URS report (1986a) are incorrect and the value actually used in the model for the dinoflagellate growth rate was 0.5 day^{-1}, which is within the range of literature values for dinoflagellate growth rates. The available literature is not site- or dominant-species-specific (e.g., Bowie et al. 1985) and covers at least an order of magnitude range for both diatoms and dinoflagellates. These uncertainties are addressed later in *Knowledge Gaps and Recommendations*.

Calibration of the phytoplankton component of the model was not completed for the autumn dinoflagellate bloom period. Because the autumn period of low DO caused by dinoflagellate blooms has yet to be quantitatively modeled, the response of these species to sewage discharges is unknown, but is assumed to be similar to the diatom bloom period (Boatman 1988). As acknowledged by URS (1986a), there is a need to improve the model to simulate periods of dinoflagellate blooms. In addition, even though the model could be modified to include nutrients other than nitrogen, in its present configuration it assumes that nitrogen is the only growth-limiting nutrient. This assumption is generally appropriate for diatoms, but may not be applicable to certain blooms of dinoflagellates and microflagellates or for situations where different nutrients limit different dominant species that occur together in Puget Sound (see *Nutrient Limitation: Micronutrients and Species Variation*). Modeling results from the URS (1986a) study showed "a 30-50 percent increase in the strength of the algal bloom relative to the no-discharge scenario for the inner portions of the inlet." Advanced wastewater treatment for removal of nutrients was chosen as a cost-effective alternative to placing the outfall outside of Budd Inlet.

The following is a summary of the strengths and weaknesses associated with using the Budd Inlet model as a regulatory tool:

Strengths:

- Comparison of model-simulated data with measured data from Budd Inlet suggests good accuracy for modeling the spring diatom bloom event
- The model contains components for simulation of biochemical interactions as well as physical transport and mixing for a multilayer system
- The model has demonstrated the ability to simulate the impact of differing outfall siting scenarios within an enclosed embayment
- Subsequent application of the model to other areas of Puget Sound (e.g., Everett Harbor) has provided realistic results (Boatman, C.D., 3 March 1990, personal communication).

Weaknesses:

- Calibration of the model for a given area and period of time requires collection of quality field data from that same area and period of time. This activity accounts for a significant portion of the total cost of setting up the model. In addition, the costs associated with running, calibrating, and interpreting the results may be as large a part of the total project cost as gathering the calibration data. Project cost estimates including the gathering of field data and calibrating and running the model range from $20,000 to $250,000 per study.
- Because of the complexity of the model, a high level of expertise is required to operate the model and interpret its results.
- The proprietary status of the URS computer source code for the model limits its availability and the opportunity to critique its structure and implementation. A computer source code for other implementations of the same basic model is available, however, and the model computations are well described.

The Budd Inlet model has been applied to other areas of Puget Sound with minimal changes. However, calibration and verification data must still be gathered from each area to which the model is applied. In addition, the calibration and verification process must still be carried out for each area and time of year. Once the model has been calibrated and verified for a selected area, simulation of new discharge scenarios for that area would require minimal effort.

Other Models and Approaches

Among the activities that have caused concern regarding their potential effects on phytoplankton populations in Puget Sound is the raising of fish in net-pens. The net-pens are placed in natural waters where dense fish stocks within the pens may contribute locally significant levels of ammonium produced as metabolic wastes. In a study to assess the effect of the proposed construction of a salmon net-pen near Dana Passage in south Puget Sound, Kiefer and Atkinson (1989) used two different models to simulate nearfield and farfield water quality variables. The nearfield model was adapted from an earlier version (Atkinson et al. 1984; Koh et al. 1984) and simulates the cycling of nitrogen through a model system consisting of an aquatic nitrogen pool, a phytoplankton crop, and a zooplankton crop. The nearfield model also includes a component that accounts for the effect of reduced light availability due to suspended sediment on phytoplankton productivity and standing stock.

The farfield model used by Kiefer and Atkinson (1989) is based on the efflux/reflux theory of Cokelet and Stewart (1985) and estimates the effect of nutrient input from the proposed net-pen on nutrient and phytoplankton in the various reaches and inlets of south Puget Sound. This same approach was used by URS (1986b) to estimate flushing in Dana Passage. In this approach, south Puget Sound is divided into a series of reaches, sills, and inlets that receive fresh water via riverine input and saltwater via exchange with the main basin through the Tacoma Narrows. The net transport in the region has been characterized by Cokelet et al. (1988) as a two-layer flow with low salinity water flowing seaward above a higher salinity landward flow. As these layers mix in shallow sill areas, part of each layer is refluxed back as part of the other layer into the reach or inlet from which it came. By measuring the riverine input, currents, and salinity, and by estimating reflux values, net transport and residence times for water quality variables may be calculated. This approach assumes that the water quality variables (e.g., nutrient concentrations) are conservative. The approach is not suitable for modeling specific outfall locations and effects in a given location, but is valuable in considering subarea or areawide effects of nutrient discharge.

Another model that has been used to simulate water quality in lakes, streams, and estuaries is the Water Quality Analysis Program 4 (WASP4). The original WASP model was developed by Dominic Di Toro, James Fitzpatrick, and Robert Thomann of Hydroscience, Inc. (Di Toro et al. 1981). The WASP4 version of the computer model was developed as part of the mandate for the EPA Center for Exposure Assessment Modeling in Athens, Georgia, to develop, maintain, and distribute water quality models (U.S. EPA 1988b). WASP4 is a dynamic compartment modeling system of variable complexity for simulating the fate, transport, and interaction of water and water quality variables in the water column and benthos of lakes, streams, and estuaries. The model can be applied in one, two, or three dimensions by supplying information on transport between model compartments. The basic WASP4 system may be augmented to simulate complex conditions by addition of existing modules. One such module is EUTRO4, which simulates interactions between nutrients, phytoplankton, carbonaceous material, and DO. Some or all of these interactions can be simulated for a given scenario at the user's discretion. The basic WASP4 model and accompanying modules, documentation, and source code (FORTRAN) can be obtained from EPA in Athens for a nominal fee. WASP4 has been proposed for use in assessing sediment contaminant impact and recovery in Puget Sound, and simulations have been run for Commencement Bay, Tacoma's City Waterway, and Bellingham Bay using WASP4 for this purpose (Chiou et al. 1991).

Model Selection

There are many options for modeling processes in Puget Sound that are potentially useful as regulatory tools. The main points to consider when choosing one or more of these options are summarized as follows:

- Ability of the model to realistically simulate the processes of concern for selected study areas
- Availability of the model and its source code, and associated costs in applying it
- Applicability of the model to many types of areas
- Amount and quality of input data required by the model, and how these data are to be acquired
- Usefulness of model results in making regulatory decisions
- Ease of model use for regulators.

Any complex numerical model requires more effort and is more costly to use than much simpler models. However, the simpler models are not as realistic and certainly cannot simulate all of the processes that the more complex numerical models can. A balance must exist between ease (cost) of model use and data requirements and simulation realism. This balance may best be obtained by either selecting a single model with the appropriate qualities, selecting a model that can be modified or operated at different levels of complexity, or selecting a set of models that produce information that can be combined to achieve the regulatory goal.

MONITORING PROGRAMS

Periodic monitoring of macronutrient concentrations (dissolved forms of nitrogen and phosphorus and some total phosphorus measurements) has been conducted for many years in Puget Sound. Monitoring of phytoplankton stocks in Puget Sound has been conducted for 1- to 2-year periods or less in the past, but there are no long-term systematic records of phytoplankton species composition or abundance. This is unfortunate because in sheltered bays, inlets, and passages, the types and numbers of dominant species of phytoplankton may exert a controlling influence on water quality conditions during the phytoplankton growing season.

Past nutrient and phytoplankton monitoring programs were discussed in previous sections of this report. After a brief summary of methods that can be used to collect phytoplankton data, this section reviews the existing nutrient monitoring protocols and Ecology's marine ambient monitoring program.

Methods of Monitoring Phytoplankton

Short-term studies of phytoplankton populations in portions of Puget Sound have been conducted (see *Overview of Phytoplankton and Nutrient Dynamics, Types of Phytoplankton)*. However, most monitoring and field studies have relied on other, more easily obtainable indicator data (e.g., surrogate indicators such as Secchi disk transparency or measures of DO saturation). Chlorophyll *a* data provide information about the gross abundance of phytoplankton but no information about species assemblages or rates of production. Typically there are inadequate records of chlorophyll *a* to allow determination of temporal trends (Tetra Tech 1988). Surrogate measures mentioned above are inadequate substitutes for chlorophyll *a* measurements or individual cell counts in some applications. In addition, surrogate indicators are often affected by other factors, confounding efforts to interpret data. For example, the solubility of oxygen in seawater is an inverse function of both temperature and salinity, but several biological and abiotic factors may contribute to departures from surface water equilibrium with the atmosphere. Emerson (1987) found the seasonal warming of the surface (mixed) layer to be the primary cause of the regular 4- to 6-percent supersaturation of oxygen in north Pacific Ocean waters. Phytoplankton production of oxygen minus plankton respiration was less important, and the effect of bubble injection by breaking waves was judged unimportant during the summer.

In the laboratory, phytoplankton abundance in some types of monospecific cultures may be quantified by automated electronic particle counters (flow cytometry and Coulter counters) or by other means. Because of varying cell shapes and sizes, these methods are unsuitable for use with natural assemblages of phytoplankton. In some research studies, primary productivity of phytoplankton is assessed through the radioactive carbon (^{14}C) assimilation method (Steemann-Nielsen 1952; Vollenweider 1974). Because of the complexity of sampling and analysis, and lack of agreement on fine points of interpretation (Petersen 1980), these methods are often not suitable for routine monitoring studies. Remote sensing of phytoplankton from airplanes and satellites holds promise for monitoring of phytoplankton standing stock in surface waters, but does not yield data on production rates, species composition, or subsurface populations.

The distribution of phytoplankton in Puget Sound and elsewhere is inherently patchy. For example, Anderson et al. (1984) found variation in neighboring stations in central Puget Sound on any specific day to average 15 percent for nutrient concentrations, 30 percent for measures of plankton biomass (chlorophyll *a*), and 40 percent for species abundance (measured by cell counts). The patchiness of phytoplankton may be related to differences in cross-channel hydrographic conditions (Barnes and Ebbesmeyer 1978), advective input from oceanic sources (Naiman and Sibert 1977), advective transport seaward in the surface layer, weather conditions, and many other factors. Margalef (1978) provides estimates of phytoplankton sampling effort required for various space and time scales to obtain reasonably accurate results.

Munson (1969) surveyed daily chlorophyll *a* distributions throughout central Puget Sound in May and found large patches of phytoplankton. Rates of patch movement and daily variability in patch size were studied by water sampling and concurrent visual observation from aircraft. The distribution and movement of patches were grossly related to surface currents and water stability (i.e., the effect of tides, river runoff, bathymetry, and weather), but the analysis did not attempt to sort out the relative effect of each influence.

Review of Existing Nutrient Monitoring Protocols

Monthly sampling of Puget Sound water quality variables is conducted by Ecology at numerous stations throughout the main channel areas and certain embayments. There are approximately 55 stations in marine waters of western Washington, with about 47 in Puget Sound and adjacent waters (see Appendix A). Since 1967, sampling has been conducted by floatplane from April to November. In 1988, sampling was expanded to include all months of the year. Recommendations made by the Monitoring Management Committee, organized by PSWQA (1988), were considered in the present review of the procedures and protocols used by Ecology.

During the preparation of this report, Ecology proposed major revisions to the existing plan along with specifically defining its goals for monitoring (Ecology 1990b). Some of the proposed changes have been implemented as of early 1991, although changes in station location, monitoring methods, and analyses have gradually taken place over the entire history of the program (Janzen, C., 23 April 1991, personal communication). To some extent, recommendations included in earlier drafts of the present report have been addressed in Ecology's proposed monitoring plan, and will be noted where appropriate below in the section *Ecology's Ambient Monitoring Program*. In addition, EPA has proposed a set of water quality monitoring protocols for Puget Sound (U.S. EPA 1990) that are important to the present discussion.

Ecology's Puget Sound database includes several variables of interest relative to nutrient-phytoplankton relationships: DIN (ammonium, nitrate, and nitrite), orthophosphate, total phosphorus, Secchi disk transparency, DO concentration, DO saturation, salinity, water temperature, and at some stations, chlorophyll *a*. From 1973 to the present, samples have been collected at the surface, at a depth of 10 meters, and, when adequate depth is available, at a depth of 30 meters. Prior to 1973, samples were collected at the surface only or at the surface and at a depth of 20 feet.

Measures of total nitrogen from the water column (sum of dissolved and particulate, organic and inorganic forms) are not available from the Ecology database. Measures of total nitrogen from combustion analysis are of use in the present context if collected concurrently with measures of total phosphorus. Measures of total Kjeldahl nitrogen are discussed by PSWQA (1988). This measure represents the sum of organic nitrogen and inorganic ammonia (APHA 1985). Total Kjeldahl nitrogen is not recommended for nutrient monitoring, as discussed later in this report.

Ecology's database includes "total" orthophosphate (unfiltered water samples, collected since 1975) and "dissolved" orthophosphate (filtered water samples, collected until about 1986 and again since October 1990). Total phosphorus measurements are also taken from unfiltered water samples and include all forms of phosphorus, both organic (dissolved and particulate) and inorganic. The use of total phosphorus or other nonconservative compounds as tracers of sewage discharges is limited. Ideally, to be an effective tracer, a substance should be inert and conservative (i.e., not subject to biological modification or sedimentation). Total phosphorus includes orthophosphate, a very labile and fast-cycling nutrient through sedimentation and food chain/plankton pathways. Phosphates are also likely to be associated with settleable solids and particulates, biasing the distribution of these compounds after discharge. However, Ketchum

(1967) suggests a method that uses total phosphorus and salinity as cotracers that may have merit under some circumstances. Total phosphorus has been suggested as a tracer for the LOTT outfall plume in Olympia Harbor (Bernhardt and Yake 1983).

As previously discussed in this report, total phosphorus and total nitrogen have been monitored in coastal areas of the world that have experienced harmful phytoplankton problems. Measurement of these variables during the winter, when algal production is minimal, could serve as a rough indicator of the total pool of each macronutrient that could eventually be incorporated into algal biomass if advective inputs are neglected. In order to investigate whether this could be a valid approach for areas of Puget Sound, available data from year-round sampling (August 1989-July 1990) of total phosphorus and orthophosphate at the surface at selected stations were plotted in Figure 14. The stations were selected to represent areas with a wide range of riverine influences and hydrographic conditions. The stations are listed below with the appropriate Ecology station code numbers and brief descriptions of their surface waters:

- **Admiralty Inlet near Point No Point (ADM003)**—Minimally influenced by rivers, with seasonally high phytoplankton production
- **Tacoma Narrows (NRR001)**—Minimally influenced by rivers, with intense vertical mixing and probably low phytoplankton production
- **Central Hood Canal at Eldon (HCB003)**—Fjord area significantly affected by rivers in the winter and by a pronounced thermocline in the summer
- **Commencement Bay (CMB003)**—The outer area of a large, central Puget Sound bay strongly affected by a riverine plume at times during the fall through spring period
- **Oakland Bay near Eagle Point (OAK004)**—A small, south Puget Sound bay affected by several relatively large creeks during the winter or during other periods of maximum runoff
- **Eld Inlet near Flapjack Point (ELD001)**—Poorly flushed, seasonally stratified bay in south Puget Sound with very small freshwater inputs.

From these plots, it is apparent that the fall through winter months (October through February) may be suitable sampling periods for detecting maximum values of total phosphorus for all areas. It is also apparent that selection of a single winter month or even several months for evaluating the maximum total phosphorus concentration may not yield consistent results among areas. In particular, Eld Inlet and Oakland Bay had peak concentrations of total phosphorus in the fall. Commencement Bay and Admiralty Inlet had equally high concentrations in the fall as in the winter. The high total phosphorus concentrations in September or October are probably a result of intrusion and upwelling of deep seawater that is known to occur in the late summer or fall (Collias et al. 1974; Duxbury 1975), although the salinity plots in Figure 14 do not indicate a strong correlation of salinity with total phosphorus concentrations in the fall. Loss of seasonal stratification and vertical mixing may also contribute to the high total phosphorus concentrations in fall. There was less-than-normal to much-less-than-normal precipitation during September and October 1989 in western Washington (SeaTac airport weather data, NOAA 1990), indicating that the high total phosphorus concentrations in fall had little to

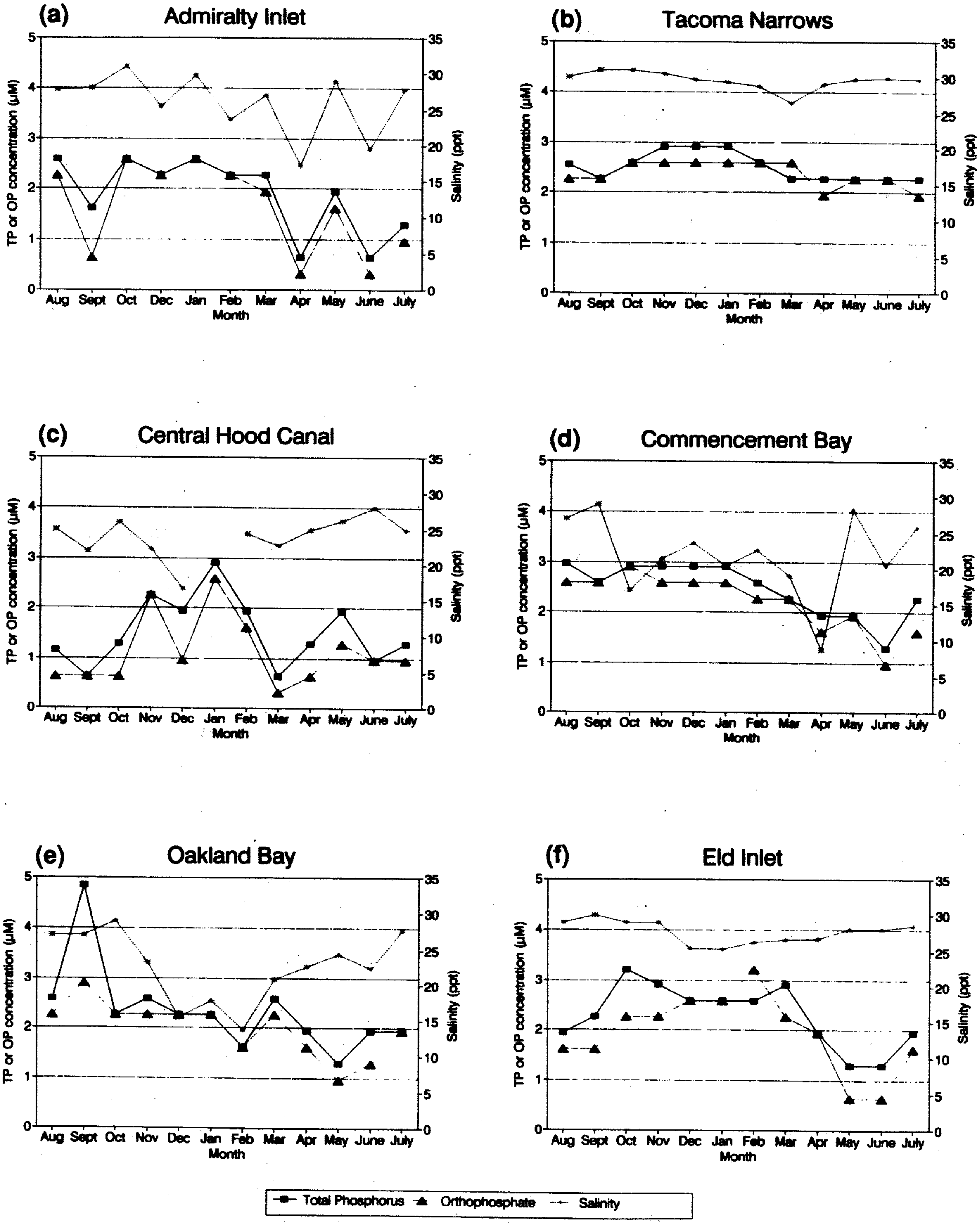

Figure 14. Total phosphorus, orthophosphate, and salinity of surface waters for selected Puget Sound locations, August 1989 to July 1990.

do with riverine inputs. It is unlikely that the high total phosphorus concentrations in the fall were the result of high phytoplankton biomass, because orthophosphate concentrations were generally high at that time too, suggesting low phytoplankton production.

If year-round data had been available for other years, averaging the data over several years may have reduced short-term variations, potentially making annual patterns more apparent [compare with Figure 4A of Bernhardt and Yake (1983), which included monthly averages for the period April-November over several years]. An October maximum of total phosphorus concentration was also reported for the three south Puget Sound inlets that they studied (i.e., Eld, Totten, and Budd inlets), but winter data were not collected.

If the total phosphorus concentration during the nonalgal growing season is to be used as one indicator of the nutrient status of an area, sampling should be conducted during the months of September to February, at least until definite annual patterns for each area are established. Moreover, the relatively close correspondence between orthophosphate and total phosphorus concentrations at most times (Figure 14) suggests that the orthophosphate concentration may be a surrogate indicator of the total phosphorus concentration during nonalgal bloom periods.

Because nitrogen pools (especially DIN) are generally more important in the control of production by marine algae, and because no extensive total nitrogen data are available from Puget Sound, no conclusions regarding its use are presented here, other than to note that it is likely a better indicator of trophic status than is total phosphorus because of the importance of nitrogen to marine algal growth.

Units used by Ecology for reporting nutrients are mg/L-N or mg/L-P. The Monitoring Management Committee of PSWQA (1988) recommends that nutrient concentrations be reported as either μg/L (mg/L divided by 1,000) or microgram atoms per liter (μg-at/L), although they note that the latter is more common for marine waters. Conversion from mg/L nitrogen to μg-at/L can be accomplished by multiplying by 1,000 (to account for the difference between micrograms and milligrams) and dividing by 14 (the molecular weight of nitrogen). Biological and chemical oceanographic nutrient research data have typically been reported as μg-at/L, also commonly referred to as micromolar (μM). Many oceanographers now report nutrient concentrations in units of micromoles per kilogram of solution (Murray, J., 29 May 1991, personal communication). However, conversion of historically collected data reported on a volumetric basis to micromoles per kilogram of solution is not generally possible because the water density is not known or has not been calculated. Federal and state standards often include nutrient concentrations in units of mg/L-N or mg/L-P, and this practice is preferred by many users of the Ecology database (Ecology 1990a).

The preservation and storage of nutrient samples has been a controversial subject. The Monitoring Management Committee recommends samples be placed in slush ice (if preserved) or filtered and frozen (PSWQA 1988). The committee recommended analysis of preserved samples within 1 week and frozen samples within 1 month. Dissolved nutrients (nitrate, nitrite, ammonium, phosphate, and sometimes silicate) from routine monitoring samples are often analyzed concurrently using shore-based autoanalyzer systems. This procedure is potentially problematic because although nitrate, phosphate, and silicate concentrations in seawater are not significantly affected by storage in freezers for up to several months (Kremling and Wenck

1986), ammonium should be analyzed as soon as possible because of the rapid losses or gains that may occur (Parsons et al. 1984a). U.S. EPA (1990) recommended that samples for ammonium analysis be analyzed within 1 week. In special studies and on oceanographic vessels, dissolved nutrients are often analyzed immediately after sample collection, avoiding the problem of cellular or detrital nitrogen being released as dissolved nitrogen during freezing or preserving (Harris 1986). Because this type of analysis is impractical for routine sampling in Puget Sound that is conducted by float plane, filtering followed by analysis or storage is now conducted as soon as possible after sample collection, but is not delayed more than 24 hours (Ecology 1990b).

Freezing of nutrient samples may have advantages over acid preservation, which was used in the past by Ecology. Acid preservation may significantly alter nitrite concentrations (APHA 1985), although nitrite concentrations are normally very low. Freezing results in very slight decreases in nitrate and orthophosphate concentrations, possibly because of biological action during freezing or thawing. Use of slush ice to store samples immediately after collection and quick-freezing procedures (when analysis is to be delayed) will, in all likelihood, improve accuracy. Use of filtrate from water samples filtered during collection of chlorophyll *a* samples is a convenient means of obtaining both filtered nutrient water and chlorophyll *a* samples in one operation.

Unlikely values of nutrient concentrations have been found in Ecology's Puget Sound database on occasion. Some values appear to be order of magnitude errors, possibly related to data entry problems, and others appear to reflect problems with sample collection or analysis. If triplicate samples were collected and analyzed, outliers could be identified objectively. Triplicates also allow for statistical testing of differences among areas. A less desirable but still useful option is the collection of triplicate samples from a subset of stations on each day. This practice has recently been used by Ecology (Janzen, C., 2 February 1990, personal communication) and now is routinely performed at quality assessment stations (Janzen, C., 23 April 1991, personal communication). However, with this approach, outliers may still occur with the nonreplicated samples, leading to uncertainty or inaccuracy in the database. Ecology (1990a) believes that the routine collection of triplicate nutrient samples from the same water bottle is not cost-effective (see *Monitoring Programs, Sample Handling, Replication, and Analysis*). PSWQA (1988), APHA (1985), Parsons et al. (1984a), and Strickland and Parsons (1972) provide further information regarding these topics.

Presently, sampling and analysis of phytoplankton species composition is not included in Ecology's monitoring program. Identification and enumeration of phytoplankton requires a trained specialist with local experience. Nishitani et al. (1988) argued that monitoring of phytoplankton species composition at certain stations would be "far more sensitive to changes in nutrient conditions than would (chlorophyll *a*) pigment concentrations which are currently monitored to determine the total abundance of photosynthetic phytoplankton." Monitoring of phytoplankton species composition and abundance at selected existing stations in bays (e.g., Sequim Bay and Carr Inlet) and open water (e.g., Admiralty Inlet) where limited historical records of phytoplankton populations are available would allow trends analyses to be performed and increase understanding of the dynamics of harmful phytoplankton species.

KNOWLEDGE GAPS AND RECOMMENDATIONS

To understand and more effectively manage potentially nutrient-sensitive areas of Puget Sound, additional information is needed regarding the dynamics of local phytoplankton populations, particularly microflagellates and dinoflagellates. At present, management agencies have no means to judge whether harmful phytoplankton blooms, extremes of DO concentrations, and certain fish kills are within the range of normally expected events or are indicators of undesirable trends. Knowledge gaps and associated recommendations related to nutrients, phytoplankton in potentially nutrient-sensitive areas, fish and wildlife mortalities, and monitoring programs are presented below. Because the priority of actions recommended below may vary among specific regulatory and monitoring programs, recommendations are not assigned any priority ranking.

LIMITING NUTRIENTS

There is a need to determine which nutrient(s) are most likely to limit the growth of locally important, harmful phytoplankton species, particularly those species that may occur or dominate in bays, inlets, and passages potentially sensitive to additional supply of nutrients. Harmful species of importance in Puget Sound include *Alexandrium catenellum*, *Gymnodinium splendens*, *Ceratium fusus*, and *Heterosigma akashiwo*. Laboratory and field studies may be designed to aid in identification of physiological coefficients needed in simulation models. Verification of laboratory studies could involve nutrient-addition bioassays using water and phytoplankton collected from areas of suspected nutrient sensitivity. Assays for dinoflagellates could include various trace metals, vitamins, and other micronutrients in combination with various forms and amounts of nitrogen and phosphorus. Laboratory techniques involving phytoplankton culture are available to conduct such work. Once nutrient dynamics of dominant species are better defined, this information, in conjunction with knowledge of the distribution of dominant species in Puget Sound, will permit a more refined analysis of phytoplankton-nutrient relationships.

PHYTOPLANKTON SPECIES COMPOSITION AND DISTRIBUTION

In addition to determining the nutrient(s) likely to limit dominant or harmful phytoplankton species, special studies of potentially nutrient-sensitive areas in Puget Sound (e.g., Budd Inlet and Lynch Cove) should include determination of phytoplankton species composition and distribution. These data are needed to help understand the species-specific dynamics of nutrient use and limitation, as well as to provide a baseline for comparison with future water-quality conditions. Other industrialized countries throughout the world (e.g., Germany, Japan, the Netherlands, and Norway) have phytoplankton monitoring programs that have been useful in detecting phylogenetic shifts toward harmful species (Smayda 1989b). Special studies of potentially nutrient-sensitive areas of Puget Sound may also be needed to determine the diel vertical distribution of phytoplankton cells and the rates of nutrient cycling relative to primary production. Further information on sediment nutrient flux during critical bloom periods is also needed. In addition to studies in potentially nutrient-sensitive areas, Campbell et al. (1977) and Anderson et al. (1984) have recommended the initiation of a long-term, but limited-scope, plankton study in central Puget Sound. Long-term monitoring of phytoplankton species at a few stations in central Puget Sound over a period of many years would be more useful than short-term sampling with closely spaced stations for understanding trends and dynamics of Puget Sound phytoplankton.

Field studies are needed to clarify the relative contributions of dinoflagellate respiration and algal decay as the causes of low DO concentrations in southern Hood Canal, inner Budd Inlet, and northern Saratoga Passage. The physical and chemical characteristics of low DO events have, in some cases, been described (e.g., Collias et al. 1974), but their biological characteristics are not well known. Some literature (e.g., Collias and Loehr 1974) assumes that algal and detrital decay are the sole causes of low DO concentrations. This assumption must be questioned in light of more recent knowledge of dinoflagellate vertical migration and their significant respiration at depth (Smayda 1979; Raven and Beardall 1981). Some information is available about sediment oxygen demand from various areas of Puget Sound (Pamatmat 1971; URS 1986a), but field studies designed to assess the relative contributions of dinoflagellate respiration, algal decay, and sediment oxygen demand to DO depletion have not been conducted.

FISH AND WILDLIFE MORTALITIES

The frequency of wild fish kills in Puget Sound is unknown, but as nutrient input increases with human population growth, hypoxic conditions and fish kills in potentially nutrient-sensitive areas could increase. As previously discussed, a primary cause of fish kills in estuarine and marine waters of Washington State is suspected to be low DO concentrations, although there is presently little empirical evidence of this in Puget Sound. Investigations of fish kills should include the immediate collection and preservation of water samples for phytoplankton analysis. Separate collections using both 1-percent buffered formalin (final concentration) and Lugol's solution are required because of the different preservation techniques for different phytoplankton species potentially responsible for fish kills. Fish specimens for pathological and clinical analysis must be collected fresh, preferably even moribund. At a minimum, surface and near-bottom measurements of DO, temperature, dissolved nutrients, and associated variables should be made during estuarine fish kills, or as soon as possible afterward. Fisheries and wildlife agencies should be prepared to rapidly collect specific tissues from fish, birds, and marine mammals subject to mass mortalities to investigate the potential involvement of PSP-causing species such as *A. catenellum* that have contributed to such kills elsewhere. Existing Ecology or WDF fish kill/fish health databases could be improved by including hydrographic data, weather and tidal data, name(s) of investigator(s), and other details.

MONITORING PROGRAMS

Recommendations for modifications to monitoring programs for nutrients and phytoplankton in Puget Sound are provided in this section. The recommendations address Ecology's ambient monitoring program, monitoring of municipal wastewater treatment plant discharges, analysis of shellfish PSP-toxicity records, and estimation of flushing of potentially nutrient-sensitive areas.

Ecology's Ambient Monitoring Program

Suggested changes to the marine water quality sampling portion of Ecology's ambient monitoring program are summarized below. The topics include sample handling, replication, and analysis; total phosphorus and total nitrogen methods; chlorophyll *a* analysis; phytoplankton species assessments; and trends analysis.

Sample Handling, Replication, and Analysis—The present collection of single samples at each depth does not allow for statistical comparison among stations, even if replicates are collected at a few stations. However, it is acknowledged that the large number of stations presently sampled by Ecology and the generally good precision and accuracy of autoanalyzer analysis support the use of single sample analysis at each station and depth. Moreover, quality control efforts, including comparisons with historical data and evaluations of field blanks, duplicates, spikes, and reference standards, improve the system's reliability (Ecology 1990a). However, special studies in potentially nutrient-sensitive bays should include at least some triplicate sampling, depending on the experimental design. The protocol could include the analysis of nutrients in the filtrate from chlorophyll *a* samples to minimize sample collection effort. Use of quick chilling and freezing methods without preservatives is recommended for handling dissolved nutrient samples.

Total Phosphorus and Total Nitrogen—Total phosphorus data could be used, in conjunction with other measures, as a historical measure of water column nutrient content for poorly flushed areas of Puget Sound. If total nitrogen analysis were conducted concurrently with total phosphorus analysis, the water column pools of each nutrient could be characterized. Total nitrogen and phosphorus measures, along with the existing dissolved inorganic nutrient analyses, could provide a powerful database for detecting undesirable shifts toward eutrophication. Total nitrogen and phosphorus analyses are more expensive than DIN and orthophosphate analyses and thus may be restricted to particular areas, special studies, or limited periods of time. As previously discussed, collection of total nitrogen and phosphorus samples only during the late summer through winter months might be a useful option in order to obtain an index of the annual maximum total nitrogen and phosphorus content before any significant uptake by phytoplankton and macroalgae during the primary growing season (spring and summer).

Total nitrogen should be analyzed by using the persulfate oxidation method, rather than through the sum of the total Kjeldahl nitrogen method and nitrate plus nitrite. Total Kjeldahl nitrogen results analytically yield the organic nitrogen plus ammonia results (APHA 1985).

Kjeldahl digestions are tedious, time consuming, and subject to contamination, and thus can exhibit poor precision (Grasshoff 1983). Alternatively, the persulfate oxidation for total nitrogen determination is efficient, reliable, and precise, and samples may be prepared for both total nitrogen and total phosphorus determinations simultaneously (Valderrama 1981). However, the persulfate oxidation may result in underestimates of DON (Jackson 1988).

Chlorophyll *a* Analysis—The frequency of nitrogen depletion in surface water used to rank the relative nutrient sensitivity of Puget Sound areas in this report provides no information regarding the production rate or standing stock of phytoplankton. Although measurement of primary productivity is not practical in routine monitoring, standing stock can easily be approximated through chlorophyll *a* measurements, which have been conducted at only a few Ecology monitoring stations. Routine monitoring of chlorophyll *a* has been recommended by Duxbury (1975) and by the Monitoring Management Committee (PSWQA 1988). It should be used during at least the April to November period in areas of frequent DIN depletion and in at least a few main basin areas that could be used as reference stations. Collection, handling, and analysis of chlorophyll *a* is relatively easy and samples may be frozen for relatively long periods. Chlorophyll *a* may be monitored along with other variables throughout the water column to yield useful information regarding the vertical distribution of phytoplankton, which is variable depending on the season and area (e.g., subsurface maxima in Carr Inlet, Dabob Bay, and possibly central Hood Canal, Tetra Tech 1988). Chlorophyll *a* data, in conjunction with water temperature, salinity, and DO measurement, may also be used to assess the relative contribution of surface heating (which decreases the capacity of water to hold oxygen) vs. net photosynthesis from phytoplankton to the degree of DO supersaturation. The unknown contribution of each is a particularly perplexing question in areas experiencing persistent, seasonal DO supersaturation (possibly related to algal production) with unexpectedly high water transparency (indicative of low phytoplankton productivity; e.g., central Hood Canal, Tetra Tech 1988; compare with Emerson 1987).

Phytoplankton Species Composition—With the exception of studies in central Puget Sound (Anderson et al. 1984) and Dabob Bay (Copping 1982), many studies in Puget Sound have not included a description of the species composition or the physiological ecology of the dominant phytoplankton species (e.g., Kruger 1979; URS 1986a), choosing instead to concentrate on chemical and physical assessments. Routine phytoplankton species monitoring, as recommended by Anderson et al. (1984) and Nishitani et al. (1988), should be added to at least a few existing routine monitoring stations in Puget Sound to track annual and long-term species variation. Such analysis is potentially a more sensitive measure of the ecological status of a body of water than are chemical measures. Computerized databases for species information storage and analysis using personal computers are being developed at the University of Washington (Horner, R.A., 22 February 1990, personal communication). The usefulness of water quality measures presently collected could be amplified by concurrent determination of phytoplankton species composition. This effort should include collection and preservation of water samples from a few potentially nutrient-sensitive or PSP-prone areas and from a few main channel areas for determination of phytoplankton species composition and vertical distribution.

Monthly sampling of phytoplankton species composition is inadequate to fully characterize the phytoplankton dynamics of an area, but collection of samples by volunteers and marine-related schools or businesses as a supplement to the regular Ecology sampling schedule could improve the situation. Additional depths of 2 and 5 meters should be added to the existing 0-, 10-, and 30-meter samples for the few phytoplankton sampling stations.

Trends Analysis—Periodic trends analysis of all existing and potentially nutrient-sensitive areas of Puget Sound could be conducted using basic hydrographic data and information about dissolved nutrient depletion of surface waters, total phosphorus, phytoplankton biomass (chlorophyll *a*), DO saturation, dominant species composition, Secchi disk depth, and the nutrient loading from tributary rivers and municipal wastewater treatment plants. These types of analyses require consideration of variations caused by patterns of weather and hydrography, such as the decadal shifts discussed in this report. Because the vast majority of DIN and orthophosphate data from Puget Sound are available within the Ecology database, efforts should be made to review the historical database for obvious outliers and miscoded data. Routine monitoring in main channels should include intensified efforts around the time of the summer and winter solstices to detect annual minimum and maximum values of nutrient concentrations and diatom abundance (PSWQA 1988). Routine monitoring in potentially nutrient-sensitive areas should also focus on the period from July to late September when dinoflagellates are most abundant. Some trends analyses are presently planned by Ecology and PSWQA as part of the Puget Sound Ambient Monitoring Program. Trends analyses should also include collection of existing information on nutrient discharge and loading from rivers and streams into potentially nutrient-sensitive areas. Much of the information is already available, but needs to be collected and integrated.

Finally, there could be improvements in the recording and reporting of fish kill information that is presently shared by Ecology (pollution-caused fish kills) and WDF (fish kills by natural causes). Although there are rapid response processes and easy access for public reporting of fish kills, no single state agency is responsible for recording historical information about all fish kills in Puget Sound. Because the cause of some fish kills is unknown, the responsible agency and reporting/recording processes are unclear. It is recommended that one agency be selected to create a standardized computer format for recording vital statistics and known information for all reported fish kills.

Wastewater Discharge Monitoring

If nutrient-removal facilities are planned for municipal wastewater treatment plants in specific areas of Puget Sound, preoperational and postoperational studies should be conducted, as previously recommended by URS (1986a). Periodic analyses of water column conditions and benthic nutrient-cycling processes in the affected areas may be required for several years, because existing sediment may provide a long-term reservoir for nutrient supply.

Ecology's existing Wastewater Discharge Information System could be useful for examining temporal changes of nutrient discharge into potentially nutrient-sensitive areas. The database presently includes estimates of the volume of some industrial and municipal discharges during dry-weather and peak-flow periods. This information, along with estimates of the concentrations of various nutrients from each system, could provide an index of nutrient loading from point sources into potentially nutrient-sensitive areas. Alternatively, a relative measure of nutrient loading can be estimated from the size of the population served by each area's wastewater treatment plant, following the method of Biggs et al. (1989).

Shellfish Toxicity Trends

Although shellfish toxicity records in Washington State have been useful in estimating general PSP trends (Erickson and Nishitani 1985; Nishitani and Chew 1988), previously collected data have not been thoroughly analyzed. In part, this is due to past practices involving irregular spatial and temporal collection procedures (PSWQA 1988). Despite these problems, trends in the onset of annual PSP events could be determined through review of data collected by the Washington Department of Health. Over a period of many years, comparison of these data with routinely collected hydrographic data from nearby monitoring stations may yield insights regarding environmental cues for cyst germination. In general, the existing Washington Department of Health program of PSP monitoring has been very effective, and some recommended improvements (PSWQA 1988) are being developed (McCallum 1991). Resource agency staff, wildlife professionals, and conservationists concerned with unexplained mass mortalities of marine birds and mammals should be educated about methods for PSP sampling and agency requirements for scientific collection of animals.

Flushing Estimates and Modeling

As an alternative or adjunct to physical transport modeling of potentially nutrient-sensitive areas, flushing rates of restricted bays, inlets, and passages could be empirically determined through a standardized field technique involving drift objects (i.e., drogues and drift sticks). Placing a large number of drift objects in areas of interest during specified tidal and weather conditions would allow each area's flushing capability to be determined. This information is required to accurately conduct all types of computer simulation modeling, as previously discussed. As the tides remove the drift objects from the area, the number that remain decreases, typically along an exponential-type curve. This method has been used in many areas in recent years, including north Skagit Bay (Ebbesmeyer et al. 1988), Port Ludlow in the vicinity of Admiralty Inlet (Ebbesmeyer 1986), northern Discovery Bay (Ebbesmeyer et al. 1988), and in waterways of Commencement Bay (Loehr et al. 1981).

Flushing of material released at a particular site within a bay (e.g., at a proposed wastewater discharge site) should be estimated under specific conditions. For example, flushing should be measured with drift sticks or shallow drogues on a series of days when the wind speed is less than 5 knots and the tidal amplitude is near the mean for the area. Several of the drift objects should be released per hour during a 24-hour period. The number remaining in the embayment should be counted at intervals to determine when 90 percent have been removed by tidal action. The time interval required to remove 90 percent of the drift objects is the "flushing time" estimate.

This method has disadvantages, however, that could limit its usefulness. One disadvantage is the inability of this method to describe flushing accurately unless studies are conducted over a wider range of weather and tidal conditions. Another disadvantage involves the limited number of release site(s) for the drift objects that are typically used. In a large body of water, there may be uncertainty about flushing from areas where drift objects are not released, tracked, or recovered. Studies in small bays and inlets, however, are less likely to encounter this problem.

Finally, the simple tidal prism model for bays and inlets that uses site-specific transport estimates should be developed and corroborated with field data as a potentially valuable tool in future ranking of potential nutrient sensitivity in Puget Sound (as discussed in *Nutrient Sensitivity Ranking: Alternative Ranking Method*).

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APPENDIX A

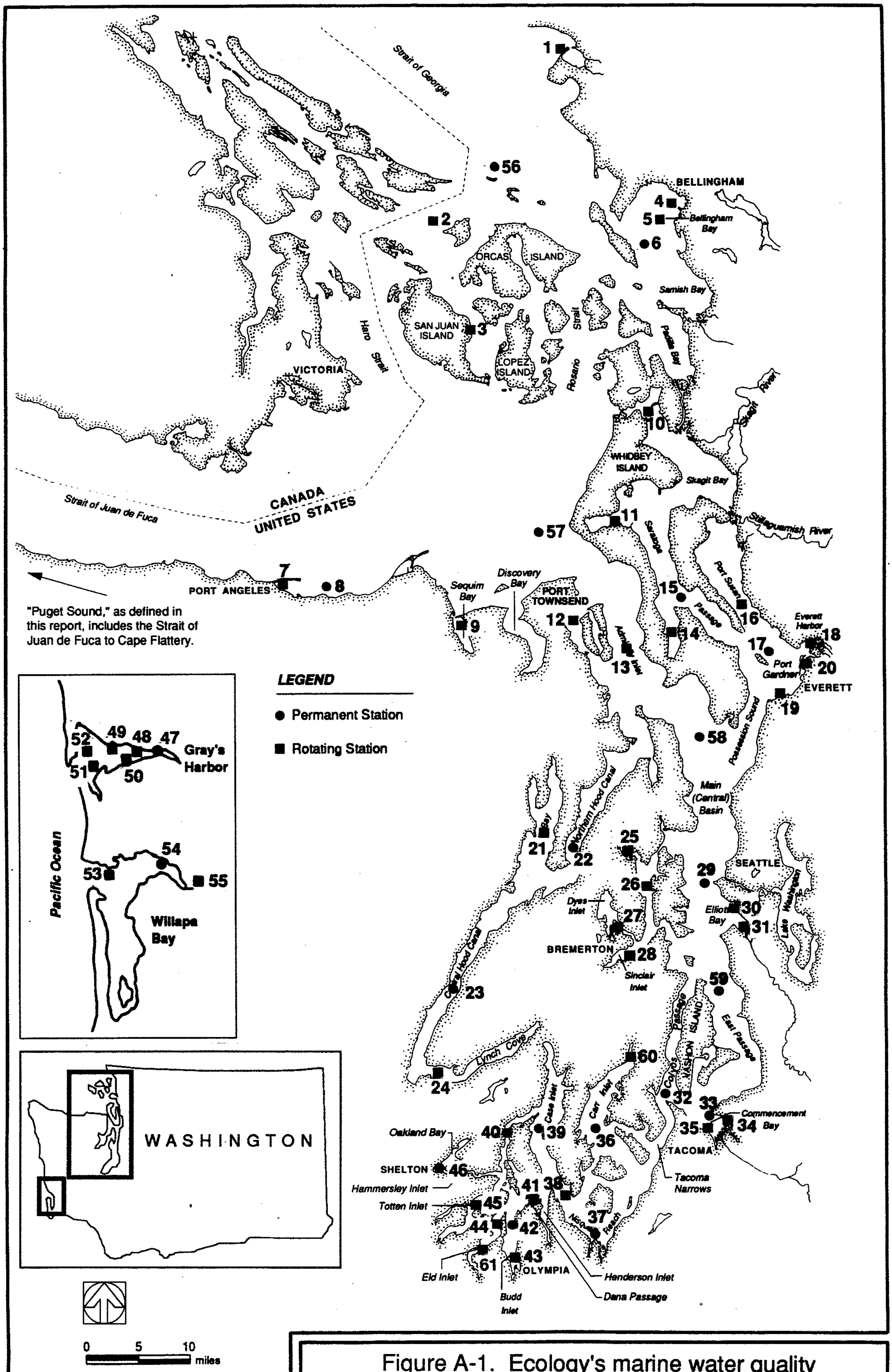
Washington Department of Ecology Puget Sound Water Quality Monitoring Stations

TABLE A-1. STATION NUMBERS, DEPTHS, AND MONITORING VARIABLES FOR ECOLOGY'S MARINE WATER QUALITY MONITORING STATIONS

Map No.	Station No.	Location	Depth (m)	D.O.	Temp	Cond	Sal ppt	pH	FC	Light %	Chl a	Nutrients
Marine Flight I												
37 P	NSQ 001	Nisqually Reach/Nisqually River	0,10	X	X	X	X	X	X	X	X	X
41 R	DNA 001	Dana Passage/Brisco Pt.	0,10,30	X	X	X	X	X	X	X	X	X
42 P	BUD 005	Budd Inlet/Oly Shoal	0,10	X	X	X	X	X	X	X	X	X
43 R	BUD 002	Budd Inlet/Near EBM	0,10	X	X	X	X	X	X	X	X	X
44 R	ELD 001	Eld Inlet/Flapjack Pt.	0,10	X	X	X	X	X	X	X	X	X
45 R	TOT 001	Totten Inlet/Windy Pt.	0,10	X	X	X	X	X	X	X	X	X
46 P	OAK 004	Oakland Bay/Shelton	0,10	X	X	X	X	X	X	X	X	X
47 P	GYS 004	Grays Harbor/Oil Dock	0,10	X	X	X	X	X	X	X	X	X
49 R	GYS 009	Grays Harbor/Moon Is.	0,10	X	X	X	X	X	X	X	X	X
50 R	GYS 008	Grays Harbor/S. Channel	0,10	X	X	X	X	X	X	X	X	X
53 R	WPA 004	Willapa Bay/Toke Pt.	0,10	X	X	X	X	X	X	X	X	X
54 P	WPA 003	Willapa River/J. Slough	0,10	X	X	X	X	X	X	X	X	X
55 R	WPA 001	Willapa River/Raymond	0,10	X	X	X	X	X	X	X	X	X
Marine Flight II												
6 P	BLL 009	Bellingham Bay/Pt. Frances	0,10	X	X	X	X	X	X	X	X	X
8 P	PAH 008	Port Angeles/Morse Creek	0,10	X	X	X	X	X	X	X	X	X
15 P	SAR 003	Saratoga Passage/East Pt.	0,10,30	X	X	X	X	X	X	X	X	X
17 P	PSS 019	Possession Sound	0,10,30	X	X	X	X	X	X	X	X	X
22 P	HCB 006	Hood Canal/King Spit	0,10,30	X	X	X	X	X	X	X	X	X
23 P	HCB 003	Hood Canal/Eldon	0,10,30	X	X	X	X	X	X	X	X	X
56 P	GRG 002	Georgia Strait/Patos Is.	0,10,30	X	X	X	X	X	X	X	X	X
57 P	ADM 002	Adm.Inlet/Port Townsend	0,10,30	X	X	X	X	X	X	X	X	X
Marine Flight III												
29 P	PSB 003	Puget Sound/West Pt.	0,10,30	X	X	X	X	X	X	X	X	X
32 P	NRR 001	Tacoma Narrows/Pt. Defiance	0,10,30	X	X	X	X	X	X	X	X	X
33 P	CMB 003	Commencement Bay	0,10,30	X	X	X	X	X	X	X	X	X
36 P	CRR 001	Carr Inlet/Green Pt.	0,10,30	X	X	X	X	X	X	X	X	X
39 P	CSE 001	Case Inlet/S. Heron Is.	0,10,30	X	X	X	X	X	X	X	X	X
58 P	ADM 003	Adm. Inlet/Possess Pt.	0,10,30	X	X	X	X	X	X	X	X	X
59 P	EAP 001	East Passage/Maury Is.	0,10,30	X	X	X	X	X	X	X	X	X
60 R	BML 001	Henderson Bay	0,10	X	X	X	X	X	X	X	X	X
61 R	ELD 002	Eld Inlet/Flapjack Pt.	Surface	X	X	X	X	X	X	X	X	X
Historical and Rotating Stations												
1 R	DRA 001	Drayton Harbor/Entrance	0,10	X	X	X	X	X	X	X	X	X
2 R	HRO 001	Hard Strait/Skipjack Is.	0,10,30	X	X	X	X	X	X	X	X	X
3 R	SJI 001	San Juan Channel/Reid Rk.	0,10	X	X	X	X	X	X	X	X	X
4 R	BLL 006	Bellingham Bay/Nun Buoy	0,10	X	X	X	X	X	X	X	X	X
5 R	BLL 008	Bellingham Bay/Post Pt.	0,10,30	X	X	X	X	X	X	X	X	X
7 R	PAH 003	Port Angeles/Edis Hook	0,10	X	X	X	X	X	X	X	X	X
9 R	JDF 005	Strait of JDF/Sequim Bay	0,10	X	X	X	X	X	X	X	X	X
10 R	SKG 001	Skagit Bay/Hope Is.	0,10	X	X	X	X	X	X	X	X	X
11 R	PNN 001	Penn Cove/Penn Cove Park	0,10	X	X	X	X	X	X	X	X	X
12 R	PTH 005	Port Townsend Harbor	0,10	X	X	X	X	X	X	X	X	X
13 R	ADM 001	Adm. Inlet/S. Whidbey Is.	0,10,30	X	X	X	X	X	X	X	X	X
14 R	HLM 001	Holmes Harbor	0,10,30	X	X	X	X	X	X	X	X	X
16 R	SUZ 001	Port Susan/Kayak Pt.	0,10	X	X	X	X	X	X	X	X	X
18 R	PSS 020	Ebey Slough/Marysville	Surface	X	X	X	X	X	X	X	X	X
19 R	PSS 008	Port Gardner/Pier 3	0,10	X	X	X	X	X	X	X	X	X
20 R	PSS 015	Snohomish River/Hwy 9	Surface	X	X	X	X	X	X	X	X	X
21 R	HCB 002	Hood Canal/Pulali Pt.	0,10,30	X	X	X	X	X	X	X	X	X
24 R	HCB 004	Hood Canal/Sisters Pt.	0,10	X	X	X	X	X	X	X	X	X
25 R	POD 006	Liberty Bay/Virginia Pt.	0,10	X	X	X	X	X	X	X	X	X
26 R	POD 005	Port Orchard/Brownsville	0,10	X	X	X	X	X	X	X	X	X
27 R	DYE 003	Dyes Inlet/Wash. Narrows	0,10	X	X	X	X	X	X	X	X	X
28 R	SIN 001	Sinclair Inlet/Shipyards	0,10	X	X	X	X	X	X	X	X	X
30 R	ELB 005	Elliott Bay/Harbor Is.	0,10	X	X	X	X	X	X	X	X	X
31 R	ELB 010	Duwamish Waterway/16th	0,10	X	X	X	X	X	X	X	X	X
34 R	CMB 010	Commencement Bay/Puyallup River	0,10	X	X	X	X	X	X	X	X	X
35 R	CMB 006	Commencement Bay/City WW	0,10	X	X	X	X	X	X	X	X	X
38 R	NSQ 002	Nisqually Reach/Devils Head	0,10,30	X	X	X	X	X	X	X	X	X
40 R	PCK 001	Pickering Passage	0,10	X	X	X	X	X	X	X	X	X
48 R	GYS 007	Grays Harbor/Rayonier	0,10	X	X	X	X	X	X	X	X	X
51 R	GYS 015	Grays Harbor/Whitcomb Fl.	0,10	X	X	X	X	X	X	X	X	X
52 R	GYS 016	Grays Harbor/Damon Pt.	0,10	X	X	X	X	X	X	X	X	X

R - rotating station

P - permanent station



"Puget Sound," as defined in this report, includes the Strait of Juan de Fuca to Cape Flattery.

Figure A-1. Ecology's marine water quality monitoring stations.

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APPENDIX B

Numerical and Percent Occurrence of Observations Within Groups of Nitrate-to-Phosphate Ratios for Individual Stations in Puget Sound

TABLE B-1. NUMERICAL OCCURRENCE OF OBSERVATIONS WITHIN GROUPS OF NITRATE-TO-PHOSPHATE RATIOS FROM SURFACE AND 10-METER DEPTHS

Underlined stations used in the Tetra Tech (1988) trends analysis. Station codes from the Washington Department of Ecology, except three stations from the Washington Department of Fisheries.
Nitrate = N; Orthophosphate = P.

	Bellingham Bay	<- @ Cannery	<- Post Pt.	P. Angeles H.	Port Gardner	Sinclair Inlet	Case Inlet	Carr Inlet
Station -->	BLL006	BLL007	BLL008	PAH003	PSS008	SIN001	CSE001	CRR001
			----------		----------	----------		----------
N & P both = 0	17	6	25	0	6	8	7	8
N=0 and p >0	21	2	14	1	2	22	17	24
other N:P < 5	18	3	17	13	4	32	31	9
subtotal	56	11	56	14	12	62	55	41
N:P 5 - 20	61	11	78	129	63	68	55	83
N:P > 20	22	2	19	37	9	19	10	8
Total	139	24	153	180	84	149	120	132

	Dabob Bay	C. Hood Canal	S. Hood Canal	Padilla Bay	Padilla Bay	Dyes Inlet	Budd Inlet	<-- same
Station -->	HCB002	HCB003	HCB004	PAD001	PAD002	DYE003	BUD003	BUD004
	----------	----------	----------				----------	
N & P both = 0	16	18	27	2	2	1	1	1
N=0 and p >0	37	44	40	1	0	6	9	2
other N:P < 5	14	20	18	0	2	45	15	8
subtotal	67	82	85	3	4	52	25	11
N:P 5to20	43	50	58	16	21	74	12	0
N:P >20	8	4	4	13	17	22	8	0
Total	118	136	147	32	42	148	45	11

	Central Budd In.	Totten Inlet	Oakland Bay	Oakland Bay	Oakland Bay	Eld Inlet	Tacoma Narrows	Port Gardner
Station -->	BUD005	TOT001	OAK001	OAK002	OAK004	ELD001	NRR001	PSS005
	----------	----------	----------	----------	----------	----------	----------	
N & P both = 0	7	3	0	0	3	0	0	2
N=0 and p >0	42	44	3	1	26	46	0	2
other N:P < 5	49	52	4	4	42	41	1	6
subtotal	98	99	7	5	71	87	1	10
N:P 5to20	52	32	6	5	27	35	116	13
N:P >20	10	2	1	6	3	4	10	21
Total	160	133	14	16	101	126	127	44

	Commencement Bay	<-- Inner	Oakland Bay	Oakland Bay	Oakland Bay	Nisqually Reach	Devils Head
Station -->	CMB003	CMB006	WDF21	WDF22	WDF23	NSQ001	NSQ002
		----------			----------	----------	----------
N & P both = 0	0	1	0	0	0	0	0
N=0 and p >0	1	1	1	0	0	2	0
other N:P < 5	11	10	6	6	3	8	3
subtotal	12	12	7	6	3	10	3
N:P 5to20	106	130	6	12	5	112	9
N:P >20	10	30	11	15	5	7	0
Total	128	172	24	33	13	129	12

TABLE B-2. PERCENT OCCURRENCE OF OBSERVATIONS WITHIN GROUPS OF NITRATE-TO-PHOSPHATE RATIOS FROM SURFACE AND 10-METER DEPTHS

Underlined stations used in the Tetra Tech (1988) trends analysis. Station codes from the Washington Department of Ecology, except three stations from the Washington Department of Fisheries.
Nitrate = N; Orthophosphate = P.

	Bellingham Bay	<- @ Cannery	<- Post Pt.	P. Angeles H.	Port Gardner	Sinclair Inlet	Case Inlet	Carr Inlet
Station -->	BLL006	BLL007	BLL008	PAH003	PSS008	SIN001	CSE001	CRR001
N & P both = 0	12%	25%	16%	0%	7%	5%	6%	6%
N=0 and p >0	15%	8%	9%	1%	2%	15%	14%	18%
other N:P < 5	13%	13%	11%	7%	5%	21%	26%	7%
subtotal	40%	46%	37%	8%	14%	42%	46%	31%
N:P 5 - 20	44%	46%	51%	72%	75%	46%	46%	63%
N:P > 20	16%	8%	12%	21%	11%	13%	8%	6%

	Dabob Bay	C. Hood Canal	S. Hood Canal	Padilla Bay	Padilla Bay	Dyes Inlet	Budd Inlet	<-- same
Station -->	HCB002	HCB003	HCB004	PAD001	PAD002	DYE003	BUD003	BUD004
N & P both = 0	14%	13%	18%	6%	5%	1%	2%	9%
N=0 and p >0	31%	32%	27%	3%	0%	4%	20%	18%
other N:P < 5	12%	15%	12%	0%	5%	30%	33%	73%
subtotal	57%	60%	58%	9%	10%	35%	56%	100%
N:P 5to20	36%	37%	39%	50%	50%	50%	27%	0%
N:P >20	7%	3%	3%	41%	40%	15%	18%	0%

	Central Budd In.	Totten Inlet	Oakland Bay	Oakland Bay	Oakland Bay	Eld Inlet	Tacoma Narrows	Port Gardner
Station -->	BUD005	TOT001	OAK001	OAK002	OAK004	ELD001	NRR001	PSS005
N & P both = 0	4%	2%	0%	0%	3%	0%	0%	5%
N=0 and p >0	26%	33%	21%	6%	26%	37%	0%	5%
other N:P < 5	31%	39%	29%	25%	42%	33%	1%	14%
subtotal	61%	74%	50%	31%	70%	69%	1%	23%
N:P 5to20	33%	24%	43%	31%	27%	28%	91%	30%
N:P >20	6%	2%	7%	38%	3%	3%	8%	48%

	Commencement Bay	<-- Inner	Oakland Bay	Oakland Bay	Oakland Bay	Nisqually Reach	Devils Head
Station -->	CMB003	CMB006	WDF21	WDF22	WDF23	NSQ001	NSQ002
N & P both = 0	0%	1%	0%	0%	0%	0%	0%
N=0 and p >0	1%	1%	4%	0%	0%	2%	0%
other N:P < 5	9%	6%	25%	18%	23%	6%	25%
subtotal	9%	7%	29%	18%	23%	8%	25%
N:P 5to20	83%	76%	25%	36%	38%	87%	75%
N:P >20	8%	17%	46%	45%	38%	5%	0%

APPENDIX C

Comparison of Surface and Subsurface Water Quality Data Used in the Nitrate-to-Phosphate Ratio Analysis

COMPARISON OF SURFACE AND SUBSURFACE WATER QUALITY DATA USED IN THE NITRATE-TO-PHOSPHATE RATIO ANALYSIS

This appendix includes water quality data for surface (0 meters), for subsurface (10 meters), and for the two depths combined that were pooled in the section on nitrate-to-phosphate ratios in Puget Sound (see Table 4). No further statistical analysis was performed, because statistical differences in the pooled data were present, in part a function of the very large number of observations used. Further, more rigorous statistical evaluation was also not performed because of inherent limitations of the data and analysis (e.g., correlative nature of the analysis, relatively high detection limits for nitrogen and phosphorus, and seasonal limits of the data).

The data in Table C-1 show essentially the same trends for all variables regardless of depth.

TABLE C-1. COMPARISON OF 0-METER, 10-METER, AND POOLED WATER QUALITY CONDITIONS FOR NITRATE-TO-PHOSPHATE GROUPS[a]

Group and Ratio Description	Depth	Water Temperature (°C)	Month (1-12)	DO[b] (mg/L)	DO Saturation (%)	PO_4^{3-} (μM)	NO_3^- (μM)	Salinity (ppt)	$NO_3^-:PO_4^{3-}$ Ratio
Group 1	Surface	14.7	6.5	10.2	116.5	0.3	0.7	23.3	NA[c]
$NO_3^-:PO_4^{3-}$ = 0	10 meters	10	6.5	10.4	115.7	0.3	0.7	26.8	NA
	Pooled data	14.3	6.5	10.2	115.9	0.3	0.7	24.0	NA
Group 2	Surface	14.5	7.2	9.8	115.0	1.3	2.3	26.3	1.7
$NO_3^-:PO_4^{3-}$ < 5	10 meters	13.3	7.2	9.1	105.2	1.5	3.5	28.2	2.2
	Pooled data	14.0	7.2	9.5	111.1	1.3	2.7	27.0	1.8
Group 3	Surface	11.5	7.5	8.9	97.4	1.6	16.1	26.6	10.6
$NO_3^-:PO_4^{3-}$ = 5-20	10 meters	11.0	7.4	8.3	91.2	1.7	16.9	28.7	10.5
	Pooled data	11.2	7.5	8.5	93.8	1.6	16.6	27.8	10.5
Group 4	Surface	11.5	7.0	8.9	96.9	0.5	15.1	24.3	31.0
$NO_3^-:PO_4^{3-}$ > 20	10 meters	10.6	6.8	8.3	90.1	0.5	17.7	28.2	39.0
	Pooled data	11.1	6.9	8.7	94.0	0.5	15.7	26.1	34.6

[a] Nitrate-to-phosphate groups are discussed in the main text and in Table 4.

[b] DO - dissolved oxygen.

[c] NA - not applicable.