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Synopsis

Although nonindigenous species are common in marine environments, and some cause significant environmental and economic impacts throughout the world, there is very little information available for the frequency or impact of invasions by nonindigenous species at high latitudes. This Pilot Study was therefore conducted over a one-year period as an initial step in defining the problem and potential risks in Port Valdez and Prince William Sound. The study briefly summarized the current state of knowledge about nonindigenous species and risk of invasions that are relevant to Prince William Sound. Although limited in scope, the study also examined the transfer of organisms into Prince William Sound that arrive in ballast water of oil tankers. This initial analysis indicates that risk of invasion exists for Prince William Sound, in that large quantities of diverse plankton is transported during spring in ballast water of tankers transiting from west coast ports. Although tankers arriving from foreign ports exchange their ballast water in open ocean, some residual foreign plankton remains in this diluted ballast water; but numbers of tankers arriving from overseas remain low at present. The available information is currently inadequate to assess the magnitude of risk for ballast-mediated invasions, because seasonal and annual variability of plankton in the ballast water has not been measured for tankers. Moreover, the potential for survival and establishment of these organisms has not been studied. Over the next two years, we will collect the necessary information for a detailed analysis of the risks, mechanisms, and patterns of species introductions for Prince William Sound. The Pilot Project and on-going research represent a cooperative and successful partnership of industry, citizen, agency, and scientific groups.

Executive Summary

Project Overview

This Pilot Study begins to assess the risk of nonindigenous species (NIS) invasion associated with oil tanker traffic and ballast water management for Port Valdez / Prince William Sound, Alaska. This study included four major components:

- Review and analysis of existing knowledge of invasions and ship-mediated transfer of species relevant to Prince William Sound.
- Analysis of plankton communities associated with segregated ballast water on tankers that arrived to Prince William Sound.
- Experimental measurements of the effect of ballast water exchange and voyage duration on plankton communities arriving on tankers to Prince William Sound.
- Characterization of plankton communities in non-segregated ballast water passing from oil tankers through the Alyeska shore-side ballast water treatment facility in Port Valdez.

Although this is a preliminary analysis of risk, the Pilot Study advances our understanding of invasion processes in many significant ways.

- Our study provides the most detailed and thorough analysis to date of plankton communities in segregated ballast water of tankers.
- This is the "first-ever" analysis of plankton in non-segregated ballast water, and especially at different stages of the Alyeska (or any other) ballast water treatment process.
- We report the first experimental and quantitative measurements of (a) the effectiveness of ballast water exchange to reduce unwanted organisms and (b) the effect of voyage duration on plankton survivorship in ballast tanks of oil tankers.
- We provide a summary of NIS known from Alaska to California that begins to examine latitudinal patterns of invasions, including invasions to 60°, for the first time.

The scope of this initial study will be expanded over the next two years to provide a more complete and detailed analysis of the risks, mechanisms, and patterns of invasion in Prince William Sound. We have now developed a strong cooperative program to address critical gaps in our understanding of invasion risks as well as facilitate information exchange and participation among a broad spectrum of industry, citizen, agency, and scientific groups.

- From a science perspective, this program will result in a comprehensive analysis of invasion processes and risks for Prince William Sound, representing the first such study in the world for a high-latitude / cold-water marine ecosystem.
- From an industry and management perspective, this program will assess the effectiveness and trade-offs involved for various management strategies that are now required in Prince William Sound, and are being promoted on a national and international scale.
- From a public perspective, this program will disseminate findings and serve as a key source of information, especially through groups like Alaska Sea Grant and the Regional Citizens' Advisory Council of Prince William Sound.

Results

Biological invasions of coastal bays and estuaries are common throughout the world and are having significant ecological and economic impacts.

- Within the lower 48 states of the U.S., 70-212 NIS per estuary are known for sites that have been explicitly surveyed for invasions, and more than 400 marine NIS are documented for these contiguous states.
- Reports of marine NIS for other global regions range from tens-to-hundreds, indicating the widespread nature of this phenomenon.
- These reports actually underestimate the full extent of invasions, because many NIS are not documented.
- Some NIS can and do significantly impact community structure and function, productivity, commercial fisheries, and human health.

Transport of coastal planktonic organisms in ballast water of commercial ships appears to be the major source of new invasions worldwide in recent years.

- Large bulk carriers and tankers can carry individually 30,000 to 60,000 metric tons of segregated (i.e., non-oily) ballast water from one port to another in voyages lasting days to weeks.
- This water often contains a rich diversity of planktonic organisms that inoculate recipient ports upon ballast water discharge.
- Single ports can receive >10,000.000 metric tons of segregated ballast water annually.

High-latitude / cold-water regions are also subject to biological invasions by many species with potential ecological and economic consequences.

- Over 100 marine NIS are known from high-latitude regions (40°-60° latitude) around the world, despite the limited effort devoted to documentation.
- These invasions have a range of impacts similar to those reported for more temperate latitudes.

A preliminary literature review and field survey for Alaska and Prince William Sound, respectively, identified 10 known NIS and many cryptogenic species (i.e., possible NIS), indicating that invasions have occurred and very limited information currently exists to evaluate NIS in Alaska.

Six major risk factors exist in Prince William Sound that may favor successful invasions by NIS.

- 600 tankers currently arrive per year to Prince William Sound and release an estimated 20,000,000 metric tons of segregated ballast water.
- The voyage duration of these tankers is usually short (3-7 days), favoring high survivorship of transported plankton.
- A pattern of repeated delivery of ballast water from the same, limited donor locations provides repeated inoculations of the same NIS.
- Environmental conditions of source ports match those in Prince William Sound for some portions of the year.
- A lack of mid-ocean exchange by most tankers of domestic origin improves transfer rate of NIS
- A large number of NIS are known from both domestic and foreign source ports of ballast water arriving in Prince William Sound.

A large quantity of taxonomically diverse plankton is released in Prince William Sound with segregated ballast water from oil tankers from domestic ports.

- During a period of high plankton productivity in May and June, segregated ballast water from domestic ports contained an average density of 7,000 large (>80 micron) planktonic organisms/m^3 across 13 different ships.
- This is equivalent to an average of 244,000,000 planktonic organisms per ship.
- We have identified a minimum of 69 different taxonomic groups in this ballast water, and a minimum average of 19 species/ship.
- Most of the 600 tankers that arrive annually to Prince William Sound come from domestic ports.

NIS are present in segregated ballast water released in Prince William Sound.

- We have identified 4 NIS arriving in ballast water from domestic ports, and many others are certainly arriving from these locations.
- The 4 NIS identified to date are all copepods from Asian waters which are now abundant in San Francisco Bay.
- Some coastal organisms are also arriving from foreign ports, despite ballast water exchange; these may include additional species that are nonindigenous to Alaska.

Ballast water exchange is effective at reducing the abundance of coastal organisms that arrive to Prince William Sound in oil tankers.

- Fewer coastal organisms appear to be present on tankers arriving from overseas which have undergone ballast water exchange.
- Over 90% of coastal plankton was removed in ballast water exchange experiments on oil tankers.

The abundance of some coastal organisms in ballast water declined with voyage duration for short-term voyages, but this relationship was not as strong as that observed in other studies.

- The densities of annelids and molluscs declined with voyage duration (3-7 days) among tankers arriving to Port Valdez from domestic ports.
- However, the total number of organisms showed no relationship with voyage duration for these same ships, differing from the negative relationship observed for longer (14-20 day), trans-Atlantic voyages of bulk grain and coal ships.
- Also, densities did not decline for most taxonomic groups over three consecutive days of sampling the same ballast water within a single ship.

A large quantity of non-segregated (or oily) ballast water arrives to Port Valdez in oil tankers.

- An average of 29.909 metric tons of non-segregated ballast water is delivered to Port Valdez per tanker, comprising 49% of the total ballast water per ship.

The density and diversity of organisms present in non-segregated ballast water as it arrives and travels through the Alyeska Ballast Water Treatment Facility is extremely low.

- Nearly all organisms present in the first 3 stages of the ballast water treatment facility (Chicksan Arms, 90s Tanks, and DAF Tanks) are dead.
- Only at the last stage (BT Tanks) were live organisms regularly detected, and these consisted primarily of nematodes and diatoms.
- It is unclear whether these nematodes and diatoms derived from ballast water or wind-borne dispersal from the local environment.

Conclusions

Prince William Sound is at some risk of invasion by NIS that arrive in segregated ballast water of oil tankers from domestic ports.

- Large, frequent, and dense inoculations of plankton in ballast water from oil tankers occur in Prince William Sound.
- This ballast water, and its plankton, derive from ports that include tens-to-hundreds of species nonindigenous to the eastern Pacific ocean.
- Some of the known NIS in Alaska also occur in San Francisco Bay, demonstrating that environmental conditions are not always a barrier to invasion by some species in current domestic source ports of ballast water.
- Surveys of Prince William Sound are not now available to assess the rate of invasions in many key habitats that are likely to be invaded by these NIS.

Ballast water exchange appears effective at reducing resident plankton on tankers from overseas, although a risk of invasion still exists.

- Preliminary data indicate >90% reduction of coastal plankton.
- Yet, 300,000 or more organisms/ship can remain from the original source port following ballast water exchange.

Despite the large volume of non-segregated ballast water delivered to Port Valdez, we surmise the risk of NIS invasion associated with oily ballast water is extremely small.

- Few to no live organisms are present in water entering the ballast water treatment facility from each oil tanker.
- The nematodes and diatoms present at the last stage may well be of local (Port Valdez) origin.
- Even if these organisms derive from ballast, the cumulative abundance and diversity of this community is effectively zero in comparison to that arriving in segregated ballast water of tankers.

Further investigation is needed to assess adequately the risk of invasion for Prince William Sound, and this effort should concentrate on segregated ballast water and existing evidence for invasion.

- Analysis of segregated ballast water from more ships is needed to sufficiently characterize associated plankton density and diversity by port, season, and voyage duration.
- Laboratory experiments should measure the viability of these organisms upon arrival and their tolerance of local environmental conditions.
- Shipboard experiments should measure the survivorship and transfer of organisms in segregated ballast water during operation under various environmental conditions and ballast water management practices.
- Intensive surveys of local biota should be focused on key taxonomic groups and habitats to measure the occurrence of NIS invasions in Port Valdez and Prince William Sound.

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TABLE OF CONTENTS

Synopsis ii
Executive Summary iii
Acknowledgments vii

Table of Contents viii

A. Introduction 1

Overview
Objectives of the Pilot Study
Approach to Project

B. Existing knowledge of NIS relevant to Prince William Sound 3

Purpose
Methods
Results
Information Exchange & Consultation 5
State of Knowledge of NIS in Marine/Estuarine Ecosystems Outside of Prince William Sound 5
NIS in Marine Ecosystems at High Latitude 8
NIS in Marine/Estuarine Ecosystems in Prince William Sound and Alaska
Hypotheses About Why NIS Are Not Evident at High Latitude
Factors Adding Risk for NIS Invasions in Prince William Sound
Known NIS in Prince William Sound and Alaskan Waters 11
Purpose
Methods
Results
Submerged Aquatic Angiosperm Plants
Macro-Algae
Invertebrates
Fish
Unsuccessful Invertebrate Introductions into Alaska
Unsuccessful Macro-Algae Introductions into Alaska
Field Surveys of NIS in Port Valdez 16
Purpose
Methods
Results
Invertebrates
Macro-Algae
Discussion
Non-Indigenous Marine/Estuarine Species with Potential for Introduction to Prince William Sound 19
Purpose
Methods
Results
High Latitude Invertebrate Introductions Outside of Alaska
Invertebrate Introductions from Alaska

High Latitude Macro-Algae Introductions
Macro-Algae Introductions from Alaska
High Latitude Micro-Algae Introductions
Discussion

C. Plankton in Segregated Ballast Water 21

Purpose
Methods
Results
Discussion

D. Experimental Analysis of Effects of Ballast Water Management Practices 27

Ballast Water Exchange Experiments

Purpose
Methods
Results
Discussion

Duration of Time in Ballast Tanks

Purpose
Methods
Results
Discussion

E. Plankton in Non-Segregated Ballast Water Passing Through the Alyeska Ballast Water Treatment Facility 30

Purpose
Methods
Results
Discussion

F. References 34

A. Introduction

Overview

Aquatic nuisance species have invaded many, perhaps most, freshwater and marine ports around the world; and ballast water from commercial shipping is increasingly recognized as the most significant vector currently for those invasions occurring (Carlton and Geller, 1993). Ballast water consists of water pumped into dedicated tanks or cargo holds/tanks for trim and stability during oceanic voyages, especially when the vessel is empty or only partially full of cargo. Ballast water is usually taken from coastal water containing a rich diversity of planktonic organisms. Ballast water is often discharged into a receiving port prior to loading cargo, inoculating the ecosystem with exotic species. If the plankton is viable and becomes established, these non-indigenous species (NIS) can cause major ecological and economic disruption in the coastal ecosystem, with numerous examples in San Francisco Bay (Cohen and Carlton, 1996), the Great Lakes (Mills et al., 1993), Chesapeake Bay (Ruiz et al., unpubl. database), and elsewhere (Ruiz et al., 1997a). In San Francisco Bay, the rate of invasion has increased to about one new NIS invasion every 12 weeks, probably as a result of increased ballast water discharge (Cohen and Carlton, 1996). Whether the invasion is Eurasian zebra mussels in the Great Lakes, Asian clams in San Francisco Bay, or ctenophores in the Black Sea, impacts of ballast introductions have been devastating and irreversible. Despite the profound impact of ballast-mediated invasions, the biological characteristics of ballast water and the factors which regulate invasion success are little studied and poorly understood. In the USA, biological characteristics of ballast water have only been studied in two port systems: Coos Bay (Carlton and Geller, 1993) and Chesapeake Bay (Smith et al., 1996; Ruiz et al., unpubl. data); and in other countries the biology of ballast water has similarly received little quantitative analysis (Carlton, 1989; however see Williams et al., 1988; Hallegraeff and Bolsch, 1992).

While the significance of ballast-mediated invasions has recently focused on temperate zone ports, little consideration has been given to NIS invasions at high latitude, despite the volume of shipping and critical importance of certain cold-water ports to the world economy and especially to U.S. energy interests. Port Valdez in Prince William Sound, Alaska, is an especially crucial region at high latitude that ships approximately 20% of U.S. domestic oil production. The terminus of the 800-mile Trans-Alaska Pipeline lies on the south shore of Port Valdez (Fig. A-1). The pipeline and terminal for loading crude oil are operated by Alyeska Pipeline Service Company and receive oil from Alaska's North Slope fields at the rate of approximately 1.35 million barrels per day. This oil is loaded into tankers at the terminal's 4 berths and transported mostly to refineries on the U.S. West Coast, but with occasional deliveries to other cold-water ports in Korea and Japan and some warm-water ports (Figs. A-2 and A-3), now that Congress has authorized sale of North Slope crude on foreign markets. Tankers have made more than 15,000 voyages through Prince William Sound to Port Valdez since the startup of the terminal in 1977. In recent years, 1987-1994, tanker arrivals to Valdez have averaged 799 per year but have declined to less than 600 per year currently (Fig. A-4). Tankers arriving to Prince William Sound discharge two types of ballast water: (1) Segregated ballast water from tanks dedicated solely to ballast water and (2) non-segregated ballast water from tanks which are used to carry petroleum products. Approximately 20 million metric tons of segregated ballast water are discharged annually by tankers into the port and sound, which is a quantity of domestic ballast water that greatly exceeds the volumes of foreign ballast water released in other U.S. West Coast ports and approaches the volumes of foreign ballast water released into New Orleans/Baton Rouge and Chesapeake Bay (Fig. A-5). All non-segregated, oily water (about 50% of total) discharged by tankers in Port Valdez must pass through the Ballast Water Treatment Facility located on shore at the Valdez Marine Terminal. Effects of the treatment plant on NIS were unknown prior to this Pilot Study. Segregated ballast water is discharged directly into the sound/port without treatment.

In light of the problem of aquatic nuisance species invading various shipping ports around the world via ballast water, the Regional Citizens Advisory Council (RCAC) of Prince William Sound, shipping industry, U.S. Fish & Wildlife Service, U.S. Coast Guard, other state and federal governmental agencies, and other citizens are concerned that Port Valdez and Prince William Sound may also be at risk of such invasions. At the root of this concern is the millions of tons of segregated ballast water discharged by these tankers into the Port and Sound each year, as well as the effluent from Alyeska's Ballast Water Treatment Facility.

Objectives of the Pilot Study

As stated in the Request For Proposals, this project was designed with the following objectives over a short (< one year) period:

- To participate in a Nonindigenous Species Working Group in the Prince William Sound;
- To review NIS literature and studies relevant to Prince William Sound, and conduct initial field survey for potential NIS in Port Valdez;
- To conduct a biological analysis of tanker ballast water and Ballast Water Treatment Facility effluent;
- To assist in the development and conduct of a one-day workshop to discuss future evaluation of NIS invasion in Prince William Sound;
- To inform and educate the RCAC, shipping industry, scientists, state and federal governmental agencies, and other citizens about this issue;
- To make recommendations based on findings, and identify specific information needs, as appropriate.

Approach to Project

There is little organized knowledge of marine/estuarine NIS at high latitudes generally or for Alaska specifically. This Pilot Study was therefore conducted over a one year period as an initial step in defining the problem and the risks of NIS in Prince William Sound. The Pilot Study briefly surveyed major elements of environmental risk for NIS in Prince William Sound, Alaska, and provided qualitative and preliminary quantitative information on the potential for ballast-mediated invasions. The Pilot Study involved 4 major components:

1. Analysis of, and communication about, existing knowledge of NIS relevant to Prince William Sound, supplemented by an initial field survey of potential NIS in Port Valdez;
2. Characterization of plankton communities in segregated ballast water coming into Port Valdez aboard oil tankers during late spring, when is abundant and presumed to be of maximal risk for introductions;
3. Experimental assessment of the effect of ballast water management practices of oil tankers (mid-ocean exchange and duration of time in ballast tanks) upon plankton communities in segregated ballast water; and
4. Characterization of plankton communities in non-segregated ballast water passing from oil tankers through the Alyeska shore-side ballast water treatment facility.

Each of these components is discussed in separate sections below.

Although this Pilot Study was limited in scope, it begins to provide important insights into the issue of NIS in a high latitude/cold water ecosystem and the role of oil tankers in transporting plankton in ballast water. The high yield of information from this initial effort derived from a special blend of cooperation and professional commitment of the partnership of private citizens, the oil and shipping industry, government managers, and scientists working closely together. Individually, several elements of this study provided the first such analyses in the world. This is the first detailed, replicated sampling of plankton communities in oil tankers. It provides the first experimental analysis of the potential for ballast management practices to reduce risk of NIS transfer in ballast water of tankers. The analysis of the ballast water treatment plant is the first of its kind. And, combined with studies in the eastern U.S., this begins to assess the importance of NIS transfer among domestic ports of the U.S. Cumulatively, these elements point to the value of cooperation for problem analysis.

The Pilot Study will be expanded in an extended analysis over a 2-year period. The proposal for the expanded study was written during the Pilot Study and submitted to the National Sea Grant Program with matching contributions from RCAC, U.S. Fish & Wildlife Service, Smithsonian Environmental Research Center, Williams College, and Alyeska Pipeline Service Company. Full funding was granted for the period from Fall 1997 to 1999. Thus, this Pilot Study forms a foundation upon which we will build a more complete and detailed analysis of the risk of invasions in Prince William Sound and the coastal zone of Alaska.

B. Existing knowledge of NIS relevant to Prince William Sound

Purpose

A major goal of the Pilot Study was to review existing information about NIS in marine ecosystems and to evaluate its relevance and quality for assessing biological invasions of high latitude/cold water regions, especially in Alaska and Prince William Sound. This review and evaluation was communicated to the constituencies of the RCAC with a variety of methods ranging from expert advice and informal conversation, telephone conferences, a public Workshop, and elements of this written report. Communication of the review and evaluation served to inform the NonIndigenous Species Working Group of the RCAC, the constituents of the RCAC as a whole, and the general public about the risk of marine invasions and the state of knowledge about ballast water transport as a vector for NIS introductions. Clearly, the focus of the analysis was risk of invasion of Prince William Sound by organisms transported in ballast water of tankers arriving at the Valdez Marine Terminal. Initially, the state of knowledge was judged to be so incomplete and poorly resolved for Prince William Sound that the analysis needed to be placed in a global perspective that conveyed information for regions which have received more intensive analyses.

Methods

To review and evaluate the existing knowledge of NIS in Prince William Sound, we used a hierarchical approach. (1) We provided a synopsis of the NIS problem in marine/estuarine ecosystems world-wide, which is based primarily on temperate ecosystems. (2) We developed an overview of reported information for NIS in marine/estuarine ecosystems at several high latitude locations around the world, which allowed us to assess whether high latitude/cold water ports generally may be subject to NIS invasions. (3) We examined known patterns of invasions by NIS in west coast source ports for tankers traveling to Port Valdez. (4) To determine whether the known NIS in Alaska provide an accurate indicator of the risk of biological invasions, we considered the factors that contribute to risk of invasion for Prince William Sound. The risk factors include the pattern of ballast water delivery to Port Valdez, and the latitudinal pattern of biological invasions for source ports along the west coast where tankers take on most of the ballast water transported to Port Valdez. (5) We assembled information about known marine/estuarine invasions of Prince William Sound and south-central to southeastern Alaska. (6) To assess whether previous workers may have overlooked NIS established in Prince William Sound, we initiated a preliminary field survey of species in habitats of Port Valdez. (7) To identify species with a risk of invasion to Port Valdez, we assembled a list of NIS which have invaded high latitude ecosystems and have the potential for ballast water transport.

In this initial analysis of the literature, we utilized state-of-the-art computer searches of bibliographic cross-references, and we reviewed our existing extensive reference library on marine/estuarine NIS world-wide. We focused on high latitude and cold water species and regions (including areas of strong upwelling). From our review, we created a taxonomic list of species for high latitude/cold water ecosystems by region. For the known introduced species along the west coast of North America that comprises the main source ports for ballast water in tankers to Port Valdez, we determined the following variables: site of introduction (southern California, San Francisco Bay, northern California, Oregon, Puget Sound area of Washington and British Columbia); native geographic range; mechanism of introduction (highlighting species introduced in ballast water); date of introduction; and reference documenting the primary source of information. This information allowed us to assess the abundance and diversity of invasions along the latitudinal gradient. It also allowed us to consider the probability of species invading multiple ports by comparing the similarity of invasive species in locations relative to San Francisco Bay, the ecosystem with the most complete analysis of NIS. By considering the similarity of NIS at locations both south and north of San Francisco Bay, we attempted to gain insight about the probability of NIS moving along this broad latitudinal gradient to Alaska.

The graded criteria (derived from J.T. Carlton, e.g., Carlton, 1979) used to determine whether each species in our database is introduced, native, or cryptogenic are described below "Cryptogenic species" cannot be identified clearly as native or introduced, and thus have unknown origin (Carlton, 1996a). Further discussion of criteria for identifying species as introductions are given in Chapman (1988), Chapman and Carlton (1991) and Eno (1996). Often a single criterion is not sufficient to designate a species as being introduced, but combinations of several factors increase the probability of an accurate reconstruction of introductions and invasions.

- Paleontological - NIS are absent from fossil record even though they are present in other locations; native species are found locally as recent fossils: cryptogentic species are not in the local fossil record, but they are not reliably fossilized generally.
- Archeological - NIS are absent from shell middens and other archeological deposits; native species are in local deposits; cryptogenic species would not be expected to be found in archeological deposits.
- Historical - NIS are not recorded by direct observation at early periods, especially by trained naturalists, but suddenly appear where trained observers did not find them previously; native species are recorded in the earliest observations of trained observers; cryptogenic species are species that were not studied by early trained observers.
- Biogeographic - NIS exhibit grossly disjunct patterns of distribution (we took care to evaluate artifacts of the distribution of biologists/taxonomists); native species have continuous geographic ranges which include Alaska/Prince William Sound or other high latitudes; cryptogenic species have poorly known distributions or "cosmopolitan" distributions.
- Ecological - NIS have habitats in close association with other NIS (co-evolved species: specialized predator-prey, commensal or host-parasite relations); native species are closely associated with other native species; cryptogenic species are more generalized, lacking close, specialized association with other species.
- Dispersal Mechanisms - NIS presence cannot be plausibly explained by natural dispersal mechanisms and have documented human-mediated mechanisms which could effect their distributions; native species have natural dispersal mechanisms and lack known human-mediated mechanisms of introduction; cryptogenic species have both natural and human-mediated mechanisms of dispersal that could account for their distribution.
- Evolutionary/Genetic - NIS have isozyme or DNA frequencies which match distant proposed source populations and are significantly different from adjacent natural populations: native species have

population genetics which blend with adjacent natural populations; cryptogenic species have not been studied with molecular techniques.

Results

Information Exchange & Consultation

The Pilot Study included participation in the RCAC NIS Work Group, providing information, analysis and advice about NIS in coastal marine systems worldwide, focusing on the risk of biological invasions of cold water/high latitude coastal ecosystems. Advice and information were conveyed to the Working Group through monthly telephone conference calls from December 1996 through November 1997. Additional consultation was provided to Mr. Joel Kopp, project manager for RCAC, throughout the course of the project.

In February 1997, we also wrote a major proposal to extend and expand the scope of the Pilot Study in response to the Request For Proposals on NIS in U.S. coastal waters from the national Sea Grant Program; subsequently the proposal has been funded for a 2 year period (1997-1999). The extended proposal provides a recommended action plan to follow up the Pilot Study, including:

- Detailed analysis of abundance and diversity of plankton organisms in segregated ballast water in tankers (N= approximately 60 ships) arriving to Port Valdez, to estimate variation by season, year, source port and duration of voyage;
- Experimental laboratory analysis of effects of temperature-salinity combinations on survivorship for certain plankton arriving to Port Valdez, to test for risk of short-term survival of plankton released with segregated ballast water into Port Valdez;
- Experimental analysis of the efficacy of mid-ocean exchange of segregated ballast water in tankers in route to Port Valdez from west coast ports;
- Experimental analysis of plankton survivorship during voyages as determined by comparing samples of segregated ballast water collected upon departure from west coast source ports and arrival to Port Valdez;
- Characterization of the fouling communities on the bottom and in the sea chests of tankers, which will be sampled in dry docks during routine maintenance;
- Review of potential NIS in existing extensive samples of organisms held in the University of Alaska Museum collections and other voucher collections for the Prince William Sound region;
- Field studies of possible NIS in communities of Prince William Sound, including fouling communities of the Marine Terminal and soft-bottom intertidal areas, which are hypothesized to have elevated risk of invasions and which have not received adequate study in the past; and
- Further literature analysis of NIS in Alaska, with assessment of biological invasions in high latitude/cold water ports.

Another major element of the process to inform and educate the Working Group was consultants' lead participation in a public Workshop organized in Anchorage by RCAC during March 1997. Presentations at the March Workshop reviewed the scope of the NIS problem and articulated the potential elements of risk for NIS in Prince William Sound. Video tape (full-length and edited, condensed versions) are available form RCAC in Valdez. A summary of the rationale and information about the risk of NIS in Prince William Sound is provided below.

State of Knowledge of NIS in Marine/Estuarine Ecosystems Outside of Prince William Sound

The significance of NIS in marine environments (including bays, estuaries, and open coasts) has received relatively little attention compared to NIS in terrestrial and freshwater habitats (Carlton, 1989). The few areas with some historical assessments of invasive species in marine/estuarine ecosystems demonstrate the potential importance of NIS invasions (Table B-1). The number of known NIS for each of five different U.S. estuaries (all those intensively studied to date) vary between 70 and 212 species.

Combined with various additional published records, we can now identify approximately 400 individual NIS from coastal estuaries of the continental U.S. (Ruiz et al., unpubl. database). Analyses for other global regions (Table B-1) indicate that NIS invasions are common throughout the world and that NIS often form a significant component of marine communities, where they can have major ecological effects and serious economic impacts. The NIS identified in these analyses, within and outside of the U.S., include a broad range of taxonomic and trophic groups that occupy diverse habitats (e.g., soft-sediment, rocky substrata, marsh surface, and water column). Estuaries and embayments appear to be particularly subject to invasions by NIS, in part because these are the sites of ports and associated shipping activities.

Despite the large number of NIS identified from some sites and regions, all available studies underestimate the actual extent of invasions for two major reasons: (a) many species simply are not examined; and (b) of those examined, the native versus non-native status often is not clear (Carlton, 1996a). Estimates for the Great Lakes and Hudson River estuary resulted from a 1-year study that focused on "accessible" taxonomic groups, or those for which historical distribution records and taxonomic identities were well established. In that limited time, however, it was possible to examine status of only a portion of the species present. By contrast, the estimate for San Francisco Bay is very comprehensive, deriving from >20 years of research (e.g., Carlton 1979a,b, 1985, 1987, 1992b; Chapman, 1988; Carlton et al., 1990; Chapman and Carlton, 1991; Cohen and Carlton, 1996). Even though that study includes "less accessible taxa" as well as conspicuous ones, the presence of "cryptogenic taxa" indicates that many NIS go undetected.

Within these and other coastal ecosystems, NIS are often numerically dominant organisms, and they can dramatically alter community function (see Ruiz et al. 1997 for review). For example, the Asian clam *Potamocorbula amurensis* achieves densities of >10,000 clams/m^2 in San Francisco Bay, where it is replacing other benthic organisms and clearing plankton communities from overlying waters (Nichols et al., 1990; Alpine and Cloern, 1992; Kimmerer, 1994; Cloern, 1996). Recently (1989), the European green crab (*Carcinus maenas*) invaded San Francisco Bay, along with Australia and South Africa, resulting possibly from ballast water (Cohen and Carlton, 1996). On the west coast, this crab has spread rapidly to the south to Monterey and north to at least Coos Bay, Oregon (Grosholz and Ruiz, 1995a,b; N. Richmond, pers. comm.). Our detailed studies of this invasion in Bodega Bay, California show that this voracious predator is drastically reducing populations of native clam and infaunal invertebrates, which also are key food items for native shorebirds, fish and Dungeness crabs (Grosholz and Ruiz, in review); this crab appears to be having similar effects in Tasmania, Australia (Thresher, 1997).

Although poorly understood, many NIS are microbial, parasitic or disease producing; and these can have major adverse impacts on marine ecosystem function and economics. One of the major diseases (*Perkinsus marinus*) decimating oyster populations in Chesapeake Bay appears to have been introduced via ballast water in the late 1950s in nearby Delaware Bay (Andrews, 1979). A parasitic barnacle (*Loxothylacus panopaei*) was introduced into Chesapeake by in the 1960s and produced epidemic infections that castrated xanthid crabs (Hines et al., 1997). Cholera (*Vibrio cholerae*) was recently transported in ballast water to Mobile Bay, Alabama, temporarily closing the local oyster fishery (McCarthy and Khambaty, 1994), and we have documented this pathogen in ballast water arriving to Chesapeake Bay (Ruiz et al., in prep.). Furthermore, such direct effects are thought to have many indirect effects on ecosystem characteristics, from food web structure to nutrient dynamics and sedimentation rates (e.g., Vitousek, 1986).

Human-mediated invasions have increased dramatically over the past century (Carlton, 1989; Mills et al., 1993; Cohen and Carlton, 1996). Human activities have allowed dispersal to occur between donor and recipient regions where natural barriers existed historically, increasing both the potential pool of species that can invade a region and the number of donor regions from which invasions occur. This increased rate of transfer and introduction of NIS among coastal regions has resulted largely from (1) movement of fouling communities on the bottom of ships; (2) movement and/or intentional release of aquaculture and fishery species along with their rich assemblages of associated (free-living and parasitic) organisms; (3) release of species associated with pet industries or management; and (4) release of organisms in ballast materials of ships (Elton, 1958; Carlton, 1979a,b, 1987, 1989, 1992a).

The relative importance of particular transfer mechanisms and source regions of NIS exhibits both temporal and spatial variation. For example, in San Francisco Bay, transplantation of oysters from the Atlantic in the late 1800s and early 1900s, and then from Japan from 1930-1960, brought many NIS associated as fouling species and with trapped sediments; whereas, ballast-mediated invasions now appear to have become the single largest source of invasions in the past 30 years (Carlton, 1979; Cohen and Carlton, 1996). In contrast, construction of canals (such as the Suez and Panama Canals, but also many smaller canals in the midwestern and northeastern states of the U.S.) has played an important role in facilitating transfers between bodies of water and drainage basins at some sites, creating bursts of invasions during certain historical periods.

It is evident that invasions continue to occur, even at heavily invaded sites, despite the centuries-long history of marine invasions associated with global ship transport. There are many reasons why invasions continue to occur, and why all potential invaders have not already arrived (Carlton, 1996b). Invasions result largely from stochastic (probabilistic) processes, rather than strictly deterministic processes that produce immediate cause and effect consequences. Moreover, changes in environmental conditions (climate) and water quality (both pollution increase and abatement) in donor and/or source regions can promote or inhibit invasion. And transport mechanisms or patterns of delivery may change as global and regional technology, commerce, and politics shift.

Despite considerable spatial variation for the importance of different NIS transfer mechanisms, the worldwide movement of ballast water appears to be the single largest source of coastal invasions today (Carlton and Geller, 1993; Carlton et al., 1995; National Research Council, 1996). Most known marine and estuarine invasions are now occurring in or near ports with international shipping traffic and are linked to ballast water as a plausible source. The U.S. and Australia each receive over 79 million metric tons of ballast water from foreign ports per year (the equivalent of 2.4 million gallons/hour; Carlton, 1995c; Hutchings, 1992, respectively). Because ballast is usually taken from bays and estuaries, with waters rich in plankton and nekton, most ships carry a diverse assemblage of organisms in their ballast water (e.g., Medcof, 1975; Carlton, 1985; Williams et al., 1988; Carlton and Geller, 1993; Smith et al., 1996), which inoculate receiving ports with large doses of larval invertebrates, fish, phyto- and zooplankton, and microorganisms.

Although coastal invasions have been widespread and continue to occur, it is not clear whether susceptibility to invasion differs among regions. Some have suggested that particular geographic sites or habitats are more invasible than others, forming "hotspots" as donor or invasion sites (Vermeij, 1991; Carlton, 1995c). Sites may be more or less susceptible to invasion due to their particular species composition, disturbance, and/or environmental conditions (Erlich, 1986; Roughgarden, 1986; Simberloff, 1986). It would be useful to compare the inoculation and invasion rates among various coastal sites, to test for correlation and possible variation in susceptibility to invasion. Unfortunately, most data available on ballast water and NIS frequencies for most coastal sites are too limited, even for many sites which are well studied. For example, San Francisco Bay has the best studied history of NIS, but has received no analysis of the characteristics of ballast water delivered there. In marine systems, only Coos Bay, Oregon (by J.T. Carlton) and Chesapeake Bay (by our research group) have received comparable comprehensive studies of NIS invasion patterns, ballast water delivery patterns, and ballast water biota. We are now comparing these data between sites.

Our preliminary findings indicate that delivery of large quantities of ballast water alone is not a good predictor of the rate of invasion of a site by NIS, nor of the type of NIS which are likely to be successful. While Chesapeake Bay receives 10 times more plankton rich ballast water annually than any other port on the U.S. east coast or than San Francisco Bay, many more NIS associated with ballast water transport appear to have invaded San Francisco Bay than Chesapeake Bay over the past 20-40 years. Rather, the delivery pattern of ballast water inoculations and the match of environmental conditions in donor and recipient regions may be critical. San Francisco Bay receives ballast water repeatedly from a limited number of the same sites (especially Japan) which have similar moderately fluctuating seasonal patterns of closely matching conditions; whereas, Chesapeake Bay receives ballast from a large number of sites spread over Europe and the Mediterranean regions, which often do not have matching environmental conditions. Importantly, we plan to extend this comparison with comparable data obtained from Prince William Sound over the next two years.

NIS in Marine/Estuarine Ecosystems at High Latitude

Although there has been no significant analysis of NIS in polar marine ecosystems, there have been a limited number of NIS surveys in high temperate latitudes between 40° - 60° and a study of the Baltic Sea, which includes a major bay that extends substantially above 60° (Tables B-2, B-3, B-4). These studies demonstrate that invasions are not limited to lower latitudes. For two regions in the northern hemisphere (Baltic Sea and United Kingdom)(Tables B-2, B-4) and one region in the southern hemisphere (Tasmania)(Table B-3), the number of known NIS at each location ranges between 32 and 54 species. For each region, the species include a broad range of taxonomic groups, and some of the invasions have generated serious concerns about their ecological and economic impacts. However, the actual impacts of these species, as well as most invasions, remain unmeasured (e.g., Ruiz et al., in review). Nonetheless, based upon reported abundances and known ecology, species such as the green crab *Carcinus maenas* (on the North American east and west coasts, Tasmania), the seastar *Asterias amurensis* (in Tasmania), and the laminarian kelp *Undaria sp.* appear likely to cause significant and irreversible changes. Furthermore, the cumulative effects of the entire NIS assemblage may cause many changes in ecosystem function that are not easily identified with any one invasion event (Cohen and Carlton, 1996).

NIS in Marine/Estuarine Ecosystems of Prince William Sound and Alaska

The numbers of NIS at high latitudes may be lower than those for temperate regions, although it is not clear whether low numbers of documented NIS reflect lack of invasion in high latitude ecosystems or lack of research focused on the invasion biology of these areas. At the outset of this Pilot Study, the number of NIS documented in Alaskan waters appeared to be lower than other high latitude/cold water ecosystems with more extensive analysis, despite the extensive environmental studies associated with the Exxon Valdez oil spill in Prince William Sound and other ecological research throughout the region.

Hypotheses About Why NIS Are Not Evident at High Latitude

We can advance hypotheses why NIS have not been as evident at high latitude as at mid-latitudes:

(1) NIS are truly rare at high latitude.

- High latitude communities may be resistant to invasion (e.g., severe seasonal stress requires specialized evolutionary adaptations not possessed by non-native species).
- Transport patterns may not have been conducive to inoculation. Shipping/ballast water is major source of rapidly escalating invasions in temperate latitudes, but perhaps neither the relatively recent (20 yrs) surge in tanker traffic to Alaska with very large ballast capacity, nor the current shift in tanker traffic to foreign ports has had time to produce invasions. Note, however, that NIS invasions mediated by ballast water have been common over the past 20 years in some cold temperate ports such as San Francisco Bay (Cohen and Carlton, 1996).

(2) NIS are actually common at high latitude, but have not yet received concentrated study by experts of invasion biology. For example, Carlton (1979) identified some 160 NIS in San Francisco Bay and Pacific northwest coast, but as of 1995 the number of NIS documented in San Francisco Bay is 212 species (Cohen and Carlton, 1995) and is now more than 220 species (J.T. Carlton, personal communication). Three years ago, the number of NIS in Chesapeake Bay was considered to be only about 25 species; yet pursuant to our on-going literature search of the historical records, we have documented >130 NIS, and the list is still growing with on-going research. Despite extensive biological/ecological assessments of coastal ecosystems associated with oil spills in Alaska, NIS probably remain inadequately studied. A review of the existing reports from past and on-going surveys from oil spill work in Prince William Sound is a good start to assessing prevalence of NIS, but such a review is probably not adequate because those surveys were designed for purposes other than detecting introduced species, and they focused on rocky shores rather than on soft-bottom and fouling communities that are most invaded in other regions (e.g., Cohen and Carlton, 1996). Most introduced species have been discovered by taxonomic experts systematically examining specimens previously identified by non-specialists or conducting field surveys of their own.

Factors Adding Risk for NIS Invasions in Prince William Sound.

To determine whether the known NIS in Alaska provide an accurate indicator of the probability for biological invasions, we considered 6 factors which contribute elements of risk for invasions of Prince William Sound:

1. Huge volume of ballast water. The greatest quantities of ballast water are transported by bulk cargo carriers and tankers (Carlton et al., 1995; Smith et al., 1996). Chesapeake Bay receives 10-fold more ballast water than other ports on the east and west coasts of the U.S. because of the high volume of bulkers arriving to the ports of Baltimore and Norfolk. Obviously, the tanker traffic to Port Valdez releases similar large volumes of ballast water (Fig. A-4). Other things being equal, larger ballast volumes mean larger inoculations of NIS.
2. Short voyage time. Our analysis of biological characteristics of ballast water arriving to Chesapeake Bay shows a marked inverse relationship between densities of organisms and length of voyage, such that ballast water after voyages of 14-24 days had more than 10-fold fewer organisms than voyages of 5-13 days (Smith et al., 1996). However, these effects may be confounded by differences in the source of ballast, which co-varies with the length of voyage (Smith et al., 1996). Voyages of tankers delivering ballast water to Prince William Sound average only 3-6 days, quite short compared to most trans-oceanic voyages that average 12-22 days. Short voyages mean that many larvae and other organisms are likely to be in good health when they are discharged. This element of risk is assessed further below in section C on "Characteristics of Plankton in Segregated Ballast Water".
3. Pattern of repeated delivery from same donor locations. Although Chesapeake Bay receives about 10-fold more ballast water than does San Francisco Bay, San Francisco Bay appears to be invaded by many more ballast-mediated NIS. This greater risk could be due to San Francisco Bay receiving repeated ballast inoculations delivered from relatively fewer ports than does Chesapeake Bay. Similarly, Prince William Sound could be at increased risk not only by the large volume of ballast water, but also by the repeated inoculation of ballast from a small set of west coast ports (Figs. A-2, A-3).
4. The match of environmental conditions of source and receiving ports. Environmental conditions in Alaska are often perceived as being harsh and inhospitable to most potential invaders from temperate latitudes where moderate conditions prevail. Obviously, temperature, light and other conditions during winter are indeed more extreme than those in temperate regions of North America and Asia. However, temperature-salinity conditions in Prince William Sound during spring and summer often approximate conditions in source ports of northwest North America, especially during productive periods of cold water up-welling. In fact, many of the native marine/estuarine species in Alaska have geographic ranges which extend to British Columbia, Washington, Oregon, and Northern California. Below in Section C on "Characteristics of Plankton in Segregated Ballast Water",

temperature-salinity conditions in segregated ballast water of tankers arriving from several west coast source ports is shown to be similar to the waters of Port Valdez during late May-early June.

5. Lack of mid-ocean exchange of ballast water delivered to Prince William Sound. Mid-ocean exchange of ballast water reduces concentrations of larvae and plankton by 50-90% (Smith et al., 1996). Exchange presumably limits the risk of invasion, as mid-ocean species are generally thought to be incapable of invading nearshore habitats. Delivery of ballast from coastal ports of the U.S. West Coast without oceanic exchange before release into Prince William Sound poses an elevated risk. Further experimental assessment of this role of mid-ocean exchange in reducing plankton abundance and diversity in ballast water is presented in Section D below on "Experimental Analysis of Effects of Ballast Water Management Practices". While ballast water exchange is required for tankers from foreign ports, the National Invasive Species Act of 1996 considers tankers from U.S. west coast ports to be domestic, coast-wise traffic that does not require exchange.
6. High frequency of known NIS - especially those transported by ballast water - in source regions of ballast coming to Prince William Sound. Some workers consider that there may be "hotspots" of invasion or donation of NIS. If such hotspots exist, certainly San Francisco Bay and other ports of the U.S. west coast qualify as having among the highest prevalences of documented ballast-mediated invasions, and these in turn form the sources donating much of the ballast water delivered to Prince William Sound. The 309 known NIS of the west coast of North America vary considerably in abundance among 6 latitudinally separate regions (southern California, San Francisco Bay, northern California, Coos Bay Oregon, northwest region from the Columbia River estuary to British Columbia, and Alaska) (Table B-5). The number of known NIS varies from 75 species in southern California to 38 species in the northwest region of Washington and British Columbia, with the largest number of 218 species occurring in San Francisco Bay. At each location along the west coast, NIS are common in a diverse array of taxonomic groups, with arthropods, molluscs, and annelids comprising major fractions of NIS at most locations (Fig. B-1). In several locations (San Francisco Bay, Northern California, Oregon), vascular plants and chordates also comprise major portions of the NIS. Much of the variation in number of NIS probably reflects the level of study and state of knowledge for each location, especially since the highest numbers occur at two locations (San Francisco Bay and Coos Bay) where J.T. Carlton has focused his past research. Many NIS occur at several locations along the west coast, indicating that invasions by the same species have occurred widely across latitudinally separate sites. In fact, all of the NIS now recognized in Alaska also occur in San Francisco Bay and other west coast regions (Table B-5).

Known NIS in Prince William Sound and Alaskan Waters

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Purpose

To identify species which already may have been introduced into Prince William Sound, we surveyed available published and unpublished in formation which may document known invasions. At this initial stage of analysis, we have attempted to document any known reports of NIS in the region, including failed or unsuccessful introductions; but we also provide comments about the potential status and quality of the information for each species.

Methods

Recognition of species as being introduced into Alaskan waters and Prince William Sound is based on the same criteria applied to other west coast locations, as presented above (Carlton, 1979; and discussed by Chapman, 1988; Chapman and Carlton, 1991; Eno, 1996). Methods of literature review and survey followed the standard approaches of electronic bibliographic searches and surveys of grey literature as described above for reviews of NIS in other regions. Personal, unpublished accounts of observations of NIS were also documented.

Results

In an initial review and literature search, we identified 12 NIS that have been reported to occur in Prince William Sound and Alaskan waters (Table B-6), plus 4 species of macro-algae which have been introduced but not established successfully (Table B-7). Although this preliminary list for NIS in Alaskan waters is small, it is of significant concern from several perspectives. First, collectively there are more species than previously recognized by other assessments (e.g., Wiegers et al., 1997). Only, the soft-shelled clam *Mya arenaria* was commonly acknowledged as an introduced species in Prince William Sound (e.g., Foster, 1989). Second, these known NIS represent a diverse array of taxonomic groups, including invertebrates, fish, and plants. Several of these are species which have histories of invasions in many places around the world, and which are associated with ecological and economic impacts. Third, the species also occupy a wide range of ecological niches: however, the abundances and realized niches of these species is not well understood for Prince William Sound and Alaska. Fourth, while the mechanisms of transport and introduction for most these species are not known for Alaska, most of them have larval or fragmented stages which may be taken up and transported by ballast water. In fact, larvae of taxonomically affiliated species are abundant in the plankton of segregated ballast water in tankers arriving to Port Valdez (see section C on "Characteristics of Plankton in Segregated Ballast Water"). Fifth, the documentation of some of these introductions is not established in the reviewed scientific literature but derived from direct observations by reliable sources (Atlantic salmon landings) and from very recent reports (Eurasian milfoil in the Juneau region). Thus, these and other invasions may be occurring without formal scientific detection. Finally, the geographic range of some of these species extends well down the west coast of North America to several source ports of ballast water for tankers, including the heavily invaded port system of San Francisco Bay. This indicates that some NIS in the temperate source ports are capable of invading Prince William Sound. We also identified cases of unsuccessful or failed introductions of NIS, primarily associated with aquaculture, indicating that multiple mechanisms of introduction are active in Alaskan waters.

Submerged Aquatic Angiosperm Plants

Myriophyllum spicatum. Eurasian watermilfoil is a freshwater species that has widely invaded tidal freshwater of estuaries as a result of release from the ornamental aquarium trade. A 1997 report by the Alaska Department of Environmental Conservation communicated by Susan Walker of U.S. Fish & Wildlife Service indicated Eurasian milfoil occurs in the Juneau area of southeast Alaska. However, this report needs further documentation. Native to Europe and Asia, *M. spicatum* can form dense unsightly beds of vegetation that may clog waterways, but it also creates refuge habitat for juvenile fish.

Macro-Algae

Codium fagile* subsp. *tomentosoides (Suringar, 1867). Hariot, 1889. In addition to the species listed (Table B-6), an algal specimen which appears to be *Codium fagile* subsp. *tomentosoides* is currently under study by G. Hansen. The specimen comes from Green Island in Prince William Sound: and if this tentative identification is validated as distinct from *C. f.* subsp. *fragile*, it will represent a new location for this widely distributed invasive species. This weedy subspecies of *Coduim fragile* is native to Japan (Cohen and Carlton, 1995). *C.* f. *tomentosiodes* was introduced to Europe in the 19th century from Asia and to New York, probably as ship fouling, around 1956. *C.* f. *tomentosoides* spread from New York, north to Maine and south to North Carolina (Carlton and Scanlon 1985, Cohen and Carlton 1995). It was first collected from San Francisco Bay in 1977 and suggested to be an introduction with ship fouling (Carlton et, al 1990, Carlton and Cohen 1995) even though nearly all other introductions to the bay from the 1970s to 1995 have been attributed to ballast water (Carlton and Cohen 1995). Sputnik weed, a common name for the subspecies, is attributed to the fragmentation of the thalli that commonly occurs during cold periods (Carlton and Scanlon 1985). Many workers have noted the striking capacity for natural dispersal of *Codium* via 1) motile reproductive cells with can be produced apomictically, 2) vegetatively by thalli fragmentation, particularly in colder months (Fralick and Mathieson 1972, Wassman and Ramus 1973b, Malinowski 1974) and whole plant buoyancy via internal gas entrapment (Bouck and Morgan 1957, Moeller 1969, Ramus 1971, Malinowski 1974). The native north east Pacific species, *Codium fragile* Suringar, 1867, ranges from Prince William Sound to Baja California (Pers. Obs., G. Hansen). Other high latitude introductions of the *Coduim fragile* complex include *Codium fragile atlanticum* (Cotton) that has spread as far north as the Orkney Islands, U.K. (Trobridge and Todd, In Review) and *Codium fragile scandinavicum* which invaded Scandinavian shores (Silva 1957).

Invertebrate Animals

We are aware of two hydroids, one clam, two crustacean amphipods and one tunicate that are reported potential or likely introductions of invertebrates to Alaska in addition to *Mya arenaria* and *Heteromastus filiformis* that we report from Port Valdez. Except for *Mya arenaria*, Carlton (1979) doubted the validity of most NIS in Alaska that he considered.

Cnidaria (Hydroids)

Syncoryne mirabilis (= *Sarsia tubulosa*) (M. Sars, 1835). Probably introduced to the northeast Pacific with fouling on wooden ships from the North Atlantic, this species is known also as *Coryne rosaria*. The early north Pacific records of *S. tubulosa* are from Puget Sound in (Agassiz 1859) and San Francisco Bay in 1860 making it, potentially, one of the earliest marine introductions to the North Pacific (Carlton 1979) and is likely to have arrived with ship fouling if it is indeed introduced. This species was collected from 90 m off of the Trinity Islands, Alaska in 1914 (Fraser 1914) and Garforth Island, Miur Inlet, Alaska (USNM 34481) in 1899 (Fraser 1937). However, we did not find this species in our preliminary survey, and it is not reported from Port Valdez. *Syncoryne mirabilis* may be misidentified in Alaska. The two northern records and all records south of Monterey are old, requiring confirmation (Carlton 1979). *Syncoryne mirabilis* occurs in the North Atlantic in Iceland and in northwestern Europe and in Japan and Russia in the west Pacific (Carlton 1979). It could be a "circumboreal neritic species" (Russell, 1953:56) extending down the four continental margins of the Arctic Ocean or a complex of species with introductions or multiple, isolated species at lower latitudes and a single, naturally distributed species at high latitudes. Carlton (1979: 237) did not consider *S. tubulosa* to be a certain introduction pending further taxonomic studies to reveal the relationships between the many populations of this taxon from around the world. It's occurrence in Alaska is uncertain.

Tubularia croacea (Agassiz, 1862). Probably introduced to the northeast Pacific with fouling on wooden ships from the North Atlantic. The Gulf of Alaska record of this species (Fraser 1937) is also doubtful. Fraser's (1937: 51) single record of this distinctive hydroid "Gulf of Alaska" gives no specific locality. *Tubularia croacea* is a common shallow-water hydroid that occurs on pilings floats and other structures on the Atlantic coast from Newfoundland to Florida and in the northwestern Gulf of Mexico (Fraser 1944: 98, Carlton 1979). It was very likely introduced into the north Pacific with ship fouling and probably again with Atlantic Oysters (Carlton 1979). Since we did not find this species in our preliminary survey and have no additional reports of it in Alaska since Fraser (1937), it is not reported from Port Valdez. This species also may be misidentified in Alaska.

Annelida

Heteromatus filiformis. The occurrence of *Heteromastus filiformis* in Port Valdez could be associated with ballast water traffic, although it is not clear when it arrived in the area. It is unlikely to have arrived in fouling communities, since it is not found in such habitats and is unlikely to have been actively introduced. *H. filiformis* is native to the Atlantic coast where its range extends from New England to the Gulf of Mexico. This species has been reported from Greenland, Sweden, the Mediterranean, Morocco, South Africa, the Persian Gulf, New Zealand, Japan, and the Bering and Chukchi Seas (Cohen and Carlton 1995). This mud-dwelling species may have been introduced to California, Oregon and Washington in the 1930s with introduced Atlantic oysters, *Crassostrea virginica* as early as the 1930s (Carlton 1979) or it may have been an early ballast water introduction (Cohen and Carlton 1995). The slow growth of oysters in Alaska results in annual oyster importations and creates a significant potential for introductions organisms that are associated with oysters. *H. filiformis* could have been introduced to Kachemak, Alaska with oysters transplanted from Willapa Bay, Washington. *H. filiformis* occurs in Willapa Bay (Carlton 1979) and it has been found in association with oysters (Wells 1961). We are unaware however, of attempts to grow oysters in Port Valdez. *H. filiformis* occurs in densities of up to 4000 individuals m^{-2} in the western half of Suisun Bay portion of San Francisco Bay (Hopkins 1986, Markmann 1986). This region of San Francisco Bay is a location where some oil tankers traveling to Valdez take on ballast water. Both the larvae and mature adults of this species are likely to flow into filling ballast water tanks in this area. Thus, ballast water transport appears to be the most likely mechanism for carrying *H. filiformis* to Alaska.

Mollusca

Mya arenaria Linneaeus1758. The occurrence *Mya arenaria* in Port Valdez is unlikely to be a result of ballast water in tanker traffic. Carlton (1979) reviewed the evidence for the introductions of this species to the northeast Pacific. *M. arenaria* is a relatively early introduction that appears to have arrived in Port Valdez long before tanker traffic began. *M. arenaria* is native to the northwest Atlantic. It has been introduced into the Pacific an Europe, Asia and the northeast Pacific. Its northeast Pacific range extends from Alaska north of the Aleutian peninsula (Carlton 1979). Despite extensive systematic research on the genus (Foster 1946, MacGinitie 1959, MacNeil 1965, and Laursen 1966), it's name.

limits of intraspecific variation, and geographic distribution are subject of considerable disagreement. This poor resolution of the species and the similarities between *M. arenaria* and *M. japonica* have caused particular difficulties in resolving the fossil occurrence of *M. arenaria* in Alaska (MacGinitie 1959, Carlton 1979a). It's endemic status in northern and western most Alaska is therefore questionable but rather clear in southeast Alaska and Port Valdez. *M. arenaria* is very likely not native in Alaska since there are no fossil records or records of it from aboriginal shell middens (Carlton 1979a). This large. edible clam may have been actively distributed throughout the northeast Pacific in the late 19th century and early into this century (Cohen and Carlton 1995) as a food source. Thus, although *M. arenaria* has a planktonic larva which could be taken up in ballast water, it is not clearly a ballast water introduction.

Venerupis phillippinarum (Deshayes, 1853). The Japanese little neck clam. Reproductive populations of this species may occur in southeast Alaska (Carlton 1979), but these have not yet been confirmed.

Crustacea

Corophium acherusicum Costa 1857. This species is probably endemic to the eastern North Atlantic and is perhaps the most widely introduced amphipod of all species in the world. No specific locality was given for a report of this species in Alaska (Crawford 1937: 617, Shoemaker 1947: 76, 1949: 76). We were unable to locate specimens from Alaska reported by Shoemaker (1947) in the Smithsonian Museum of Natural History collections, therefore, Shoemaker's reports may be based on Crawford (1937). The unidentified *Corophium* in the University of Alaska Museum collections (code 616915020000) should be closely examined to determine whether they are possibly *C. ascherusicum*. However, this nearly cosmopolitan species is common in southern British Columbia (Bousfield and Hoover 1997, Carlton 1979) and occurs at high latitudes of the North Atlantic, in North America and Europe (Lincoln 1979), New Zealand (Hurley 1954) and Japan (Ishimaru 1984). Hurley (1954) reports that the distribution of *C. acherusicum* "traces out some of the major shipping routes" of the 19th and 20th centuries. *Corophium insidiosum* has been introduced to nearly every location in the world where *Corophium acherusicum* has by the same mechanisms and is morphologically similar. Moreover, *C. insidiosum* is more commonly reported in the northern end of the confirmed northeastern Pacific ranges (Carlton 1979). Since, if *C. insidiosum* is clearly established in Prince William Sound (see below), the records of *C. acherusicum* in Alaska should be regarded with caution. Nevertheless, Crawford's (1937) indication that he had examined the Alaskan specimens of *C. acherusicum* in the same paper in which *C. insidiosum* is described confirms that this species was in Alaska despite the absence of subsequent reports.

Corophium insidiosum Crawford, 1937. This species, like *C. acherusicum*, is nearly cosmopolitan *C. insidiosum* commonly occurring in southern British Columbia (Bousfield and Hoover 1997, Carlton 1979) and at high latitudes of the North Atlantic, in North America and Europe (Lincoln 1979), New Zealand (Hurley 1954) and South America (Alonsa de Pina 1997). Bousfield and Hoover (1997: 114) erect the new genus *Monocorophium* from *Corophium* and describe a new species, *Monocorophium carlottensis* from 29 lots containing 290 specimens collected from Prince William Sound and S. E. Alaska through the Queen Charlotte Islands. We continue with the old epithet *Corophium* pending acceptance of this new designation by other workers. Bousfield and Hoover (1997) identify *C. carlottensis* in collections as far south as British Columbia and *Corophium insidiosum* from British Columbia, Washington and south. Bousfield and Hoover (1997) thus recognize *C. carlottensis* as northern and "distributionally non-overlapping" that of *C. insidiosum*. They distinguish *C. carlottensis* from *C. insidiosum* Crawford, 1937 by its smaller size, "more tightly setose antenna 2", the relatively greater length of article 6 of pereopod 7 and the lack of setae on the anterior margins of segment 4 of pereopods 3 and 4. *Corophium carlottensis* appears otherwise identical to *C. insidiosum*. The variation among specimens of *C. insidiosum* from a single live cultured population Hawaiian *C. insidiosum* and from specimens collected from Yaquina Bay, Oregon completely overlaps every combination of dense to light setation on the antennae and anterior margins of segment 4 of pereopods 3 and 4 of *C. carlottensis*. Additionally, the relative length of article 6 of pereopod 7 illustrated for *C. carlottensis* (Bousfield and Hoover 1997: fig. 29) is indistinguishable from that of *C. insidiosum* (Bousfield and Hoover 1997: fig. 26). We hope to conduct further morphological comparisons and conduct reproductive viability tests between the Yaquina Bay, Hawaiian and Alaskan populations. There is no evidence that *C. carlottensis* is a good species at present (Chapman *et al.* in prep.)

Urocordata (Tunicates)

Ciona intestinalis (Linnaeus, 1767). Ritter's (1913) record (USNM 5633, 1903,) of this species in southeastern Alaska does not provide a specific locality (in Carlton 1979). Records of this species in British Columbia are few and relatively old, warranting re-examination; and there are no firm Washington or Oregon records (Carlton 1979). Carlton (1979) therefore concludes that this species is only confirmed in California south.

Vertebrate Animals, Fish

Salmo salar. Atlantic salmon have been introduced to supplement salmon fisheries in numerous locations around the world, including various locations of the U.S. by the United States Fish Commission. Often these intentional introductions have not become established successfully; however the species' range in Europe extends northward well within the Arctic Circle, indicating that it has potential for establishment in Alaskan waters where it could compete with native species. Atlantic salmon are reared in commercial pen-culture in Puget Sound, British Columbia and apparently Alaska. Some of these fish escape from the pens are caught by commercial and sport fishermen in isolated instances. Atlantic salmon have be landed in recent years at the small boat harbor in Valdez (R. Benda, personal communication). Thus far, this species does not appear to have established a self-sustaining population in Alaska.

Unsuccessful Invertebrate Introductions Into Alaska

Crassostrea gigas (Thunberg, 1795). We are aware only of attempts to establish the Japanese Oyster in Ketchican Bay for aquaculture, which have been unsuccessful. Growth and reproduction of this warm water species in cold Alaskan waters appear too limited to maintain local populations. Local growers depend on annual introductions of new spat for each year's harvest (Foster 1991).

Unsuccessful Macro-algae Introductions to Alaska

Four species of macro-algae have been introduced into Alaskan waters in association with aquaculture activities, but these have not established successfully (Table B-7).

Macrocystis integrifolia Bory. The giant macro-alga *Macrocystis integrifolia* was introduced from California by egg fishermen of Prince William Sound to provide sites for herring eggs until its introduction was outlawed in the 1990s. This species has not been successfully established within the Sound.

Macrocystis pyrifera (L.) C. Agardh 1820. Since the introduction of *M. integrifolia* into Prince William Sound was outlawed in the 1990s, *Macrocystis pyrifera* from southern Alaska has been used exclusively to provide sites for herring eggs. This species has not been successful in the Sound.

Pachymenia carnosa Introduced cultures of *Pachymenia carnosa* held in open net-pens in southern Alaska for aquaculture have not resulted in the establishment of self maintaining populations.

Porphyra yezoensis Introduced cultures of *Porphyra yezoensis* held in open net-pens in southern Alaska for aquaculture have not resulted in the establishment of self maintaining populations.

An additional algal species of *Ascophyllum* has also been introduced unsuccessfully into waters off British Columbia (Table B-7). These unsuccessful introductions are easier to document than accidental releases, because they have been associated with aquaculture operations which provide recorded activities. They illustrate the point that many introductions may be occurring by various mechanisms, but many such introductions often do not result in populations becoming established successfully.

Field Surveys for NIS in Port Valdez.

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Purpose

To determine whether nonindigenous species have already become established within Port Valdez without being detected by earlier biological surveys, we conducted an initial collecting survey of intertidal invertebrates and algae from several habitats. Our initial collecting effort focused on mudflat and fouling communities, because these habitats have not had as much study as most other habitats of Port Valdez, and because NIS in other, better studied locations often invade such habitats (e.g., Cohen and Carlton 1996). However, Port Valdez is characterized by reduced salinities and comparatively low productivity due to low nutrients and high turbidity in glacial melt that flows into the fjord. We now hypothesize that habitats in Prince William Sound, which are characterized by higher biotic diversity, higher salinities and higher nutrients, may be more vulnerable to invasions than low nutrient waters within the Port. Tankers often begin releasing segregated ballast water into Prince William Sound well before they reach Port Valdez, providing inoculations to extensive areas outside the confines of the Port. Our opportunity to sample habitats outside Port Valdez were very limited due to constraints of time and access during the Pilot Study.

Survey Methods

A preliminary survey of native and nonindigenous intertidal flora and fauna of parts of Port Valdez and Valdez Arm was conducted during late May - early June, 1997. The Duck Flat, immediately east of Valdez, was sampled primarily on 22, 26 May and 3 June of 1997. The rocky intertidal zone of Anderson Cove at the southwest entrance to the Valdez Harbor, the rocky and mudflat tidal areas of Sawmill Bay at the northwest entrance to Valdez Harbor, and the float fauna of the Valdez small boat harbor were sampled on 31 May 1997. Organisms were collected by hand or from sediments washed on an 0.5 mm mesh sieve. Invertebrates were preserved in 10% formalin in the field and later transferred to 70% ethyl alcohol. Half of the specimens of each macro-alga species were immediately preserved in 5% formalin and the remaining half were pressed and dried.

Jeff Cordell, Fisheries Unit, University of Washington, Seattle WA identified harpacticoid copepods and all other Crustacea were identified by John W. Chapman. Polychaetes were identified by Leslie Harris, Los Angeles County Museum of Natural History, Los Angeles, CA, and Faith Cole, US EPA, Newport, OR. Molluscs were identified by James T. Carlton and John W. Chapman. Macro-algae were identified by Gayle I. Hansen. We compared our species list with Wiegers et al. (1997) summary of previous taxonomic surveys of the mudflat and rocky intertidal flora and fauna of Port Valdez to provide an independent estimate of the relative diversities of nonindigenous, cryptogenic and native species in Port Valdez. This comparison was used also to indicate the sufficiency of all surveys combined for indicating the total diversity of the area.

Recognition of species as being introduced into the Port Valdez/Prince William Sound area is based on the same criteria applied to other west coast locations, as presented above (Carlton, 1979; and discussed by Chapman, 1988; Chapman and Carlton, 1991; Eno, 1996).

Results

Invertebrate Survey

We collected 49 benthic invertebrate species, not counting insects (Table B-8). Twenty-six of the 49 species (53%) were reported for Port Valdez previously by Wiegers et al. (1997) and 23 (47%) are new. Most of the new records are in taxa such as gammaridean amphipods that were not identified in previous surveys, and only *Shaerosyllis brandhorsti, Limnoria ligatum and Danielssenia cinctus*, of our survey, are similar to other species reported previously (Table B-8). The large proportion of new benthic invertebrate records (Table B-8) appears to result from incomplete collecting in the Port. Wiegers et al. (1997) report the distinctive commensal clam *Orobitella rugifera*, which attaches to the abdomen of the mud shrimp *Upogebia pugettensis*. However, *U. pugettensis* was not reported in their summary list and we did not find the distinctive holes of this long-lived species (Posey *et al.* 1991). We also collected several specimens of the conspicuous purple shore crab *Hemigrapsus nudus* for the first time but did not find *Hemigrapsus oregonensis*, an equally distinctive shore crab reported previously. Thus, significant changes in benthic community structure may occur in the area over time and despite the preliminary nature of our survey, the diversity of intertidal flora and fauna of the Port appears to be underestimated.

The geographic range and overlap with southern species by the Alaskan fauna is significant, indicating suitability of Port Valdez for other species from more temperate locations. Of the combined 73 species with known ranges collected from all surveys of the Port, only 6 (8%) appear endemic only to Alaska (Table B-8). Twenty-two (30%) of the species occur as far south as Washington and 29 (40%) occur as far south as California. Of the 89 benthic invertebrates collected, 15 (17%) are cryptogenic, having disjunct geographical or cosmopolitan distributions but origins which are not adequately resolved. Two species, the polychaete worm *Heteromastus filiformis* (Claparede, 1864) and the clam *Mya arenaria* Linneaus, 1758, clearly appear to be introduced.

Macro-algae Survey

Twenty-one macrophyte species and an unidentified tubular filamentous diatom were collected in the field survey (Table B-9). Three macrophytes, *Blidingia chadefaudii, Neorhodomela aculeata* and *Neorhodomela oregona*, were newly recorded for the Port. All three are similar to other species reported previously from the Port suggesting even these species may have been collected previously but misidentified. These preliminary results suggest that the macro-algae diversity of Port Valdez is better known than the intertidal invertebrate diversity. Nevertheless, endemism among the macrophytes is even less than among the invertebrates. None of the 101 species included in the analysis are endemic to Alaska (Table B-9). Of the 102 macrophytes, 24 occur in Australia, 60 in Japan, 85 in Washington, 76 in

Oregon, 70 in California and 43 occur in Great Britain. Including only species that occur in all 6 geographical regions, 21 (21%) of the 101 species analyzed are cryptogenic.

Twenty-nine of the 101 macrophyte species of Port Valdez (Table B-8) can survive unattached (in which plant fragments section off and drift free) (Norton and Mathiesson 1983). This process is unlikely to be noticed without systematic observations and, thus, is probably underestimated in general. Thus, if algal fragments are entrained in ship ballast systems, a broad diversity of macrophyte species in Port Valdez have the potential to be transported in ballast water. However, macro-algae are seldom reported from ballast water (Carlton and Geller, 1993), and accidental introductions are generally associated with fouling on ship hulls (Carlton 1979a) or transplantation of species for aquaculture purposes or for packing (Cohen and Carlton 1995). Norton and Mathiesson (1983) have discovered numerous algal species that can survive in an unattached state for days to weeks, and these species are likely candidates for ballast-water transport. Even the fragments of many taxa can release spores that colonize the new environments or reattach by entangling in other algae where they can mature and reproduce. Small algae or algal fragments could be easily overlooked in ballast water studies, and many of the cryptogenic algal species in Port Valdez could have been transported there in this way.

Discussion

The small proportion of NIS found in Port Valdez differ strikingly from the large proportions of NIS that have been collected in surveys of other ports of the northeast Pacific such as Puget Sound and San Francisco Bay (Carlton 1979, Cohen and Carlton 1995). However, the high incidence of cryptogenic species suggests that the total number of nonindigenous species (invertebrates at 17% and macro-algae species at 21% of total species) in Port Valdez may be underestimated. Carlton (1996) estimates conservatively that 37% of the species of San Francisco Bay, one of the most intensely invaded ecosystems in the world, are cryptogenic. Many cyptogenic species also occur in Alaska, and some will undoubtedly will be recognized as nonindigenous species as their local, regional, and global distributions are better resolved. In addition, the low species overlap between our survey and previous surveys indicates that many more species are likely to occur in Port Valdez than are presently reported, and/or that the biota is changing. The large proportion of new invertebrate species collected in the Port in this small survey (Table B-8) indicates that many unreported taxa may remain. The low overlap among algal species collected in the three separate surveys (Calvin and Lindstrom 1980, Wiegers *et al.* 1996, Chapman and Hansen 1997, and Hansen 1989, respectively) indicates changing diversity over time. These differences are likely due to the seasonality of macro-algae in Alaska. The appearance of all but extremely successful new taxa arriving in the port would be difficult to detect with the background data at hand. A more extensive survey of the biota will correct this problem by providing missing information on the distributions, abundances and diversity of the biota. The number of NIS discovered may increase as the sampling effort increases.

The taxonomic resolution of the local biota is presently insufficient to estimate whether the large numbers of cryptogenic species are likely to be predominantly introduced or native. The tremendous species overlap between the Port Valdez biota and geographically areas remote from Alaskan waters greatly complicates the analysis. Active ballast water introductions of many genetically distinct intraspecific populations from Washington, Oregon and California could occur, for instance, without detection. The presence of genetically distinct, introduced populations of a species, would be a significant effect of ballast traffic. Thus, the genetic composition of some of the more abundant species in the Port, such as *Mytilus trossulus,* should be compared to more southern populations for this possibility. Increasing the resolution of the taxonomic and biogeographical information on the Port Valdez biota may be even more valuable. These data are critical for establishing the numbers of NIS, their ecological significance, their likely origins, timing and mechanisms of introduction. Clear resolution of the local and regional distributions of the biota over time will allow tests of the criteria for introduced species and resolution of many of the presently cryptogenic species.

Non-Indigenous Marine/Estuarine Species with Potential for Introduction to Prince William Sound

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Purpose

To identify non-indigenous species with potential for introduction into Prince William Sound, we reviewed the literature for species which have demonstrated introductions into high latitude ecosystems, especially via ballast water transport. We do not consider the absence of previous introductions of a species (which applies to most species) as an indication of low potential for introduction via ballast water. Furthermore, the introduction and subsequent abundance of a species in a new location provides little information on its expansion in a new geographic area. The green crab expansion on the east coast of North America progressed over many decades but this species is advancing up the west coast of North America in less than a decade. The Asian estuary clam, *Potamocorbula amurensis* has not expanded its range since arriving in San Francisco Bay, yet its populations there are extremely abundant (Carlton *et al.* 1990). However, focusing upon high latitude species which are known to invade, and which have introductions apparently by transport in ballast water, provides an indication of the taxonomic and ecological diversity of species with demonstrated potential for invading an ecosystem such as Prince William Sound.

Methods

We surveyed the literature for known introduced species of the world that have native populations at latitudes greater than 40° north or south and that appear to have a significant potential for transport into Alaska via ballast water traffic. The primary criterion for transport potential is the likelihood of entrainment into and short-term survival in ballast tanks. Spionid polychaetes, decapod crustaceans and molluscs, for instance, produce planktonic larvae, zoea and veligers that are common in ballast water samples (Carlton and Geller 1993, pers. obs.). Peracaridan crustaceans are active swimmers at night as adults (Williams and Bynum 1972) and are also common in ballast water (Carlton and Geller 1993, pers. obs.). Micro-algae are common in plankton of coastal waters. These taxa thus have significant potential for ballast water transport to Port Valdez.

Macro-algae are included even though they are rare in ballast water samples (Carlton and Geller 1993), were not collected in this ballast water survey, and are rarely associated with ballast water dispersal. The principal mechanisms usually associated with accidental introductions of macro-algae are fouling on the hulls of ships or aquaculture related transplantations of species such as oysters and as packing material (Carlton 1979, Carlton and Scanlon 1985, Cohen and Carlton 1995, Trowbridge 1995).

However, fragmentation can function as a mechanism of dispersal in algae (Norton and Mathieson 1983). These fragments can become entangled in suitable locations after discharge and mature. These fragments can also release spores or gametes if they have reproductive bodies which can also lead to colonization of new areas. These fragments could be entrained in ballast water and transported to new locations. Thus, ballast water could be an important mechanism for transporting macro-algae since even a small number of such fragments could be sufficient to colonize new areas. These fragments would easily be missed in a ballast water survey that does not coincide with seasons of maximum fragmentation, principally in the fall.

Results

High Latitude Invertebrate Introductions Outside of Alaska

We include 32 NIS invertebrates in our list of potential ballast water introductions of Alaska (Table B-10). These species represent a wide array of taxonomic and ecological diversity. The potential impact of an invasion by any of these species is difficult to predict for Port Valdez/Prince William Sound.

Invertebrate Introductions from Alaska

Adult red king crab, *Paralithodes camtschatica* from the northern Pacific were successfully introduced to the Barents Sea by the Soviet Russions between 1961 and 1969. The Barents Sea populations was estimated to be at least 500,000 by 1995 (Olsvik 1996). It's environmental impact in the Barents Sea is unknown. The seastar *Asterias amurensis* appears to be native to Alaskan waters and has been introduced into Tasmania, where the Australian government is concerned about its predatory impacts on subtidal communities and food webs. These introductions suggest that important invertebrate species are capable of introduction into high latitude waters equivalent to those in Alaska.

High Latitude Macroalgae Introductions

We include 21 potential macrophytes as potential species for introduction to Alaska via ballast water (Table B-11). As for invertebrates, the diversity of algal species with potential for invasion is high; and potential impacts are difficult to predict.

Macroalgae Introductions from Alaska

Several species of macroalgae from Alaskan waters have been exported and successfully introduced into other cold-water locations of the world (Table B-12), indicating that algal species are capable of introduction into high latitude ecosystems compatible with those in Alaska.

High Latitude Micro-algae Introductions

Harmful phytoplankton blooms in coastal ecosystems are a growing concern world-wide (Taylor, 1987), and phytoplankton are common in ballast water (Carlton and Geller 1993). We include 11 species of micro-algae that may have potential for ballast water introduction into Alaska (Table B-13). All but three of these species already occur in the North Pacific.

Discussion

The pool of foreign species from locations with potential for introduction into Alaskan waters and Prince William Sound is large and diverse. The array of species which already have successfully invaded a cold-temperate and boreal waters around the world represent nearly every major taxonomic group and ecological niche in marine and estuarine waters. Many other species do not have recorded introductions but are also at risk for transport in ballast water. While the odds of successful introduction into Alaska are impossible to calculate accurately for any given species, this diversity of known introductions clearly indicates that a demonstrable risk of invasion occurs in Prince William Sound.

C. Plankton in Segregated Ballast Water

Purpose

To begin an evaluation of the magnitude and diversity of biota transferred to Prince William Sound in the segregated ballast water of oil tankers, we sampled and analyzed ballast water on tankers arriving to the Alyeska Marine Terminal during a 2 week time period, 23 May - 6 June 1997. We selected this time period to correspond to a season of relatively high reproductive activity and plankton abundance in waters of the northern hemisphere. Also, we predicted that conditions in Prince William Sound are most conducive for invasion during the spring/summer, when water temperatures and productivity may most closely match ballast water source regions to the south.

This portion of our study focused entirely on the segregated ballast water of tankers that arrived to Port Valdez. All but one of the vessels that we sampled arrived from domestic ports. This exception, an arrival from Korea, underwent open ocean ballast water exchange, a management practice used by tankers arriving from foreign ports to reduce the abundance of NIS. Here, we discuss our analysis of abundance and diversity for plankton communities associated with segregated ballast water as it usually arrives from domestic and foreign ports. In the next section we discuss ballast water exchange in greater depth.

Methods

We sampled a total of 16 oil tankers arriving to Valdez between 22 May and 5 June 1997. Although we attempted to collect ballast water from every tanker that arrived during this time period, some of the vessels with double bottom tanks could not be sampled easily without disruption of ship operations and modification of our sampling protocol. We therefore sampled only a portion of ships with double bottom tanks, as well as all other tankers arriving with ballast water. For these vessels, we applied our established methods for qualitative and quantitative analysis of biota transported in ballast water, which evolved from methods pioneered by J.T. Carlton (e.g., Carlton and Geller, 1993; Smith et al., 1996). During the sampling period, we adapted our standard methods to ship operations and ballast management practices for tankers at the Valdez Marine Terminal. Our protocol consisted of collecting the following information and samples:

- Ship and ballast management information: Last port of call, number of tanks by type, capacity of tanks, amount of segregated and non-segregated ballast water on board, source(s) of ballast water, age of ballast water, date of arrival, ballast management practices;
- Physical variables of ballast water: Water temperature and salinity were measured (surface and 10m depth) for each tank sampled (as below), collecting ballast water with a Niskin bottle through the Butterworth hatches; oxygen (O_2) concentration was not measured because previous extensive analysis of ballast water tanks in other cargo ships indicated that O_2 concentrations rarely varied and were not appreciably lower than saturation (Smith et al., 1996).
- Biological samples of ballast water: Plankton samples were collected by towing a standard plankton net (80 micron mesh, 30 cm diameter) vertically through the entire height of the water column in each ballast tank; access to ballast tanks was obtained through the Butterworth hatches; at least 2 tanks were sampled for each ship, when ballast water was present and accessible, and two plankton tows were collected for each tank; the height of each plankton tow was measured to the nearest 10 cm.
- Additional observations and opportunistic samples: Upon initiating sampling of ballast tanks, we routinely examined the surface waters to look for large, mobile biota (e.g., fish) and organisms

attached to the sides of tanks; we often take opportunities to collect any such organisms observed, as well as bottom sediments, since these are usually missed in our plankton tows.

- Physical variables of port water: Shipside water temperature and salinity were measured (surface and 10m depth) usually within an hour of sampling ballast water of most vessels; the samples were collected from the berth platform (within 50m of the ship), using a Niskin bottle.

Most plankton samples were returned to the laboratory and examined initially within an hour of collection to assess condition of organisms present. More specifically, we examined each plankton sample with our dissecting microscopes (10-40x), temporarily set-up in the Quanterra chemistry laboratory at the Valdez Marine Terminal, to provide a qualitative assessment of plankton viability, diversity of plankton species, and categorical abundance of major taxonomic groups. Each sample was washed carefully into a finger bowl for examination, and the presence of each morphologically distinct taxonomic group was noted. For each taxon identified, we estimated the categorical abundance as rare (1-10 individuals/sample), common (10-100 individuals), or abundant (>100 individuals). For each taxon, the percent of individuals alive was estimated by evaluating their morphological integrity, movement, and activity; although status of some organisms (e.g., diatoms or eggs) was difficult to discern with confidence during a brief screening. After initial microscopic examination, the plankton samples were preserved in 5% buffered formalin for later identification and enumeration of organisms (as below). Only those plankton samples from last two ships sampled (*Prince William Sound* and *S/R Benecia*) received no live analysis. These samples were preserved directly after collection.

Quantitative analysis of the preserved samples was conducted at the Invasions Biology Laboratory of the Smithsonian Environmental Research Center (SERC) in Edgewater, Maryland. Samples were concentrated on an 80 micron sieve and washed into a finger bowl for identification and enumeration. Each whole sample was examined using a stereo microscope, and all morphologically distinct taxa were identified to the lowest taxonomic level. For many groups that included larval invertebrates (e.g., bivalves, gastropods), identification could not progress beyond gross taxonomic groups; further identification can only be accomplished with intensive culture of larvae to adult stages, upon which taxonomy is based, or the use of molecular probes. For other groups that include adult stages (e.g., copepods), we sought species-level identifications. For those taxa present in abundances < 100 individuals per sample, the total number of individuals were recorded. For abundant taxa (> 100 individuals/sample), samples were split using a Folsom plankton splitter to achieve counts between 10-100 individuals per subsample (usually splits of 1/8 to 1/32). For organisms in split samples, two subsamples were counted.

Throughout this section, we treat ship as the level of analysis and replication, because the source of ballast water and ballast management practices were similar for all ballast tanks within ships, except for those cases of experimental exchange on two vessels (see next section). Although our replicate plankton samples from each of two tanks per ship provide some important information on variation within ships, these are not statistically independent (since the ballast water originates from the same source and time) and mainly provide greater confidence in estimating plankton communities per ship. Here, we use mean density measures that derive from replicate tanks and tows, and we plan to address explicitly within-ship variance during our 2-year study.

Taxonomic identification of plankton has followed a standard protocol. For those groups of organisms that can be identified using the life stages present in ballast water samples (as discussed above), we made an initial identification based upon our current knowledge and literature that was immediately available to us at SERC. For many copepods, we were able to discern genera without much difficulty. Enumeration proceeded based upon the lowest discernible taxonomic units, and representative specimens were vouchered for taxonomic verification and, wherever possible, species-level identification. These voucher specimens were sent to taxonomists at the Smithsonian Institution's

National Museum of Natural History and elsewhere for such verification and identification. To date, only a portion of the voucher specimens have completed this process for full verification and identification, and we report these results where available.

Results

Of the 16 ships sampled during the Pilot Study, 15 carried and discharged segregated ballast water from domestic source regions (Table C-1); the sixteenth ship (*O/S Washington*) was delivering diesel fuel to the port. Most of these vessels and their ballast water came directly from the ports of Long Beach (CA; 2 vessels), San Francisco Bay (CA; 4 vessels), and Puget Sound (WA; 6 vessels). A single vessel and its ballast water came from each Portand (OR) and Cook Inlet (AK). Only two vessels came from outside the west coast of North America: one from Barber's Point, Hawaii and one from Yosu, Korea. Only the vessel from Korea conducted ballast water exchange on all of its tanks, and two of the ships arriving from San Francisco Bay conducted experimental exchange of ballast water in two tanks upon our request.

The 15 ships arriving with segregated ballast water carried an average of 30,311 (s.e. = 3,634) m^3 of segregated water, or 73% of their total capacity (Table C-1). With the exception of those 3 ships that underwent some ballast water exchange, all of the segregated ballast water derived from the last port of call. The average age of ballast water was 5.3 days old, ranging from 1 to 10 days. The average temperature and salinity of this ballast water was 13.0 °C and 27.6 ppt, respectively (Table C-2). There was no evidence of stratification within segregated ballast tanks, as salinity and temperature varied less than 1 unit between measures at the surface and 10m.

The temperature and salinity of surrounding waters at the time of ballast water discharge was similar to that of the sampled ballast water, although Port Valdez exhibited significant temperature and salinity stratification. The temperature ranged from 10-14 oC at the surface and 5-9 oC at 10m depth, and the salinity varied between 20-29 ppt at the surface and 29-32 ppt at 10m (Table C-2). Mean values for field and ballast water measures varied less 5°C and 9ppt (Fig. C-1). Although this represents an overall significant difference for both salinity and temperature among measures (temperature: $F=44.24$, $df=2,38$, $p< 0.001$; salinity: $F=27.22$, $df=2,38$, $p<0.001$), the ballast water did not differ from the surrounding surface waters for temperature or from 10m depth for salinity, using a multiple range test.

To date, we have identified a minimum of 69 different taxonomic groups arriving in the segregated ballast water of tankers from domestic ports (Table C-3). This greatly underestimates the species diversity, as many of the categories include multiple species. Some of this will become evident as we obtain further verification and identification of vouchers, but most larvae cannot be identified to species without culturing them to maturity.

Our quantitative measures of abundance underscore the high level of variation that existed among ships, even over a very short time period. There was as much variation in ships arriving from different domestic ports as those arriving from the same port (Table C-3). For example, the S/R Baton Rouge and Chevron Mississippi each delivered ballast from Anacortes, but exhibited extreme differences in the densities of Owenid Polychaetes (688 vs. 1 per m^3), bivalves (2,897 vs. 109 per m^3), barnacle cyprids and nauplii (1,450 vs. 13 per m^3), and many other taxa.

Some of the differences in plankton abundance among ships may result from variation in age of the ballast water, since survival may have been lower in the older water of the Chevron Mississippi (7 days versus 4 days for the Baton Rouge; Table C-1). There was a significant decline in the density of annelids and molluscs with increasing age of ballast water from domestic, continental sources (Fig. C-2), when excluding the 1-day old water from Cook Inlet (labelled as AK). However, a similar decline in total number of organisms was not apparent for these same ships. Although it is probable that some variation in both density and diversity of plankton among ships also resulted from differences in communities that were entrained in the ballast tanks, the relative contribution of initial conditions and survivorship to observed patterns is unknown at present.

It is evident that species nonindigenous to both Alaska and North America are arriving to Prince William Sound in segregated ballast water from domestic sources. Although we have only verified the species-level identification of a fraction of our vouchers from the vessels, we have identified at least 4 NIS of copepods arriving from domestic ports. In Table C-3, copepods identified originally as *Oithona* spp. include at least two different copepod species (*Limnoithona* sp. and *Oithona davisae*), and *Pseudodiaptomus* spp. includes at least two nonindigenous species (*P. forbesi* and *P. marinus*) (expert identifications by Frank Ferrari and Chad Walter, National Museum of Natural History). These copepods arrived in multiple vessels from San Francisco Bay, which was invaded by these copepods from Asia during the 1980s (Ferrari and Orsi, 1984; Orsi and Walter, 1991). It also appears that some of these NIS may also be arriving to Prince William Sound from other domestic ports. We are now in the process of separating the species complexes for *Oithona* and *Pseudodiaptomus* in each sample, to obtain counts of the individual nonindigenous and native species which are very difficult to discern based upon gross morphology.

The large volume of segregated ballast water and high density of organisms that derive from domestic source ports, which are heavily invaded by NIS, indicate a high probability of releasing NIS into Prince William Sound. For the 13 tankers sampled and analyzed that originate from domestic, continental ports (Table C-4)., these carried:

- An average of 31,755 m^3 of segregated ballast water;
- An average of 7,000 organisms m^3 in the segregated ballast water;
- A minimum diversity of 19.13 species per ship (excluding unresolved species complexes or the identification of most larvae).

For this time period (late May - early June), we estimate approximately 244,000,000 organisms/ship arrive to Prince William Sound in segregated ballast water, multiplying the total volume of segregated ballast by the density of organisms (Table C-4). Roughly 600 tankers arrive to Prince William Sound per year currently (Tom Colby, pers. comm.). Although the density of plankton likely declines from fall to spring, a large transfer of plankton is occurring on an annual basis from domestic ports with tens to hundreds of known species that are not native to North America (Tables B-1 and B-5), as well as native species that are not established in Alaska.

For the single tanker we sampled arriving from a foreign port, the density of coastal organisms was significantly lower than that for domestic traffic (Table C-5, Fig. C-3). More specifically, the densities measured for polychaetes, bivalves, and barnacles were all at least an order of magnitude lower than the average for the 13 ships from domestic ports analyzed to date, falling outside of the 95% confidence interval for these taxa. Copepods were a conspicuous exception to this pattern, as the total number of organisms were not significantly different between domestic and this foreign arrivals. The differences in relative abundances of these taxonomic groups between domestic versus foreign ships may result from open-ocean ballast water exchange, which can reduce densities of coastal organisms and increase densities of oceanic copepods and phytoplankton (see next section). Although the same genera of copepods (e.g., *Oithona*) appear for both domestic and foreign vessels, these include multiple species that may differ between sources. Alternatively, but not mutually exclusive, the low relative abundance of coastal organisms on the foreign arrival may also reflect (a) low initial density and (b) low survivorship on the 10-day voyage - the longest for any of these ships sampled during this Pilot Study.

The only surprise in analysis of the foreign arrival was in the abundance of larval barnacles, gastropods, and bryozoans. Although abundances of these coastal organisms were low compared to that for domestic arrivals (Table C-5), they represent a significant residual population when considering the total volume of segregated ballast water. Assuming a homogenous distribution among ballast tanks, our estimates of 10 organisms per m^3 for each snails, bryozoans, and barnacles as residual coastal plankton suggests that > 250,000 individuals of each species were delivered with 25,000 m^3 of ballast water by this vessel (Table C-1). While these residual organisms may result from very dense initial populations that have undergone severe reductions during the exchange (see next section), some of the larvae may also be generated by resident adult populations within the ballast tanks themselves.

As with the ballast water from the foreign arrival, domestic ballast water from Hawaii on *the OMI Columbia* was relatively depauperate of plankton compared to ballast water of domestic, continental origin (Table C-3). However, unlike the foreign arrival, this vessel did not undergo ballast water exchange. Thus, the low plankton density reflects either a low initial density or poor survivorship, but we cannot distinguish between these potential causes at present.

Discussion

Quantitative analysis of segregated ballast water from the first 15 tankers demonstrates that these ships deliver relatively large inoculations of planktonic organisms to Prince William Sound on both an individual and cumulative basis. Most tankers arrive to Port Valdez from other domestic ports, and carry segregated ballast water of domestic origin from bays and estuaries. The average density of planktonic organisms (7,000 individuals/m^3) that we measured in this ballast water was roughly 10 times higher than previous studies from Coos Bay, Oregon and Chesapeake Bay, Maryland (Carlton and Geller, 1993; Smith et al., 1996). These densities, combined with the volume of ballast water per tanker and the number of tanker visits per year, indicate a large-scale transfer of organisms to Prince William Sound is occurring.

The high densities of planktonic organisms present in our quantitative analyses of domestic ballast water may result from any combination of factors. First, this water was only entrained for a short period of time, 3-7 days. In contrast, the ballast water sampled in other studies was often 7-10 days old, or older, and plankton density has been shown to decline with increasing age of water (Smith et al., 1996; Wonham et al., 1996). Second, the collection of ballast water samples in Valdez coincided with a probable time of peak plankton abundance in the northern hemisphere, whereas previous studies have measured and averaged plankton abundances year-round, including significantly lower densities in winter. Nonetheless, even controlling for seasonal variation, we surmise the annual transfer of organisms is large relative to many port systems within the U.S., based upon magnitude of ballast water involved (e.g., Carlton et al, 1995; Fig. A-4).

Although we detected a significant relationship between voyage duration (age of water) and plankton density among ships in this study, it was not as strong as reported elsewhere. For example, repeated sampling of the same ballast water within a ship has shown significant and exponential mortality over only a few days for densities of both individual taxa and total organisms (Gollasch et al., 1995; Wonham et al., 1996). However, although Smith et al. (1996) found a significant relationship of plankton density and duration of voyage among voyages that included a much larger range of 5-25 days, no relationship was evident for short voyages of 5-10 days. It is possible that initial variation in densities at the time of ballasting may swamp a stronger relationship of plankton survival with duration of voyage over this short range of 3-7 days for domestic voyages. The relative importance of initial communities versus survivorship in the observed variation among ships, compounded by voyages of various durations, can only be tested by comparing ballast water communities in the same tank at the start and finish of each voyage (however, see also below for discussion of repeated measures over a 3-day period).

The pattern of transfer for plankton communities on tankers arriving to Port Valdez from foreign ports is very poorly resolved. Although plankton densities from the first and only tanker sampled to date from a foreign port carried few coastal organisms, relative to arrivals from domestic ports, it is premature to draw any conclusions about the magnitude of transfer or effectiveness of exchange. This set of tankers from domestic ports illustrates the variation in plankton communities of segregated ballast water without exchange. Thus, we must increase the sample size to adequately understand the general patterns and variation associated with ballast transport from foreign ports.

For arrivals from both foreign and domestic ports, our focus thus far has been restricted to planktonic organisms that are sampled by an 80 micron mesh net. However, other organisms are certainly entrained in ballast tanks that deserve serious consideration as potential invaders. First, from our research on ballast water arriving to the Chesapeake Bay, we have documented the presence of mobile macrofauna (adult fish, crabs, shrimp) that are often missed by plankton nets (Smith et al., 1996; Ruiz et al., unpubl. data). We did in fact observe fish swimming in ballast tanks of at least 3 of the tankers sampled, although we only captured one in a plankton tow (Table C-3). Second, sediments in ballast tanks of tankers can apparently accumulate quickly (as they must be removed at regular intervals) and are likely colonized by many benthic organisms that include juvenile and adult invertebrates, as well as resting stages of amoebae to dinoflagellates and various invertebrates (Munson et al., 1996; Smith et al., 1996; Ruiz et al., unpubl. data). Third, small zooplankton and phytoplankton are certainly present that are not included in 80 micron net samples. Fourth, microorganisms such as bacteria and viruses are probably the most abundant organisms in ballast water arriving to Prince William Sound. For example, in the Chesapeake Bay, we routinely measure densities of 10^6 bacteria and 10^7 viruses per ml of ballast water, including many genera of potential pathogens. We plan to assess the abundance and diversity of the first two groups during our 2-year study, and will seek additional opportunities to explore the latter two groups.

We have confirmed 4 NIS (all copepods) arriving in domestic ballast water of tankers to Port Valdez, and many more are surely delivered from both domestic ports and overseas that are not yet established in Alaska, but we cannot yet estimate the potential to invade or potential impacts for most species. The potential to invade is in part a function of physiological or environmental tolerance, which we will measure using laboratory experiments over the next two years with organisms collected from ballast tanks upon arrival to Port Valdez. Invasions are also a function of inoculation density. We will also estimate inoculation density of key taxa on tankers arriving to Port Valdez (from both domestic and foreign ports) over the next 2 years to test for a relationship with invasion success measured through faunal surveys.

D. Experimental Analyses of Ballast Water Management Practices

Purpose

To understand the consequences of various ballast water management practices for the transfer and potential risk of invasion for NIS in Prince William Sound, and more generally, we initiated two types of experimental measures in the Pilot Study. The first type of experiment measured the effect of open-ocean ballast water exchange on the density and diversity of organisms present in segregated ballast tanks, and the second type of experiment measured the effect of time (or voyage duration) on survivorship of organisms in segregated ballast water. As with our other analysis of segregated ballast water, these experiments occurred in May-June 1997, corresponding to a season of relatively high reproductive activity and plankton abundance in waters of the northern hemisphere.

Methods

Ballast Water Exchange Experiments

We measured the effect of ballast water exchange on the abundance and diversity of organisms in ballast tanks by comparing plankton samples from tanks that underwent exchange with those on the same ship that were not exchanged. More specifically, two pairs of wing tanks were selected per vessel that initially contained water ballasted from the same source location, and at the same time. One tank (port or starboard) of each pair was selected in advance for ballast water exchange in open ocean, 500-600 miles offshore, during transit from the source port to Valdez. Upon arrival to Valdez Marine Terminal, each of the four tanks per ship was sampled following the same standard procedure described for segregated ballast water.

The exchange experiments were conducted on two separate vessels: the *S/R Long Beach and S/R Benicia*. Each vessel arrived to Valdez directly from San Francisco Bay, after a 4-6 day voyage (Table C-1). Each vessel carried segregated ballast water from its last port of call, and each vessel exchanged its ballast in the two designated tanks offshore of British Columbia in depths of >2,000m (Table D-1). Although both tankers used the same flow-through method (instead of empty-refill method) for ballast water exchange, the *S/R Benicia* replaced 100% of its tanks' volumes and the *S/R Long Beach* replaced 300% of its tanks' volumes. These differences in the volume of ballast water exchanged were selected to begin measuring the relationship between volume exchanged and replacement of coastal biota.

Quantitative analysis and taxonomic identification of plankton samples followed the same standard procedure as described above for segregated ballast water, and we compared the plankton communities of exchanged versus non-exchanged ballast tanks for each vessel. Again, we treated each ship as the level of replication, estimating a mean density of each taxon by similar management condition (based upon the mean of 2 tanks, using replicate plankton tows within tank to obtain tank-specific estimates) and comparing the percent differences between these means.

Plankton Survivorship Experiment

We measured the effect of time on plankton density, using repeated sampling over multiple days within the same segregated ballast water tanks of one tanker, to estimate the survivorship of various taxa during voyages of different duration. In this initial experiment, we measured changes in two different ballast water tanks aboard the *Arco Juneau*, following its arrival to Port Valdez on 2 June 1997 with 4-day old ballast water from Puget Sound, Washington (Table C-1). For each of three consecutive days, we took 2 replicate plankton samples from each tank, following the standard sampling protocol described for segregated ballast water.

Results

Ballast Water Exchange Experiments

For both the *S/R Long Beach* and *S/R Benecia*, ballast water exchange was associated with reduced densities of coastal organisms compared to non-exchanged tanks on the same vessels (Table D-2). The *S/R Benicia* had noticeably fewer taxa, and often lower densities, in its non-exchanged tanks compared to the *S/R Long Beach*. However, for those taxa present on the *S/R Benecia*, the effect of exchange on entrained biota appeared very similar to that on the *S/R. Long Beach* (Figs. D-1 and D-2), despite a 3-fold difference in the amount of ballast water exchanged on the two vessels. Using the difference in salinity values between exchanged and non-exchanged tanks (Table D-1), we estimated the efficiency of exchange on *the S/R Benecia* as only 60% (= [35ppt -exchanged salinity] / [35ppt - non-exchanged salinity] x 100), whereas that for the *S/R Long Beach* was between 70 - 100%. In contrast, we estimated that the percent reduction of coastal organisms during exchange varied between 70-100% on each ship for those common taxa (>10 individuals / m^3; Fig. D-1), with the lowest percent reduction appearing for the *S/R Long Beach*.

Despite the apparent overlap between tankers in estimated percent reduction for the common coastal taxa, our data suggest that the 300% exchange on the *S/R Long Beach* resulted in somewhat higher reductions of coastal organisms than the 100% exchange on the *S/R Benecia*. All but one of the coastal taxa exhibited reductions > 95% on the *S/R Long Beach*. The exception was the copepod genus *Oithona*, for which measures may be inaccurate due to an oceanic species that could have replaced the coastal congeners, obscuring a higher efficiency. Further taxonomic scrutiny of these samples is now underway. In comparison, three of the five coastal taxa exhibited reductions < 90% for the *S/R Benecia*.

In contrast to coastal plankton, the abundance of some common oceanic taxa exhibited even larger differences, but in the opposite direction, between exchanged versus non-exchanged ballast tanks on these two vessels (Table D-2, Fig. D-2). This was most extreme for discoid diatoms, dinoflagellates in the genus *Ceratium*, and foraminefera, which were 200-10,000 % more abundant in exchanged tanks.

Plankton Survivorship Experiment

Our plankton analysis of segregated ballast for the first sample date indicated that the *Arco Juneau* contained unusually high densities of crab larvae compared to the average across all ships (260 versus 25 individuals/m^3, respectively), while many other taxa appeared to be of roughly average densities (Table C-4). In total, there were approximately 12 taxonomic groups with abundances sufficient to measure changes in density over time, given the level of variation among samples. Interestingly, this includes groups that are known to have relatively high impact as invaders (crabs, clams, and snails), as well as a nonindigenous species of copepod (*Oithona* sp.).

There was no clear pattern of change in the abundance of organisms in each tank of the *Arco Juneua* among the 3 sample dates (approximately 48 hours; Table D-3). Some taxa, such as gastropods exhibited an order-of-magnitude decline, although it is not clear whether this was due to mortality or metamorphosis (and colonizing the bottom of tanks). Most taxa did not exhibit an appreciable change in abundance, given the high level of variation among samples. Also, some taxa increased in abundance, resulting from metamorphosis of early larval stages into later stages (e.g., the copepods *Acartia* sp. and *Limnoithona* sp.) or possibly changes in distribution within the tanks (e.g., crab zoea).

Discussion

Our results from these initial ballast water exchange experiments provide the first measures of exchange efficiency for oil tankers and suggest that this management strategy may be effective at reducing the transfer of nonindigenous species by this class of vessels. The efficacy of exchange is roughly similar to the few existing measures for other types of commercial and military vessels. With chemical and particle tracers, we have estimated a >90% efficiency for exchange on vessels that use an empty-refill method of exchange (Wonham, 1996; Ruiz et al., 1997b). In addition, Rigby et al. (1993) measured exchange efficiencies of 95% for water (using dye tracers) and between 75-95% for phytoplankton on a vessel using a flow-through method of exchange. Finally, a collaborative study between Australian and New Zealand researchers has just made similar measures for one voyage across the Tasman Sea; the results are not yet available, but the outcome appears to be similar in the efficiency measures (J. Hall, pers. comm.).

Despite the general similarity of results among these ballast exchange studies, it is important to recognize that these few studies (usually of only one vessel each) comprise all of the direct measures throughout the world on effectiveness of ballast exchange. This handful of vessels represents a fraction of the classes and designs in operation. Even these existing measures have only been made under a limited range of conditions (e.g., temperature, salinity, etc.) and have usually focused on a small subset of the taxa known to be entrained in ballast tanks (e.g., Carlton and Geller, 1993; Smith et al., 1996).

There is almost certainly variation in the effect of exchange among vessel types, "habitats" within tanks (e.g., bottom vs. surface vs. mid-water), and species which include a broad spectrum of sizes, mobilities, and densities. For example, resting stages of dinoflagellates and other organisms can accumulate on the bottom of ballast tanks, along with sediments and many other organisms, that may be relatively difficult to remove via ballast exchange compared to planktonic organisms, due to density and surface cohesion. This may explain the high densities of toxic dinoflagellates, up to 300 million viable cysts per ship, reported from the bottom sediments of cargo vessels entering Australia (Hallegraeff and Bolsch, 1991, 1992); these resting stages are also found commonly on the bottom of ballast tanks entering U.S. ports (Kelly, 1993; Smith et al., 1996).

At present, the relationships between the amount (or percent) of ballast water exchanged within a tank and (a) reduction of resident biota or (b) influence on invasion success remain unresolved. Our preliminary data suggest a slightly greater reduction of organisms with a 3-fold increase in volume exchanged. There are insufficient data for these tankers, or any other ships, to establish the rate function of decreasing biota with increasing exchange or to define an asymptote in this relationship (below which the return per unit effort diminishes. Intuitively, reduction of number of propagules released will diminish invasion success, and the shape of this function likely varies among species, depending upon particular aspects of biology and ecology. Yet, we cannot now evaluate whether a 90% reduction of organisms during ballast exchange results in a substantial decrease in invasion rate, or whether the residual 1-10 organisms/m3 that we observed (equivalent to 30,000 - 300,000 organisms per ship) represent a significant risk of invasion.). In any case, the effect of density or inoculation size on invasion success is unknown. Thus, although our two experimental measures of ballast exchange on tankers represent a significant advance in present knowledge, they provide only the initial steps in assessing the general patterns, efficiency, or consequences associated with ballast exchange.

Although limited to only one ship, the repeated measures of plankton densities in ballast tanks of the Arco Juneau suggest that survivorship of many taxa is relatively high compared to other studies (e.g., Wonham 1986). The variation among samples is relatively high, but it is clear that densities did not decline exponentially. We cannot draw broader conclusions about comparative survivorship among taxa. Despite some apparent differences, this may result from differences in rates of development, metamorphosis, and colonization of bottom sediments. Also, there may be shifts in the distribution within the water column that affects density estimates. It is clear that the community is highly dynamic and that future work should estimate the relative importance of mortality, benthic colonization, and changes in water column distribution to the overall changes observed.

We plan an ambitious series of experiments over the next two-year study (1997-1999) to extend these initial measures of ballast water exchange efficacy and plankton survivorship in oil tankers.

E. Plankton Characteristics of Non-Segregated Ballast Water Passing Through the Alyeska Ballast Water Treatment Facility

Purpose

Tankers arriving to Port Valdez, Alaska, carry two types of ballast water: segregated ballast water in tanks dedicated to ballast management; and non-segregated ballast water in tanks which are also used to carry oil. The oily ballast water is required to be treated by a shore-side Ballast Water Treatment Facility of the Valdez Marine Terminal to remove and recycle petroleum compounds before the water is discharged into Port Valdez. Plankton communities in non-segregated ballast water passing through the Ballast Water Treatment Facility were sampled to determine if non-indigenous species (NIS) are being released into Port Valdez after treatment. To determine whether the Treatment Facility affected plankton communities in oily ballast water, non-segregated ballast water was sampled and compared before and after the treatment process and following each of the two intermediate stages of treatment. Plankton communities in these samples were characterized for abundance and diversity of morphologically distinct taxa in the same manner as biological sampling of segregated ballast, so that: the plankton communities in segregated and non-segregated ballast water could be compared; effects of each stage of treatment could be assessed for plankton passing through the plant; and the plankton communities entering the Treatment Facility could be compared to the plankton leaving it.

Methods

During May 23-June 4 1997, non-segregated ballast water discharged from 11 tankers arriving to Port Valdez was sampled from the Alyeska Ballast Water Treatment Facility. The First Mate of each ship was interviewed upon arrival to the Valdez Marine Terminal to determine the quantities of segregated and non-segregated ballast water transported during the voyage. Ballast water management practices were recorded on standardized data sheets to characterize ballast quantities by tank, location of water uptake, and date of loading. After recording the ballast water management data, samples of the discharged non-segregated ballast water were obtained from 4 stages of the Treatment Facility to determine abundance, diversity and viability of planktonic organisms at each stage of the process (Fig. E-1):

(1) Chicksan Arms, which connect the tankers to the piping system of the Facility;
(2) 90s Tanks, which receive the ballast water and allow it to settle for a period of ca. 4-12 hours so that oil separated from water by difference in specific gravity, allowing the floating oil fraction to be pumped off and recycled, while the residual oil-water mixture was sent to the second stage of treatment;
(3) Dissolved Air Filtration (DAF) Facility, which injects micro-bubbles of air into the water after adding a polymer, causing petroleum chemicals both to adhere to the foam and to volatilize; and
(4) Biological Treatment (BT) Tanks, which culture petroleum-metabolizing microbes in large outdoor ponds, so that their biological activities may remove remaining oil chemicals before the ballast water is finally discharged into Port Valdez through a diffuser pipe located at about 180 ft depth just offshore of Berth No. 3.

By sampling from the Chicksan arms, plankton of non-segregated ballast water could be characterized for each ship before it entered the treatment process. Wherever possible, the same parcel of water was sampled through time as it passed subsequent stages of the Treatment Facility; however, in most cases water from more than one ship (sometimes from several ships) was co-mingled in the 90s tanks and subsequent stages as standard practice of the Facility. Therefore, co-mingled water was often sampled at the subsequent stages of processing. Although this allowed us to test adequately for organisms at the later stages of the treatment process, effects of the process could not be tracked through the plant for specific parcels of water containing plankton from individual ships.

Sampling was timed to collect water during the middle of its transfer to the next stage. Thus, sampling of the Chicksan Arms began after pumping off the ship was well underway. Sampling the 90s Tanks occurred after the settlement period was completed and water was being transferred to the DAF Facility. Water leaving the DAF Facility was sampled after it had reached a midpoint in its transfer to the BT Tanks. And water from the BT Tanks was sampled at the overflow point as it passed into the discharge pipe.

Water at each point was collected as duplicate samples. For each of the first 3 sampling points, water was collected from spigots designed for the purpose of collecting water samples from the piping system of the Treatment Facility. Water from these spigots was collected in 5 gallon (0.006 m^3) plastic buckets. For each sample of the Chicksan arms, the first 5 buckets of water were discarded to clear residual water and/or oil in the pipe. The next 10 buckets of water (total sample = 50 gallons, 0.06 m^3) were poured through a 80-micron mesh plankton net supported over a drain to catch the filtered water. The plankton retained by the net was collected from the cod-end jar; care was taken to wash any plankton retained on the net into the sample jar. The duplicate samples of concentrated plankton were returned promptly to the Quanterra water chemistry laboratory on the Terminal for further processing. Water from the 90s Tanks and DAF Facility was sampled similarly to the Chicksan Arms, except that water was collected from the DAF Facility without discarding the first 5 buckets before filtering through the plankton net, because it was evident that the piping system had essentially no residual water in it. The Biological Treatment Tanks were sampled with duplicate plankton tows pulled vertically from near the bottom of the tank up through 4.5 m of water column with a net 30 cm in diameter (total sample volume = 0.32 m^3). For each sample at each point, the temperature and salinity of the water were measured in the collecting bucket with a calibrated alcohol thermometer and refractometer, respectively.

Within 30 minutes of delivery to the Quanterra laboratory, the water samples were inspected under a microscope (10-40X zoom magnification) for qualitative assessment of plankton viability, and for initial determination of diversity and "abundance category" of the major taxonomic groups of plankton. Each sample was washed carefully into a finger bowl, and all major morphologically distinct categories of taxa were identified. For each taxon identified, we estimated its categorical abundance as rare (present, but <10 individuals), common (10-100 individuals), and abundant (>100 individuals). For each taxon, the percent of individuals alive was estimated by evaluating their morphological integrity, movement and activity. However, viability of discoid diatoms was often difficult to determine with confidence. After initial microscopic examination of the fresh samples, they were either preserved for later detailed quantitative analysis (if they contained numerous organisms) or discarded (if they contained such low diversity and abundance of organisms that they were characterized adequately when fresh). Preserved samples were fixed in 5% buffered formalin solution and transported back to the Invasions Biology laboratory at the Smithsonian Environmental Research Center in Edgewater, Maryland.

Quantitative analysis of the fixed samples in the SERC laboratory used the following procedure. Samples were concentrated on an 80 micron sieve and washed into a finger bowl. Each whole sample was examined carefully under a stereo microscope (10-40X zoom magnification) and all morphologically distinct categories of taxa were identified. For taxa present in abundances of less than 100 individuals per sample, the number of individuals in the whole sample were counted. For abundant taxa (>100 individuals per sample), the samples were split with a Folsom plankton splitter to achieve counts of 10-100 individuals per subsample (splits of 1/8 to 1/32). For organisms in split samples, two subsamples were counted.

Results

Non-segregated ballast water comprised a mean of 29,692 metric tons (range 0-60,305) per ship or 49% (range 0-75%) of the total ballast water on board oil tankers sampled upon arrival to Port Valdez during the study period (Table E-1). The source locations for the non-segregated ballast water that was sampled included the major port systems and adjacent waters for Valdez tanker traffic: Los Angeles/Long Beach, California; San Francisco Bay, California; Portland, Oregon; Puget Sound, Washington. A few ships also arrived from other locations, including: Barbers Point, Hawaii; and Korea.

A total of 70 samples of non-segregated ballast water was collected from the Ballast Water Treatment Facility). Oily ballast water from 11 tankers was sampled with duplicate collections at the Chicksan Arms (N=22 samples total). Due to co-mingling of water, eight pairs of samples were collected as water departed from the 90s Tanks (N=16 samples total), from water leaving the DAF Facility (N=16 samples total), and from the BT Tanks (N=16 samples total).

Temperature of the water samples averaged 11.5°C (range 10.75-15°C) (Table E-2). Salinity averaged 31 ppt (range 13-35 ppt) (Table E-2). Temperature and salinity of duplicate samples were quite similar, varying less than 1°C and 1 ppt.

A total of 23 taxa/morphological stages of live and dead organisms were identified among the 70 samples (Table E-3). However, the percent occurrence of individual taxa in individual samples ranged from 0-94%. The diversity of taxa changed in the sample from sequential stages of treatment, with highest diversity occurring in Chicksan Arm samples and lowest diversity occurring in the DAF Facility samples. Dominant taxa in the samples were various stages of copepods, discoid diatoms, and nematodes. While the prevalence of discoid diatoms remained relatively constant in occurrence in samples from sequential stages of treatment, the occurrence of copepod stages declined and nematodes increased in occurrence in the sequence of treatment. Overall, the diversity of taxa was low compared to that of segregated ballast samples (see above sections).

Quantitative counts of total organisms (live and dead) in the samples showed similar patterns of low diversity (Table E-4). However, mean density of total organisms increased significantly in the sequential stages of the Treatment Plant (ANOVA, $F_{3,19}$=60.70, P<0.0001): 7 organisms per m^3 in the Chicksan Arm samples; 45 organisms per m^3 in 90s Tank samples; 21 organisms per m^3 in DAF Facility samples; and 285 organisms per m^3 in the BT Tank samples. Numerically dominant taxa were discoid diatoms and nematodes. Compared to densities in the Chicksan Arm samples, discoid diatoms increased 5-8 fold in density at intermediate treatment stages and about 20 fold in the BT Tanks, but variability was high and the differences were not significant (ANOVA, $F_{3,19}$=1.99, P=0.15). Nematodes, which averaged less than 0.02 individuals per m^3 in Chicksan Arm samples, 0.5 individuals per m^3 in 90s Tank samples, and 0.3 individuals per m^3 in DAF Facility samples, increased significantly by 4 orders of magnitude in density to 100 individuals per m^3 in the BT Tank samples (ANOVA, $F_{3,19}$=40.71, P<0.0001). All other taxa combined averaged less than 1 individual per m^3 in the samples from Chicksan Arms, 90s Tanks, and DAF Facility; and in BT Tanks the combination of taxa other than discoid diatoms and nematodes increased significantly to about 50 organisms per m^3 (ANOVA, $F_{3,19}$=12.90, P<0.0001). Thus, these densities also strongly indicate that diversity and abundance of organism in the non-segregated ballast water were low as it passed through the Chicksan Arms and remained relatively low in the treatment stages of the 90s Tanks and DAF Facility. The increase in abundance of organisms occurred mainly in the BT Tanks, and the increase was primarily attributable to discoid diatoms and nematodes.

Viability of organisms in the samples was very low (Figure E-2). For all taxa combined other than nematodes and diatoms, viability was nearly 0%. For nematodes, an average of about 35% of individuals were alive in the BT Tanks. While viability of diatoms was difficult to evaluate, we estimated that about 15% of diatoms in the samples from the Chicksan Arms, 90s Tanks and DAF Tanks were alive; and about 40% of the diatoms in BT Tanks were alive.

Discussion

The diversity and abundance of planktonic organisms in non-segregated ballast water discharged from the tankers were low as water left the ships and entered the Treatment Facility. Plankton diversity and abundance remained low throughout the treatment process. However, discoid diatoms and nematodes increased markedly in abundance in the Biological Treatment stage, probably reflecting a process of culturing of these organisms in the BT Tanks.

The source of these two taxa is not evident. They could originate at low abundances from the non-segregated ballast water, and then increase under conditions of the BT Tanks. Alternatively, these taxa are readily transported by wind and may simply colonize the BT Tanks, where they find favorable conditions for population growth. Despite the increase of these two taxa in the BT Tanks, their apparent viability was low in the samples collected from water on the way to the discharge pipe. The viability of all other planktonic organisms was low at all stages of the treatment process, indicating that conditions in the non-segregated ballast water and/or the Ballast Water Treatment Facility was not favorable for survival of plankton.

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