Density Matters:

Review of Approaches to Setting Organism-Based Ballast Water Discharge Standards

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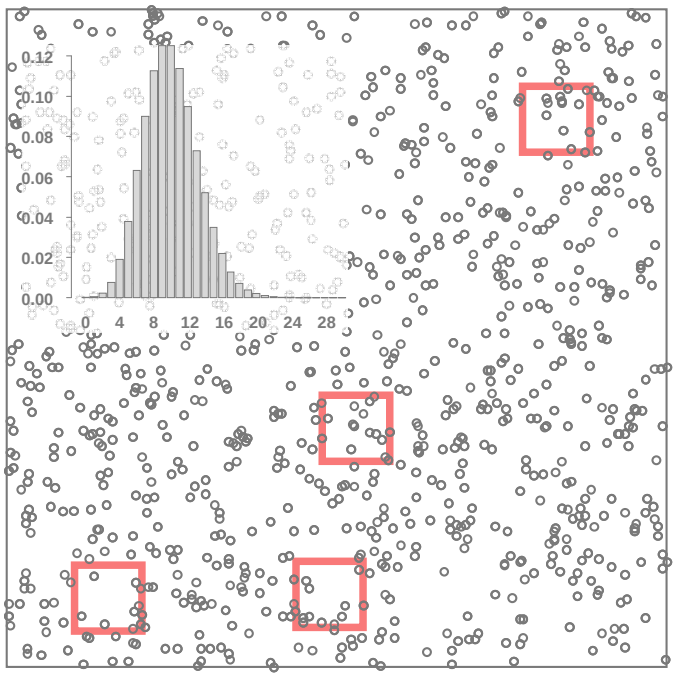

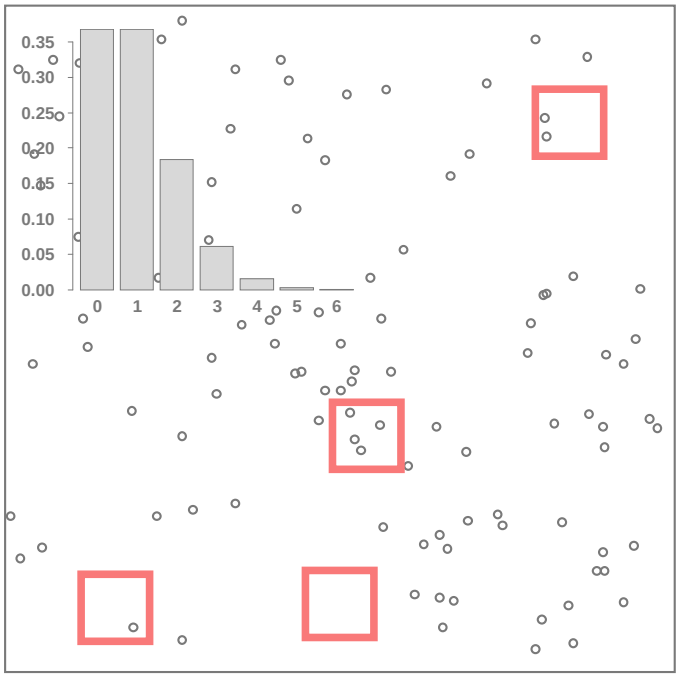

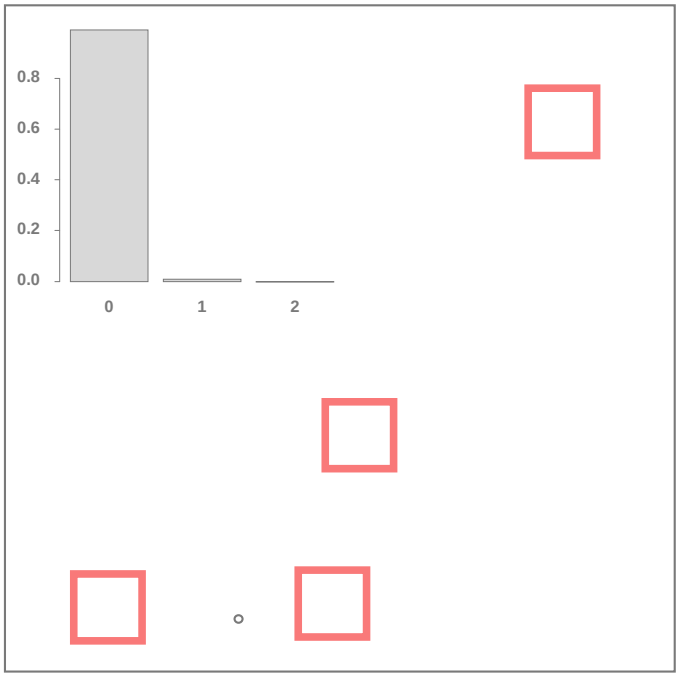

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EXECUTIVE SUMMARY

As one of the major vectors by which invasive species invade the coastal waterbodies and the Great Lakes, ballast water discharges from ocean-going ships are a major environmental threat to the Nation's waters. Recognizing the importance of ballast water as a vector for invasive species on a global scale, in February 2004 the text of an international ballast water treaty was negotiated through the International Maritime Organization (IMO). The IMO has proposed organism-based ballast water discharge standards for different size classes of organisms (Table ES-1). While this represents a major accomplishment, there is concern that the IMO standards are not sufficiently protective. Accordingly, the United States Coast Guard (USCG) just released proposed Phase I (equal to the IMO standards) and Phase II (1000 more stringent then the IMO standards) standards for the waters of the United States (Table ES-1). Additionally, California and other states have implemented or have proposed state standards more stringent than those proposed by the IMO (Table ES-1).

Historically, the EPA had excluded discharges incidental to the normal operation of vessels (including ballast water) from the need to obtain an NPDES permit. However, that exclusion from the NPDES permitting program was successfully challenged in court, and as a result, was vacated by the U.S. District Court for the Northern District of California. In light of the court decision, in December 2008 EPA issued a general NPDES permit (known as the "Vessel General Permit" or "VGP") that contains, among other things, standards for ballast water discharges from non-recreational vessels. The Office of Water currently is undertaking development of organism-based discharge standards for ballast water discharges for use in the future reissuance of the VGP. To help ensure it uses a scientifically sound approach in that effort, the Office of Water is seeking an objective and independent scientific opinion on approaches for deriving these standards and has requested that a National Academy of Sciences (NAS) expert panel evaluate the technical merits of approaches to generating the standards.

To assist the NAS technical review, this report evaluates the potential approaches to generating national organism-based discharge standards. Because of data available, we focus on the >50 micron organism class in our review. On the basis of ecological principles we identified six previous approaches to developing standards and developed a new one, the per capita invasion probability approach, which is described here. The approaches are:

1) Reduction in Propagule Supply Based on Expert Opinion/Management Consensus
2) Zero Detectable Living Organisms
3) Natural Invasion Rates
4) Reaction-Diffusion Models
5) Population Viability Analysis (PVA) Models
6) Per Capita Invasion Probability
7) Experimental Studies

Although not an approach to setting standards per se, sampling issues need to be considered when assessing the practicality of verifying that a discharge standard has been met either in test facilities for purposes of regulatory approval of a treatment system or as part of compliance monitoring of vessel discharges. Additionally, the sampling protocol, particularly the volume of

Table ES-1: Existing or proposed international and national ballast water discharge standards applicable to the waters of the United States and examples of state standards. All organism dimensions are for the "minimum dimension". Standards for the >50 micron and >10 - ≤50 micron classes are for "viable" or "living" organisms. Note that Phase II of the Coast Guard standard can be implemented incrementally. The date for the implementation of the final California standard is 2020. The California standards are instantaneous standards while those for Wisconsin are daily averages. NPRM = Notice of proposed rule making. IMO = International Maritime Organization. cfu = "colony forming units"

Organism Class	IMO D-2 Standard	USCG NPRM Phase I	USCG NPRM Phase II	WI State Standard	CA Interim Standards	CA Final Standard
Organisms >50 microns	<10 per m^3	<10 per m^3	<1 per 100 m^3 (< 0.01 per m^3)	<1 per 10 m^3 (<0.1 per m^3)	"No detectable living organisms"	"Zero detectable living organisms"
Organisms >10 - ≤50 microns	<10 per ml	<10 per ml	<1 per 100 ml (<0.01 per ml)	<1 per 10 ml (<0.1 per ml)	≤1 per 100 ml (<0.01 per ml)	"Zero detectable living organisms"
Organisms ≤10 microns	No standard	No standard	<10^3 bacteria/100 ml <10^4 viruses/100 ml	No standard	< 10^3 bacteria/100 ml < 10^4 viruses/100 ml	"Zero detectable living organisms"
Escherichia coli	<250 cfu per 100 ml	<250 cfu per 100 ml	<126 cfu per 100 ml	<126 cfu per 100 ml	<126 cfu per 100 ml	"Zero detectable living organisms"
Intestinal enterococci	<100 cfu per 100 ml	<100 cfu per 100 ml	<33 cfu per 100 ml	<33 cfu per 100 ml	<33 cfu per 100 ml	"Zero detectable living organisms"
Toxicogenic *Vibrio cholerae* (serotypes O1 and O139)	<1 cfu per 100 ml or 1 cfu per g wet wt. zooplankton	<1 cfu per 100 ml	<1 cfu per 100 ml	No standard	<1 cfu per 100 ml or <1 cfu per g wet zoological sample	"Zero detectable living organisms"

water sampled, defines the actual risk level associated with any standard based on "zero detectable living organisms". Accordingly, we address the statistical considerations of the volume of water that needs to be sampled when estimating the concentrations of organisms in ballast water discharges.

The potential utility and limitations of each of the approaches to generating national discharge standards is briefly discussed below and summarized in Table ES-2.

Reduction in Propagule Supply Based on Expert Opinion/Management Consensus: Several of the proposed discharge standards, including the IMO standards, were based on a combination of expert opinion and management consensus. As used here "expert opinion" refers to technical recommendations for ballast water standards from experts in the areas of invasion biology and related life sciences made without the explicit use of a quantitative invasion model. "Management consensus" is used to capture decisions made utilizing this expert opinion in additions to inputs from experts in other disciplines, such as shipping and engineering, risk managers, as well as state, national, non-governmental organization (NGO), and industry representatives. Thus, management consensus decisions in the "real world" incorporate components of risk assessment, risk management, and lobbying.

The major advantage of expert opinion is that it is possible to address complex issues even with limited data and in the absence of quantitative models, which then can be evaluated in a risk management context. Expert opinion/management consensus was successful in generating the IMO organism-based standards despite the uncertainties in the invasion process itself and the politics inherent in any international treaty. This was a "watershed" accomplishment and a critical step toward reducing new invasions via ballast discharges. The question remains, however, as to whether the IMO standards are sufficiently protective. In part, this question arises because the expert opinion/managerial consensus approach does not allow a rigorous evaluation of the process or how the final decisions were reached. In light of these limitations and the continued increase in our scientific understanding, we recommend that future development of standards rely more heavily on quantitative models than qualitative expert opinion. If expert opinion is used as a major input into the development of national standards, we suggest that a formal process be used to reduce the limitations or biases of expert opinion. Additionally, we suggest that experts in a diverse range of biological, shipping, and engineering fields be consulted.

Zero Detectable Living Organisms: California and other states have adopted or proposed standards with the goal of "zero detectable living organisms" in ballast water discharges. California's standards will be adopted in two phases, with an interim standard of "no detectable living organisms" >50 microns in ballast discharged from ships constructed in 2010 to 2012 and a final standard in 2020 of "no detectable discharge" of zooplankton, phytoplankton, protists, bacteria, or viruses in ballast discharges for ships constructed beginning in 2020 (Table ES-1). The stated rationale for the California standard was "The scientific basis for a standard of discharging no exotic organisms is that exotic organisms, unlike conventional chemical pollutants, can reproduce and increase over time, persist indefinitely and spread over large regions. Thus, very large, widespread and long-term impacts could potentially result from the discharge of a small number of individual organisms — in some cases as few as a single mated

Table ES-2: Comparison of approaches to generate national, organism-based discharge standards for >50 micron organisms in ballast discharges. Assessment is based on current implementation; potential modifications are identified when appropriate. "Reality check" is used to denote that the approach could be used to help evaluate whether predictions from other approaches fall within a realistic range. "Recommend for national standard development" is our assessment of whether the approach should be considered for generating quantitative organism-based discharge standards at the national level.

Approach / Attribute	**Expert Opinion / Management Consensuses**	**Zero Detectable Organisms**	**Natural Invasion Rate**	**Reaction – Diffusion**	**Population Viability Analysis**	**Per Capita Invasion Probabilities**	**Experimental**
Current implementation generates quant. standards	Yes	Yes	Yes (prelim. for CA)	No (volume based)	No (relative comparison)	Yes	No
Apparent range of uncertainty in standard	10,000 fold (range of conc. proposed in IMO negotiations – 0.01 to 100 org m^{-3})	10,000 fold (upper possible conc. w/1L vs. 10 m^3 sample)	100-fold (3 experts) or 10,000-fold (our analysis)	About 200 fold (approx. range in "max. safe release volumes")	<2 fold (w/12 spp. in ballast) to 10,000 fold (multiple voyages – our analysis)	6-fold (among coasts) or 12-fold (w/Great Lakes)	NA
Key data needs for generation of quant. standards	Unknown since decision process not transparent	Development of statistically rigorous sampling protocol	Natural invasion rates in range of ecoregions	Instantaneous population growth rates for a range of taxa	Instantaneous population growth rates & instantaneous variance of the population growth rate for a range of taxa	None	Extensive experimentation w/range of taxa
Assumes linear dose response	Unknown since decision process not transparent	NA (does not assume a dose response)	Yes	No	No	Yes	NA (does not assume a dose response)
Incorporates invasion risk from multiple species in a discharge	Yes?	Yes	Yes	No?	Yes	Yes	No
Incorporates invasion risk from multiple ship discharges	Yes?	Yes	Yes	No	No (modify to incorporate multiple ships?)	Yes	No
Based on historical invasion rates	No	No	Yes	No	No	Yes	No
Based on population dynamics	No	No	No	Yes	Yes	No	Yes

Approach / Attribute	Expert Opinion / Management Consensuses	Zero Detectable Organisms	Natural Invasion Rate	Reaction – Diffusion	Population Viability Analysis	Per Capita Invasion Probabilities	Experimental
Applicable to all taxa and guilds	Yes?	Yes	Yes? (depends on taxa included in analysis)	No (limited to short-lived holoplanktonic species)	Yes? (depends upon which species the pop. data can be obtained)	All	No (limited to taxa adaptable to experiments)
Separates risk assessment from risk management	No	No?	Yes	Yes	Yes	Yes	Yes
Published in peer-reviewed scientific literature	No	No	No	Yes (extensive literature on reaction-diffusion models)	No (extensive literature on PVA models)	No (in process)	Yes (individual experiments)
Recommended for national standard development	No (use as "reality check")	No	No (possible use as "reality check")	No (use as "reality check" for holoplanktonic species)	Yes (if sufficient pop. data available for predictions of actual vs. relative risk)	Yes	No (use as "reality check" and test assumptions)

pair, or in the case of asexually-reproducing species, a single individual." From this perspective, the only biologically safe standard is no discharge of exotic organisms.

While it sounds protective, the zero detectable organism standards suffer from several technical limitations. The first is that unless the entire ballast water discharge is sampled, it has to rely on samples of the discharge, and the degree of protection depends directly on the sampling protocol. If a small volume is used to evaluate whether the discharge meets the standard, the sample may contain zero detectable organisms, but the true concentration of organisms may be quite high. For example, even with a relatively high concentration of 100 organisms m^{-3}, only about 10% of 1 L samples will contain one or more organisms. The general point is that more organisms may be released in ballast discharge using a stringent standard paired with a poor sampling protocol than a more lenient standard paired with a stringent sampling protocol.

The second limitation is the feasibility of developing ballast water treatment systems that can remove all organisms while operating within the constraints of a ship. It is beyond the purview of this report to evaluate ballast water treatment systems; however, we did assess California's review of existing treatment systems. They rated a system as having "potential" if no organisms were detected in a laboratory, land-based, or ship-based test if "at least one replicate in compliance with the performance standards". In other words, a system was considered to have "potential" as long as it did not fail the standard in 100% of the replicates. A reanalysis of the data summarized by California showed that with the exception of one system (SeaKleen®), all systems failed a moderate to high percentage of the replicates and/or they were not tested in all three modes (laboratory, land-based, and shipboard testing). While the results for SeaKleen® are promising, the extent of testing does not meet the minimum IMO requirements under their G8 guidelines and it has not been registered by EPA for use in treating ballast water under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). The general point is that approved treatment systems capable of removing all larger (>50 micron) organisms are not likely to be available in the near term, much less systems that can remove all microbes and viruses.

Because of these various issues, we recommend that the zero detectable discharge standard approach not be used at the national level. If, however, zero detectable standards are considered at the national level, it is critical to define the sampling protocols to be used to verify ballast water treatment systems and in compliance monitoring. Without this information, the meaning of the standard is undefined, making it impossible to assess the actual risk or to enforce it in a scientifically defensible fashion.

<u>Natural Invasion Rates:</u> The natural invasion rate approach was proposed by Dr. Andy Cohen in an August 7, 2005, memo to the California Ballast Water Treatment Standards Committee. As noted by the California State Lands Commission, "this approach is based on numerous assumptions that create a high level of uncertainty for its application to performance standards that will have regulatory impacts." Because of these uncertainties, California did not use the natural invasion rate approach to set their state standards. Nonetheless, the natural invasion rate approach is worth examining since it addresses generating ballast water discharge standards in a novel way.

The rationale for the natural invasion rate approach is that marine/estuarine ecosystems are subject to a very small natural rate of invasion from rare events when species drift or raft across oceans and then become established in new locations. A ballast water discharge standard that resulted in an invasion rate approximately equal to the natural rate would be "reasonably close to the natural rate and possibly within the normal range of variation, and thus would be reasonably protective of the environment." To generate a discharge standard from natural invasion rates, four parameters are needed: 1) natural invasion rate; 2) historical invasion rate from ballast discharges; 3) organism concentrations in ballast water; and 4) speciation rate. Of the four parameters, the major limitation is estimating the natural invasion rate with any degree of certainty; indeed, three invasion experts at the California meeting differed by 100-fold in the their estimates of this rate. Our analysis suggests that the full range of uncertainty could be as much as 10,000 fold in the standards depending upon estimates of ballast water organism concentrations, whether additional taxa are included in the analysis, and whether natural invasions from other areas in the North West Pacific region are considered. Additionally, our analysis of the literature indicated a much greater genetic exchange across the Pacific than suggested by the low estimates of natural invasion rates in the Cohen analysis. Because of these uncertainties, we do not believe the natural invasion approach is suitable for the development of national ballast water discharge standards.

Reaction-Diffusion Models: Reaction-diffusion models predict the concentration of a "substance" that is simultaneously influenced by diffusion which dilutes it and by some type of reaction affecting its concentration. The basic assumptions of this family of models in terms of invasions are: 1) they model continuous time and space; 2) there is local random movement of individuals; and 3) population dynamics are deterministic. When applied to ballast water, the two competing processes are the dilution of the ballast water containing the introduced organisms, which rarefies the populations, and the population growth rate of the organisms in the ballast discharge. If the dilution of the species is too fast, the population goes extinct.

The primary use of reaction-diffusion models in invasion biology has been the theoretical analysis of the pattern of invasion spread of terrestrial invaders. The only published example of a reaction-diffusion model applied to ballast water is that of Drake et al. (2005). Using changes in relative population densities, Drake et al. (2005) predicted the maximum safe volume of ballast water that could be exchanged. Because these predictions are volume based, they can not be used to generate organism-based standards. On a more general level, our analysis indicates that violation of the assumption that species are passively distributed is likely to result in a substantial underestimation of the likelihood of establishment of a species. In particular, benthic species whose larval and/or juvenile phases actively settle out of the water column are much more likely to become established than predicted from dilution models. Thus, in aquatic environments, diffusion models are primarily limited to predicting invasions of small, holoplanktonic organisms, such pelagic copepods. Because of this limitation, diffusion models do not appear to be suitable for generating concentration-based discharge standards applicable to the wide range of taxa found in ballast water.

Population Viability Analysis (PVA) Models: Population viability analysis (PVA) models are a family of population growth models commonly used to predict the extinction probability of endangered species. The basic premise of PVA models is that any population undergoing stochastic growth has a certain probability of going extinct even if it is presently showing positive growth. In general, the smaller the population size, the slower the population growth rate, or the larger the variation in population growth rate, the greater the probability of extinction. When used with nonindigenous species, the objective is to predict either the time to extinction or the probability of extinction for an invader, where extinction is the converse of establishment of a new invader.

A PVA model was used in the USCG Draft Programmatic Environmental Impact Statement (DPEIS), which we reviewed in detail because it is part of the technical analysis used by the USCG in setting their proposed rules and because it is the only study that has used a PVA model to address generating ballast water standards. The key parameters that need to be estimated in the form of the PVA model used in the DPEIS are the instantaneous population growth rate, instantaneous variance of the population growth rate, and the critical population density (the threshold at which the population is considered "extinct"). The strategy taken in the DPEIS was to evaluate different discharge standards by predicting the relative increase in the probability of extinction based on the fractional reductions in the number of organisms per cubic meter of ballast discharge among the different standards. This is a relative approach and as such does not generate organism-based discharge standards. The PVA analysis was conducted both for single species and for multiple species in a ballast discharge, though we contend that the latter, which predicts the risk of any invasion occurring from a discharge, is the more ecologically relevant analysis.

A potentially confusing strategy in the DPEIS was to compare relative decreases in organism concentrations resulting from different standards to the full range of organism concentrations found in unmanaged ballast water and ballast water after exchange. In several cases, the use of these extreme ranges obscured the long-term benefits from reducing organism concentrations. Our reanalysis suggests that the relative reduction in risk was greater than indicated by some of the analyses in the DPEIS. Additionally, values for several of the parameters in the DPEIS were not well justified. For these reasons, and because the analysis was based on relative risks among treatment alternatives, we suggest that the DPEIS analysis should not be used to generate new national standards. However, versions of PVA models that predict actual (vs. relative) risk of invasion may be a viable approach to generating organism-based standards. The limitation to developing such models is the lack of instantaneous population growth rates and the instantaneous variances of the growth rates for a range of taxa. While it may be possible to estimate instantaneous population growth rate through various methods, long-term population studies are needed to estimate the instantaneous variance of the population growth rate. To assist in generating these population vital rates, we identify a number of sources summarizing long-term population studies with marine/estuarine organisms. If new PVA are used to generate new national standards, a comprehensive sensitivity analysis should be conducted. In particular, a range of instantaneous growth rates and instantaneous variances in growth rate should be explored, with the ranges based on an extensive review of population dynamics.

Per Capita Invasion Probability: During the process of generating this synthesis, the authors developed a new method of generating ballast water discharge standards that appears to resolve many of the limitations associated with the other approaches. Based on the general consensus that an increase in propagule supply increases the likelihood of invasion, we developed a "per capita invasion probability" (PCIP) approach to estimate the likelihood of invasion based on historical invasion rates and calculated ballast-associated propagule pressure. The PCIP is the per year probability that an individual non-native propagule discharged from ballast water will become established as a new nonindigenous species in a specified waterbody. Using a linear dose response assumption, the PCIP is calculated from the historical number of potential ballast-mediated invasions in a specified waterbody over a defined time period, the average annual total ballast discharged at that location during this time period, and the estimated organism concentration in the discharged ballast water. Once a PCIP is calculated from historical data, it can be used to predict the rate of new ballast-associated invasions in a waterbody with a projected ballast discharge volume and organism concentration. By altering the organism concentration, it is possible to generate risk scenarios predicting the number of new invaders for different discharge standards.

Historical invasion rates were estimated for the period from 1986 to 2005 and ballast discharge rates were obtained from the National Ballast Information Clearinghouse for 2005 to 2007, which represent the most complete records after mandatory ballast water reporting was instituted. A distribution of organism concentrations in unmanaged ballast water was obtained from published estimates and a simulation conducted to predict a range of possible organism concentrations. Using these inputs, an analysis was conducted for the East, Gulf, and Pacific coasts of the coterminous United States as well as for 17 individual coastal estuaries. In addition, a preliminary analysis was carried out for the Great Lakes. Predictions across individual estuaries showed high variation, possibly due to secondary invasions from other estuaries or ports. The three coast-wide estimates, which eliminate the uncertainty with secondary invasions, showed only a 6-fold variation even with the large differences in environments, donor regions for invaders, and intensity of nonindigenous species surveys. Risk diagrams were then constructed that illustrate the relationship of the likelihood of invasion to organism concentrations and ballast water discharge volumes, which allow risk managers to assess the risk with different discharge standards and safety factors.

As with any method, the per capita invasion rate approach makes a number of assumptions. The approach may underestimate the risk of invasion from asexual and parthenogenic species. It also assumes no change in the invasion potential of new invaders or in the invasibility of a specific waterbody over time. These types of uncertainties can be addressed by risk managers by adding a safety factor to the predictions. Because this approach is based on relatively well-known input values and allows risk managers to generate organism-based standards, we recommend that the per capita invasion probability be considered for the development of national standards.

Experimental Studies: Laboratory and field experiments can be used to quantify the likelihood of invasion under controlled environmental conditions and dosing scenarios. Over the last decade both the number and sophistication of such experiments have increased using both freshwater and marine organisms. However, we conclude that it is impractical to derive discharge standards from laboratory or field experiments because of the: 1) impracticality of

adequate replication to quantify rare events; 2) limitation in the number and types of species than can be experimentally manipulated; and 3) artificiality and simplification of laboratory experiments and, to a lesser extent, field experiments. The real power of these experiments is to advance the theory of propagule supply and to evaluate and parameterize different types of population models.

Statistical Considerations in Estimating Concentrations of Organisms in Ballast Discharges: The stringent discharge standards that have been proposed will require estimating very small concentrations of organisms in ballast water. At these low densities, very large volumes of water must be sampled to find enough organisms to begin to estimate the actual concentration. To assess the requirements for a statistically rigorous sampling protocol, we assumed a random (Poisson) distribution of organisms in a set of samples. We then calculated the upper possible concentration (UPC) of organisms based on one-tailed 95% confidence intervals when zero organisms are detected in a range of sample volumes. For a 1 L sample with no organisms, the UPC was almost 3000 organisms m^{-3} while for a 10 m^3 sample the UPC was 0.3 organisms m^{-3}. Thus, even if no organisms are detected in a very large sample (10 m^3), the actual concentration could be 30 times greater than the USCG Phase II standard of 0.01 m^{-3}. As large as these volumes are, they likely underestimate the volumes needed if the organisms are aggregated or clumped.

Based on our analysis, it is apparent that instituting standardized sampling protocols is a critical component of implementing ballast discharge standards. One possible strategy is to require the large sample sizes required for high statistical power during the validation of treatment systems, in particular with land-based testing facilities. Practical considerations may limit the role of compliance monitoring to detecting gross violations, though detection of poor performing ships would be improved if there was a global repository of compliance test results for individual ships so as to track compliance over time with multiple samples. It is important to note that these analyses assume that the goal of discharge standards is to directly regulate the concentration of organisms in ballast discharges using “average based sampling”. However, if “maximum instantaneous” discharge standards are used, then additional statistical factors must be considered because the results will be very sensitive to the sample number and volume.

I. INTRODUCTION

Henry Lee II

Objectives and Scope of Report:
The U.S. Environmental Protection Agency (EPA) is currently evaluating organism-based ballast water discharge standards (= performance standards or effluent limits), where organism-based standards are based on the concentrations of viable organisms in the discharged ballast water. To support this effort, the objectives of this report are to: 1) summarize approaches that have been used or proposed to establish organism-based ballast water discharge standards that prevent and/or protect aquatic ecosystems from ballast mediated invasions and 2) assess the potential utility and limitations of these methods. While not an original objective, during the process of synthesizing these approaches we developed an approach to the generation of discharge standards that we believe offers a practical alternative (Section VIII: Per Capita Invasion Probabilities). The purpose of our review is to provide the technical background for the U.S. EPA and a National Academy of Sciences (NAS) expert panel to evaluate the technical merits of approaches to generating effluent limits for living organisms in ballast water discharges. The review focuses on organisms $\geq$50 microns, which includes most holoplanktonic organisms (e.g., calanoid copepods), pelagic species such as fishes, and larval stages of benthic organisms. We focus on this size class because most of the theoretical, empirical, and experimental studies have focused on these larger organisms. To the extent possible, we assess whether an approach is potentially applicable to organisms in the 10-50 micron size class, such as phytoplankton and protozoa. The human health endpoints for microbes and viruses are beyond the scope of this document but a brief overview of the approach used to establish microbial ballast water standards is given in Appendix A. We also do not review the efficacy or practicality of various ballast water treatment systems, which have been addressed elsewhere (e.g., Lloyd's Register, 2008; Gregg et al., 2009).

On the basis of ecological principles, we identified seven general approaches to generating organism-based ballast water discharge standards for organisms >50 microns, each of which is evaluated:

1) Reduction in Propagule Supply Based on Expert Opinion/Management Consensus
2) Zero Detectable Living Organisms
3) Natural Invasion Rates
4) Reaction-Diffusion Models
5) Population Viability Analysis (PVA) Models
6) Per Capita Invasion Probability
7) Experimental Studies

Although not an approach to setting standards per se, sampling issues need to be considered when assessing the practicality of verifying that a discharge standard has been met either in test facilities for purposes of regulatory approval of a treatment system or as part of compliance monitoring of vessel discharges. As discussed in Section IV, the sampling protocols define the actual risk levels associated with the "zero detectable living organisms" approach. Accordingly,

we address the statistical considerations of the volume of water that needs to be sampled when estimating the concentrations of organisms in ballast water discharges.

Niche or species distribution models that predict the potential distribution of species based on environmental conditions are not considered. While these models can be used to predict current and future distributions of an individual non-native species after it has invaded (e.g., Peterson and Vieglais, 2001; Herborg et al., 2007; Reusser and Lee, 2008), they do not address the likelihood of invasion via ballast water discharges.

Nonindigenous Species Background:
Introductions of nonindigenous species (NIS)[1], also known as aquatic nuisance species (ANS), are recognized as one of the major environmental stressors in freshwater and marine/estuarine ecosystems. Examples of individual invasive species having deleterious impacts on aquatic systems include the zebra mussel (*Dreissena polymorpha*) in the Great Lakes and other freshwater systems (Drake and Bossenbroek, 2004) and the European green crab (*Carcinus maenas*) on both the Atlantic and Pacific coasts (Carlton et al., 2003). Other indicators of the prevalence of nonindigenous species are their dominance in benthic communities in the San Francisco Estuary (Lee et al., 2003) and the large number of invaders found on the East, Gulf, and Pacific coasts of the United States (e.g., Ruiz et al., 2000; Wonham and Carlton, 2005). It is beyond the scope of this document to review the effects of nonindigenous species on aquatic systems, and the reader is referred to previous reviews on the ecological, human health, and economic impacts of invasive species (e.g., McMichal and Bouma, 2000; Pimentel et al., 2005; Lodge et al., 2006). Additionally, implications of invasions of nonindigenous species on the ability of the EPA to achieve its environmental goals and mandates as of 2000 were reviewed by Lee and Chapman (2001).

Nonindigenous species can potentially invade aquatic systems through a variety of mechanisms (Ruiz and Carlton, 2003). Of these potential routes, shipping, including both ballast water discharges and hull fouling, is the primary vector for biological invasions in the Great Lakes (Duggan et al., 2003) and most marine/estuarine ecosystems (Ruiz and Carlton, 2003; Hewitt et al., 2009) with the notable exception of the Mediterranean where Lessepsian invasions through the Suez canal is the major invasion mechanism (Galil and Zenetos, 2002). In the past century, the increase in shipping traffic as well as the reduced time for transoceanic voyages has increased the number and abundance of nonindigenous species arriving in new environments around the world (Ruiz et al., 1997). This increasing propagule supply appears to have increased the rates of invasions in a number of aquatic ecosystems (e.g., Cohen and Carlton, 1998; Holeck et al., 2004; but see Costello and Solow, 2003 and Drake et al., 2005).

Of the sub-vectors associated with shipping, ballast water is a major source of nonindigenous species in both the Great Lakes and most marine/estuarine environments (Carlton and Geller 1993, Carlton 1996; Fofonoff et al. 2003a; Holeck et al., 2004; Hewitt et al., 2009). When ships

[1] The terms "nonindigenous species" and "non-native species" are used to denote species that were introduced via anthropogenic vectors into a novel location with no specific connotation of ecological, human health, or economic impacts. "Invasive species" is used to denote a nonindigenous species for which there is evidence of an adverse impact on ecological, human health, or economic endpoints. (Executive Order 13112; http://www.invasivespeciesinfo.gov/laws/execorder.shtml).

take on ballast water to compensate for changes in load, vast assemblages of aquatic organisms are collected and then discharged into subsequent ports. This international transfer of organisms is massive – untreated ballast discharges can contain thousands of organisms per cubic meter (Minton et al., 2005) and the total foreign ballast discharged in the United States in 2004-2005 was over 73 million metric tons (Miller et al., 2007; also see National Ballast Information Clearinghouse (NBIC), http://invasions.si.edu/nbic).

IMO Ballast Water Treaty and Proposed USCG Standards Background:
The first approach to managing this vector was to implement mid-ocean ballast water exchange, where ballast was exchanged either through flow-through of the ballast or empty-and-refill. Ballast water exchange, and similar treatment for vessels declaring "No Ballast on Board" (NOBOBs), have been shown to reduce the number of living organisms in ballast water tanks which are adapted to living in both freshwater and coastal/estuarine environments, thereby reducing the risk of invasion (Gray et al., 2007; Locke et al., 1993; McCollin et al., 2007; Ruiz and Reid, 2007; Cordell et al., 2009; see summary of 4.3 in U.S. EPA, 2008a). Though ballast water exchange and saltwater flushing may reduce the risk of invasion, a number of studies have shown that ballast water exchange was not sufficiently effective or consistent in reducing organism concentrations in ballast water especially in coastal/estuarine environments (e.g., Locke et al., 1991; see summary in Section 4.3.2 of USCG, 2008). Additionally, in many cases, it is not safe for vessels to conduct ballast water exchange, given constraints of design and construction.

In response to this concern, national and international efforts began to evaluate other options for managing ballast water discharges. Ultimately, a key decision was made to base ballast water discharge standards on the concentration of organisms in discharged ballast water, rather than on the percentage of ballast water exchanged during mid-ocean exchanges. Through the Marine Environment Protection Committee (MEPC) of the International Maritime Organization (IMO), an international ballast water treaty ("The International Convention for the Control and Management of Ships' Ballast Water and Sediments, 2004") was initiated to reduce the spread of nonindigenous species through ballast water transport (IMO, 2004a). An overview of the IMO international convention can be found in Gollash et al. (2007). A key section of the IMO treaty (Regulation D-2) sets standards for the maximum concentrations of organisms allowed in discharged ballast water based on different size groups of organisms (Table 1). The treaty has not yet entered into force, and while recognized as a major step forward, the IMO standards are considerably above those proposed by the United States (<0.01 organisms m^{-3}; IMO, 2004b). In response to concerns that the IMO standards were not sufficiently protective, a number of states, such as California and Wisconsin, have initiated or passed more stringent discharge limits for ballast water.

The United States Coast Guard (USCG) responded to this concern by preparing a draft Programmatic Environmental Impact Statement on ballast water discharges (USCG, 2008) and then proposing a two phase implementation of discharge standards (USCG, 2009). The proposed USCG Phase I standards are equivalent to the IMO D-2 standards, while Phase II is 1000-fold more stringent (Table 1). Additionally, California and some other states have proposed alternative standards with the ultimate goal of "no detectable" discharges of organisms in ballast water (Table 1).

Table 1: Existing or proposed international and national ballast water discharge standards applicable to the waters of the United States and examples of state standards. All organism dimensions are for the "minimum dimension". Standards for the >50 micron and >10 - ≤50 micron classes are for "viable" or "living" organisms. Note that Phase II of the Coast Guard standard can be implemented incrementally. The date for the implementation of the final California standard is 2020. The California standards are from the California State Lands Commission (2009). The Wisconsin standards are from the Wisconsin Department of Natural Resources (2010). The California standards are instantaneous standards while those for Wisconsin are daily averages. NPRM = Notice of proposed rule making. IMO = International Maritime Organization. cfu = "colony forming units"

Organism Class	IMO D-2 Standard	USCG NPRM Phase I	USCG NPRM Phase II	WI State Standard	CA Interim Standards	CA Final Standard
Organisms >50 microns	<10 per m^3	<10 per m^3	<1 per 100 m^3 (< 0.01 per m^3)	<1 per 10 m^3 (<0.1 per m^3)	"No detectable living organisms"	"Zero detectable living organisms"
Organisms >10 - ≤50 microns	<10 per ml	<10 per ml	<1 per 100 ml (<0.01 per ml)	<1 per 10 ml (<0.1 per ml)	≤1 per 100 ml (<0.01 per ml)	"Zero detectable living organisms"
Organisms ≤10 microns	No standard	No standard	$<10^3$ bacteria/100 ml $<10^4$ viruses/100 ml	No standard	$< 10^3$ bacteria/100 ml $< 10^4$ viruses/100 ml	"Zero detectable living organisms"
Escherichia coli	<250 cfu per 100 ml	<250 cfu per 100 ml	<126 cfu per 100 ml	<126 cfu per 100 ml	<126 cfu per 100 ml	"Zero detectable living organisms"
Intestinal enterococci	<100 cfu per 100 ml	<100 cfu per 100 ml	<33 cfu per 100 ml	<33 cfu per 100 ml	<33 cfu per 100 ml	"Zero detectable living organisms"
Toxicogenic *Vibrio cholerae* (serotypes O1 and O139)	<1 cfu per 100 ml or 1 cfu per g wet wt. zooplankton	<1 cfu per 100 ml	<1 cfu per 100 ml	No standard	<1 cfu per 100 ml or <1 cfu per g wet zoological sample	"Zero detectable living organisms"

U.S. EPA Regulatory Background:
Under the Clean Water Act (CWA), the U.S. EPA has the responsibility for managing the National Pollutant Discharge Elimination System (NPDES) Program (see http://cfpub.epa.gov/npdes/home.cfm?program_id=45). Under the NPDES Program, all facilities that discharge pollutants from any point source into waters of the United States generally are required to obtain an NPDES permit (U.S. EPA, no date; U.S. EPA, 1996; see also CWA § 301(a)). Since the early 1970's, EPA regulations (40 C.F.R. 122.3(a)) had excluded discharges incidental to the normal operation of vessels (including ballast water) from the need to obtain an NPDES permit (see http://www.epa.gov/owow/invasive_species/ballast_water.html). However, that exclusion from the NPDES permitting program was successfully challenged in court, and as a result, was vacated (struck down) by the U.S. District Court for the Northern District of California (*Northwest Envtl Advocates et al.* v. *United States EPA,* No. C 03–05760–SI (December 17, 2008) (vacatur of 40 C.F.R. 122.3(a) as of February 6, 2009)). For a further description of the lawsuit, see also, *Northwest Envtl. Advocates v. EPA*, 537 F.3d 1006 (9th Cir. 2008). In light of the court decision, in December 2008, EPA issued a general NPDES permit (known as the "Vessel General Permit" or "VGP") that contains, among other things, effluent limits for ballast water discharges from non-recreational vessels (U.S. EPA, 2008b; http://cfpub.epa.gov/npdes/home.cfm?program_id=350). Specifically, "The 2008 Vessel General Permit (VGP) regulates discharges incidental to the normal operation of vessels operating in a capacity as a means of transportation. The VGP includes general effluent limits applicable to all discharges; general effluent limits applicable to 26 specific discharge streams; narrative water-quality based effluent limits; inspection, monitoring, recordkeeping, and reporting requirements; and additional requirements applicable to certain vessel types. Recreational vessels as defined in section 502(25) of the Clean Water Act are not subject to this permit. In addition, with the exception of ballast water discharges, non-recreational vessels less than 79 feet (24.08 meters) in length, and all commercial fishing vessels, regardless of length, are not subject to this permit." (U.S. EPA, 2008b)

EPA currently is undertaking development of organism-based effluent limits (discharge standards) for ballast water discharges for use in the future reissuance of the VGP. To help ensure it uses a scientifically sound approach in that effort, EPA is seeking an objective and independent scientific opinion on approaches for deriving these standards. As part of the effort to achieve that objective, this document synthesizes potential approaches to generating organism-based discharge standards. This synthesis is a component of the risk assessment process. As pointed out by EPA's Office of the Science Advisor, "The primary purpose of a risk assessment is to inform the risk manager's decision making process. The primary purpose of a risk assessment is not to make or recommend any particular decisions; rather, it gives the risk manager information to consider along with other pertinent information." (U.S. EPA, 2004a). Accordingly, it is not the purpose of this document to propose specific discharge standards, which is a risk management decision that incorporates additional factors potentially including existing laws, social factors, economics, and feasibility.

Challenges in Setting Organism-Based Discharge Standards:
Predicting the rate of invasion into specific water bodies from ballast water and/or other vectors with a high degree of accuracy is one of the most complex problems in applied ecology. The invasion process can be viewed as a series of stages, ranging from the initial entrainment of a potential invader in its native environment to its establishment and spread in a novel location (e.g., Sakai et al., 2001; Ruiz and Carlton, 2003). Each of these stages is confounded with its own suite of complexities and uncertainties, with examples listed in Table 2 (also see Ruiz et al., 2000). The purpose of listing these complexities/uncertainties is not to imply that the problem is insurmountable but rather to help set reasonable expectations about what is possible in the near-term given the nature of the problem and the state of the science. Realistically, development of discharge standards will require a number of simplifying assumptions, and the 1000 fold range in quantitative standards for the >50 micron class (Table 1) reflects, to a large part, differences in the assumptions made. Therefore, one of the objectives of our review is to identify the major stated and implied assumptions of each of the approaches and whether they tend to under- or overestimate the likelihood of invasion via ballast water. Additionally, with this level of complexity we suggest that it is unrealistic to expect development of highly predictive, mechanistic models in the foreseeable future. However, we believe it is possible to generate standards that are protective of the environment under most situations by making "conservative" assumptions, using safety factors similar to those used in ecological risk assessments for pollutants, and/or by setting the standards based on the upper confidence limits of predictions of invasions. The risk management challenge will be to set standards that balance the level of protection afforded versus their technological feasibility and economic viability.

Table 2: Examples of complexities and uncertainties confounding the prediction of invasion rates via ballast water.

Ports, Ship Routes, and Ballast Water

- Over 100 ports in the United States and its territories, ranging from the sub-Arctic to the tropics, both receive foreign ballast water discharges and donate ballast to foreign ports.
- Over 1000 foreign ports across nearly every biogeographic ecoregion that are potential sources of ballast water discharged in the United States.
- Changes in dominant source regions as trade routes are modified in response to changes in the economies at regional, national, and global scales.
- Ship voyages that span multiple foreign and domestic ports and biogeographic regions.
- Mixing of ballast water from multiple waterbodies and/or biogeographic ecoregions within a single voyage.
- Changes in absolute and relative densities of species within ballast tanks during a voyage.
- Different voyage durations and effects on concentrations within ballast water tanks.
- Among-ship variation in organism concentrations and ballast discharge volumes.

Vectors and Propagules

- Stochasticity in the mix of species originally entrained in ballast water in the source waterbody.
- Species that can invade via multiple vectors (polyvectic invaders).
- Uncertainty in the nature of the propagule dose-response relationship for any particular species at any place or time.
- Secondary invasions into a waterbody from other regional waterbodies.

Invasibility of Recipient Waterbody

- Extent of environmental matching between the donor and recipient regions and uncertainty in how to quantify similarity among environments.
- Seasonal changes in invasibility within a waterbody.
- Long-term changes in invasibility within a waterbody due to environmental trends (e.g., increase in nutrient loading).
- Long-term changes in invasibility within a waterbody due to climate change.
- Differences in invasibility among different waterbodies and biogeographic ecoregions.
- Differences in existing pool of NIS among waterbodies (resulting in whether a specific non-native species represents a “new” invader to the waterbody).

Establishment and Spread of New Invader

- Uncertainty regarding population dynamics at low densities
- Competitive interactions with existing flora and fauna.
- Predator/prey interactions with existing flora and fauna in the invaded ecosystem.
- Feedback between existing NIS and establishment of new NIS (biological meltdown).
- Determining whether a NIS is actually established within a waterbody or ecoregion.

Taxonomy and Sampling Biases

- Underestimation of the extent of invasion within a waterbody because of taxonomic difficulties in identification of new invaders.
- Differences in extent of invasion among waterbodies or regions because of different sampling efforts and/or taxonomic expertise.

II. PROPAGULE SUPPLY DOSE-RESPONSE AND ALLEE EFFECTS

Henry Lee II and Deborah A. Reusser

Two major factors driving the likelihood of invasion are the nature of the propagule supply dose-response relationship and Allee effects on population dynamics. We first discuss Allee effects and then propagule dose-response relationships before addressing specific approaches to setting ballast water standards.

Allee Effects:
Allee effects are reductions in the per capita population growth rate in sparse populations. Such depressions in individual growth rates in rarefied populations may occur due to several potentially interacting mechanisms (see Stephens and Sutherland, 1999; Courchamp et al., 1999; Berec et al., 2006; Kramer et al., 2009) including:

1) Mate limitation (i.e., difficulty in finding a mate at low densities).
2) Genetic inbreeding and loss of heterozygosity.
3) Demographic stochasticity of a small population, which may result from random fluctuations in sex ratios and/or birth rates or environmental perturbations.
4) Increased predation due to less effective or lack of predator swamping.
5) Increased predation due to less effective cooperative defense against predators.
6) Absence or reduction of cooperation in social species, including cooperative feeding.
7) Absence or reduction in habitat alteration that increases fitness of recruits.
8) Increased dispersal away from areas of low density.

A distinction is made between "weak" and "strong" Allee effects (Taylor and Hastings, 2005; Kramer et al., 2009) (Figure 1). A weak Allee effect depresses the per capita growth rate at low densities, but the per capita growth rate remains positive. In comparison, a strong Allee effect results in a negative per capita growth rate below a threshold density, referred to as the "critical density". With deterministic population growth models, the population will go extinct after it falls below its critical density unless there is immigration of new individuals.

Allee effects are predicted to have major impacts on the likelihood that an invader will become established and on its rate of spread (e.g., Lewis and Kareiva, 1993; Drake, 2004; Taylor and Hastings, 2005). Unfortunately, there is limited empirical evidence regarding the role of Allee effects in natural populations, or the densities at which they might occur. In a review of Allee effects with marine organisms, Gascoigne and Lipcius (2004) found little evidence for widespread Allee effects in marine populations, though they did find "suggestive observations" with exploited fish and shellfish populations, as well as with broadcast spawners, a common breeding type among marine/estuarine invaders. In a more complete meta-analysis of natural populations, Kramer et al. (2009) concluded that Allee effects have been documented in a range of taxa, including mollusks, arthropods, and chordates (including three classes of vertebrates). They also concluded that there was evidence that these effects occurred through at least six of the mechanisms listed above.

In assessing the likelihood of Allee effects in populations of non-native species introduced via ballast water discharges, it is important to recognize that the density of any individual species will be very low given the proposed discharge standards (Table 1). This density will be further reduced by dilution of the ballast water in the receiving water. As an upper case example assume: 1) total discharge concentration of zooplankton equal to the IMO standard of 10 organisms m^{-3}; 2) the most abundant species constitutes 50% of the discharged individuals; and 3) a 10-fold dilution of the ballast water when discharged into the receiving water. Under this scenario, the density of the most abundant species is 0.5 organisms m^{-3} in the receiving water. As a lower case scenario, assume: 1) a total zooplankton concentration proposed in Phase II by the USCG of 0.01 organism m^{-3}; 2) the most abundant species constitutes 10% of the discharged individuals; and 3) a 50-fold dilution of the ballast water. With this scenario, the density of the most abundant species is 0.00002 individuals m^{-3}. These two scenarios are for planktonic species, but the low discharge standard concentrations and dilution in the receiving waters would also result in low densities of benthic organisms.

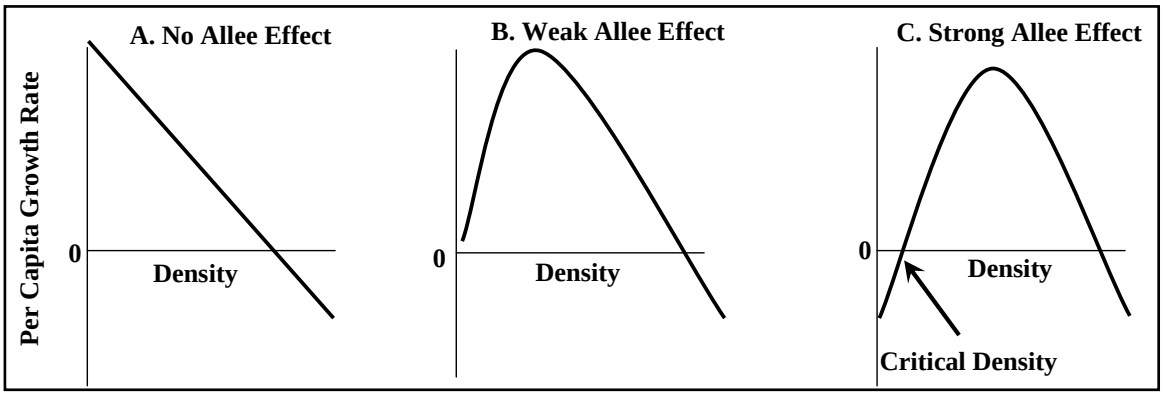

Figure 1: Illustrations of Allee effects on per capita population growth. The per capita rate declines at higher population densities in all three scenarios due to intraspecific interactions such as competition. Scenario A illustrates the case where there is no Allee effect and the per capita rate increases at lower densities. Scenario B illustrates a weak Allee effect where there is a decline in the per capita growth rate at lower population densities but the growth rate does not became negative. Scenario C illustrates a strong Allee effect where the per capita growth rate declines below 0 at population levels below the "critical density". (Modified from Taylor and Hastings, 2005)

Propagule Supply Dose-Response:

The concept that invasion risk decreases with decreasing propagule supply is the fundamental assumption behind the IMO and USCG ballast water performance standards. This assumption is supported by a wide body of empirical, theoretical, and experimental evidence showing that invasion success increases with an increase in propagule supply, either by a higher concentration of organisms in an inoculation and/or by an increase in the frequency of inoculations (e.g., Simberloff, 1989, 2009; Ruiz et al., 2000; Kolar and Lodge, 2001, Ruiz and Carlton, 2003; Lockwood et al., 2005; Johnston et al., 2008). The difficulty is that the nature of the dose-response relationship (Figure 2) is unknown, and "we cannot predict the corresponding change in invasion success in terms of either the type (general shape) of the response or the specific magnitude (slope) of the response" (Ruiz and Carlton, 2003).

While it is not possible to predict the exact shape of the dose-response, two generalities are possible in context of generating discharge standards. First, there is likely to be a saturation dose beyond which any increase in the number of organisms is unlikely to increase invasion success. In most cases, organism concentrations are likely to be well below this saturation value. The second generality is that the linear dose response is likely to be a reasonably protective first approximation for many, if not most, species and densities. At low concentrations, actual invasion probabilities are likely to be lower than that predicted from a linear dose response because of Allee effects and stochastic events. At higher organism concentrations, response slopes that are steeper than the linear model (e.g., curves a and b in Figure 2) imply some type of positive intraspecific facilitation that increases the likelihood establishment. While there are examples of intraspecific facilitation in freshwater and marine/estuarine species (e.g., Leslie, 2005; Nilsson et al., 2006), they appear to be the exception rather than the rule and do not appear to be sufficiently strong to result in invasion probabilities substantially greater than the linear model.

There are, however, important exceptions to the generalization that establishment is unlikely at very low densities. In experimental studies with freshwater cladocerns, Bailey et al. (2009) found that the probability of establishment of the parthenogenic *Daphnia retrocurva* can be >0.1 with an inoculum density of only 1 individual m^{-3}. Simberloff (2009) cites several cases of mammals and insects where release of just a few individuals resulted in establishment of a non-native species. One sobering example is that all of the Indian mongooses (*Herpestes auropunctatus*) in the West Indies were initially derived from just five females and four males. However, Simberloff goes on to cite the "Noah fallacy" proposed by Jim Carlton - that a single breeding pair suffices for an introduction to take hold and spread. While recognizing the cases where a minute propagule supply was responsible for a successful invasion, Simberloff concludes, "if we think probabilistically (and invasion biology is largely a probabilistic science), the metaphor of Noah's fallacy is correct in spirit, because for most if not all species the probability of such an event is small, even vanishingly small, and larger propagule sizes drastically increase the probability of establishment." In terms of setting ballast water discharge standards, the possibility that a single mated female or parthenogenic individual may result in a successful invasion needs to be acknowledged. However, the only standard that would completely eliminate this possibility is the discharge of sterile water, which not even the "zero detectable organism" standard can provide because of the impracticality of collecting a sufficient ballast water sample to detect a zero concentration with a high confidence (see Sections IV and X). Thus, all practical standards contain some risk of invasion, though to varying extents they can substantially reduce this risk as discussed below.

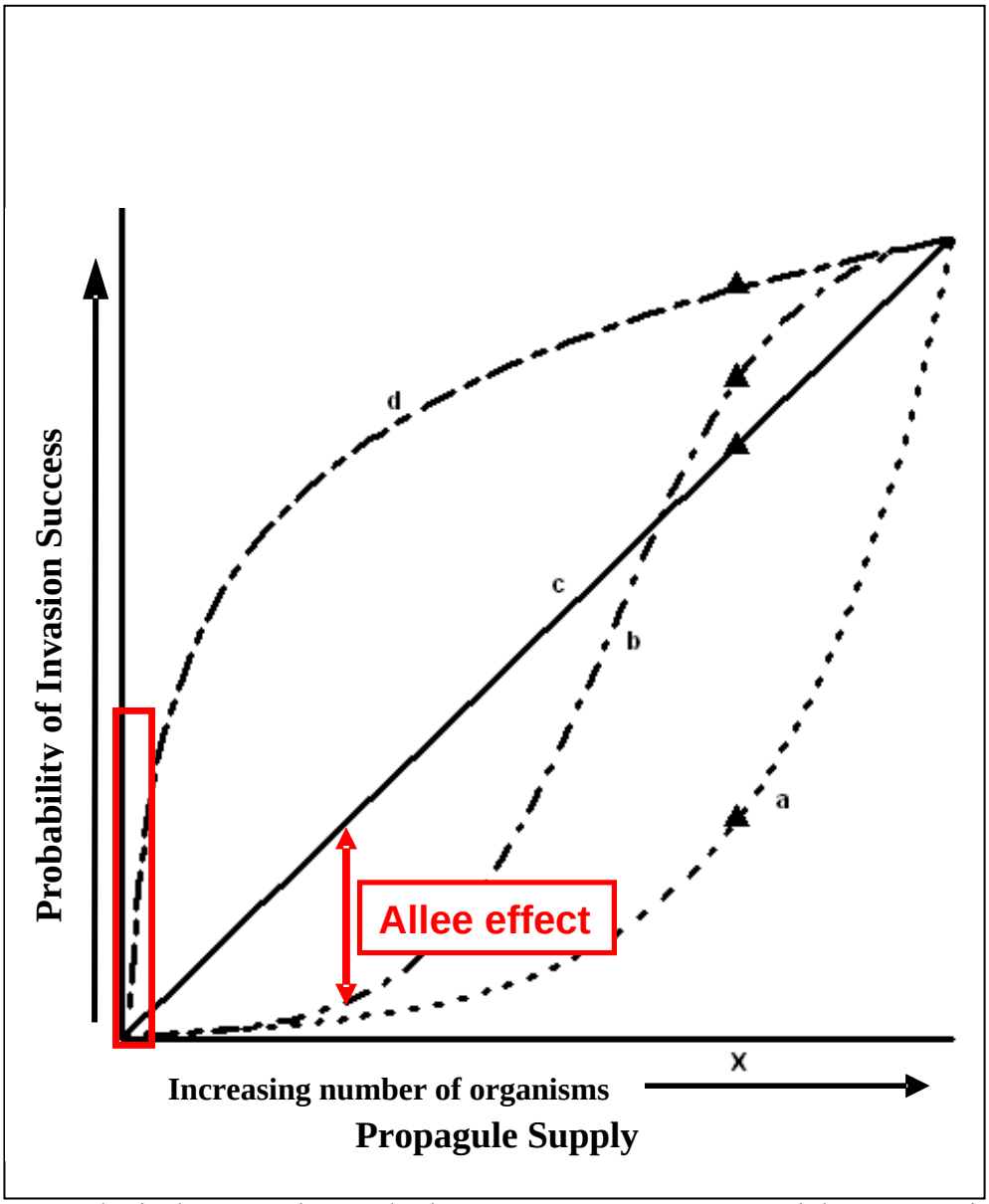

Figure 2: Hypothetical propagule supply dose-response curves. Potential responses include; a) exponential; b) sigmoid; c) linear; and d) logarithmic or hyperbolic. The triangles denote the range in invasion probabilities predicted at a single propagule dose (X) for different response models. The exponential and sigmoid models demonstrate the possible influence of Allee effects on invasion dynamics, and the double-headed arrow shows the reduction in invasion probability, relative to the linear model, due to Allee effects. The box on the left illustrates that the propagule doses associated with the proposed discharge standards (Table 1) are likely to be very low. (Modified from Ruiz and Carlton, 2003).

III. REDUCTION IN PROPAGULE SUPPLY BASED ON EXPERT OPINION/MANAGEMENT CONSENSUS

Henry Lee II

Overview:
Several of the proposed discharge standards were based on a combination of expert opinion and management consensus. As used here "expert opinion" refers to technical recommendations for ballast water standards from experts in the areas of invasion biology and related life sciences made without the explicit use of a quantitative invasion model. "Management consensus" is used to capture decisions made utilizing this expert opinion as well as inputs from experts in other disciplines, such as shipping and engineering, risk managers, as well as state, national, non-governmental organization (NGO), and industry representatives. Thus, management consensus decisions incorporate components of risk assessment, risk management, and lobbying. With homage to G. E. Hutchinson (1965), the consensus process can be characterized as a scientific opera staged by experts in a political theater.

Perhaps the apogee of a consensus driven process was the derivation of the IMO D-2 standards for the >50 micron and 10-50 micron size groups. (IMO standards for microbes and viruses were derived from existing human health criteria and thus had a different origin.) As discussed below, there were several meetings of national and international invasion experts prior to and during the IMO convention evaluating the scientific merits of possible standards. At the treaty convention itself, however, the vast majority of the delegates were not invasion experts and, as is true of any international treaty negotiation, the delegates had a wide range of agendas. Thus, the scientific recommendations from the invasion experts were only one of a suite of factors going into deriving the IMO standards. Additionally, both national and state bills have been drafted (e.g., S. 1578 (110th Congress), "The Ballast Water Management Act of 2007;" see also, accompanying S. Rept. 110-269) with performance standards apparently reflecting an expert opinion/management consensus approach. We suggest that this includes California's "zero detectable discharge" approach as well, though it is discussed separately because of the ambiguity regarding the exact concentrations of the standards.

It is beyond the scope of this document to attempt to decipher the management consensus decision making process at the IMO treaty negotiations or in the derivation of the proposed/existing national bills or state regulations. Rather, we will address the scientific benefits/limitations of an expert opinion approach to generating recommendations for risk managers. We will also summarize the expert opinion process leading up to the IMO convention as an example.

Rationale:
The major advantage of expert opinion is that it is possible to address complex issues even with limited data and in the absence of quantitative models. Additionally, expert opinion can draw upon types of knowledge and experience that is difficult or currently impossible to quantify in a model. Finally, decisions generated using expert opinion inputs provide a focus for guiding future research and management strategies. Because of all the complexities associated with

generating ballast water standards (see Table 2), expert opinion has been a key type of scientific input into the generation of ballast water standards to date.

One of the authors (HL) participated in several technical workshops and IMO meetings where much of the discussion focused on estimating organism concentrations in unexchanged ballast water (see Figure 3 and MEPC, 2003a) and what reduction in these concentrations would be ecologically protective. The basic premise driving the expert decision process was that "less is better" and the greater the reductions in propagule supply, the lower the risk of invasion. No quantitative invasion models were used, though the expert consensus was that the discharge standard needed to be substantially below that normally achieved through ballast water exchange. Additionally, in the U.S.-sponsored workshops, the practicability of achieving the expert-derived concentrations was not explicitly considered. Thus some of the values generated via expert opinion should be viewed as conceptual end-points rather than achievable near-term goals.

To document the expert opinion decision making process, we will briefly summarize the sequence of events that lead up to the IMO standards (Table 1). Some of the first national and international meetings addressing ballast water standards were a pair of workshops held by the U.S. Coast Guard on the East and West coasts in 2001 (USCG, 2002a) and an IMO GloBallast workshop in London also in 2001 (Raaymakers, 2002). Drawing on these workshops, the USCG published the "Advance notice of proposed rulemaking; request for comments" in the Federal Register in March of 2002 (USCG, 2002b). In this notice, they listed four possible standards:

> "S1. Achieve at least 95% removal, kill or inactivation of a representative species from each of six representative taxonomic groups … (GLOBALLAST PROPOSAL ''A''.)
>
> S2. Remove, kill or inactivate all organisms larger than 100 microns in size. (GLOBALLAST PROPOSAL ''B''.)
>
> S3. Remove 99% of all coastal holoplanktonic, meroplanktonic, and demersal zooplankton, inclusive of all life-stages (eggs, larvae, juveniles, and adults). Remove 95% of all photosynthetic organisms … (COAST GUARD WORKSHOP PROPOSAL ''A''.)
>
> S4. Discharge no organisms greater than 50 microns in size, and treat to meet federal criteria for contact recreation … (COAST GUARD WORKSHOP PROPOSAL ''B''.)"

In 2003, an International Workshop on Ballast Water Discharge Standards was hosted by the State Department and the USCG in cooperation with the National Science Foundation (NSF) in Washington, DC. Workshop participants included IMO representatives and technical experts from seven countries (MEPC, 2003a). The synthesis suggestion from this workshop was a standard of <1 organism m^{-3} by 2006 for the >50 micron size group. The workgroup provided two alternative recommendations for >10 – 50 micron organisms by 2015 as either "No detectable viable organisms" or "< 1 org./100 MT" (= 0.01 organisms m^{-3}).

In January of 2004, the United States submitted a recommended discharge standard for zooplankton of <0.01 organisms m^{-3} to the IMO (IMO, 2004b). The rationale for this value was

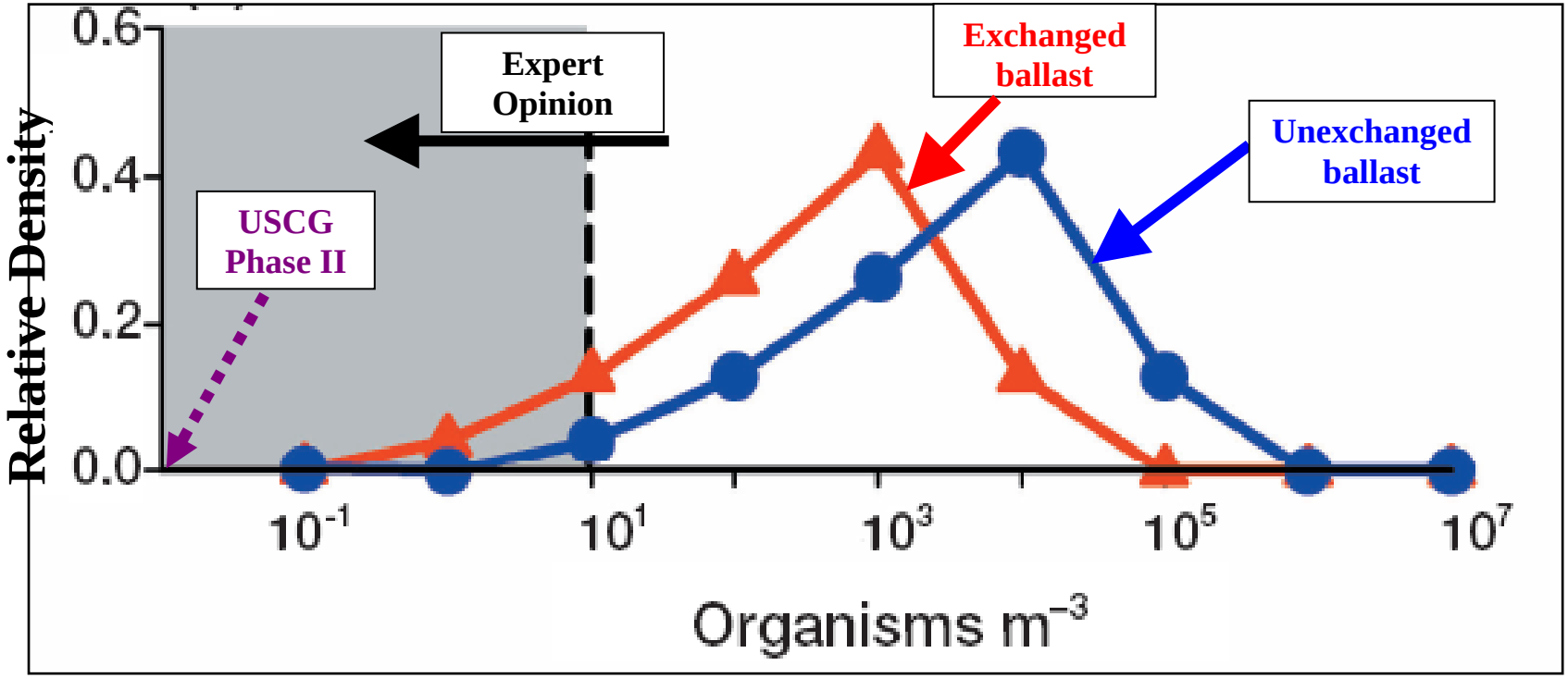

Figure 3: Relative density distributions of zooplankton (>80 micron) in unexchanged ballast water (blue line) and after theoretical ballast water exchange (red line). The dashed line indicates the IMO standard of <10 organisms m^{-3} for >50 micron organisms and the gray area indicates concentrations that meet the IMO standard. The "Expert Opinion" arrow pointing to the left illustrates the basic assumption that lower organism concentrations would reduce invasion risk. The "USCG Phase II" arrow points to the proposed standard of 0.01 organisms m^{-3} for organisms >50 microns. (Modified from Minton et al., 2005).

based on the large number of organisms that would be discharged even at these low concentrations and the additive densities from multiple ship discharges. The example they gave was, "if the ICES figure of an average of 4.6/l [organisms] is used, a vessel with 10,000 m^3 of ballast water would discharge 46,000,000 zooplankton. This vessel would actually be carrying 4,600 zooplankton/m^3, and in the absence of treatment would discharge a total of 46,000,000 zooplankton. Even if treated to reduce the concentration by 4 orders of magnitude [= 0.46 organisms m^{-3}], this single vessel would still potentially discharge 4,600 living organisms into a harbour or estuary. Given that many ports and estuaries receive multiple vessel visits from the same regions over the course of days and weeks, the cumulative number of organisms introduced will be quite a bit larger. For these reasons the United States urges the Conference to adopt less than 0.01/m^3 as the concentration standard for zooplankton."

In February of 2004, the IMO adopted by consensus "The International Convention for the Control and Management of Ships' Ballast Water and Sediments, 2004" (IMO, 2004a) with the specific standards given in Annex D-2 of the Convention as listed in Table 1. There is no discussion in the Convention or the diplomatic conference's Records of Decision of the Plenary as to how these values were settled upon. The reality is that the final IMO standards represent a negotiated compromise between the more stringent standards proposed by the U.S. and some other countries and the less protective standards (100 organisms m^{-3}) proposed by several other countries. Note that the standards in the Convention will enter into force 12 months after ratification by 30 nations, representing 35% of the world shipping tonnage. As of October 2, 2009, 18 countries representing 15.36% of the world's shipping have ratified the treaty. The United States has not yet signed or ratified.

Assumptions and Limitations:

While the IMO standards were developed with input from experts, the numbers ultimately adopted reflect a negotiated outcome among the many countries with differing views that participated in the Diplomatic Conference negotiations. This is not uncommon, as in general, decisions generated through an expert opinion/management consensus approach tend to mix risk assessment, risk management, and politics. This makes it extremely difficult, or impossible, to parse exactly how a decision was made, which in turn, makes it difficult to update the decision based on new information, or even to identify what new scientific information would be required to modify a decision.

A related issue is the lack of documentation on how management consensus decisions are made. One exception is the State of California that provides detailed documentation of their process (http://www.slc.ca.gov/Spec_Pub/MFD/Ballast_Water/Ballast_Water_Default.html).

A general limitation of the expert opinion approach is that it is dependent upon which experts are involved, making it difficult to reproduce the decision making process. A related limitation is that one, or a few, outspoken experts may drive the decision making process at the expense of exploring alternative ideas. This effect can be minimized or eliminated when experts respond via a questionnaire rather than within a workshop setting or with the use of an effective facilitator.

A group of experts restricted to a narrow field of specialization tend to look to at problems through the "lenses" of their particular expertise. Such differences not only reflect differences in the knowledge base of the individuals, but also their values. While such specialization is both appropriate and required, the reality is that additional factors, including economics, feasibility, and timeliness, are likely going to be important considerations when making risk management decisions. Accordingly, we suggest that the best approach would be for experts to provide a range of suggested standards accompanied with details on the ecological risks associated with the different standards. This would allow the risk managers to weight better all the cost-benefits of different standards.

With the exception of certain structured approaches (e.g. Delphi Method), the decision making process is less transparent when it is based on expert opinion in comparison to models. In particular, it is difficult to capture the implicit assumptions that go into an expert's decision, which in turn makes it difficult to assess the validity of the decision or to reproduce the decision making process.

Kuhnert et al. (2009) identified ten "key heuristics, judgments or mental operations that can result in bias when eliciting information from experts" for ecological models. One example was that experts can overestimate the accuracy of their beliefs or underestimate the uncertainty. Another type of bias referred to as "anchoring and adjustment" is the "tendency for groups to anchor around (any) initial estimates and adjust their final estimate from this value irrespective of the initial estimates' accuracy." These authors present several methods to minimize such biases that are summarized below.

Recommendations/Conclusions:

Expert opinion/management consensus was successful in the face of the uncertainties in the invasion process itself and the politics inherent in any international treaty in generating the IMO organism-based discharge standards. This was a "watershed" accomplishment and a critical step toward reducing new invasions via ballast discharges. The question remains, however, as to whether the IMO standards are sufficiently protective. In part, this question arises because the expert opinion/managerial consensus approach does not allow a rigorous evaluation of the process or how the final decisions were reached. In light of these limitations and the continued increase in our scientific understanding, we recommend that future development of standards should rely more heavily on quantitative models. Use of invasion models will not remove the need for expert knowledge (e.g., what models and data to use, etc.) nor will it eliminate the need for risk managers to make difficult decisions weighing environmental risks versus other considerations. However, use of such models will make the process more transparent, more repeatable, and help to generate standards with defined levels of risk and associated uncertainty.

If expert opinion is used as a major input into the development of national standards, we suggest that the recommendations by Kuhnert et al. (2009) be considered to help formalize the process: 1) use multiple experts in a normative setting to avoid overconfidence; 2) pool expert beliefs with a mechanism for separating variability from ignorance; 3) calibrate the expert opinions to ensure that the experts report what they actually mean; 4) incorporate a feedback and comparison process that allows experts to discuss and revise their opinions as well as comparing their assumptions of the methodology with their beliefs; 5) utilize a methodology that allows the

experts to respond in a non-threatening manner; and 6) design the elicitation process around the statistical methods that will be used to analyze the data and investigate the impact of this information on the model outcomes. To this, we add that experts in a diverse range of biological, shipping, and engineering fields be consulted.

IV. ZERO DETECTABLE DISCHARGE

Henry Lee II

Overview:
The state of California and other states have adopted or have proposed standards with the goal of "zero detectable living organisms" in ballast water discharges. In this assessment of zero detectable organisms, we will focus on the California standards as they are the best documented of the efforts (see Dobroski et al., 2009a and http://www.slc.ca.gov/Spec_Pub/MFD/Ballast_Water/Ballast_Water_Default.html). California's standards will be adopted in two-phases, with an interim standard of "no detectable living organisms" >50 microns for ships constructed in 2010 to 2012, depending upon vessel size (Table 1). The proposed final standard is "no detectable discharge" of zooplankton, phytoplankton, protists, bacteria, or viruses for ships constructed beginning in 2020 (Table 1). While California considered the natural invasion rate approach (described in Section V), it was not used in establishing the standards and the "no detectable discharge" standard is a special case of the expert opinion/management consensus approach discussed in Section III.

Rationale:
The principal legal authority for California to set these standards is the Coastal Ecosystems Protection Act of 2006. As noted in the Notice of Proposed Regulatory Action (www.slc.ca.gov/Spec_Pub/MFD/.../Art_4-7_2009_NOPR.doc), "Current California law requires that vessels manage ballast water to reduce the discharge of NIS into California waters. The performance standards for the discharge of ballast water prescribed by Article 4.7 are necessary to minimize the transport of NIS into and throughout the waters of the State of California." (Amendments to Article 4.7 entitled "Performance Standards for the Discharge of Ballast Water for Vessels Operating in California Waters"; Updated August 31, 2009).

The environmental rationale for the zero detectable discharge standard given by the "Report and Recommendation of the California Advisory Panel on Ballast Water Performance Standards" (www.slc.ca.gov/Spec_Pub/MFD/Ballast_Water/Documents/Appendix_A.doc) was, "The scientific basis for a standard of discharging no exotic organisms is that exotic organisms, unlike conventional chemical pollutants, can reproduce and increase over time, persist indefinitely and spread over large regions. Thus, very large, widespread and long-term impacts could potentially result from the discharge of a small number of individual organisms—in some cases as few as a single mated pair, or in the case of asexually-reproducing species, a single individual. From this perspective, the only biologically safe standard is no discharge of exotic organisms." This rationale was also discussed by Cohen (2005 in Appendix 3 of the California Panel Report). Note that this rationale implies that no organisms should be discharged (actual zero discharge), which can be substantially different than the "zero detectable discharge" of the California regulation, which is entirely dependent upon the sampling regime used.

Another rationale for setting stringent standards is to "force" technology development. In a letter to California Lt. Governor Bustamante and the California State Lands Commission, The Ocean Conservancy (TOC) stated, "During the [Ballast Water] Committee's work, TOC sought higher

standards because the existence of such standards – combined with a competitive marketplace for ballast water treatment products – would motivate the rapid development of technology appropriate for meeting them."
(http://www.slc.ca.gov/Spec_Pub/MFD/Ballast_Water/Documents/Appendix_C.pdf).

California states that their ballast water standards should be interpreted as instantaneous standards rather than averages over the entire discharge. As a result, if any individual sample from a discharge exceeds any of the standards, this would be grounds for finding non-compliance and it is unnecessary to show non-compliance in multiple samples or in mean values (Dobroski et al., 2009a). California justifies the instantaneous standards based on the 2005 draft MEPC G2 sampling guidelines. However, the final MEPC G2 guidelines were subsequently revised to instead suggest an average method (J. Lishman, pers. comm., November, 2009). It is worth noting that while California cites the earlier G2 guidelines as a justification, any zero based standard is inherently an instantaneous standard since once any sample contains an organism the discharge has failed the standard.

Assumptions and Limitations:
A true zero discharge of all size groups eliminates the risk of invasion via ballast water, assuming perfect compliance and no equipment failures. However, perfect compliance and no failure is practically, if not theoretically, impossible, particularly for microbiological organisms unless ballast water is discharged into a land-based treatment facility or ships are redesigned to eliminate the need to discharge ballast water (see Gregg et al., 2009 for discussion of ballast-free ships). Thus, ignoring all the other issues mentioned below, there will still be some level of risk associated with the proposed California standards resulting from equipment and human failure.

A major limitation with "zero detectable" discharge standards is that they are undefined in the absence of a quantitative sampling protocol, and depending upon the sampling protocol, the actual risk may be considerably higher than that associated with other standards. For example, as discussed in Section X, when zero organisms are detected in a 1 liter sample, the actual ballast water concentration could be as high as 3,000 organisms m^{-3} based on the 95% confidence interval (1-tailed). For a 10 m^3 sample (=2641 gallons) with no detected organisms, the ballast water concentration could be as high as 0.3 organisms m^{-3} (see Table 15). Thus, with a small (1 liter) sample with no organisms, the actual concentration could be as much as 300-fold higher than the IMO standard, while for a 10,000-fold larger sample the actual concentration could be 30-fold higher than the proposed USCG Phase II standard. Without a statistically-robust sampling protocol to quantify the detection limits during both testing of treatment systems and compliance monitoring, it is impossible to conclude that the zero detectable discharge standard is actually any more stringent than the other standards.

These problems with a zero detectable discharge standard for ballast water have been previously identified by various expert panels. For example, the summary of the 2003 "International Workshop on Ballast Water Discharge Standards" (MEPC, 2003a) included the following two points:

> "Experience following passage of the United States Clean Water Act showed that an absolute standard of "zero discharge" was an unrealistic/unworkable concept – detection limits have always been a problem."

> "Setting a specific detection limit means that an actual concentration will be allowed for the testing protocol, therefore it might be better to specify the (acceptable) concentration as determined by the selected test protocols, rather than to use the expression "zero detectable" in the standard. This concept could be specified in the testing protocol guidelines."

Other major challenges are whether the putative low concentration associated with the "zero detectable" standard is economically viable and/or technologically feasible given the constraints of ship operations. These challenges are likely to be especially acute meeting the standards for microbiological organisms. It is beyond the scope of this document to conduct a review of the technical approaches to ballast water treatment methodologies but it is worth noting the scope of the challenges such systems face. Large tankers can carry in excess of 200,000 m^3 of ballast and the rates of ballasting and deballasting can be as high as 20,000 m^3 hr^{-1} (NRC, 1996; Wright, 2007). Treatment systems must fit within the confined spaces available on ships, continue to operate under the demanding conditions of ocean voyages, not be so complex that the crew can not operate them effectively, and pose no risk to the crew or environment. As pointed out by Gregg et al. (2009), "Effectively eliminating the risk of ballast water mediated invasions still remains a monumental technological and economical challenge."

In evaluating the practicality of the "zero detectable" standard, the State of California conducted a review of ballast water treatment systems using available information (Dobroski et al., 2009a, b) in terms of whether they presently meet the California standards or showed the potential of meeting them. They conducted a review for the >50 micron group, 10 - 50 micron size group, *E. coli*, intestinal *Enterococci*, and *Vibrio chlorae*. They initially included viruses, but concluded that there was no widely accepted technique or proxy for enumerating them and dropped them from the evaluation. They noted that their review was hampered by the lack of detailed testing data, inconsistency in the testing methodologies, and differences in the scale and location in which the tests were conducted (e.g., lab based vs. land based vs. shipboard). They also noted that much of the available data have not been subject to a review by an independent scientific organization.

Based on the January 2009 review (Dobroski et al., 2009a), 15 systems were considered to have the potential to meet the California standard of zero detectable discharge for the >50 micron size class (see Table 3). In an October 2009 update to this review (Dobroski et al., 2009b), the MH Systems was also listed as having potential. However, it is critical to note that California listed a system as having the "potential" to meet their standards if it had "at least one replicate in compliance with the performance standards" (Dobroski et al., 2009a). In other words, a system was considered to have "potential" as long as it did not fail the standard in 100% of the replicates, which is the least stringent criterion possible. Failure of these systems could be due to several factors, such as mechanical problems, inherent variability in the efficacy of the system, the system working in one test mode but not another (e.g., working in a land-based testing facility but failing on a ship), or statistical variation in the results based on a inadequate sampling regime. Regardless of the cause for failures, the California criterion for "potential" is much less stringent than the criteria to meet approval through the Marine Environment Protection Committee (MEPC) of the IMO. The G8 MEPC Guidelines for Approval of Ballast Water Management Systems provide that to obtain type-approval, a system needs to satisfy the IMO Regulation D-2 standards in three consecutive valid shipboard test cycles and five consecutive valid land-based cycles (MEPC, 2008a).

To gain a better insight into the performance of these treatment systems, we analyzed their failure rates among replicate trials as presented in Appendix B1 of Dobroski et al. (2009a) (Table 3). It was not possible to conduct this analysis with the more limited data presented in the October update (Dobroski et al., 2009b), so the MH Systems treatment system is not included. With the exception of SeaKleen®, all systems failed a moderate to high percentage of the replicates and/or they were not tested in all three modes (laboratory, land-based, and shipboard testing). While the results for SeaKleen® are promising, they are only based on one laboratory test, two land-based tests, and one ship-based test, which do not meet the minimum G8 requirements mentioned above. Additionally, there are concerns over residual toxicity from SeaKleen®, as well as its effectiveness against bacteria, resistant resting stages, and sediment-dwelling organisms (Gregg et al., 2009). SeaKleen® has not been registered by EPA for use in treating ballast water under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), and thus is not currently approved for such use in the United States. Furthermore, it has not received final type approval under G8 requirements or final approval from IMO for use as an active substance. We identify these issues not criticize SeaKleen® but to point out the gap between identifying a system with "potential" and having an approved system. Finally, our analysis is based only on the percentage failure as reported in Dobroski et al. (2009a); it would take a detailed statistical review of the sampling protocols used in testing these systems to ascertain the statistical confidence of detecting a zero discharge (see Section X) and thus what level of confidence to place in the reported system successes.

Dobroski et al. (2009a) concluded based on their review that "at least two treatment systems have demonstrated the potential to comply with California's performance standards. Many additional systems are close to completing system performance verification testing and will soon have data available for review. Commission staff expects that before 2010 several systems will be ready to meet California standards." Our assessment is not as optimistic, especially since it is now 2010 and no systems meeting the California standards have been approved by IMO. While predictions about technology development are littered with embarrassing prognoses (Ken Olson founder of DEC computers: *"There is no reason anyone would want a computer in their home."*), our view is that it is unlikely that any practical ballast water treatment system will approach an actual zero discharge of organisms, defined here as concentrations substantially less than the USCG Phase II standards, in the near term, in particular for microbes and viruses. Of course it is possible to achieve a "zero detectable" standard simply by using an inadequate sampling protocol with insufficient statistical power. Again, this emphasizes the need to have quantitative sampling protocols with adequate sample volumes and replication to quantitatively assess these systems.

Recommendations/Conclusions:
As discussed above, "zero detectable" discharge standards are undefined in the absence of a quantitative sampling protocol, and depending on the sampling protocol, the actual risk may be considerable higher than that associated with other standards. Therefore, we recommend that it not be used at the national level as an approach for deriving environmentally protective limits on concentrations of living organisms in ballast water.

If zero detectable discharge standards are considered as a possible approach for national standards, part of the technical analysis should include an assessment of the relative risk associated with the zero detectable discharge standards versus risk associated with the USCG Phase II standards

Table 3: Failure rates of the systems listed as having the "potential" to achieve the California discharge standard for >50 micron organisms (Dobroski et al., 2009a). Failure is defined as detection of organisms in a test sample. The failure rate was calculated as the percent of the replicate tests that failed the criterion using the data in Appendix B1 of Dobroski et al. (2009a). The Hitachi system was listed as achieving the California standard in Table VI-1 of Dobroski et al. (2009a) but no results were given in Appendix B1. The RWO Marine Water Tech. was tested with *Artemia* cysts only. Systems that had a 100% failure rate for the >50 micron organisms may have passed the standard for another size group. The status of the type and active substance approval are from Dobroski et al., 2009a, Dobroski et al., 2009b, and Gregg et al. (2009). The commercial names of the systems are given in parentheses. NT = not tested. NA = approval for active substance not applicable. Note that the number of samples or sample volume used in the validation testing was not reported by Dobroski et al. (2009a, b).

System	**Failure Rate for Laboratory Testing (# Tests)**	**Failure Rate for Land-based Testing (# Tests)**	**Failure Rate for Shipboard Testing (# Tests)**	**Type Approval[1]**	**Active Substance Approval[2]**
Alfa Laval (PureBallast)	100% (1)	25% (12)	40% (5)	Yes	Final
Ecochlor (Ecochlor BW Treatment System)	0% (2)	NT	0% (1)	No	Basic
Greenship (Sedinox)	NT	0% (5)	NT	No	Final
Hamann Evonik Degussa (SEDNA)*	0% (2)	16% (19)	20% (5)	Yes (Germany)	Final
Hitachi (Clearballast)	?	?	?	No	Final
Hyde Marine (Hyde Guardian)	100% (1)	50% (4)	100% (4)	Yes	Basic
MARENCO	33% (3)	NT	NT	No	NA
Mitsui Engineering (Special Pipe)	NT	100% (4)	100% (1)	No	Basic
NEI (Venturi Oxygen Stripping (VOS))	NT	80% (5)	75% (4)	Yes (Liberia)	NA
Nutech 03 Inc. (SCX 2000, Mark III)	100% (3)	67% (3)	33% (3)	No	No

System	Failure Rate for Laboratory Testing (# Tests)	Failure Rate for Land-based Testing (# Tests)	Failure Rate for Shipboard Testing (# Tests)	Type Approval	Active Substance Approval
OceanSaver (OceanSaver BWMS)	NT	86% (14)	67% (12)	Yes (Norway)	Final
OptiMarin (OptiMarin Ballast System)	100% (1)	38% (13)	100% (7)	No	NA
RWO Marine Water Tech. (CleanBallast)	0% (1)	NT	NT	No	Final
SeaKleen	0% (1)	0% (2)	0% (1)	No	No
Severn Trent DeNora (BalPure)	NT	40% (5)	NT	No	No
TechCross (Electro-Cleen)	NT	27% (11)	0% (3)	Yes (Korea)	Yes

1) Type Approval: Type approval is granted by Flags states following successful equipment performance during land based and ship board testing in accordance with the IMO G8 Guidelines to verify treatment system efficacy, safety, design, construction, operation, and function (MEPC, 2008a).

2) Active Substance Approval: Active Substance Approval is granted by MEPC, not the Flag state, and is required by Convention Regulation D-3 for those treatment systems that make use of Active Substances (biocides) to comply with the Convention's Regulation D-2 standards. Active Substance Approval relates to the environmental and safety aspects of the system's use of biocides and is conducted in accordance with the IMO G9 Guidelines (MEPC, 2008b). Approval typically is given in 2 stages, first "basic" approval, and then "final" approval. Systems subject to Active Substance Approval also must undergo type-approval testing by Flag states under the IMO G8 Guidelines.

*****: Harmann Ag ceased work on its ballast water treatment system after it was discovered that the biocide Perclean was more toxic in cold waters and in freshwater than initially assumed (Lloyd's List, February 9, 2010).

(USCG, 2009) and/or standards derived from the per capita invasion approach (Section VIII). This analysis should include an assessment of the number and impacts of historic invaders in coastal waters and the Great Lakes that the USCG or PCIP standards might not have prevented, in particular asexual and parthenogenic invaders, in comparison to the likelihood that the zero detectable standards would have prevented their introduction, assuming some practical sampling protocol for the zero detectable standards.

If zero detectable standards are considered at the national level, it is critical to define all aspects of the sampling protocols for verification of ballast water treatment systems and for compliance monitoring. (see Section X).

V. NATURAL INVASION RATES

Henry Lee II

Overview:
The natural invasion rate approach was proposed by Dr. Andy Cohen (San Francisco Estuary Institute) in an August 7, 2005, memo to the California Ballast Water Treatment Standards Committee, with a follow-up addendum with corrected values. The memo and addendum are Appendix 5 and 6, respectively, in Appendix A of Falkner et al. (2006). As noted by the California State Lands Commission (Falkner et al., 2006, page 21), "this approach is based on numerous assumptions that create a high level of uncertainty for its application to performance standards that will have regulatory impacts. … The proposed approach had been neither published nor peer reviewed and was thus not known or widely accepted by the scientific community." Because of these uncertainties, they adopted the zero detectable organism approach instead (see Section IV). Even though not adopted by California, the natural invasion rate approach is worth examining since it addresses generating ballast water discharge standards in a novel way.

Rationale:
The rationale for the natural invasion rate approach is that marine/estuarine ecosystems are subject to a very small natural rate of invasion from rare events when species drift or raft across oceans and then become established in new locations. A ballast water discharge standard that resulted in an invasion rate approximately equal to the natural rate would essentially double the natural invasion rate but would be "reasonably close to the natural rate and possibly within the normal range of variation, and thus would be reasonably protective of the environment" (Cohen, 2005 in Falkner et al., 2006). Cohen further assumed that such a standard would be "reasonably protective of the various environmental, recreational and economic beneficial uses of California's waters."

Calculation of Discharge Standard Based on Natural Invasion Rates:
As discussed in Appendices 5 and 6 of Falkner et al. (2006), development of a discharge standard (= concentration standard) resulting in a ballast water invasion rate approximately equal to the natural rate requires that the concentration of organisms in ballast discharges needs to be reduced "by the ratio between the natural invasion rate and the invasion rate due to the discharge of untreated and unexchanged ballast water." This ratio is referred to as the Reduction Factor:

Equation 1: $$\text{Reduction Factor} = \frac{\text{Natural invasion rate}}{\text{Invasion rate due to untreated and unexchanged BW}}$$

Where:
BW = ballast water

Cohen assumes a linear dose-response for propagule pressure (Fig. 2, line c) so that the ballast water standard that would result in an invasion rate approximately equal to the natural rate of invasion is:

Equation 2: Discharge Standard = Concentration of organisms in untreated & unexchanged BW X Reduction Factor

Therefore, to calculate the discharge standard, three values are needed: 1) organism concentrations in untreated ballast water; 2) a rate of invasion resulting from discharge of untreated ballast water; and 3) a natural invasion rate. For organism concentrations, Cohen assumes concentrations in untreated ballast water to be on the order of 10^2-10^3 per m^3 for organisms >50 microns, 10-10^2 per mL for organisms between 10 and 50 microns, and 10^8-10^9 per 100 mL for organisms <10 microns.

For the rate of invasion from ballast water, Cohen focused on the San Francisco Estuary for which he has extensive experience (e.g., Cohen and Carlton, 1995; Cohen and Carlton, 1998; Cohen 2005). From 1961 to 1995, which is prior to the California and USCG regulations requiring mid-ocean ballast water exchange, he estimated the rate of invasion as 3.7 species per year, with an increase to 5.2 species per year during 1991 to 1995 (Cohen and Carlton, 1998). The fraction of these invaders assumed to have been introduced via ballast water discharges was 0.7-1.7 species per year for the period 1961 to 1995 and 1.6-3.2 species year for the period 1991 to 1995. Cohen makes the argument that these numbers underestimate the actual rate of invasion because of: 1) new invaders that have not yet been collected; 2) new invaders that have not yet been identified as exotic species (e.g., misidentified as a native species); and 3) species that have been collected but whose invasion status is uncertain (cryptogenic species). Cohen estimates that these factors could increase the invasion rate by 50 to 100%. Cohen is correct in asserting that these factors are likely to result in an underestimation of the true invasion rate (e.g., Ruiz et al., 2000; Carlton, 2009), and while the actual extent of underestimation is not known, increasing the observed invasion rate by 50% to 100% does not seem unreasonable.

These rates only capture invasions into the San Francisco Estuary, and Cohen assumes that including all of California would increase the rate by at least another 50 to 100%. Implicitly this assumes that there are potentially as many unique invaders in the rest of California as have been found in San Francisco. This validity of this assumption was not assessed, though it would be possible to synthesize the existing California invasion records (e.g., http://www.dfg.ca.gov/ospr/about/science/misp.html) to determine how many California invaders are not found in the San Francisco Estuary or were first reported from areas other than San Francisco. Nonetheless, based these assumptions, the invasion rate for all of California from ballast water was estimated by Cohen as 2 to 7 species per year during the 1961 to 1995 period and 4 to 13 species per year during the 1991 to 1995 period.

Estimate of Natural Invasion Rate on Pacific Coast:
The third input value needed is the natural invasion rate, which is the most difficult to estimate. A natural invasion event is defined as a "marine organism that is transported across an ocean by drifting, rafting or some other natural, irregular and rare transport mechanism and becomes established initially as a disjunct, isolated population in waters on the other side" (Falkner et al.,

2006). In assessing the prevalence of natural invasion rates, Cohen excluded several groups of organisms that would "inflate" the natural invasion rate. Pelagic organisms that have "regular, natural genetic exchange between populations on opposite sides of the ocean" were excluded. Such species would include pelagic copepods, many of which have trans-Pacific or trans-Atlantic distributions (see http://copepodes.obs-banyuls.fr/en/index.php). Additionally, species that have continuous ranges on both sides of the ocean (e.g., boreal species that occur from northern Japan across the Aleutians and into British Columbia) were excluded. Another group that was excluded was species that have disjunct, transoceanic populations "that are relics of formerly genetically-continuous populations". Finally, Cohen excluded species that have teleplanic larvae, which are larvae that have a long residence times as plankton and may be transported across oceans (e.g., Scheltema, 1986; Scheltema and Williams, 1983). There is no discussion of how to identify species that consist of relict populations, what constitutes a continuous range on both sides of the ocean, or the larval duration defining a species as teleplanic. Thus, the taxa that potentially would be included in such an analysis are not well defined.

Cohen's equation to calculate the one-way invasion rate is:

$$\text{Equation 3:} \quad \text{Natural invasion rate} = \frac{0.5 \times \text{The number of species common to both sides of the ocean that are thought to result from natural invasion}}{\text{The length of time it takes for isolated populations to become morphologically distinct}}$$

The natural invasion rate as defined by this formula is for one side of the ocean and multiplying the number of species common to both sides by 0.5 inherently assumes that there is an equal natural invasion rate in both directions. The logic of dividing by time for isolated populations to evolve into separate species is not discussed but appears to be an attempt to account for species that successfully invaded but are no longer "common to both sides of the ocean" because they evolved into a new species. For example, given 100 species in common and a speciation rate of 0.75 million years, the adjusted natural invasion rate (for one side of the ocean) would be 66.7 invaders per million years versus 50 invaders if no adjustment for speciation had been made. [Note that for speciation rates >1 million years, the time needs to be entered into Equation 3 as years and not million of years otherwise the formula decreases the invasion rate below the observed rate.]. In any case, Cohen assumes that it takes 1 million years for isolated species to become morphologically distinct without giving any documentation.

Then based on a "review of the biogeographical literature and other relevant data", Cohen estimated that the number of fish and invertebrates common to both sides of the Pacific Ocean resulting from natural invasions is ≤100 species per million years. Two other invasion experts, Dr. Jim Carlton and Dr. Greg Ruiz, estimated ≤10 species per million years and ≤1000 species per million years, respectively (Table 4). While it was stated that a review of the biogeographic literature was conducted, the only reference given was Vermeij's (1991) estimate that the Northeast Pacific mainland had been invaded by 11 gastropod species from the Line Islands in the Central Pacific over the last 2 million years (a one-way invasion rate). This results in a natural invasion rate of 5.5 species per million years (corrected value from Appendix 6 of Falkner et al., 2006). The assumptions inherent in these estimates are discussed below.

Table 4: Estimates of natural invasion rates, resulting discharge limits, and adjusted discharge limits. Adjustment factors are the extent to which the discharge limit would be increased because of the identified factor. The first three estimates are from invasion experts who participated in the CA Advisory Panel on Ballast Water Performance Standards (Falkner et al., 2006). These estimates are adjusted based on the mean IMO organism concentrations for zooplankton compared to that used by Cohen. The second approach to adjusting the discharge rates is to adjust the natural invasion rate from Vermeij (1991) for gastropods from the Line Islands that invaded the Northeast Pacific. The reduction factors for Vermeij's natural invasion rate are derived from Equation 1 using Cohen's range in the estimate of yearly ballast water invaders in California. The taxonomic adjustment increases Vermeij's rate based on the estimated relative proportion of gastropods to total number of invertebrate and fish species. The two species pool adjustments further increase Vermeij's rate based on the presumed increase in the number of natural invaders when considering the entire Central Polynesia Province or all the ecoregions within the Northwest Pacific and Indo-West Pacific. For reference, the IMO standard for >50 micron size class is organisms is 10 organisms per m^3 while the USCG Phase II is 0.01 organisms per m^3.

Expert / Type of Adjustment	Number of one-way natural invasions per 10^6 years / Extent of adjustment	Reduction Factor	Discharge limits per m^3 for organisms >50 microns	Discharge limits per ml for organisms 10-50 microns	Discharge limits per ml for organisms <10 microns
J. Carlton	≤10	10^{-6}	10^{-4} to 10^{-3}	10^{-5} to 10^{-4}	10^{2} to10^{3}
A. Cohen	≤100	10^{-5}	10^{-3} to 10^{-2}	10^{-4} to 10^{-3}	10^{3} to 10^{4}
G. Ruiz	≤1,000	10^{-4}	10^{-2} to 10^{-1}	10^{-3} to 10^{-2}	10^{4} to 10^{5}
Ballast water conc. adjustment to mean IMO conc.	4.6 to 46 fold increase	NA	$4.6\ 10^{-4}$ to $4.6\ 10^{0}$ (0.00046 - 4.6 org. m^{-3})	NA	NA
Vermeij (1991)	5.5	$4.2 - 28 \times 10^{-7}$	$4.2\ 10^{-5}$ to $2.8\ 10^{-4}$ (0.00004 – 0.0003 org. m^{-3})	NA	NA
Taxonomic adjustment for all inverts & fish	10.6 fold increase	NA	$4.45\ 10^{-4}$ to $2.97\ 10^{-3}$ (0.00045 - 0.003 org. m^{-3})	NA	NA
Species pool within Central Polynesia Province	2 fold increase	NA	$8.90\ 10^{-4}$ to $5.94\ 10^{-3}$ (0.00089 - 0.0059 org. m^{-3})	NA	NA
Species pool within Western Pacific & Indo-West Pacific	10 fold increase	NA	$8.9\ 10^{-3}$ to $5.94\ 10^{-2}$ (0.0089 - 0.059 org. m^{-3}	NA	NA

As mentioned, the only quantitative estimate of a natural invasion rate was for gastropods from the Line Islands to the Eastern Pacific (U.S. Pacific coast). The Line Islands are located 2,500 kilometers south of Hawaii in the central Pacific, and the 5,400 km expanse of deep ocean between the Line Islands and the Clipperton Islands off western Mexico constitute the "East Pacific Barrier" (EPB), the single largest oceanic barrier in the world (e.g., Scheltema, 1988; Collin, 2003). Thus a natural invasion rate derived from the Line Islands presumably represents the "worst case" scenario (i.e., lowest natural invasion rates). No similar analysis was conducted to estimate natural invasion rates from subtropical/tropical Asia and Indo-West Pacific or the Northwest Pacific (northern China, Japan, Korea, or Russia) to the U.S. and Canadian Pacific coasts. Natural invasion rates were not calculated from Europe to the U.S. East Coast.

Discharge Standards Derived from Natural Invasion Rates:
Depending upon the ballast water concentrations and the natural invasion rate used, the discharge standards based on the natural invasion rate for the >50 micron size class reported in Falkner et al. (2006) ranged from 0.1 to 0.0001 organisms per m^3 (Table 4). Natural invasion rates for smaller organisms were not addressed in Falkner et al. (2006) but the reduction factors for the >50 micron invaders were applied to 10-50 micron and <10 micron groups, based on the implicit assumption that there had been a similar number of natural invasions for these smaller taxa. This resulted in ranges of discharge standards of 0.01 to 0.00001 organisms per ml and 100 to 10,000 organisms per ml for the two smaller size classes, respectively (Table 4).

Evaluation of Natural Invasion Rates and Adjustment Factors:
In this section we evaluate several of the assumptions inherent in estimating natural invasion rates and suggest some adjustment factors to these rates. We use these adjustment factors to first modify the range in discharge limits from the expert estimates and second to derive a new discharge limit based on modifying the invasion rate from Vermeij (1991) (Table 4).

Adjustment of Ballast Water Organisms Concentrations to IMO Mean

Cohen uses ballast water organism concentrations in untreated ballast water of 10^2-10^3 per m^3 for organisms >50 microns. In comparison, the IMO baseline study (MEPC, 2003b) reported a mean zooplankton concentration of 4640 m^{-3} (also see Figure 3 from Minton et al., 2005). Because the calculated discharge standards increase linearly with higher organism concentrations (see Equation 2), the estimates of 10^2-10^3 per m^3 potentially underestimate the lower and upper discharge limits. Adjusting the upper and lower discharge limits from the three experts by the IMO ballast water concentration results in a range of discharge limits of 0.00046 – 0.46 organisms m^{-3}. The lower value is still more than an order-of-magnitude lower than the USCG Phase II standard while the upper value is almost 50-times greater than the Phase II standard.

Taxonomically Adjusted Natural Invasion Rate

The number of natural invasions will depend, in part, upon the total number of species available for invasion (i.e., the species pool). The natural invasion rate from Vermeij's (1991) work is based solely on gastropods, and thus substantially underestimates the potential species pool. To adjust this rate to be taxonomically inclusive of all macroscopic taxa in near-coastal ecosystems, we estimated the ratio of total number of gastropods to total number of near-coastal invertebrates and fishes. The invertebrate numbers were taken from the recently revised "*The Light and Smith Manual: Intertidal Invertebrates from Central California to Oregon*" (Carlton, 2007). The

preface (page xi) to the *Light and Smith Manual* states that "over 3700 species are keyed or discussed in this fourth edition" and this value was used for the total number of intertidal and near-shore invertebrates. A total of 376 benthic gastropods was determined by counting the number of gastropod descriptions in *Light and Smith Manual* exclusive of the pelagic gastropod families. While we are unaware of a total species inventory for the Line Islands, a total of 281 coastal fishes have been estimated from Kiritimati, one of the Line Islands (Sandin et al., 2008).

Based on these values, gastropods constitute 10.2% of the intertidal and near-coastal invertebrate species in northern California and Oregon. Accordingly, Vermeij's rate of 5.5 gastropods per million years was multiplied by 9.8 to account for all invertebrate taxa, resulting in an estimated natural invasion rate of 54.1 invertebrates per million years. Adding the number of fishes estimated from Kiritimati to the total species count results in gastropods constituting 9.4% of the total fauna. In turn, this results in an upward adjustment of 10.6 fold, generating a natural invasion rate of 58.5 species per million years. Thus, Vermeij's rate for gastropods likely underestimates the natural invasion rate by about 10-fold, assuming all taxa have approximately equal probability of natural invasion. These are approximate corrections as ideally the invertebrate ratio would be based on a total species list from the Line Islands, the fish estimate would be based on all the islands within the Line Islands group, and all taxa such as macroalgae would be included. Even with these limitations, we believe that these taxonomic adjustments more closely capture the potential species pool for natural invasion than only using the number of gastropods.

Biogeographic Analysis of Potential Species Pool for Invasion

The size of the potential species pool available for natural invasions depends not only on the taxa included in the analysis but also the geographical area considered to represent potential donor regions. Vermeij's estimate is based only on the Line Islands, which contain approximately 250 known gastropod species (Vermeij, 1991). While it would take a major effort to conduct a detailed review of the number of potential invaders in the entire Western Pacific, it is possible to use the number of distinct biogeographic regions as a relative indicator of the number of unique species available for invasion.

In the "Marine Ecosystems of the World" (MEOW) hierarchical biogeographic schema, "ecoregions" are the smallest biogeographic breakout which are contained within larger "provinces" (Spalding et al., 2007). The Line Islands are part of the "Line Island Ecoregion" which is contained within the "Central Polynesia Province". The Central Polynesia Province is composed of two additional ecoregions (Cook Islands and Samoa Islands), which are approximately the same distance from the U.S. Pacific Coast as the Line Islands. The Cook Islands contain 377 extant native gastropod species (search conducted at http://cookislands.bishopmuseum.org/search.asp), while the number of marine gastropods in the Samoa Islands is apparently unknown. Though there is likely some overlap of species among the three ecoregions, the total number of potential gastropod invaders within the entire Central Polynesia Province is greater than from the Line Islands Ecoregion alone, as indicated by the 50% greater number of gastropods in the Cook Islands. Presumably, this increase in the available species pool would also apply to other taxonomic groups as well. In lieu of a detailed biogeographic analysis, we suggest that the total number of potential invaders in the entire Central Polynesia Province is at least 100% greater than the Line Islands alone.

In addition to these tropical island ecoregions, there have likely been natural invasions to the U.S. Pacific Coast from the subtropical/tropical ecoregions of Asia and the Indo-West Pacific as well as the temperate/boreal ecoregions of the Cold Temperate Northwest Pacific Province (northern China, Korea, Japan, and Russia). It is beyond the scope of this document to attempt a biogeographic synthesis of these areas, but it is suffice to say that these regions harbor an extensive number of species. The South China Sea contains more than 3500 fish species (Kwang-Tsao et al., 2008) and more than 20,000 species are listed in an inventory of China's seas (Zongguo, 2001). While the distance of these areas from the U.S. Pacific Coast may have limited the number of natural invasions migrating directly across the Pacific Ocean, it is possible that species "hop scotched". For example, cold adapted species from the six ecoregion making up the Cold Temperate Northwest Pacific Province may have initially colonized the Aleutian Islands before migrating to the Gulf of Alaska and then southward to cold temperate ecoregions of Washington, Oregon, and northern California. The main point is that estimates based on only the Line Islands are likely to substantially underestimate the total number of natural invasions and thus result in artificially low discharge standards. The extent of this underestimate is not known, but given the small size of the Line Island Ecoregion compared to all the potential donor ecoregions, it is possible that it is at least 10-fold.

Evidence for Transoceanic Interchange:
Independent of the adjustments to the natural invasion rates, there are several lines of evidence indicating that transoceanic migrations are not as rare as originally hypothesized. For example, a reasonably high percentage (13%) of the gastropod species from the Line Islands has invaded offshore islands in the Eastern Pacific (Vermeij, 1991). It is possible that these species are not found on the mainland of California and Mexico because of environmental mismatches rather than an absence of dispersal. Additionally, in a review of tropical trans-Pacific shore fishes, Robertson et al. (2004) reported 80 species that likely migrated eastward to the tropical Eastern Pacific and 22 species of shore species that likely migrated westward from the tropical Eastern Pacific.

Another line of evidence for transoceanic transport is the genetic similarity in a number of trans-Pacific species. In a study of 20 reef fish morphospecies found on both sides of the Pacific, Lessios and Robertson (2006) found that 18 of the 20 had high genetic overlap. They concluded that the similarity in these 18 trans-Pacific species was maintained by recurrent gene flow between the populations on the two sides of the Pacific. Additionally, these authors had previously found "massive breaching of the EPB" in two species of sea urchins (Lessios et al. 1998, 2003). Thus Lessios and Robertson (2006) concluded that while the EPB was generally an effective barrier in separating species in the Northwest and Northeast Pacific, it should be considered a "sporadically permeable filter." This conclusion is supported by a study of calyptraeid gastropods (slipper shells) (Collin, 2003). Collin found *Bostrycapulus* species on both sides of the Pacific Ocean, leading her to conclude that the Eastern Pacific Barrier is "somewhat permeable to some calyptraeids". These genetic studies indicate that there is periodic mixing of populations across the Eastern Pacific Barrier, and that such transport is not as "irregular and rare" as assumed in generating the natural invasion rates in Table 4.

A final line of support for the potential for transoceanic dispersal is the increasing appreciation of ocean dispersal as an important factor in determining organism distributions (de Queiroz,

2005) and the importance of rafting as a transport mechanism in particular (e.g., Thiel and Gutow, 2005). As stated by de Queiroz (2005), "If vicariance biogeography was a revolution, we are now in the midst of a counterrevolution, driven primarily by new evidence in favor of oceanic dispersal."

Assumptions and Limitations:
The natural invasion approach has only been described in the memo and addendum to the California Ballast Water Treatment Standards Committee. As such, the assumptions and input values have not been adequately vetted nor have they been peer reviewed. As mentioned, there is no discussion of how to identify species that consist of relict populations, what constitutes a continuous range on both sides of the ocean, the larval duration defining a species as teleplanic, or documentation for assuming a speciation rate of 1 million years.

An inherent assumption of the natural invasion rate approach is that a doubling of this rate would be sufficiently protective. This is a central assumption and it needs to be supported through a review of appropriate ecological models and/or empirical studies. We caution that "argument by analogue" (e.g., comparison of the effects of invasive species to the effects of a two-fold increase in atmospheric CO_2) provides no real scientific support for the adequacy/inadequacy of a ballast water standard equivalent to the natural invasion rate. Furthermore, the decision as what constitutes an adequate level of protection is a risk management issue, not a component of the risk assessment. For example, risk managers could decide that an invasion rate equal to the natural rate constitutes too great a risk and set a standard based on one-half or one-quarter the natural invasion rate. Conversely, they could decide that a rate twice the natural rate would provide sufficient protection. In any case, the role of this approach, and all the science-based models reviewed in this document, is to provide risk managers with scientifically-sound and transparent estimates of the rate and/or effects of invasion based on different ballast water concentrations.

Our review suggests that the analysis by Cohen underestimated the rate of natural invasions, which then results in an artificially low discharge standard. Specifically, the following would all result in higher natural invasion rates: 1) including taxonomic groups in addition to gastropods; 2) including the additional potential invaders (species pool) from other ecoregions within the Western Pacific; and 3) using higher mean organism concentrations in ballast water. Our conclusion that natural invasion rates were underestimated is consistent with recent genetic studies showing that the East Pacific Barrier is "semi-permeable". Based on these data, we suggest that the estimate of <1000 natural invasions per million years from Falkner et al. (2006) (Table 4) is the most defensible rate for natural invasions, and potentially may still underestimate the rate.

Estimating natural invasion rates is likely to have high uncertainty as indicated by the 100-fold difference among just three invasion experts (Table 4). With the available evidence, it appears that this approach will not generate discharge standards with less uncertainty than those developed using other approaches.

The natural invasion rates used to generate the reduction factors were based on macrofaunal invaders (>50 microns). Application of these reduction factors to the 10-50 and <10 micron

groups, which primarily consist of phytoplankton and protozoa, introduces additional uncertainty especially given the differences in rates and vectors for natural dispersal of these smaller taxa especially for the microbes (e.g., Finlay, 2002). A separate analysis would have to be conducted for these groups, perhaps focusing on diatoms because of the availability of fossil records.

The natural invasion rate approach assumes a linear dose-response for propagule pressure. As discussed in Section II, this assumption should be adequate for many species and densities.

The approach assumes that there is an equal invasion rate in both directions across the Pacific (the 0.5 multiplier in Equation 3). This assumption seems unlikely for species transported to/from from the Line Islands. Because of the much greater shoreline, there is a much higher probability that an eastward traveling propagule would encounter the Pacific Coast of the United States compared to the probability that a westward traveling propagule would encounter the Line Islands, or other islands in the central Pacific.

The calculated natural invasion rate will vary inversely with the speciation rate (Equation 3). Thus, the speciation rate is a key factor in estimating natural invasion rates, and another source of uncertainty. However, there is no discussion of the importance of this parameter, and the one-million year rate used by Cohen is not justified. Rather than using an unsupported value, the growing theory on evolutionary rates (e.g., Allen and Gillooly, 2006) as well as empirical studies using genetic techniques (e.g., Wares, 2001) should be consulted to generate a range of scientifically-defensible rates.

The calculation of the natural invasion rate does not account for species that successfully crossed the oceanic barrier and became established (e.g., survived for 10 generations) but eventually went extinct. A number of nonindigenous species have shown dramatic population crashes (e.g., Simberloff and Gibbons, 2004) so extinction of some fraction of the natural invaders is possible if not likely. Excluding these extinct invaders artificially lowers the natural invasion rate, and thus results in a lower discharge standard.

As with all the approaches that rely on historic invasion rates, the possibility that a nonindigenous species may have invaded via secondary vectors and/or hull fouling or another vector instead of ballast water potentially inflates the ballast-water invasion rate. Incorrectly assigning invaders to ballast water reduces the reduction factor (Equation 1) which in turn reduces the discharge standard.

The geographic scope of the current analysis is the state of California, which is a political entity and is not defined by any specific set of biological or environmental conditions. By the MEOW biogeographic schema, California encompasses two coastal provinces and three ecoregions. Thus the current analysis mixes a number of different areas which likely have different natural invasion rates as well as numbers of ballast-mediated invasions.

Before the Wisconsin glacier, natural invasions into the freshwater bodies of the current Great Lakes region were presumably minimal and less than that for the Pacific Coast. After the retreat of the glaciers, there was relatively rapid population of at least some lakes and ponds (e.g., Daniels and Peteet, 1998), though it is not clear whether this would be considered "natural

invasions" or a re-colonization process. Thus, it is not clear that ecologically relevant natural invasion rates could be generated for the Great Lakes or whether the standards resulting from an analysis of coastal regions would be applicable to the Great Lakes.

Recommendations/Conclusions:
We conclude that given the lack of peer review, high level of uncertainty and number of unsupported assumptions, the current range of values based on the natural invasion approach should not be used to generate discharge standards. Furthermore, given the inherent uncertainties with the approach, we do not believe that even a revised analysis should be used to generate national discharge standards. It is worth noting, however, that after adjusting for IMO ballast discharge rate, the upper estimate of the ballast water discharge standard derived from the natural invasion approach is about 50-fold greater than the USCG Phase II standard.

Because the natural invasion rate approach is the only technique that attempts to define an ecologically acceptable invasion rate other than 0, values from a revised analysis could be used as an informal benchmark for comparisons with values generated by other methods. The purpose of such comparisons would be to put results from other methods in context with our current understanding of natural invasion rates. Such a comparison would require that all the techniques use similar assumptions regarding ballast water discharge volumes and organism concentrations.

If the natural invasion rate approach is to be considered either as a formal approach to developing discharge standards or as an informal benchmark for other approaches, it is critical that it be further developed and reviewed not only by invasion biologists but also paleontologists, biogeographers, and geneticists working on connectivity among transoceanic populations. Any future development should address the limitations mentioned above, especially those that are likely to reduce the discharge standards artificially. Such an analysis should develop estimates of uncertainty around the predictions.

Any further development should expand the geographic range considered. Instead of assessing invasion rates across a political entity (California) it would be more ecologically relevant to generate estimates by the biogeographic ecoregions making up the U.S. Pacific Coast. If each ecoregion was evaluated independently, it would then be possible to generate confidence intervals around the suite of estimates. Within the north Pacific, Hawaii provides a "natural experiment" on rates of colonization and speciation, and such an analysis could draw on the efforts to document the biodiversity of the Hawaiian Islands (Eldridge, 2006) as well as their evolutionary history (Price and Clague, 2002). Additionally, natural invasion rates should be evaluated on the East Coast of the United States, especially given the large number of amphi-Atlantic species (e.g., Vermeij, 2005).

VI. REACTION-DIFFUSION MODELS

Henry Lee II and Deborah A. Reusser

Overview:
Reaction-diffusion models predict the concentration of a "substance" that is simultaneously influenced both by diffusion (dilution) and by some type of reaction affecting its concentration (e.g., chemical reaction, population growth). Their applications to biological systems have been reviewed by Okubo and Levin (2002) and Sexton et al. (2009) while their application to invasions is discussed in Kinlan and Hastings (2005). The basic assumptions of this family of models in terms of invasions (Kinlan and Hastings, 2005) are: 1) they model continuous time and space; 2) there is local random movement of individuals; and 3) population dynamics are deterministic. The primary use of reaction-diffusion models in invasion biology has been the theoretical analysis of the pattern of invasion spread of terrestrial invaders, with the models usually predicting a linear rate of spread under the most common assumptions. The only published example of a reaction-diffusion model being applied to determining ballast water standards that we are aware of is that of Drake et al. (2005).

Application to Ballast Water Discharges by Drake et al. (2005):
Drake et al. (2005) developed a reaction-diffusion model with an Allee effect to predict the probability of establishment of species based on the volume of ballast water released. Note that this approach predicts "acceptable volumes" of ballast water and does not directly use or predict concentrations of organisms in the ballast water. Thus, it does not directly generate an organism-based discharge standard.

The form of the model used by Drake et al. (2005) to predict the change in the density of a species released via a ballast water discharge was:

Equation 4: $$\frac{\partial u}{\partial t} = D\nabla^2 u + f(u)$$

Where:
u (relative density) = local population density scaled by carrying capacity (i.e., between 0 and 1)

t = time

D = diffusivity of discharged ballast water (m^2s^{-1})

∇ = Laplace operator defining the spatial gradient over two dimensions

$f(u)$ = model describing local population growth (change in relative density/time)

In Equation 4, the first set of terms is the "diffusion" component of the model which models the dilution of the individuals in the water column over time. The second function captures the simultaneous population growth, which is the "reaction" component. By normalizing population

size to carrying capacity, the model does not predict a population concentration but rather changes in relative population size over time due to the combined effects of dilution and population growth (see Lewis and Kareiva, 1993).

Using the cubic population model from Lewis and Kareiva (1993), the local growth of a species subject to an Allee effect was modeled as:

Equation 5: $f(u) = ru(1-u)(u-a)$

Where:
r = intrinsic rate of population increase (day^{-1})

a = Allee "critical density" (unitless).

The form of the relative population growth rate as a function of population size from Equation 5 is illustrated in Figures 4 and 5. In this model, an Allee effect occurs when $0 < a < 1$, where a represents the fraction of the carrying capacity below which the detrimental effects of a low density result in negative population growth (Lewis and Kareiva, 1993). As can be seen in Figure 4, inclusion of a mild Allee effect has a minor effect on population growth over most densities. It is only at very low relative densities (Figure 5) that the Allee effect results in a noticeable decrease in relative population growth. The decline in growth rate above a relative density of about 0.7 is due to negative intraspecific interactions.

Assuming a mild Allee effect and an initial population density substantially above the critical density (a), the necessary and sufficient conditions for the establishment of a population of an introduced species in terms of area occupied is:

Equation 6: $R_{\min} = \frac{1}{1/2 - a}\sqrt{\frac{D}{2r}}$

Where:
$R_{\min}$ = radius of the initially occupied area from the ballast discharge (m).

To convert this area into a volume, Drake et al. (2005) assumed that the ballast water was dispersed within a 10 m deep zone. Based on this assumption and setting the radius of the cylinder to $R_{\min}$, they calculated $V_{\max}$, the "maximum volume of [ballast] water that may be released to maintain the risk of population establishment at or below a level that would be specified by policy." The risk of population establishment was calculated by utilizing different values of r as described below.

Using the data from Figure 17 of Blueweiss et al. (1978), Drake et al. (2005) generated a regression between body mass and population growth rate (r) (their equation not given). They then estimated the maximum per capita population growth rate, $r_{\max}$, using the upper 0.01, 0.001, and 0.0001 confidence levels of the regression. These upper confidence levels were used as a

method to establish a range of "risk tolerances", which represent the probability that a species would become established. Based on Lewis and Kareiva (1993), they then set r_{max} as approximately equal to $4 \times r$ or $r = r_{max}/4$. Substituting r_{max} for r in Equation 6 results in:

Equation 7: $$R_{min} = \frac{2}{1/2 - a}\sqrt{\frac{D}{2r_{max}}}$$

Based on the r_{max} from body size and for given "*a*" (critical density) and *D* (diffusivity), it is possible to calculate R_{min}. Assuming a 10 m depth (*d*), it is then possible to calculate V_{max}, the maximum volume of ballast water that can be discharged at a specified risk level (from the confidence level of the *r*):

Equation 8: $$V_{max} = \pi R_{min}{}^2 d \qquad V_{max} = \pi R_{min}{}^2 d$$

Where:
V_{max} = maximum volume of ballast water discharge for specified risk level (m^3)

d = depth (m)

Parameter Estimation:
Drake et al. (2005) assumed an Allee effect equal to *a* = 0.01. With this threshold, the population experiences negative growth from an Allee effect at <1% of its carrying capacity (Figure 5). While considered a "mild" Allee effect (i.e., "*a*" is a small fraction of the carrying capacity), it may actually be an important factor at the discharge standards that have been proposed (e.g., 0.01 to 10 organisms > 50 microns per m^3).

As mentioned, a single depth (d) of 10 m was assumed for all ballast water discharges. V_{max} scales linearly with depth so the "exact value is not hugely important". The authors used two horizontal diffusivity values in their calculations, 0.02 $m^2 s^{-1}$ and 0.3 $m^2 s^{-1}$, the minimum and maximum values reported from a study of lakes. These values are substantially lower than those found in many estuarine/marine systems, which can have diffusivities over 1000 $m^2 s^{-1}$ (e.g., Banas et al., 2004; also see Figure 5 of Drake et al., 2005). Because the probability of invasion decreases as diffusivity increases, the use of these lower values is protective of exposed marine/estuarine conditions. However, as noted by the authors, the lake diffusion values may not be protective in enclosed harbors which physically restrict diffusion (see their Figure 5). Note that the diffusivity values in $m^2 s^{-1}$ need to be multiplied by 86,400 to convert them to m^2 day^{-1} so that the units are consistent with the intrinsic rate of growth (day^{-1}) when used in Equations 6 and 7.

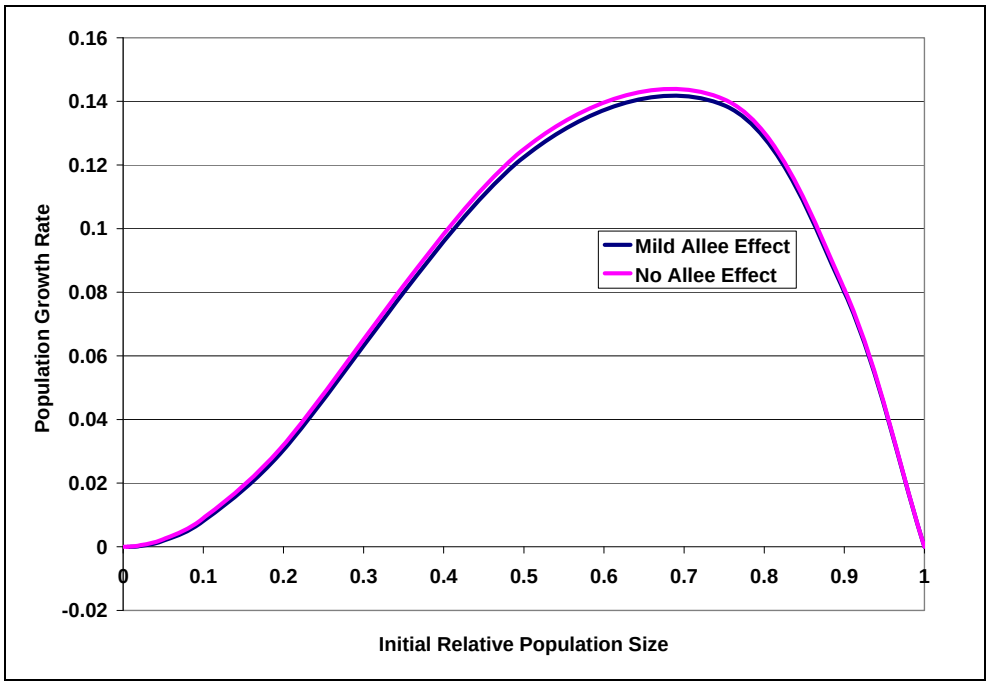

Figure 4: Example of relative population growth rate based on the cubic population model from Lewis and Kareiva (1993) with and without a mild Allee effect ($a = 0.01$). The relative population size is the population of a species at a given time in relation to that species carrying capacity. The decrease in growth rate at high relative population is due to negative intraspecific interactions.

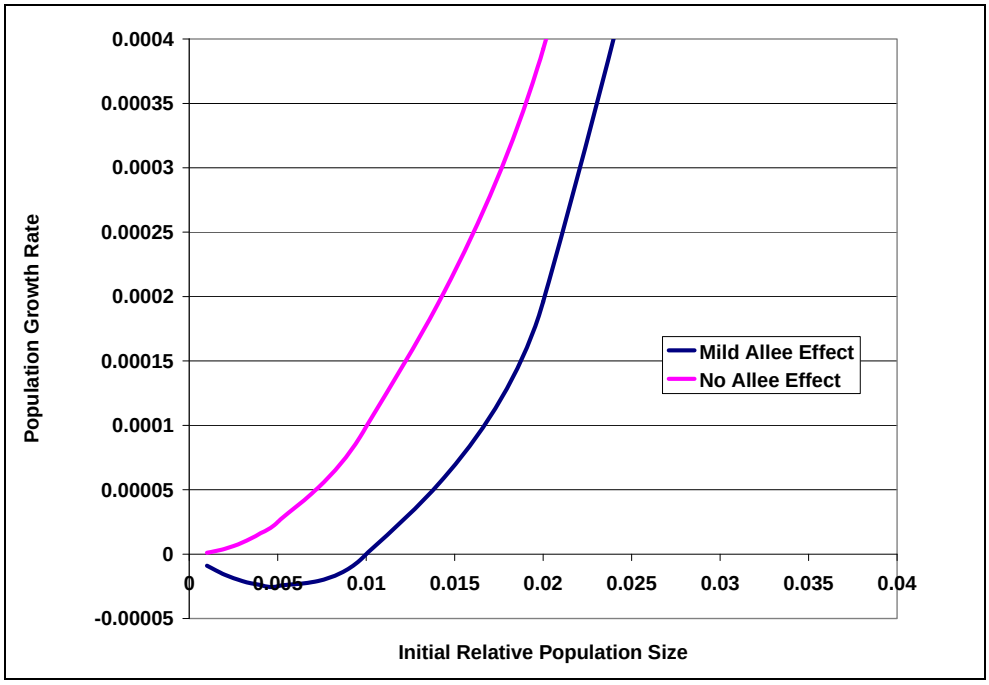

Figure 5: Enlargement of the relative population growth rate based on the cubic population model from Lewis and Kareiva (1993) with and without a mild Allee effect ($a = 0.01$). The population growth becomes negative below a relative density of 0.01 due to the Allee effect.

Drake et al. (2005) use risk tolerance values of p = 0.01, p = 0.001, and p= 0.0001 to represent the "chance of establishment per introduction" to bracket different levels of protection. These risk tolerance values are derived from the upper confidence levels around the allometric relationship between body size and intrinsic rate of population increase (*r*). The validity of the resulting values is discussed below.

Assumptions and Limitations:
The major limitation of the analysis by Drake et al. (2005) for development of discharge standards is that it generates acceptable volumes of ballast water discharges rather than risks associated with the discharge of different organism concentrations. Therefore, it can not be used to generate organism-based discharge standards. We initially attempted to convert their analysis to a concentration basis, but this would require, at the minimum, estimates of carrying capacities for the species in the ballast discharge, which are unknown for nearly all the invertebrates and larval fishes entrained in ballast water. However, as discussed under Recommendations/ Conclusions, it would be possible to use a different form of a reaction-diffusion model or other types of dilution models to predict changes in organism concentrations resulting from dilution.

A general limitation of using reaction-diffusion models to develop ballast water discharge standards is that they only apply to small holoplanktonic species (such as calanoid copepods), that spend their entire adult life span in the water column and that are passively transported by currents. Species with pelagic larvae (e.g., most polychaetes and mollusks) that actively settle out of the water column violate several key model assumptions including: 1) individuals are passively distributed by currents; 2) species complete their life span within the water column; and 3) population dynamics are rapid compared to redistribution through diffusion. Pelagic species such as fish that remain within the water column but which actively swim violate the assumption of passive dispersal and, in nearly all cases, the assumption that population dynamics are rapid compared to diffusion. Because holoplanktonic species make up a relatively small fraction of the total marine/estuarine invaders (see Ruiz et al., 2000; Wonham and Carlton, 2005), this family of models can address only a subset of potential invaders in these systems. Even in the Great Lakes, zooplankton only constitutes 6 of the 37 (16%) fish and invertebrates introduced via "shipping, Ballast Water" (calculated from data at http://www.glerl.noaa.gov/res/Programs/ncrais/docs/great-lakes-list.xls).

Drake et al. (2005) generated a range of "acceptable" ballast water volumes for "invasion risk tolerances" of 0.01, 0.001, and 0.0001. The risk tolerance represents the probability of an invasion of an unknown species; so a risk of 0.0001 means that there is a 1 in 10,000 chance that an invader will become established. These risk tolerances are the probabilities that a single species will become established. However, as discussed in more detail under the PVA model (Section VII), the key environmental question is not whether any particular species will become established but rather whether any of the multitude of species in a ballast discharge will successfully invade. This multi-species risk is calculated as the risk of a single species <u>not</u> invading raised to the power of the number of species in the ballast discharge. Assuming 100 species in a ballast discharge and an individual species' risk tolerance of 0.0001, the probability of a single species not invading is 0.9999; when this is raised to the 100th power, the result (0.99) is the probability of all 100 species not invading. With this multi-species scenario the probability of no species invading is about 1 in a hundred. Thus, even the lowest invasion risk tolerance

value used by these authors results in a high risk of invasion when considering all the species potentially present in a ballast discharge.

It is not possible to use lower risk tolerances in this model because of how they were determined. The risk tolerance levels were generated from the upper confidence limits of "*r*" from the regression of intrinsic rate of growth versus body size. For example, to establish the acceptable volume of ballast discharge at an invasion risk tolerance of 0.0001, they used the intrinsic rate of growth equal to the upper 0.0001 confidence level for the particular size of organism in Equation 7. While an innovative approach, the problem is that the intrinsic growth rates associated with the upper 0.001 and 0.0001 upper confidence levels are unrealistically high. For example, back-calculating from their equations using a risk tolerance of 0.0001, we obtained an intrinsic growth rate (r) greater than 18 day^{-1}. This value is at least an order of magnitude greater than nearly all metazoans, and even for ciliated protozoans the highest value was 6.3 day^{-1} (Taylor and Shuter, 1981). Thus, this approach to setting different protection levels is limited to risk tolerance levels around 0.01, a very high, and presumably unacceptable, invasion risk.

The authors state that the derivation of Equation 6 assumes that the original release density u_0 is "considerably above the Allee threshold a". Given the low proposed discharge standards (0.01 – 10 organisms m^{-3}) it is possible that ballast water concentrations of individual species will not be "considerably" above Allee thresholds. However, it is not clear whether this assumption is actually required for the derivation of the equation or simply that ballast discharge densities below the Allee threshold result in negative growth in Equation 2 and thus result in "relatively little threat of invasion".

The solution in Equation 6 "obtained from Lewis and Kareiva (1993) relies on the assumption that population dynamics are relatively fast compared to organism redistribution through diffusion" (Drake et al., 2005). To evaluate this assumption, the authors conducted numerical simulations to evaluate the potential effects on their results. Based on these simulations, the authors concluded that their model would underestimate the acceptable ballast water volume for larger species (= species with slower population growth rates). From their Figure 3, biased estimates occur for organisms larger than about 0.05 grams, which they list as fish and ctenophores. The lower boundary of adult size for amphipods, decapods, copepods, and ostracods is listed as less than 0.05 grams, and thus have unbiased estimates. They do not state how biomass is measured, but we assume that it is wet weight.

Recommendations/Conclusions:
The work by Drake et al. (2005) can not be used to generate organism-based discharge standards since it is based on "relative densities" to predict acceptable volumes of ballast discharge. However, it should be possible to generate reaction-diffusion models addressing ballast water discharges that utilize actual densities rather than relative densities, though this would require estimates of species specific population vital rates. Alternatively, it may be possible to link population growth models with models simulating dilution of pollutant discharges, such as Visual Plumes or CORMIX2 (see http://www.epa.gov/waterscience/standards/mixingzone/resources.html#models). In this case, the dilution models would be "turned on their head" and the ballast discharge would occur at the surface rather than from depth. These simulation models are "mature" and allow for inclusion of

real world complications not readily captured in analytical models, such as density differences between discharged and receiving waters. Without further analysis, however, it is not clear to what extent the existing dilution models would have to be modified to model ballast discharges.

The more germane question is how much effort should be devoted to diffusion models in general for the generation of organism-based discharge standards. Violation of the assumption that species are passively distributed is likely to result in a substantial underestimation of the likelihood of establishment of a species. In particular, benthic species whose larval and/or juvenile phases actively settle out of the water column are much more likely to become established than predicted from dilution models. Thus, in aquatic environments, diffusion models are primarily limited to predicting invasions of small, holoplanktonic organisms. Because of this limitation, diffusion models do not appear to be suitable for generating concentration-based discharge standards applicable to the wide range of taxa found in ballast water.

While not suitable as a general approach to generating discharge standards, results from diffusion models with holoplanktonic organisms can be used to help elucidate the role of population dilution in initial establishment. Such an analysis may help explain why there are relatively few copepod invaders in marine/estuarine systems even though they make up a substantial portion of the fauna in ballast water (e.g., Lavoie et al., 1999; Levings et al., 2004).

VII. POPULATION VIABILITY ANALYSIS (PVA) MODELS

Henry Lee II

Overview:
Population viability analysis (PVA) models are a family of population growth models commonly used in the conservation field to predict the extinction probability of endangered species (Beissinger and McCullough, 2002; Morris and Doak, 2002). The basic premise of PVA models is that any population undergoing stochastic growth has a certain probability of going extinct even if it is presently showing positive growth. In general, the smaller the population size, the slower the population growth rate, or the larger the variation in population growth rate, the greater the probability of extinction. There are three general types of PVA models: 1) count-based PVA; 2) demographic PVA; and 3) spatially explicit PVA. The count based PVA is the simplest, and utilizes historical census data to estimate population growth rate and variation assuming all individuals are identical. The diffusion approximation of Dennis et al. (1991) is the simplest of the count-based PVA models and is based on two parameters -- the instantaneous population growth rate and instantaneous variation in the population growth rate. The diffusion approximation is most suitable when there is a lack of detailed life history information. Demographic PVA models are based on population projection matrices that incorporate size- or age-specific demographic vital rates, and thus incorporate differences among age/size groups. Spatially explicit PVA models are the most complex and incorporate population migration and colonization into and out of areas.

There is growing recognition that PVA models are a potential tool to predict the establishment and spread of nonindigenous species (Andersen, 2005). When used with nonindigenous species, the objective is to predict either the time to extinction or the probability of extinction for an invader, where extinction is the converse of establishment. Recently, PVA models have also been evaluated in laboratory experiments on population dynamics to gain insights into the invasion process (see Section IX). In this section, we examine the PVA analysis conducted in the USCG Draft Programmatic Environmental Impact Statement (DPEIS; USCG, 2008). We detail this analysis both because it is part of the technical analysis used by the USCG in setting their proposed rules (USCG, 2009) and because it is the only study that we are aware of that used PVA models to directly address ballast water standards. However, as discussed below, the formulation used in the DPEIS is not the only possible PVA methodology to addressing the risks associated with ballast discharges.

PVA Model Used in USCG Risk Assessment for Single Species Scenario:
The DPEIS used the diffusion approximation model (Dennis et al., 1991). The strategy taken in the DPEIS was to evaluate different discharge standards by predicting the relative increase in the probability of extinction based on the fractional reductions in the number of organisms per cubic meter of ballast discharge. This is a relative approach and it was not the objective of the DPEIS analysis to predict the actual probability of invasion associated with any specific organism.

In their analysis, the DPEIS listed five potential treatment alternatives, which for the >50 micron size group were:

Alternative 1: No Action (no ballast water treatment is implemented and ballast water exchange is the preferred option if vessels can conduct it).

Alternative 2: <10 organisms m^{-3} (= IMO standard)

Alternative 3: <1 organisms m^{-3} (= $1/10^{th}$ IMO standard)

Alternative 4: <0.1 organisms m^{-3} (= $1/100^{th}$ IMO standard)

Alternative 5: 0 organisms (= sterilization)

Alternative 1 (No Action) was taken as the baseline against which the other treatments were compared. Ranges in organism concentrations for both unexchanged and exchanged ballast water were used to establish this baseline. Alternative 5 was not formally analyzed because no invasions would occur with sterilization. The DPEIS did not analyze the USCG Phase II standards (= $1/1000^{th}$ IMO standard), but when possible we include such an analysis for the >50 micron organisms.

The remainder of this sub-section will detail the diffusion model and input parameters used in the DPEIS for a single species analysis, which implicitly assumes that all the individuals in a ballast discharge are of single species. The parameters used in the PVA model are given in Table 5, and for all population rates we assume a time unit of a day. The reader is referred to Sections 4 and 5 of Appendix A of the DPEIS for a more detailed derivation of the equations.

The simplest model of population dynamics incorporating stochastic variation is:

Equation 9: $dX(t) = \mu dt + \sigma dW(t)$

In this model, dW(t) is a normal random variable that adds randomness to the population dynamics. The larger the value of dW(t) and/or the larger the instantaneous standard deviation of the population, the larger the swings in population size, and the more likely that the population will drop to the critical population threshold (n_e). Populations with negative growth ($\mu < 0$) will go extinct regardless of initial population size (N(0)) and are not further considered. For populations with positive growth ($\mu > 0$), the probability that a population with an initial size of N(0) will go extinct (i.e., reach the critical population threshold) is:

Equation 10: $p_e = \exp(-2\,\mu\,\text{‘d’} / \sigma) = e^{(-2\,\mu\,\text{‘d’} / \sigma)}$

[Note: Equation 10 is a direct reproduction of Equation 3 in the DPEIS (App. 4, p. A-25); we assume this represents a typographical error and that the authors meant to write:

$p_e = \exp(-2\,\mu\,\text{‘d’} / \sigma^2)$

This equation indeed can be rearranged to Equation 5 in the DPEIS (our Equation 11).]

Table 5: Parameters used in the PVA models in the DPEIS.

$N(0)$ = population size at time 0 (initial population size) $N(t)$ = population size at time t $X(t) = \log N(t)$ (log of population size at time t) μ = instantaneous population growth rate σ^2 = instantaneous variance of the population growth rate σ = instantaneous standard deviation of the population growth rate $dW(t)$ = normal random variable with mean 0 and variance 1 (to add random variation in population dynamics) n_e = critical population threshold at which the population is considered "extinct" (quasi-extinction). The critical threshold is assumed to be 1 individual in the DPEIS for the single species scenario, which is the smallest possible value. $x_e = \log n_e$ p_e = probability that a population with initial size $N(0)$ will go extinct 'd' = $X(0) - x_e = \log (N(0)/n_e)$ Log of the ratio of the initial population size to the critical population threshold. (Note that we put quotes around d to differentiate it from "delta" in the rate equations) $c = 2\mu / \sigma^2$ = "biological parameter" (ratio of instantaneous growth rate to instantaneous variance in growth rate; see Equation 85 of Dennis et al., 1991) exp = exponential function f = fractional decrease in the initial population size ($N(0)$) due to a ballast water treatment. f is calculated as the ratio of the total number of organisms discharged under a particular management Alternative to the number discharged under Alternative 1 (No Action option). $p_e(f)$ = probability of extinction as a function of the fractional decrease in initial population size f_e = fractional effect on extinction probability (p_e) of reducing initial population size ($N(0)$) by the factor f. Equal to ratio of probability of extinction with the fractional decrease (f) in initial population size to the probability without the decrease (= $p_e(f) / p_e$). f_r = the proportion of the mean rate of successful introductions relative to that under Alternative 1 (No Action option) DE = number of discharge events when calculating joint probabilities of no establishment of a single species from multiple identical discharge events $q(m)$ = probability that at least one species is successfully introduced in the multiple species scenario

Assuming positive growth, the corrected version of Equation 10 can be rewritten as:

Equation 11: $p_e = (n_e / N(0))^c$ Where $N(0) \geq n_e$

This equation predicts the probability of extinction (i.e., an invader not becoming established) based on the initial propagule supply (N(0) = organism concentration in ballast * volume of ballast discharged). However, these predictions require quantitative estimates of instantaneous population growth and variance for the specific species. As discussed in the DPEIS, such values are not available for most marine/estuarine or freshwater fishes and invertebrates. Additionally, the specific species composition in foreign ballast discharges is not known. The strategy taken in the DPEIS to circumvent these limitations was to calculate relative changes in the probability of extinction as a function of fractional decreases (f) in the initial population size resulting from different levels of ballast water treatment.

To calculate the relative fractional reductions in initial population size (f) by treatment type, the DPEIS first estimated the range of organism concentrations in unexchanged ballast water and the range that these concentrations would be reduced by ballast water exchange. The range in concentrations in unexchanged ballast water includes concentrations below the IMO limit, thus the range in the percent removal from ballast exchange includes 0 (no reduction). As discussed below, ballast water concentrations below the IMO standard are considered a rare event. From these estimates, they calculated a range of initial population sizes for Alternative 1, which were used to calculate the range in fractional decreases in initial population size in the other treatment alternatives (Table 6).

Because the DPEIS used the lower and upper bounds of organism concentrations in their analyses, there is a wide spread in the fractional decreases for the ballast water treatment alternatives, including 1 (= no reduction). We believe more representative fractional decreases for the >50 micron size group can be calculated from the relative decrease in median propagule doses from ships undergoing ballast water exchange (BWE) versus the doses based on the IMO standards (data from Table 1 of Minton et al., 2005). Under this scenario, the IMO standard resulted in a median fractional decrease slightly more than an order of magnitude (0.094) compared to exchanged ballast water. (A similar comparison to the dose from unexchanged ballast resulted in a fractional decrease of slightly more than 100-fold.) Based on this, we then reduced the fractional decreases in Alternatives 3 and 4 and the USCG Phase II standards each by an order of magnitude, representative of the changes in their ballast water concentrations (Table 6).

Estimates of the biological parameter "c" are needed to translate the fractional decreases in Table 6 to f_e, the factor by which the reduction in initial population size increases the extinction probability. The parameter c ($=2\mu / \sigma^2$) is based on the ratio between instantaneous growth rate and its instantaneous variance and is a critical variable determining the probability of extinction (see Equation 85 of Dennis et al., 1991). As pointed out in the DPEIS, the value of c can vary substantially among taxa and with environmental conditions. They address this uncertainty by using a range of 0.001 to 0.1, though they do not give a detailed justification for these values. A value of 0.1 means that the variance of the instantaneous growth rate is 20 times greater than

Table 6: Ranges in the fractional decreases (f) in initial ballast water population size generated by treatment Alternatives 2 - 4 relative to the range of population sizes in Alternative 1 used by the DPEIS. A value of 0.1 signifies that the initial population size was reduced to 1/10th of the size in Alternative 1 while a value of 1 indicates that there was no decrease relative to Alternative 1. The bolded value for the >50 micron size class for Alternative 2 is a recalculated fractional decrease based on the reduction in median propagule dose associated with the IMO standard versus that associated with exchanged but untreated ballast (concentrations from Minton et al., 2005). The recalculated fractional decreases for the other alternatives based on proportional decreases relative to the IMO standard are also bolded. NC = not calculated in the DPEIS.

Alternative	**Fractional Decrease (f) (10-50 micron taxa)**	**Fractional Decrease (f) (>50 micron taxa)**
Alternative 2 (IMO)	0.1 – 1	0.001 – 1 **(0.1)**
Alternative 3 (1/10th IMO)	0.01 – 1	0.0001 – 0.1 **(0.01)**
Alternative 4 (1/100th IMO)	0.001 – 1	0.00001 – 0.01 **(0.001)**
USCG Phase II (1/1000th IMO)	NC	NC **0.0001**

mean instantaneous growth rate, while a value of 0.001 means that the variance is 2000 times greater. Populations of small invertebrates can be highly variable, but without a quantitative review of their population dynamics it is unclear whether variances on the order of 1000 fold greater than the mean growth rate are representative of many or most species likely to be discharged in ballast water. The significance of these high variances (= low values of c) is that they increase the probability of extinction (Equation 11) because larger population variation increases the likelihood that the population will drop below the critical population threshold (n_e).

Once f and c are estimated, it is then possible to calculate the probability of extinction resulting from the fractional decrease in the initial population size (f):

Equation 12: $p_e(f) = f^{-c}\, p_e \;=\; f^{-c}\,(n_e / N(0))^c$

The effect of reducing the initial population size discharged under each of the treatment alternatives on the probability of extinction can be expressed by f_e, the ratio of the extinction probability with and without the fractional decrease in initial population size resulting from the treatment alternative:

Equation 13: $f_e = f^{-c} = p_e(f)/p_e$

The DPEIS used these formulas to evaluate the sensitivity of extinction probabilities to a range of values of c (their Table 5-2). For small values of c they noted the relative insensitivity of extinction probability to the density of organisms in the ballast. We have recalculated the probabilities (Table 7) and believe that the apparent insensitivity to initial densities needs to be re-examined. In this recalculation we used the assumptions in the DPEIS of a critical population threshold of 1 organism and a ballast discharge volume (used to calculate N(0)) of 10,000 m^3. In Table 7, we report the values with three decimal places (versus two in their Table 5-2) so that it is possible to see that decreasing organism density does in fact increase the likelihood of extinction. Additionally, the apparent insensitivity to organism density is partially due to the high extinction probabilities (>97%) with low values of c, so that there is not "much room" to increase the probability of extinction.

Another key point is that the "small" differences in extinction probabilities become important when considering the joint extinction probabilities of multiple species (discussed below) or with discharges from multiple ships. To explore the risk associated with multiple ship discharges, we calculated the probability that a species would not become established assuming 10 independent, identical ballast water discharges of 10,000 m^3. The probability of extinction resulting from 10 independent discharges of the same species is calculated by raising the probability that the species did not become established in a single event to the 10th power.

Table 7: Probability of extinction (p_e) expressed as a function of the initial organism concentrations (>50 microns) in ballast water and the "biological parameter" c for a single species. Extinction probabilities are calculated from Equation 11 based on a single discharge event of 10,000 m^3 with a critical population threshold of 1 organism. The probability that the species becomes established is 1 minus the probability of extinction given in the table, thus the higher the value, the lower the risk of invasion. While the probabilities of extinction are given as actual values, the data are most appropriately analyzed as relative differences among organism concentrations or values of c. Modified from Table 5-2 of the DPEIS, including adding the organism concentration for the USCG Phase II standard.

	Initial Organism Concentration (>50 microns) in Ballast Water (organisms m^{-3})								
c	10^{-2} (USCG Phase II)	10^{-1} (1/100th IMO)	10^{0} (1/10th IMO)	10^{1} (IMO)	10^{2}	10^{3}	10^{4}	10^{6}	10^{8}
0.001	0.995	0.993	0.991	0.989	0.986	0.984	0.982	0.977	0.973
0.01	0.955	0.933	0.912	0.891	0.871	0.851	0.832	0.794	0.759
0.1	0.631	0.501	0.398	0.316	0.251	0.199	0.158	0.100	0.063

The probability of the species becoming established is 1 minus the joint probabilities of extinction across the multiple voyages. This approach is similar to considering the risks from multiple species (discussed below), and the general formula for multiple voyages is:

Equation 14: probability of a species becoming established from multiple, identical discharges $= 1 - p_e^{DE}$

Where:
DE = number of identical, independent discharge events of the same species.

Results from our analysis of probability of extinction of a single species from 10 identical discharge events are given in Table 8. Considering the risk of multiple discharge events highlights the importance of reducing organism concentrations. For example, at the lowest value of c (0.001), the calculated risk of invasion at the Phase II standard is 0.045 versus 0.1087 for the IMO standard, more than a two-fold reduction in risk with the more stringent standard. A similar two-fold difference in invasion risk was also seen with these standards with a c value of 0.01. The key point from results given in Tables 7 and 8 is that reductions in the organism concentrations in ballast water result in ecologically significant relative decreases in the invasion risk even for species and environments with a naturally low invasion probability (= species in environments with low value of c).

Table 8: Probability of extinction (p_e) as a function of the initial organism concentrations in ballast water and the "biological parameter" c for a single species for 10 identical, independent discharge events. Extinction probabilities are calculated from Equation 11 based on 10 discharge events each of 10,000 m^3 with a critical population threshold of 1 organism. The probability that the species becomes established is 1 minus the probability of extinction given in the table, thus the higher the value, the lower the risk of invasion. While the probabilities of extinction are given as actual values, the data are most appropriately analyzed as relative differences among organism concentrations or values of c.

	Initial Organism Concentration (>50 microns) in Ballast Water (organisms m^{-3})								
c	10^{-2} (USCG Phase II)	10^{-1} (1/100th IMO)	10^{0} (1/10th IMO)	10^{1}	10^{2}	10^{3}	10^{4}	10^{6}	10^{8}
0.001	0.955	0.933	0.912	0.891	0.871	0.851	0.832	0.794	0.759
0.01	0.631	0.501	0.398	0.316	0.251	0.199	0.158	0.100	0.063
0.1	0.010	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000

The DPEIS then goes on to analyze the results as the range in the factor by which the extinction probability would be increased compared to Alternative 1 (versus the absolute values in Table 7). This extinction probability factor (f_e) was calculated from Equation 13 for Alternatives 2-4. Their ranges in f_e for the >50 micron size class are replicated in Table 9 along with our analysis for multiple voyages. For the multiple ship voyages, we calculated f_e from $p_e(f)/p_e$ (see Equation 13). We first calculated p_e for a single voyage based on the assumptions of an organism concentration of 1,000 m^{-3} after ballast water exchange, a discharge volume of 10,000 m^3 per voyage, and a critical population threshold of 1 organism. The $p_e(f)$ were calculated using the same assumptions and the organism concentration associated with each treatment. Then both extinction probabilities for a single voyage were raised to the 10th power and the ratio calculated. The results in Table 9 from a single voyage show that for the lowest c (high variance compared to growth rate), the treatment alternatives do not increase the probability of invasion. As discussed above, this is a consequence, in part, of the high rate of extinction for species with a high population variance. For the species with a lower variance (higher c), the extinction rates can increase two to three fold with the additional reduction of organism concentrations in the ballast.

Table 9: Factor by which the probability of extinction would be increased compared to Alternative 1 (f_e) for >50 micron organisms. The values in Column I represent the f_e for the range in fractional decrease values (f) and in c for a single voyage calculated in the DPEIS. Column II is our calculations of f_e for 10 independent voyages using only the range in c. Modified from Table 5-3 of the DPEIS.

Alternative	**c**	**f**	**I. Single Voyage f_e (range in f & c)**	**II. Multiple Voyages f_e (range of c)**
Alternative 2 (IMO)	0.001 – 0.1	0.001 – 1.0	1.0 – 2.0	1.047 – 100
Alternative 3 (1/10th IMO)	0.001 – 0.1	0.0001 – 1.0	1.0 – 2.51	1.072 – 1000
Alternative 4 (1/100th IMO)	0.001 – 0.1	0.00001 – 1.0	1.0 – 3.16	1.097 – 10,000
Phase II USCG (1/1000th IMO)	0.001 – 0.1	-	-	1.122 – 100,00

With multiple voyages using the median reduction in concentration, there is a relatively small increase in the extinction rate, about 5% to 12%, with the low values of c. However, with the high values of c, the extinction rate increased by orders of magnitude, 100 to 100,000 times, compared to concentrations associated with exchanged ballast water. This result suggests that the importance of decreasing organism concentrations in the ballast becomes increasingly important when there is a likelihood of multiple ships discharging the same organisms within a port. The analysis of the multiple voyages was not part of the DPEIS and we consider these results preliminary. Nonetheless, they suggest that further analysis of the risk of invasion from multiple voyages is warranted.

The final analysis in the DPEIS for single species was to calculate f_r, the mean rate of successful introductions for a treatment relative to Alternative 1. Recalling that the probability of introduction is 1- probability of extinction, f_r is calculated as:

Equation 15: $$f_r = \frac{1 - p_e(f)}{1 - p_e}$$

The numerator in this equation is the probability that invaders will become established with the organism concentration associated with the treatment alternative while the denominator is the extinction probability under Alternative 1. As before, the extinction probabilities were calculated assuming a critical population threshold (n_e) of 1 organism, a ballast discharge of 10,000 m^3, and range of values for c and organism concentrations. The ranges in f_r for the two size fractions from the DPEIS are given in Table 10. We also analyze the ratio of successful invaders using the p_e calculated using a concentration of 1000 m^{-3}, the approximate modal organism concentration after ballast water exchange (from Figure 2 in Minton et al., 2005).

Table 10: Mean rate of successful introductions for treatment alternatives relative to Alternative 1. The ranges in the first two columns are from the DPEIS and were derived from a range of both organism concentrations in unexchanged or exchanged ballast water and a range of c values (Table 5-4 of the DPEIS). To better focus on long-term trends, we compared the treatment alternatives to a single organism concentration of 1000 m^{-3} for ballast water exchange (BWE) and a range of value for c. The smaller the value, the greater the relative reduction in invasion risk compared to Alternative 1 or BWE.

Alternative	Single Voyage: 10 – 50 micron organisms (DPEIS range)	Single Voyage: >50 micron organisms (DPEIS range)	Single Voyage: >50 micron organisms (Comparison to BWE)
Alternative 2 (IMO)	0.92-1.0	0.50-1.0	0.71 – 0.85
Alternative 3 (1/10th IMO)	0.67-1.0	0.50-1.0	0.57 – 0.75
Alternative 4 (1/100th IMO)	0.67-1.0	0.41-1.0	0.43 – 0.62
Phase II USCG (1/1000th IMO)	-	-	0.29 – 0.46

The conclusions in the DPEIS from their values in Table 10 are:

> "The reduction in the mean rate of successful introductions is the complement of the ranges of values presented above. As a result, the reduction in the mean rate of successful introductions, as compared to the No Action Alternative under:
>
> • Alternative 2 is expected to range between no reduction and an 8% reduction, and no reduction and a 50% reduction for smaller and larger organisms, respectively;
>
> • Alternative 3 is expected to range between no reduction and a 33% reduction, and no reduction and a 50% reduction for smaller and larger organisms, respectively;
>
> and
>
> • Alternative 4 is expected to range between no reduction and a 33% reduction, and no reduction and a 59% reduction for smaller and larger organisms, respectively."

As mentioned above, we believe the use of the median reductions in concentrations compared to exchanged ballast water rather than the ranges in the DPEIS give a more representative picture of the long-term improvement due to the treatment alternatives. For Alternatives 2-4, the use of the median organism concentrations indicates a 15% to 57% reduction in the introduction rate of >50 micron organisms. For the USCG Phase II standard, the predicted relative reduction in invasion rate is 54% to 71%.

PVA Model Used in USCG Risk Assessment for Multiple Species Analysis:

The analysis detailed above predicts the relative effects of the alternative treatment options on the invasion probability of a single species discharged in ballast water. The DPEIS also

conducted a multiple species scenario, which addressed the probability of invasion by any of the multiple species in a ballast discharge. In other words, this analysis asks "What is the probability that at least one of the species in a ballast discharge will successfully invade?" Similar to the calculation of the probability of establishment for a single species with multiple discharges (Equation 14), the simplest equation to predict the probability that at least one of the multiple species discharged from a single ballast event is introduced is:

Equation 16: probability that at least one species becomes established = $1 - p_e^n$

Where:
n = number of species introduced in a single ballast water discharge that are not already successfully established in the waterbody.

This equation assumes that all species are independent and that they all have the same extinction probability. The second of these assumptions would nearly always be violated because of different densities of species. The more realistic equation used in the DPEIS is:

Equation 17: $q(m) = 1 - \prod_{j=1}^{n} p_j(m)$

Where:
q(m) = probability that at least one species is successfully introduced (= becomes established)

$p_j(m)$ = probability that species *j* is not successfully introduced in a single ballast water discharge under treatment option m based on the density of species j

To address the different densities of species, the DPEIS calculated the relative abundances of the n species using a geometric model. Additionally, because many of the species may be rare, and thus close to the critical population threshold, they compared the probability of extinction with $n_e = 1$ and $n_e = 100$. Finally, in this section they also considered different organism population sizes resulting from unexchanged and exchanged ballast.

For brevity sake, we will not detail the steps in this analysis, and the reader is referred to Section 5 of Appendix A of the USCG Draft Programmatic Environmental Impact Statement (USCG, 2008). Rather we will present the final relative treatment efficiencies for Alternatives 2-4 compared to initial organism populations equivalent to unexchanged and exchanged ballast water discharges. From these population sizes and the assumption of 12 different species within the ballast discharge, they calculated the probability (q_m) of at least one successful introduction of a species from the ballast discharge. Probabilities that no species would successfully invade for the treatment alternatives were then determined relative to those with unexchanged ballast water or exchanged ballast water (Table 11).

Table 11: Relative efficiencies of Alternatives 2, 3 and 4 that no species successfully invades from a ballast discharge compared to unexchanged ballast water or after ballast water exchange (BWE). The analysis assumes 12 unique species in the ballast discharge. The critical population threshold (n_e) is equal to 1 or 100 organisms. Modified from Table 5-9 in the DPEIS.

	$n_e = 1$		$n_e = 100$	
Alternative	Unexchanged	BWE	Unexchanged	BWE
2 (IMO)	52%	37%	78%	63%
3 (1/10th IMO)	73%	64%	94%	90%
4 (1/100th IMO)	88%	85%	100%	100%

The conclusion that the DPEIS draws from this analysis is, "The specific reduction depends upon the alternative selected and the size class of the organism considered, but the modeling results for multiple species support the conclusion that more stringent treatment alternatives will substantially reduce the likelihood of new ballast water introductions" (DPEIS, page A-42). This analysis also emphasizes the importance of evaluating the total risk for the multiple species in ballast discharges. Finally, the reduced risk at the higher critical population threshold value illustrates the importance of this value in driving the results from PVA models, at least at the very low population densities that will be associated with the proposed ballast water standards.

Assumptions and Limitations:

Use of PVA models are not without their critics (e.g., Ludwig, 1999; Fieberg and Ellner, 2000; Coulson et al., 2001). Coulson et al. (2001) "doubt the general claim that they can be accurate in their ability to predict the future status of wild populations." More optimistically, in a review of 271 time series representing 46 taxa, Holmes et al. (2005) concluded that diffusion approximations did a reasonably good job at predicting proportional and severe population declines. They were not as good at predicting true extinction. Some of the criticisms of PVA models are blunted when the PVA models are used to evaluate relative differences among treatment alternatives rather than to predict quantitative extinction probabilities, as was noted in the DPEIS (USCG, 2008). However, analyzing relative differences precludes the use of the models to directly develop organism-based discharge standards.

Values for several of the parameters in the DPEIS are not well justified, in particular the values for c and the critical population threshold. These are critical parameters that drive the conclusions, and any future PVA modeling effort needs to justify the input values better.

The multi-species scenario was only run for the >50 micron size class in the DPEIS. As data become available, a similar analysis should be attempted with the 10-50 micron size class. Additionally, the importance of the number of species used in the analysis should be explored, as the assumption of only 12 unique taxa in the DPEIS seems low given the diversity of phytoplankton and zooplankton found in ballast water (e.g., Choi et al., 2005; Cordell et al., 2009).

In several cases, the use of extreme ranges for input parameters in the DPEIS analysis obscured the long-term benefits from reducing organism concentrations. In particular, in the single species analysis, the ranges in organism concentrations of Alternative 1 bracketed the treatment concentrations, resulting in the erroneous conclusion that the treatment alternative would offer no improvement. It is true that some ships may have essentially no organisms in their ballast but this is a rare event (see Minton et al., 2005), and on the average ballast water treatment will substantially reduce organism concentrations, and hence risk. While the DPEIS notes this in their multi-species analysis, it was not apparent in the tables or in the discussion in the single species scenario.

The greatest practical limitation in developing PVA models for marine/estuarine organisms is deriving high quality values for the instantaneous population growth rates and variation from long-term population trends. Diffusion models have primarily been applied to birds and mammals based on 10+ years of population monitoring. Such long-term population data for the marine/estuarine organisms likely to be discharged in ballast water (e.g., phytoplankton, holoplanktonic zooplankton, and benthic species with pelagic larvae) are rare. Even when long-term monitoring data are available, population estimates for marine/estuarine organisms may display a higher sampling variability than found with birds and mammals, which can affect parameter estimation (Holmes et al., 2005; Holmes, 2004).

Recommendations and Conclusions:
Given the current state of the science and data availability, PVA diffusion models are appropriate tools to estimate the relative effectiveness of different ballast water treatment alternatives. While recognizing the substantial insights into relative treatment efficiencies provided by the DPEIS (USCG, 2008), we recommend that any new efforts using PVA models should begin anew rather than building upon the models in this document. We make this suggestion, in part because of the difficulty we had in following some of the specific procedures in the DPEIS and because presenting the results in terms of the total range of ballast water organism concentrations tended to obscure the benefits of the treatment alternatives. Additionally, an independent assessment may suggest a modified approach.

The use of PVA models to generate quantitative predictions of invasion success (versus relative treatment efficiencies) is less clear. The advantage of such models is that they would provide quantitative invasion risks for proposed discharge standards. The greatest limitation is the current lack of quantitative population vital rates. Accordingly, before initiating any quantitative PVA modeling study, we recommend that a dedicated effort be undertaken to extract estimates of population growth rates and variances from long-term studies of marine/estuarine species. One obvious source are the commercial catch statistics, but this would require separating population variability from variability due to fishing related mortality and/or changes in fishing effort. There are, however, other data that would not have these confounding effects. Eckert (2003) synthesized 570 population time series for 170 invertebrate species, with the durations ranging from one to 39 years while Eckert (2009) collected 786 population time series greater than 2 years for 226 species in the Gulf of Alaska. Desmond et al. (2002) report on an eleven year record of fish and invertebrates in Southern California. We are less familiar with zooplankton, but even a cursory scan of the literature suggested that long-term records exist for several estuarine and marine copepod species (Jossi et al., 2003; Pershing et al., 2004). Not all

these studies will be suitable for deriving vital rates, but with sufficient effort it should be possible to generate ecologically realistic ranges for population growth and variability for a suite of species across a range of taxa and habitats.

Whether assessing relative treatment efficiencies or quantitative risk probabilities, any future PVA analyses should focus on multiple species scenarios rather than modeling a single species. That is, the analysis should address the question "what is the likelihood of any species from a ballast discharge becoming established?" rather than "what is the likelihood of any particular species becoming established?" The former is the critical ecological and regulatory issue.

A comprehensive sensitivity analyses should be part of any new PVA modeling. In particular, a range of instantaneous growth rates and instantaneous variances in growth rate (the "c" parameter as used in the DPEIS formulation) should be explored, with the ranges based on the review of population vital rates mentioned above. Another important factor that should be evaluated is the critical population density. While a full range of values should be used for all the input parameters, it is critical that the interpretation of the results explicitly consider the likelihood of particular values so as not to obscure the general trends with rare events, such as ships with organism concentrations below the proposed standards.

Any new PVA modeling should evaluate the full range of potential discharge standards including the proposed USCG Phase II standards. Since the USCG may implement more stringent standards in an incremental fashion, we suggest that standards equivalent to $1/10^{th}$ and $1/100^{th}$ of the IMO standards (e.g., standards of 1 and 0.1 organisms per m^3 for the >50 micron class) also be evaluated.

VIII. PER CAPITA INVASION PROBABILITIES

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Overview:
As discussed in Section 2, there is a general consensus that an increase in propagule supply increases the likelihood of invasion. Based on this premise, we developed a "per capita invasion probability" (PCIP) approach to estimating the likelihood of invasion based on historical invasion rates and calculated ballast-associated propagule pressures. The PCIP is the per year probability that an individual non-native propagule discharged from ballast water will become established as a new nonindigenous species in a specified waterbody. Using a linear dose response assumption, the PCIP is calculated from the historical number of potential ballast-mediated invasions in a specified waterbody over a defined time period, the average annual total ballast discharged at that location during this time period, and the estimated organism concentration in the discharged ballast water. We focus on the >50 micron size class because sufficient data are available to calculate the PCIPs for multiple waterbodies and coasts, though the approach can be applied to the 10-50 micron size class if the data are available. We calculate coastal estimates of PCIPs for the East, Gulf, and Pacific coasts of the coterminous United States as well as individual PCIP values for 17 coastal estuaries. Additionally, we include a PCIP value for the Great Lakes as a preliminary assessment of whether standards developed for the marine/estuarine systems would be protective of freshwater systems.

An advantage of this approach is that it can be used to generate quantitative discharge standards because it directly relates the risk of invasion to ballast water organism concentrations. It is important to note, however, that because of the complexities involved with the invasion process (Table 2), our objective was not to find a highly predictive relationship between the calculated propagule supply and site-specific invasion rates. Rather, our objective was to "cut through" the complexities to develop an approach to allow risk managers to generate discharge standards based on defined assumptions and risk levels.

Calculation of Per Capita Invasion Probabilities:
The per capita invasion probability (PCIP) is calculated as:

Equation 18 : $PCIP = N_h / (D_h * C_h)$

Where:
PCIP = per capita invasion probability (new invading species * organism^{-1})
N_h = historical annual invasion rate of potential ballast-associated invaders for a waterbody (new invading species * year^{-1})
D_h = historic annual foreign ballast discharge rate into a waterbody (m^3 year^{-1})
C_h = historic concentration of organisms in ballast water discharged into a waterbody (organisms * m^{-3})

As mentioned, the PCIP is the probability that an individual propagule, or organism, discharged in ballast water will become established as a new nonindigenous species within the waterbody.

For example, if one new nonindigenous species became established within a waterbody in which a total of a million individual organisms were discharged in a year, the per capita invasion probability would be 10^{-6}. Because the PCIP only accounts for new invaders, it does not address the issue of multiple invasions of currently existing nonindigenous species into a waterbody.

This model assumes a linear dose-response, with the number of invaders increasing proportionally with larger ballast water organism concentrations and/or greater volumes of ballast water discharged. Accordingly, after calculating a PCIP from a historical invasion rate, it is possible to predict the number of new, unique invaders per year for a given ballast water organism concentration and ballast water volume:

Equation 19: $N_p = PCIP * D_p * C_p$

Where:
N_p = predicted annual invasion rate of potential ballast-associated invaders for a waterbody (new invading species * $year^{-1}$)
D_p = predicted annual foreign ballast discharge rate into a waterbody (m^3 $year^{-1}$)
C_p = predicted concentration of organisms in ballast water discharged into a waterbody (organisms * m^{-3})

Foreign Ballast Water Discharge Rates for Coastal Waterbodies and the Great Lakes:
Historical average annual foreign ballast discharge rates (D_h in Equation 18) were used to calculate the total propagule supply. Discharge rates for coastal waterbodies were obtained from the Smithsonian Institution ballast water database (see the National Ballast Information Clearinghouse, http://invasions.si.edu/nbic/search.html). Estimates for the contiguous East, Gulf, and Pacific coasts were generated from discharge records from all ships discharging foreign ballast into coastal ports on the respective coasts. Only ballast identified as coming from a foreign source was included. The values in Table 12 are the average of the yearly rates for the period 2005 to 2007, which was chosen because it occurs after the implementation of mandatory ballast water reporting and represents the most complete discharge records available. Average annual foreign discharge rates were also calculated from 2005 to 2007 for 17 coastal ports, representing a cross section of small to large ports. Because the foreign ballast was calculated on a per tank basis, the movement of undischarged foreign ballast among ports can be estimated. That is, by following foreign ballast by tank it is possible to account for foreign ships that initially entered one port but did not discharge their ballast until they visited another port. Foreign discharge values for multiple ports within a waterbody were summed for a total discharge volume for a waterbody, including freshwater ports in larger systems (e.g., Columbia River). For the Great Lakes, the National Biological Invasion Shipping Study (Reid and Carlton, 1997) reported a total annual foreign ballast water discharge into the Great Lakes of 1,395,461 metric tons in 1991. This is before mandatory ballast water exchange, which was initiated in the Great Lakes in 1993.

Estimates of Organism Concentrations in Ballast Water:
Organism concentrations in ballast water discharged in coastal waters (C_h in Equation 18) were estimated from Minton et al. (2005), who reported zooplankton (> 80 microns) concentrations in

Table 12: Historical number of invaders (N_h), foreign ballast discharge volumes (D_h), number of ships discharging foreign ballast, and per capita invasion probabilities (PCIP) for the East, Gulf, and Pacific coasts of the United States, 17 coastal ports, and the Great Lakes. The number of coastal invasions is the number of non-native invertebrates and macroalgae >50 microns first reported from 1981 to 2006 that were possibly introduced via ballast water and considered established. The total number of invaders in the coastal ports includes marine, brackish, and freshwater species, while the total without freshwater excludes the freshwater invaders. The foreign ballast discharges for the coastal waterbodies are the annual averages of 2005 to 2007 and include marine, brackish, and freshwater ports within the waterbody. Per capita invasion probabilities for the coastal waterbodies are given for a range of possible values, including the lower quantile (0.025), median, and upper quantile (0.975), based on the simulation estimates of organism concentrations among the ships discharging into a waterbody. The number of invaders for the Great Lakes is given for both macrofauna and phytoplankton for the period 1960 to 1988, while the ballast water discharge volume is for 1991. The sum of the discharge volumes and number of ships from the 17 ports is less than the coastal averages because all ports were included in the coastal values. FW = freshwater.

Waterbody	Total # Invaders / Total # w/o FW species	Average Annual Foreign Ballast Water Discharge Vol. (m^3 $year^{-1}$)	# Ships with Foreign Ballast Water 2005-2007	PCIP (lower 0.025 quantile)	PCIP (median)	PCIP (upper 0.975 quantile)
East Coast	**40**	**7,407,832**	**12,860**	**4.00E-11**	**4.31E-11**	**4.64E-11**
Charleston	13/12	281,160	563	3.05E-10	3.70E-10	4.46E-10
Chesapeake	17/14	3,011,982	1315	3.85E-11	4.51E-11	5.28E-11
Jacksonville	14/13	130,296	791	7.48E-10	8.58E-10	9.83E-10
Miami	4/4	578,482	2515	5.04E-11	5.51E-11	6.02E-11
Narragansett Bay	13/13	21,030	19	2.38E-09	5.41E-09	1.35E-08
Portsmouth	9/9	6,377	10	3.26E-09	1.54E-08	6.16E-08
Gulf Coast	**18**	**19,605,340**	**11,821**	**6.98E-12**	**7.31E-12**	**7.67E-12**
Corpus Christi	5/5	1,254,845	621	2.65E-11	3.18E-11	3.84E-11
Galveston	4/4	748,136	778	3.53E-11	4.28E-11	5.22E-11
Pensacola	3/3	1,121	8	8.72E-09	2.45E-08	7.88E-08
Tampa Bay	7/1	734,718	923	5.37E-11	6.54E-11	7.88E-11
Pacific Coast	**67**	**14,788,369**	**5998**	**3.41E-11**	**3.61E-11**	**3.83E-11**
Columbia River	22/12	5,533,618	1759	2.89E-11	3.17E-11	3.47E-11
Coos Bay	22/22	583,517	87	2.18E-10	3.04E-10	4.40E-10
Humboldt Bay	29/29	5,539	10	1.42E-08	5.24E-08	1.85E-07

Waterbody	Total # Invaders / Total # w/o FW species	Average Annual Foreign Ballast Water Discharge Vol. (m^3 $year^{-1}$)	# Ships with Foreign Ballast Water 2005-2007	Per Capita Invasion Probability (lower 0.025 quantile)	Per Capita Invasion Probability (median)	Per Capita Invasion Probability (upper 0.975 quantile)
Los Angeles / Long Beach	31/31	2,676,874	1693	8.20E-11	9.23E-11	1.05E-10
Puget Sound	23/21	3,960,438	1167	4.12E-11	4.64E-11	5.23E-11
San Diego Bay	23/21	31,271	112	4.20E-09	5.92E-09	8.52E-09
San Francisco Estuary	53/45	1,548,116	1015	2.33E-10	2.74E-10	3.22E-10
Great Lakes – Macrofauna	17	1,395,461	Unknown	NA	9.10E-11	NA
Great Lakes – Phytoplankton	14	1,395,461	Unknown	NA	NA	NA

unmanaged ballast water in 354 ships of various types (see Figure 3). Similar values were reported in a survey of 429 ships of multiple vessel types that had no ballast water exchange or treatment (MEPC, 2003b). Both of these studies showed that organism concentrations in untreated ballast water can vary by orders of magnitude among ships. For example, about 3.8% of the ships reported by Minton et al. (2005) had organism concentrations less then 10 m^{-3} while about 1.1% of the ships had concentrations greater than 50,000 m^{-3}. Thus, the actual propagule dose a waterbody receives will depend on the distribution of organism concentrations among the ships discharging within the system.

Because the distribution of organism concentrations in ballast water of is highly skewed, the mean concentration may over or underestimate the true propagule pressure depending upon the concentrations in the specific set of ship discharging within the waterbody. Consequently, rather than estimating PCIP values using the mean concentration of organisms we performed a simulation to estimate PCIP values from a range of possible propagule pressures. The simulation was performed by randomly assigning each ship discharging foreign ballast in a waterbody a concentration of organisms, selected from the distribution of values reported by Minton et al. (2005; their Figure 3a). The randomly selected concentration was then multiplied by the volume of foreign ballast discharged by that particular ship (see Table 12 for number of ships in each waterbody). The values for each ship within a waterbody were summed, generating a total propagule dose from which the PCIP value was calculated. This process was repeated 10,000 times to create a distribution of PCIPs for each waterbody from which the lower (0.025), median, and upper (0.975) quantile values were determined (Table 12). Figure 6 shows the range of PCIPs for the Pacific Coast generated with this method. Using a range of possible PCIP values allows us to make predictions that do not underestimate the risk of invasion, which might occur if only the mean concentration of organisms is used. (Note that with a fixed historical invasion rate, higher PCIP values result from lower discharge values since the same number of invaders occurred with a lower propagule pressure.) Because we did not have individual ship records for the Great Lakes during 1991, we could not generate the PCIP distributions and instead used the mean ballast water organism concentration from the IMO baseline study (4640 m^{-3}, MEPC, 2003b) to calculate the PCIP for the Great Lakes.

Estimates of Historical Invasion Rates:

The total numbers of invaders reported between 1981 and 2006 were synthesized for the contiguous United States Pacific Coast, East Coast, and Gulf Coast as well as for 17 individual coastal waterbodies (Table 12). The 1981 to 2006 time period is before the implementation of mandatory mid-ocean ballast water exchange for coastal waterbodies, allowing the use of the estimates of organism concentrations in unexchanged ballast. A 25 year time period was chosen to smooth out short term variations in invasion rates as well as variations in monitoring efforts. A longer time period also helps to mitigate effects of the lag between an actual invasion event and when the species is first discovered (e.g., Costello and Solow, 2003).

The number of invaders is based on non-native invertebrates and macroalgae >50 microns; fishes and vascular plants were not included. Besides being reported in each coast or waterbody within the 25 year window, the species included in the analyses had to be considered established and potentially introduced via ballast water. The coastal invaders were classified into three salinity tolerance regimes: marine/estuarine (>20 psu), brackish (0.5-20 psu), and freshwater (<0.5 psu). This broad classification allows an evaluation of the importance of freshwater invaders in river-dominated estuaries such as the Columbia River. Because of the poor resolution between native

versus nonindigenous phytoplankton species in coastal waters (Carlton, 2009), no attempt was made to estimate the number of invaders in the 10-50 micron size class. The number of invaders was generated from the Smithsonian Institution invasive species database (Fofonoff et al. 2003b) and the majority of the East, Gulf, and Pacific invaders and their vectors are listed in Appendix A of Ruiz et al. (2000).

The 1960 to 1988 time period was chosen for the Great Lakes because it is before the implementation of mandatory ballast water exchange in 1993. During this interval, a total of 17 macrofaunal ballast-associated invaders was reported (http://www.glerl.noaa.gov/res/Programs/ncrais/docs/great-lakes-list.xls, accessed September 26, 2009), resulting in an invasion rate of 0.58 invaders per year. This rate is based on all shipping-related invaders as well as three macrofaunal invaders with unknown vectors. The invasion rate for phytoplankton was similar (Table 12), resulting in a total rate of slightly more than 1 invader per year which is similar to that reported by Ricciardi (2006).

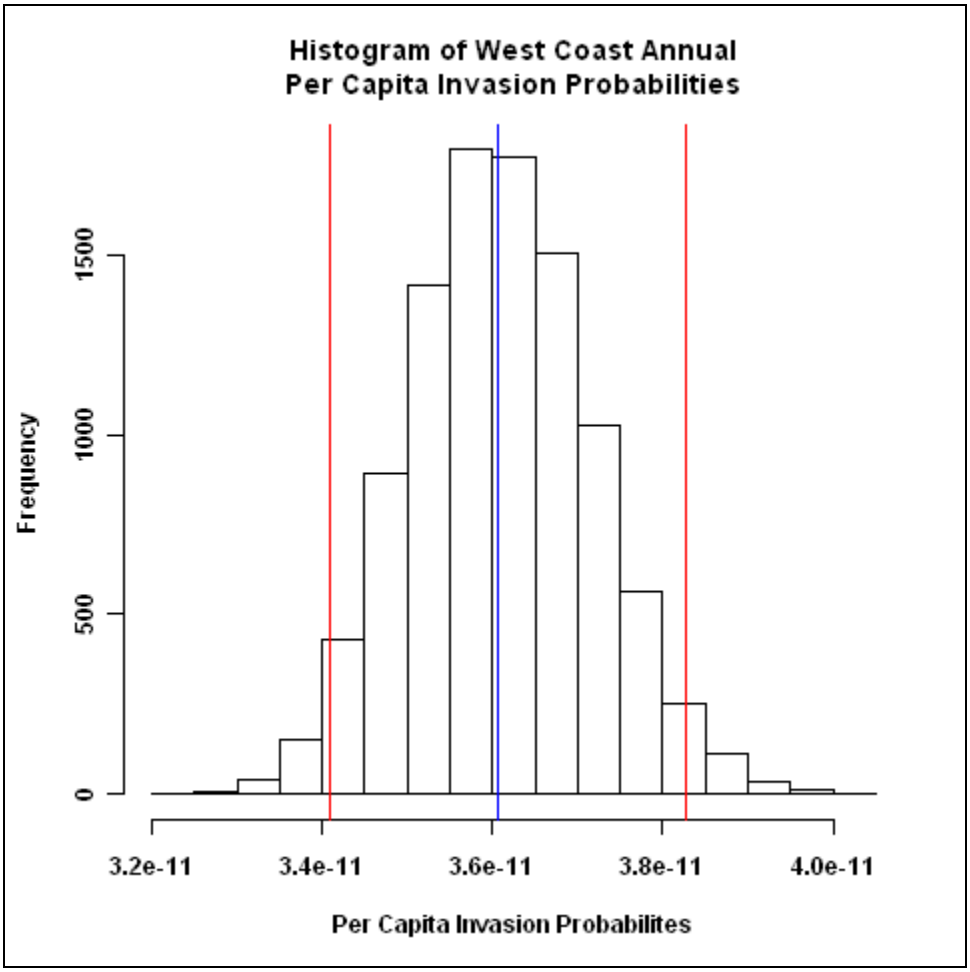

Figure 6: Distribution of per capita invasion probabilities (PCIPs) for the Pacific Coast based on 10,000 random simulations of organism concentrations among the 5998 ships discharging foreign ballast. The red lines indicate the lower 0.025 quantile and the upper 0.975 quantile while the blue line indicates the median. Approximately 95% of the values fall between the red lines.

Uncertainties in Historical Invasion Rates and Safety Factors:
Of the three parameters going into the calculation of a PCIP, the historical invasion rate has the greatest uncertainty and it is worth exploring both the sources of this uncertainty and whether it tends to over or underestimate future invasion rates. One source of this uncertainty is that many coastal nonindigenous species can potentially invade through multiple vectors, such as both ballast water and hull fouling (e.g., Fofonoff et al., 2003a). Inclusion of these "polyvectic" invaders (Ruiz and Carlton, 2003) in the historic invasion numbers in Table 12 potentially inflates the ballast-associated invasion rate, resulting in an artificially high PCIP. Because of differences in the relative importance of different vectors among estuaries, uncertainty related to multiple vectors is probably greater when comparing among estuaries than for the coast-wide estimates. For example, San Diego Bay, which has a high invasion rate relative to the ballast discharge volume, is the home to the largest naval base on the Pacific Coast consisting of approximately 54 naval ships. Ballast discharges from military ships are not included in the volumes in Table 12, but most naval ships tend to discharge relatively small amounts of ballast (see Table 2 in Appendix A of U.S. EPA, 1999), which suggests a higher propagule pressure from hull fouling in San Diego. Hull fouling may also be relatively more important in smaller ports that have low ballast discharge rates but a relatively large number of commercial fishing and recreational boats with no foreign ballast.

Secondary invasions could also inflate estimates of historical ballast-associated invasion rates in individual waterbodies. After the primary invasion and establishment of a new NIS into a biogeographic region, the invader may spread within the biogeographic region via secondary invasions from the initially established population. Likely mechanisms for secondary invasions include ballast water discharges and hull fouling via intracoastal commercial traffic emanating from the infected waterbody (e.g., Simkanin et al., 2009; Cordell et al., 2009) as well as hull fouling on recreational boats. Secondary invasions may also occur via natural dispersal mechanisms, such as currents and rafting, as suggested by occurrence of soft-bottom NIS in Pacific Northwest estuaries with no ballast discharges or oyster aquaculture (Lee et al., 2006; Lee, unpublished data).

An important source of uncertainty that could result in underestimating PCIP values is the underestimation of historical invasion rates. Carlton (2009) identified 12 sources of error leading to invader underestimation including unknown, unreported, misclassified, and rare invaders. In some parts of the world, such as Denmark, South Africa, and Chile where no invasions prior to mid-nineteenth century are recognized, the number of known invaders could be underestimated by as much as 5 to 10 times (Carlton 2009). For California, Cohen (in Falkner et al., 2006) suggested that unrecognized invaders could increase the invasion rate by 50% to 100%. A recent analysis of California invaders lists 457 cryptogenic species versus 358 nonindigenous species (California Dept. of Fish and Game, 2009); the California invasion rate would more than double if all these cryptogenic species were actually nonindigenous. While some of these cryptogenic species are likely unrecognized native sibling species (e.g., Knowlton, 1993), the high number of cryptogenic species in California suggests that the reported number of invaders may underestimate actual numbers by 50% to 100% within the United States.

Other sources of uncertainty could also cause us to underestimate the risk of introducing new invaders through ballast discharges: the relationship between propagule pressure and the

probability of invasion could be steeper than the proportional relationship we assume, in particular at very low concentrations (curve d in Figure 2); survival in ballast tanks could improve if voyage durations decrease due to faster ships; and waterbodies may become increasingly susceptible to invasion due to climate change or other environmental changes. While it is not possible to quantify the total uncertainty from these various sources, safety factors on the order of 5 to 20-fold have been proposed when calculating the risk to endangered and threatened species from exposure to pesticides (U.S. EPA, 2004b), and similar ranges could be used in the generation of discharge standards (see Equations 20 and 21). We strongly suggest using a single safety factor rather than multiplying a string of individual safety factors for each source of uncertainty, which quickly results in unrealistic values (see Chapman et al., 1998).

Among Port Patterns of Invasion Risk:
There is considerable range in the PCIP values among the 17 individual ports both along a single coast and across coasts (Table 12). The largest difference is between the Humboldt Estuary and Columbia River, a more than 1600-fold difference. We suspect these among-estuary differences are due to a suite of non-exclusive factors. Part of this range may reflect differences in the invasibility among waterbodies, whether due to differences in biotic resistance or local environmental drivers. For example, the lower invasion probability in the Columbia River compared to other large Pacific Coast ports may be partially explained by wide seasonal and tidal salinity fluctuations (e.g., Hickey et al., 1998) that largely limit estuarine invaders to euryhaline or freshwater species.

One pattern observed on all three coasts is that the smaller ports had more invaders than expected from the amount of foreign ballast water, which resulted in higher PCIP values. Humboldt Bay, a small port in northern California, had only ten ships discharging foreign ballast from 2005 to 2007 (Table 12). Even with this small ballast input, Humboldt had the third largest number of invaders of the 17 estuaries, only exceeded by the San Francisco Estuary and the Los Angeles/Long Beach port. It is possible that these smaller ports have a greater invasibility than larger systems, but we suggest secondary invasions and invasions via mechanisms other than foreign ballast water discharges are relatively more important in these systems, which inflate the PCIP values. In particular, Humboldt Bay's proximity to the San Francisco Estuary and the prevailing northward oceanographic currents along the coast from San Francisco Estuary (particularly in El Niño years) may provide one mechanism of secondary invasion (Grosholz, 1996; Behrens Yamada et al., 2005) in addition to intracoastal shipping.

We evaluated the potential effect of polyvectic species and secondary invasions on the invasion rate in Humboldt by removing NIS from the Humboldt list if they: 1) had been observed in Pacific Coast estuaries that do not receive ballast water discharges; 2) were found on the outer coast; and/or 3) had a potential vector other than ballast water. Of the 29 potential ballast-water invaders reported from Humboldt between 1980 and 2005, the introduction of only two could not be explained by mechanisms other than foreign ballast water discharges in Humboldt. The corresponding PCIP value (median = 3.58E-09) with the reduced invader list is only about 5% of the value when all potential invaders are included. We suspect that secondary invaders and polyvectic invaders also inflate the PCIP values in the other small ports. Another issue for estimating invasion probabilities in small estuaries is the large statistical variability in estimates

based on small sample sizes. Consequently, ports with small amounts of ballast discharge will have high PCIP values even with the occurrence of a single ballast associated invader.

Because of these factors, we believe the PCIP values for the moderate to large ports are more reliable, with moderate/large ports defined as those having an average annual foreign discharge volume of $\geq$100,000 m^3. This threshold was chosen because of a distinct break in ballast discharge volumes that occurs between 31,271 m^3 (San Diego) and 130,296 m^3 (Jacksonville). The 12 moderate/large ports contribute 99.67% of the total ballast from the 17 estuaries. The range in PCIP values among these moderate to large ports is only about 28-fold compared to the more than 1000-fold range when the small ports are included.

Discharge standards can be generated for individual ports by rearranging Equation 19 to calculate the organism concentration in ballast water (C_p) associated with a projected ballast discharge volume (D_p), acceptable risk as represented by the number of new invaders per year (N_p), PCIP value from Table 12 or otherwise calculated, and a safety factor:

Equation 20: $C_p = N_p/(D_p * PCIP * \text{Safety Factor})$

Safety factor = number $\geq$1 (unitless)

What value to use for the PCIP in Equation 20 is a risk management decision. The 0.975 quantile represents an upper probability that a propagule discharged from ballast water will become established as a new invader based on the distribution of organism concentrations in the ships discharging into the port/estuary. The median represents an "average" probability of establishment based on the "average" organism concentration in the ships. Similarly, the inclusion and size of any safety factor is also a risk management decision. Because it is in the dominator, the safety factor is set to 1 if no adjustment is made for uncertainties.

Because of the uncertainties surrounding invasion rates for single estuaries, we believe a better alternative is to base the standard on a specified confidence interval (e.g., upper 95% CI) around the PCIP values for the 12 moderate/large ports. An advantage of this approach is that it incorporates the among estuary variation in PCIP values in the calculation of the discharge standard. Using this approach, the formula to calculate the discharge standard is:

Equation 21: $C_p = N_p/(D_p * PCIP_{CI} * \text{Safety Factor})$

$PCIP_{CI}$ = probability that a single propagule from ballast discharge will become established as a new invasive species; calculated for a given confidence interval estimate of PCIP for the 12 moderate to large ports.

PCIP values for 12 individual ports can be based on the 0.5 (median) or 0.975 quantile estimates from the simulations of organism concentration for each ship, or some other quantile value from the randomization. Additionally, different confidence levels can be used for $PCIP_{CI}$. Table 13 gives the 90%, 95%, 99%, and 99.9% upper confidence intervals generated for the 12 moderate and large ports around the median and 0.975 quantile values. These are two-tailed confidence intervals so, for example, 5% of the values are larger than the 90% confidence interval values.

Assuming a doubling of the annual ballast water discharge rate on the Pacific Coast to 30 million m^3 (see Table 12), an acceptable risk as represented by an invasion rate of one new invader per thousand years, the upper 99.9% confidence interval value for the 0.975 quantile PCIP for the Pacific Coast, and a 10-fold safety factor, the discharge standard becomes:

Equation 22: $C_p = (1x10^{-3}$ invader/yr) / ($30x10^6$ m^3 ballast water/yr * $5.90x10^{-10}$ invader/organism * 10) = 0.006 organisms m^{-3}

The resulting discharge standard of 0.006 organisms m^{-3} is similar to the USCG Phase II standard for >50 micron organisms (0.01 organisms m^{-3}). The value derived from Equation 22 is based on a number of protective assumptions, including doubling the current Pacific Coast ballast discharge volume, using the 0.975 quantile for the estimate of PCIP, using the upper 99.9% CI value, and including a 10-fold safety factor. Modifying the assumptions changes the discharge standard to varying degrees, and one way to visualize the "regulatory landscape" is to plot the invasion probabilities as a contour plot, or "risk diagram", as a function of ballast water discharge volumes and organism concentrations. (Note that while the risk diagrams include long-range predictions, invasion rates for 1000 to 10,000 years in the future are best interpreted as indicating a very low probability of invasion rather than quantitative predictions.) Figure 7 shows the risk diagrams for the Pacific Coast based on three different safety factors (1, 10, and 20), using the PCIP value for the 99.9% confidence interval of the 0.975 quantile value from the 12 moderate/large estuaries. We consider these risk diagrams as a complement to Equation 21, and the R code (R Development Core Team, 2008.) to generate these diagrams based on different input values is given in Appendix A.

Table 13: $PCIP_{CI}$ estimates based on upper 90%, 95%, 99%, and 99.9% confidence intervals around the median and 0.975 quantile PCIP values for the 12 moderate to large estuaries in Table 12.

	Upper 90% CI	Upper 95% CI	Upper 99% CI	Upper 99.9% CI
Median	3.48E-10	3.77E-10	4.41E-10	5.34E-10
0.975 quantile	3.71E-10	4.05E-10	4.80E-10	5.90E-10

Coastal Patterns of Invasion Risk:

Due to the significant potential for secondary invasions, we believe the best alternative to developing discharge standards is to use Equation 20 with PCIP values derived from the aggregated data for an entire coast. The aggregated coastal data eliminate the uncertainty associated with secondary invaders as the historical invasion rate is based on the number of unique invaders to a coast so no invader is counted more than once. This approach is supported by the small variance in PCIP values among the coastal regions. In particular, there is only a 19% difference between the East and Pacific coasts (Table 12). The Gulf Coast PCIP is less than 6-fold smaller than the East or Pacific coasts, while the PCIP value for macrofauna for the Great Lakes is about 2-fold larger than those for the East and Pacific Coasts. Thus, even when comparing across three different coasts and the Great Lakes, there is only slightly more than a 12-fold range in the PCIP values. This relatively small range across diverse environments with different ballast discharge volumes and donor regions indicates that the analysis at this spatial scale captures many of the sources of variation.

We focus our analysis on the Pacific Coast because the extensive research on the distribution of NIS in this region (e.g., Cohen and Carlton, 1995; Cohen et al., 2001; Lee et al., 2003: deRiveria et al., 2005; California Dept. Fish Game, 2009) produces the most complete historical invasion rate. Using the same inputs for an acceptable risk level, ballast water discharge volume, and safety factor as for the estuary calculation (Equation 22), and the upper 0.975 quantile PCIP value specific to the Pacific Coast, the discharge standard becomes:

Equation 23: $C_p = (1x10^{-3}$ invaders/yr) / ($30x10^6$ m^3 ballast water/yr * 3.83 10^{-11} invaders/organism * 10) = 0.087 organisms m^{-3}

Based on this set of assumptions, the discharge standard for >50 micron organisms would be approximately 100-fold lower than the proposed IMO standard, about 9-fold higher than the Phase II USCG standard, and about 10-fold higher than the standard derived from the multiple estuaries (Equation 22). As another example we set the acceptable risk at one new invader per 100 years, the safety factor to 2, and use the median PCIP value instead of the upper quantile. With these less protective assumptions, the standard is 4.6 organisms m^{-3}, about 2-fold lower than the IMO standard. Both of these predictions are illustrated as risk diagrams in Figure 8.

Assumptions and Limitations:
The approach described here has not been subject to peer review. However, we have a draft of a paper and our goal is to submit it to a peer-reviewed journal in 2010.

As with any approach used to establish ballast water discharge standards, the per capita invasion probabilities make a number of assumptions. We list the major assumptions in Table 14 along with an assessment of how they affect the calculation of the PCIPs and the discharge standard derived from these probabilities.

The PCIP values for the smaller ports are substantially higher than those for systems with moderate to large ballast discharge volumes. As discussed, we believe this is largely a result of secondary invasions inflating the presumed ballast-associated invasion rate. However, if the higher invasion rates are actually a result of the smaller ports having a greater invasibility, the standards generated from the coast values or the moderate/large ports would not be protective of these systems. Another way that the present analysis could underestimate risk is by failing to account for the introduction of species that can become established with a single or very small number of individuals, such as a parthenogenic species. As discussed in Section II, the only absolute protection against such invaders is a true zero discharge standard.

Our analysis is limited to organisms >50 microns, though the PCIP approach is theoretically applicable to smaller size classes. The practical limitations, however, are the difficulty in distinguishing native from nonindigenous protozoa, phytoplankton, and microbes and the corresponding lack of data on historical invasion rates. As pointed out by Carlton (2009), “no introduced diatoms, dinoflagellates, or other phytoprotists are recognized in San Francisco Bay, at either the morphospecies or genospecies level” despite the abundance of phytoplankton in ballast water. However, it would be possible to conduct an analysis for the Great Lakes given the reported historical invasion rate for phytoplankton (Table 12) if an estimate for the historical ballast water phytoplankton concentrations can be obtained.

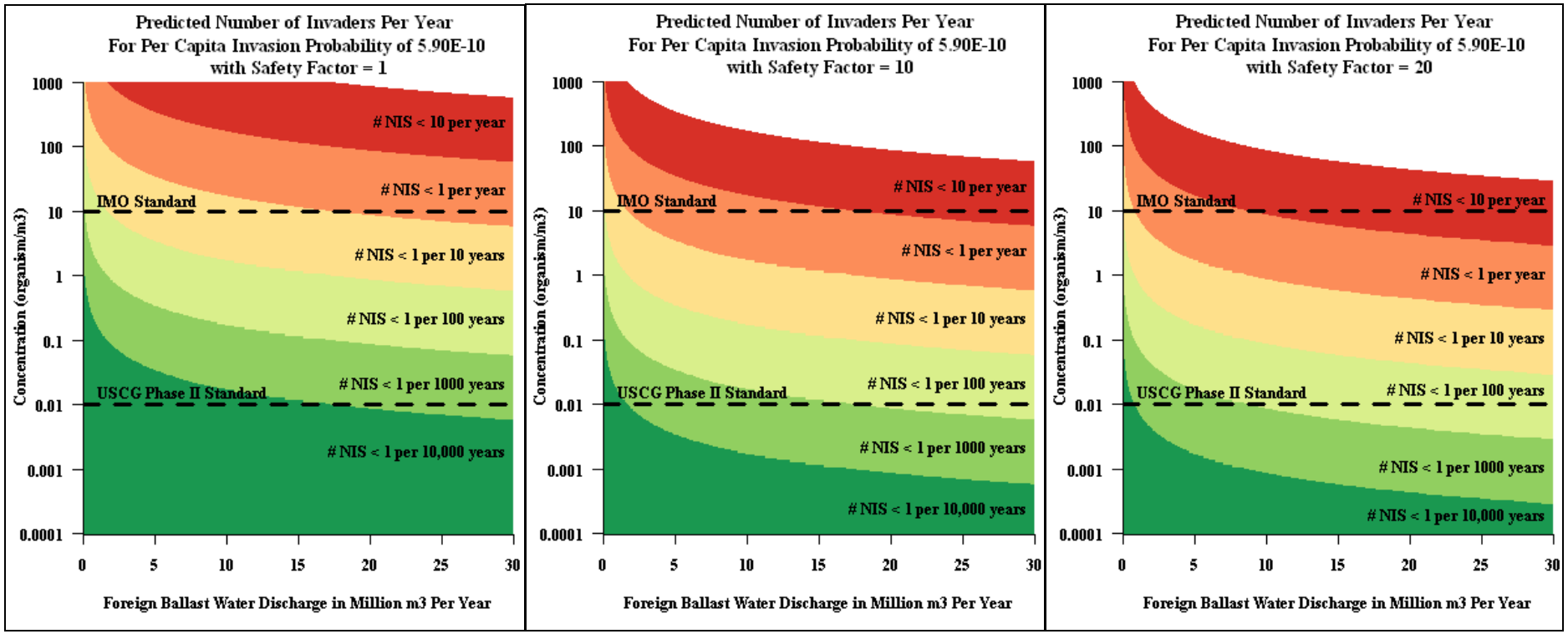

Figure 7: Risk diagrams for the Pacific Coast illustrating the effect of three different safety factors (1, 10, and 20). Calculations are based on the 99.9% confidence interval of the 0.975 quantile value of PCIP from the 12 moderate to large estuaries. A safety factor of 1 means that there was no adjustment for the uncertainties.

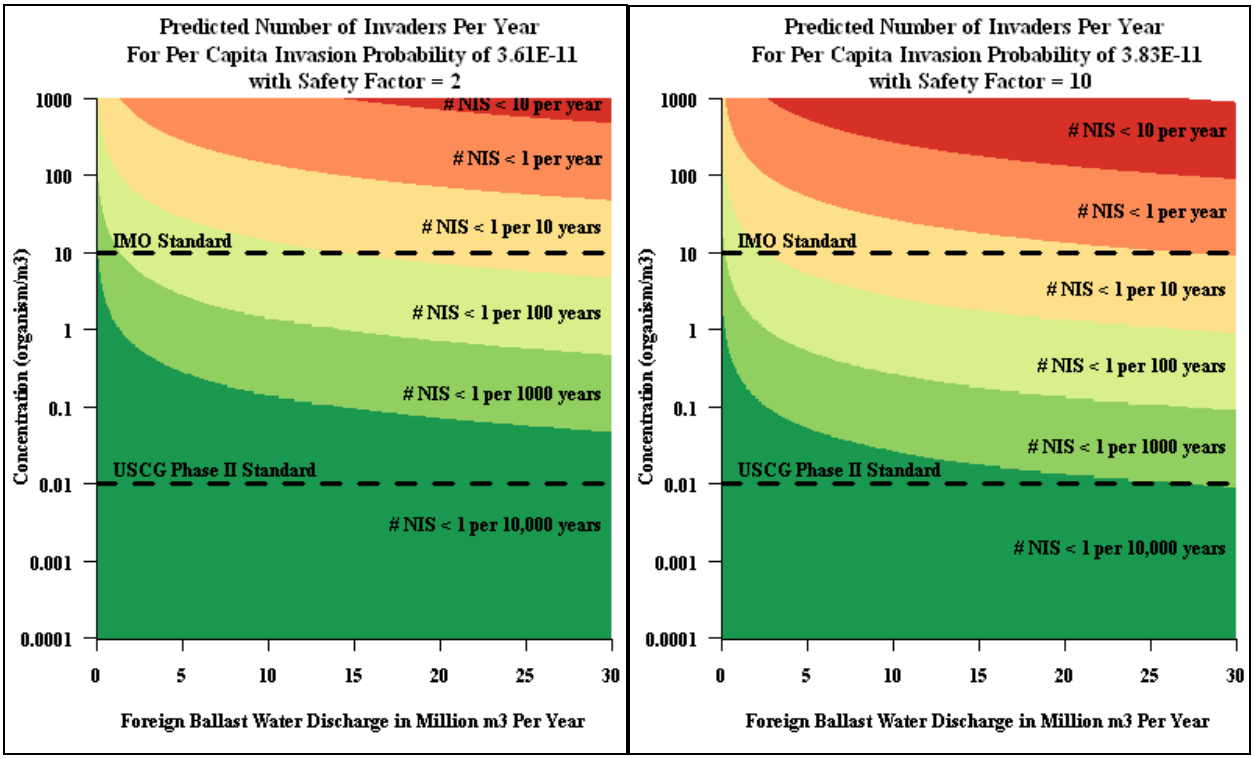

Figure 8: Risk diagrams for the Pacific Coast based on less protective (left diagram) and more protective (right diagram) assumptions. The risk diagram on the left is based on the median PCIP for the Pacific Coast and a safety factor of 2. The diagram on the right is based on the upper 0.975 quantile PCIP value and a safety factor of 10.

By using past invasion rates to predict future rates, fundamental assumptions of the per capita probability approach is that neither the invasion potential of any new invaders or the invasibility of the waterbody itself will change in the future. Actually, these assumptions apply to nearly all the approaches (e.g., use of previously measured population vital rates in PVA models) but the issue is more apparent when using historical rates. If the best colonizers tended to invade first, then the PCIPs derived from these historical data would over predict the number of new invaders for a given propagule pressure. However, the apparent increase in the rate of invasion in a number of aquatic ecosystems (e.g., Cohen and Carlton, 1998; Holeck et al., 2004) is the opposite of what would be expected if there had been a general decrease in the virility of new invaders. Changes in the invasibility of aquatic ecosystems are more difficult to assess. In particular, environmental change associated with climate change is a "wild card" for all the approaches to setting discharge standards. Development due to port expansion could also change the invasibility of a system. Probably the only practical near-term solution is to incorporate a safety factor in anticipation of such changes. Over a longer-term, it is possible to periodically evaluate PCIP values for a coast to determine if there have been any substantial changes.

Recommendations/Conclusions:

The per capita invasion probability approach attempts to cut through the "Gordian Knot" of uncertainties associated with predicting ballast water invasions, and Equations 20 and 21 and the

risk diagrams (Figures 7 and 8) can be used to set organism-based discharge standards. As with all approaches, however, there are a number of assumptions (see Table 14). Accordingly, our strategy was to develop an approach that allows risk managers the option to develop discharge standards with different risk levels based on different sets of assumptions. Specifically, the following inputs can be set: 1) acceptable invasion risk as measured by an invasion rate; 2) ballast water discharge volume; 3) use of PCIPs based on median ballast water organism concentration or upper quantile values; 4) median or an upper confidence interval around the PCIP with the among-port analysis; and 5) magnitude of any safety factor.

The uncertainty around the parameters going into the per capita invasion probability model is relatively small. Even with the historical invasion rate, the uncertainty is only on the order of 2-fold for the Pacific Coast. In comparison, our analysis suggests that there are much greater levels of uncertainty in the population vital rates that are needed for reaction-diffusion or PVA models. Additionally, the model does not have to be parameterized for each species or type of species as with the population modeling approaches. Finally, the data going into the per capita probability approach are readily understandable by managers and the public, which is beneficial in gaining acceptance for any ballast water discharge standard.

Of the three approaches to setting discharge standards (PCIP from individual estuaries; values based on upper confidence intervals from distributions of PCIPs about individual estuaries; PCIP values based on aggregated coastal values), we suggest that the coastal approach has the lowest inherent uncertainty. Furthermore, since most invaders spread along the coast, analysis at this scale is ecologically appropriate. Because of the extensive effort in documenting invaders on the Pacific Coast, the PCIP values for the Pacific Coast are the most reliable and we recommend using this coast to generate discharge standards for marine and estuarine ports within the United States.

The PCIP value for macrofauna for the Great Lakes is about 2-fold larger than those for the East and Pacific coasts, suggesting that there may be a greater likelihood of any individual propagule becoming established as a new invader in the Great Lakes. However, less complete data were available for ballast discharge volume and organism concentrations, and we consider the calculations for the Great Lakes a preliminary analysis. While there is the complicating factor of mandatory ballast water exchange after 1993, it may be possible to generate more up-to-date data for an analysis using the PCIP approach with a detailed study on the Great Lakes. As mentioned above, a study focused on the Great Lakes may also allow an analysis on phytoplankton invasion rates.

Secondary invasions appear to be an important source of uncertainty. To understand the role of secondary invasions better, future surveys for nonindigenous species should not only focus on the larger ports but should also include smaller ports and estuaries with no foreign ballast input. Additionally, further studies of the role of intracoastal shipping and ballast discharges are needed to help elucidate their role in spreading invaders into smaller ports with minimal foreign ballast water discharges.

Table 14: Major assumptions of the per capita invasion probability (PCIP) approach to setting ballast water discharge standards.

Assumption	Effect on Estimate of Per Capita Invasion Probability	Effect on Discharge standard	Mitigation Approaches
Linear dose-response	Likely over estimates invasion probability for many sexual species due to Allee effects; potentially under estimates for asexual and parthenogenic species.	Protective against most sexual invaders; possibly under protective for asexual and parthenogenic species.	Use upper bound estimates for input values and/or safety factor to account for cases when dose-response is more than linear.
Secondary invasions did not contribute to historical invasion rate.	Inflates PCIP to the extent that invaders did not invade via foreign ballast water discharged into the waterbody.	Erroneously results in too low discharge standard.	Exclude small ports from analysis and/or conduct analysis on a coastal scale.
Polyvectic invaders actually invaded via ballast water.	Inflates PCIP to the extent that polyvectic invaders were introduced via some vector other than foreign ballast.	Erroneously results in too low a discharge standard.	Analysis on coastal scale would correct if species invaded via ballast water anywhere on coast.
Exclusion of small ports from across-port calculations.	Generates more accurate PCIPs if invasions in small ports from secondary vectors. Artificially decreases PCIP if actual primary invasions into the small ports.	Depends whether invasions in small ports are from primary or secondary vectors.	Conduct analysis on a coastal scale so that all ports and invaders included.
No change in the invasion potential of new invaders over time.	Decrease in viability of new invaders results in PCIPs based on historical rates over predicting new invasions.	Erroneously results in too low a discharge standard. .	No adjustment unless further data indicates actual change in invader viability.
No change in invasibility of waterbody over time.	Either increases or decreases PCIP depending upon type & magnitude of environmental changes in waterbody.	Protective or under protective depending upon the type & magnitude of changes.	Use upper bound estimates for input values and/or safety factor to account for changes in environment.

IX. EXPERIMENTAL APPROACHES

Henry Lee II

Overview:
Laboratory and field experiments can be used to quantify the likelihood of invasion under controlled environmental conditions and dosing scenarios. Such experiments may represent the cutting edge in invasion science, at least in the Popperian sense, and the frequency of experiments has increased over the last decade (see review of extinction studies in experimental populations by Griffen and Drake, 2008). It appears that freshwater studies have primarily used laboratory experiments while field experiments are used more frequently with marine/estuarine species. Examples of laboratory experiments with freshwater organisms include those by Drake and his collogues (e.g., Drake and Lodge, 2004; Drake, 2006; Griffen and Drake, 2008; Drake and Griffen, 2009). An example of a freshwater field experiment is Bailey et al. (2009) who used field enclosures to parameterize and evaluate the diffusion approximation PVA model. Their results indicated that the proposed IMO standards for >50 micron organisms would reduce the probability of establishment of certain parthenogenic species by three fold. Examples of marine/estuarine field experiments include the studies on the recruitment of native and nonindigenous bryozoans (Clark and Johnston, 2005; Piola and Johnston, 2009).

Assumptions and Limitations:
The successful introduction of any specific species is a rare event. For example, it took the green crab about another century to invade the Pacific Coast after invading the East Coast (Carlton and Cohen, 2003). Quantifying the probability of such rare events is generally impractical using experiments. The main problem is that the number of samples becomes prohibitively large when attempting to quantify probabilities of events with likelihoods of 10^{-3} to 10^{-6}. This is the classic problem when attempting to determine the carcinogenic potency of a compound using laboratory exposures.

Some of the experiments have used high propagule doses, which biases the results to the right side of the propagule supply curve (Figure 2). Experimentally testing the recruitment of a species into an established community at the density in the USCG Phase II standard (0.01 organisms m^{-3}) will prove especially challenging.

Most marine/estuarine species as well as many freshwater species are difficult to culture and spawn in the laboratory. This limits the experiments to the aquatic "white rats" (e.g., use of *Daphnia magna* in Drake and Griffen, 2009). Such species are often "opportunistic" and thus are unlikely to be representative of the full breadth of potential invaders in foreign ballast.

All the freshwater experiments that we are aware of have used planktonic organisms, presumably because of the ease of culture and manipulation. Many of the marine experiments have used bryozoans or barnacles. We are unaware of any studies that have evaluated propagule supply with soft-bottom species, such as polychaetes. This taxonomic limitation potentially biases the results from experiments.

The main advantage of experiments, the tightly controlled environmental and biotic conditions, is also one of its main limitations. The world is much more complex than can be simulated in a beaker or even in a field enclosure. This "dumbing down" of nature in experimental studies may be why "Results from laboratory experiments often conflict with field studies" (Griffen and Drake, 2009).

Recommendations/Conclusions:
We believe it is impractical to derive discharge standards using the experimental approach because of the: 1) impracticality of adequate replication to quantify rare events; 2) limitation in the number and types of species than can be experimentally manipulated; and 3) artificiality and simplification of laboratory experiments and, to a lesser extent, field experiments.

The real power of the laboratory and field experiments is to advance the theory of propagule supply, test the assumptions of the various invasion models, and parameterize the population models that predict the probability of invasion. The recent work by Bailey et al. (2009) and Britton-Simmons et al. (2008) are good examples of how experimental studies can be coupled with population models.

X. STATISTICAL CONSIDERATIONS IN ESTIMATING THE CONCENTRATION OF ORGANISMS IN BALLAST WATER DISCHARGES

Melanie Frazier, Henry Lee II, and Deborah A. Reusser

Overview:
In the previous sections, we evaluated the potential utility and limitations of several approaches for generating ballast water discharge standards; here we address the statistical issues associated with monitoring organisms at very low concentrations. This is not an approach for setting standards; however these issues must be considered when assessing the practicality of verifying a discharge standard either in test facilities or as part of compliance monitoring. The stringent discharge standards that have been proposed by various agencies will require estimating very small concentrations of organisms in ballast water. This will be challenging due to the inherent stochasticity of sampling. Furthermore, at low densities, very large volumes of water must be sampled to find enough organisms to begin to estimate concentration. Understanding the limitations and requirements of sampling will help inform the development of protocols that ensure discharge standards are adequately implemented.

Stringent discharge standards are environmentally appealing because they are very protective; however, they present challenges because it is difficult to estimate low concentrations through sampling. The U.S. Coast Guard recently proposed a Phase II discharge standard of <0.01 organisms m^{-3} for organisms >50 µm, a standard 1000 times more stringent than the IMO's (USCG proposal is currently in the Federal Register, 74 Fed. Reg. 44632, August 28, 2009). Some states, such as New York and California, have proposed discharge standards of "zero detectable organisms" (Dobroski et al., 2009a). We explore some of the statistical issues that must be considered for either ship-board testing in the field or type-approval testing of treatment systems in controlled facilities. We do not address the logistics of sampling ship ballast water, which are described in references such as Lemieux et al. (2008) and Wright (2007). We also use a "best-case-scenario" approach: we limit our focus to organisms > 50 µm in size, which are the easiest to quantify; we assume no human or equipment error, such that all organisms in a sample volume are counted; and for most scenarios, we assume organisms are randomly distributed in the ballast discharge.

Rationale:
Currently, a great deal of effort is being devoted to selecting a discharge standard that adequately protects against invasive species. An important aspect of testing whether ballast discharges meet these standards, during either the testing of treatment systems or compliance monitoring of individual ships, is developing sampling protocols that are adequate for a discharge standard. For example, a standard of "zero detectable organisms" may seem very protective, but in reality, the degree of protection depends on the sampling protocol. If a small volume is used to evaluate whether the discharge meets a standard, the sample may contain zero organisms, but the true concentration of organisms may be quite high. For example, even with a relatively high concentration of 100 organisms m^{-3}, only about 10% of 1 L samples will contain one or more organisms. Furthermore, even if zero organisms are detected in a 1 L sample, the upper possible

concentration, based on a 95% confidence interval, is about 3,000 organisms m^{-3}. More information about these calculations is presented below. The general point is that more organisms may be released in ballast discharge using a stringent standard paired with a poor sampling protocol than a more lenient standard paired with a stringent sampling protocol. For these reasons, some researchers claim that "part of establishing the criteria is defining the required sampling plan" (Jarvis, 2000).

The current lack of consistent sampling protocols makes it difficult to compare among existing and proposed standards. Even if a single discharge standard is adopted, without consistent sampling protocols the outcome of different ballast management programs will vary dramatically. Furthermore, the efficacy of different ballast treatment technologies cannot be compared without consistent sampling protocols (Phillips, 2005). In this chapter, we explore some of the statistical aspects of estimating the concentration of organisms in ballast discharge based on techniques that involve counting the living organisms in a random sample volume. In Appendix C, we describe some tools that can be used to develop statistically sound sampling protocols.

Sampling Ballast Water Discharges:
Given current methodologies, it is not possible to count every single organism in a ballast discharge (i.e., the "population"); consequently, we must use sampling techniques to estimate the true concentration. Due to the stochastic nature of sampling, multiple samples taken from the same population will have varying numbers of organisms due to random chance. However, if we know how organisms are distributed in their environment, this uncertainty can be estimated and taken into consideration during the development of sampling protocols. A sample taken from a ship's ballast discharge may have a higher concentration than the standard even though the true concentration of organisms is less than the standard. In this situation, if we failed to take into account the inherent stochasticity of sampling, ships that do not violate the standard would be unfairly penalized. Conversely, a sample may have a lower concentration than the discharge standard even though the true concentration of the discharge exceeds the standard. In this situation, if sampling protocols are inadequate then many ships that exceed the standard would not be detected.

Two questions that must be answered to develop adequate sampling protocols, are 1) how many organisms must we observe in a sample before we feel reasonably confident that we can identify ships in violation of the standard; and 2) how few organisms must we observe before we can feel reasonably confident that a ship does not violate the standard? The answers will depend on the size of the sample, the true concentration of organisms, the discharge standard, and the definition of "reasonable confidence".

The answer also depends on how organisms are distributed in the discharged ballast water. The best-case scenario, from a sampling perspective, is a random distribution (Figure 9A), meaning that organisms occur independently of one another. A random distribution will occur in well-mixed ballast water. This distribution may be unlikely because organisms are often aggregated to some extent in their environment. There are several biological reasons for this phenomenon. Organisms may be responding to similar environmental cues, resources or physical forces, such as gravity (Figure 9B); they may be actively seeking conspecifics (Figures 9B and C); or, for

organisms with fast population growth rates, reproduction may occur at a faster rate than diffusion or convection away from each other (Figure 9C). Different mechanisms can lead to varying patterns of aggregation, which can have different consequences for sampling. Murphy and colleagues (2002) have shown that the abundance of zooplankton varies with depth in the ballast tanks, indicating that at least one type of aggregation in ballast discharges is likely.

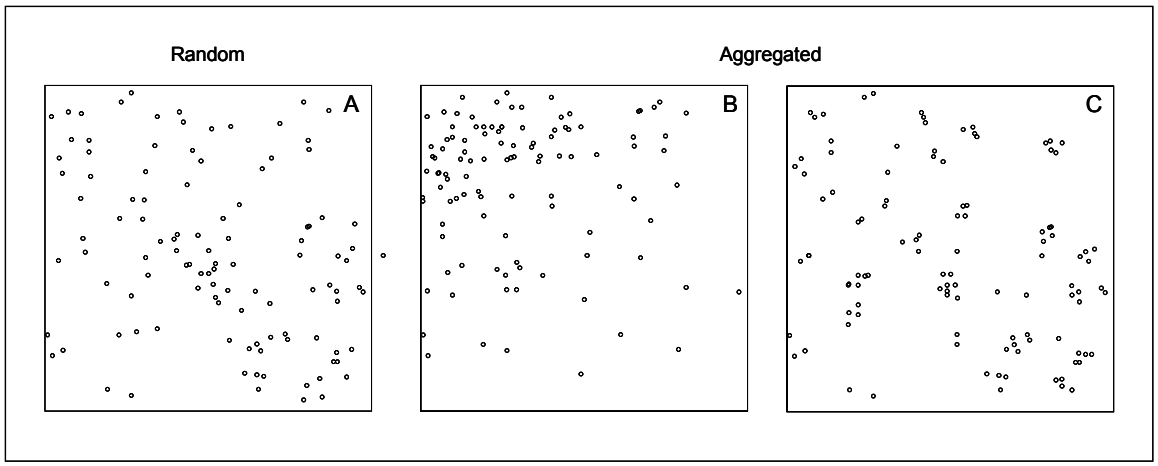

Figure 9: An example of a random distribution (A, Poisson) and two possible variations of aggregated distributions (B and C).

For most of the analyses in this report, we assume that organisms are randomly distributed in ballast tanks and the discharges. From a practical perspective, this was the only option because we do not possess data that can be used to estimate the degree of aggregation in ballast water. Furthermore, Elliott (1971) argues that assuming a random distribution is a reasonable starting point because the Poisson is the default, or null, hypothesis, and therefore, should be assumed until rejected by testing. Elliott also makes the point that for benthic organisms low density populations are effectively randomly distributed in regard to sampling, and therefore a random distribution is often a suitable hypothesis. Whether this applies to organisms discharged in ballast water is unclear. The values presented in this chapter are probably optimistic because: 1) almost all organisms demonstrate at least some aggregation; and 2) for aggregated populations, larger volumes must be sampled to obtain reliable estimates of concentration.

Further, we assume that the samples are "taken from the discharge line, as near to the point of discharge as practicable, during ballast water discharge whenever possible", as recommended in the final MEPC G2 ballast water sampling guidelines (MEPC, 2008c), and as such are representative of the actual concentrations discharged. Important aspects of developing sampling protocols may be determining the extent that organisms are aggregated in ballast discharges and whether samples of ballast water discharges are representative of the total number of organisms discharged. We discuss some aspects of sampling aggregated populations later in this chapter.

Sampling Poisson Distributions:
For randomly distributed populations, the Poisson distribution can be used to determine the probability that a given number of individuals will occur in a sample given the true concentration of organisms (see Table 15 for definitions). This information provides the statistical basis of sampling protocols for randomly distributed populations. A defining characteristic of the Poisson

distribution is that it is defined by a single parameter, λ, which describes both the mean and variance of the expected counts per unit of sampling effort, thus $\lambda = \mu = \sigma^2$. Lambda can be a real number, and in regard to ballast sampling, it can be interpreted as the true concentration of organisms in the ballast discharge.

For a randomly distributed population the variance increases at exactly the same rate as the mean. This differs from the normal distribution which has two parameters, the mean and variance, which can vary independently of one another. The expected mean and variance scale isometrically with sampling effort. If sample volume doubles, the expected mean and variance of the sample will also double. For these reasons, ten 1 m^3 samples do not provide more information than a single 10 m^3 sample when a population is randomly distributed. However, the first sampling scenario (i.e., ten 1 m^3 samples) provides the data for independently estimating variance, which can be used to determine whether a population is randomly distributed versus aggregated. If the population is randomly distributed, then the mean and variance from multiple samples should not be significantly different from one another (see Elliott, 1971, for more information). An increase in sampling effort, either by taking more sample units or increasing sample volume, improves the average estimate of λ. Ultimately, if all the discharged ballast water was sampled the concentration would equal λ.

Given λ, the probability of N organisms occurring in a sampling unit (individual sample of ballast discharge) is

Equation 23: $P(N) = \dfrac{e^{-\lambda}\lambda^N}{N!}$

For example, for a true concentration of 15 organisms m^{-3}, the probability of getting 10 organisms in a sampling unit (i.e., 1 m^3) is

$$P(10) = \frac{e^{-15}(15)^{10}}{10!} = \frac{(3.06\times10^{-7})\times(5.77\times10^{11})}{10\times9\times8\times7\times6\times5\times4\times3\times2\times1} = \frac{176399}{3628800} = 0.0486$$

Although the true concentration of organisms in the ballast tank is 15 organisms m^{-3}, there is a 4.9% chance that a 1 m^3 sample unit will contain 10 organisms (there is about a 12% chance that the sample unit will contain ≤10 organisms). The estimate of parameter λ is represented by the statistic *m*, which, in this sample, equals 10 organisms m^{-3}. This estimate of λ is low given the true concentration of 15 organisms m^{-3}. As the sample volume increases, the sampling statistic, *m*, will on average provide a better estimate of λ.

In the above example, the probability of N events occurring in a sampling unit is determined by λ which represents the average number of events expected to occur per sampling unit. By assuming a constant sampling unit, λ represents an average count with no associated units. However, λ is often expressed as a concentration (organisms m^{-3}) by dividing the average expected count by the volume of the sampling unit. An alternative parameterization of the Poisson distribution can be used which expresses probabilities in terms of the number of events per sampling effort rather than sampling unit. In this case, λ is replaced by the true concentration of organisms, *c*, times the sample volume, *v*,

Equation 24: $P(N) = \frac{e^{-cv}(cv)^N}{N!}$

This equation is more flexible because it allows the volume of the sample to vary, and it emphasizes that even with a constant population density the Poisson distribution of sample counts can change by sampling larger or smaller volumes (Bolker, 2008).

Table 15: Definition of statistical terms.

Population	In this case, all the >50 μm organisms in the entire volume of a ship's ballast discharge.
Sample	In this case, a random sample of a ship's ballast discharge in which all >50 μm organisms are counted.
Count	Number of organisms in a sample. This value is an integer.
Poisson	A distribution that describes the probability of a given number of "events" (counts, individuals, arrivals, etc) occurring in a unit of time/space if the events are independent of each other. A defining characteristic of the Poisson is that the mean and variance of the expected sample counts are equal ($\lambda = \mu = \sigma^2$). If organisms in a ballast tank are randomly distributed, then sampling probabilities can be modeled using the Poisson.
λ	Lambda, the single parameter of the Poisson distribution, which is a rate describing the average number of events expected to occur per unit of time/space. In this case, it also describes the true concentration of organisms in the discharged ballast water. This parameter is a real number.
m	A statistic estimating λ, calculated from the average number of events observed during sampling. In this case, *m* is an estimate of the true concentration of organisms in the discharged ballast water based on sampling.
Negative Binomial	A probability distribution often used to model aggregated populations ($\sigma^2 > \mu$).
θ	Theta, dispersion parameter for the negative binomial distribution. Highly aggregated populations have smaller θ values, and as θ approaches infinity, the negative binomial approximates the Poisson distribution.
Taylor's Power Law	An alternative to the negative binomial for modeling aggregated populations

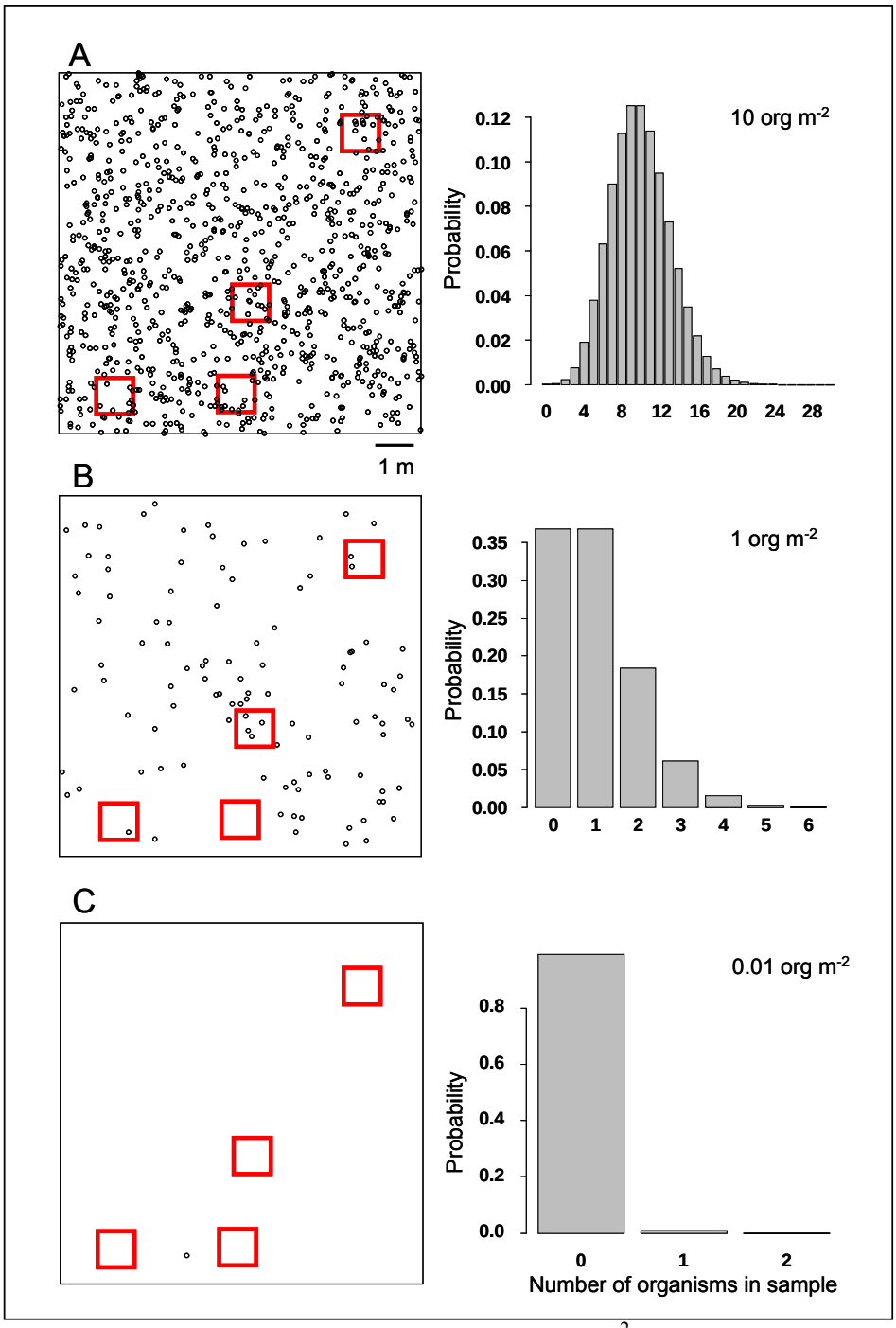

Figure 10: Probability distributions for random samples of 1 m^2 for a randomly distributed population with 10 (A), 1 (B), or 0.01 (C) organisms m^{-2}. Red squares represent random samples. The data are displayed in terms of area with units of m^2, but the probabilities are the same for volumes. Plots on the right indicate the probability that a 1 m^2 sample will contain a given number of organisms. At low concentrations, the concentration of organisms is likely to be estimated as 0 organisms m^{-2}, unless very large volumes are sampled.

From equations 23 or 24, probability distributions can be obtained that describe the probability of a sample containing a specific number of organisms given λ and the sample volume (Figure 10). If 1 m^3 of ballast is sampled from the discharge with a concentration of 10 organisms per m^3, the sample could theoretically contain any number of organisms from zero to positive infinity (or in the case of a finite volume such as a ballast discharge, the total number of organisms in the discharged ballast), but about 95% of samples will contain 4 to 17 organisms (Figure 10A).

The shape of the Poisson probability distribution, for a fixed sample volume, changes with λ. For a concentration of 10 organisms m^{-3} and a sample volume of 1 m^3, the probability distribution for the number of organisms in a random sample is very similar to a normal distribution (Figure 10A, right). However, there are some key differences between the Poisson and normal distribution. For the Poisson distribution: 1) the model is bounded at 0, indicating there is zero probability of a negative count; 2) when the mean number of organisms in a sample is small (less than 10 or so organisms) due to low concentrations or relatively small sample volumes, the frequency distribution is skewed, with a tail to the right; and 3) the variance can not vary independently of the mean. As the concentration decreases, the frequency distribution becomes increasingly skewed (Figure 10 from A to C) and the probability of obtaining a sample with zero organisms becomes very likely. For concentrations of 1 organism per m^3, the probability of a 1 m^3 sample volume containing 0 organisms is 36.8% (Figure 10B). For a concentration of 0.01 organisms per m^3, the probability is about 99% (Figure 10C). One of the general challenges of sampling at low concentrations is a large number of samples will have zero detects. In these cases, the estimated concentration will likely remain at zero until enormous volumes of ballast discharge are sampled.

Some Sampling Scenarios:

In this section, we translate the information from the theoretical probability distributions into specific sampling scenarios. We hope to illustrate some of the challenges inherent in sampling, as well as to aid in the development of sampling protocols that meet the goals of regulatory agencies. For the following analyses, we use the conditions presented in Table 16.

Table 16: Conditions for ballast water sampling scenarios (unless otherwise stated).

1) We interpret the ballast performance standards as establishing the allowable concentration of organisms in the entirety of the ballast discharge. Based on this interpretation, the purpose of sampling is to estimate the concentration of organisms in the whole ballast discharge (i.e. "average-based sampling"). An alternative interpretation (Dobroski et al. 2009a) is that the standards are "instantaneous" and consequently, a ship is non-compliant if any samples exceed the discharge standard. These two interpretations of the enforcement of performance standards are equivalent only in the case where the standard is zero detectable organisms. Sampling protocols based on "instantaneous sampling" must consider additional statistical factors because the results will be sensitive to the number and volume of the samples.
2) Sampling probabilities can be modeled using the Poisson distribution either because organisms are randomly distributed in ballast discharge, or alternatively, continuous integrated sampling is used to obtain the sample (method used by the land-based testing facilities of the U.S. Coast Guard).
3) We assume ALL organisms in a sample are counted with no human or equipment error. Therefore any variation among the samples from the same population is due to the natural stochasticity of sampling.
4) For current ballast sampling techniques, the "sample volume" can be obscured by the steps required to collect and count the organisms. The sample volume must be calculated from the total volume of ballast that is filtered (i.e., concentrated) and the volume of filtrate that is subsampled. The specific steps for sampling can vary, but one technique (Lemieux, 2008) involves filtering a known quantity of ballast water through a net to capture > 50 µm organisms (Gollasch, 2006). The organisms are then rinsed from the net and resuspended in 1 L of water. From this diluted filtrate, several aliquots of 1 mL are collected to enumerate the number of organisms. If 100 m^3 of ballast is filtered, then the filtrate is diluted with 1 L of water, and the organisms from 20 – 1 mL aliquots are counted, then the total sample volume is 2 m^3 (20 mL of aliquot /1000 mL filtrate × 100 m^3 filtered ballast = 2 m^3), not 100 m^3.
5) Sometimes we report organism counts and sample volumes rather than concentration. These can be converted to concentration by dividing the total number of organisms by the total volume of the sample.

One of the primary problems of sampling low density populations is that large volumes of ballast discharge must be sampled to have a reasonable probability of detecting any organisms. From equation 24, the probability of getting 0 organisms in a sample is e^{-cv}, and therefore, the probability of getting 1 or more organisms is $1- e^{-cv}$. We used this expression to calculate the probability of detecting ≥1 organism for a series of concentrations and sample volumes (Table 17). For a concentration of 0.01 organisms m^{-3} about 300 m^3 of ballast must be sampled to have a 95% probability of detecting at least one organism. For relatively small sample volumes, the probability of detecting an organism is low even at relatively high concentrations. If a 1 L sample is taken from a population with a concentration of 100 organisms m^{-3}, organisms will be detected in fewer than 10% of the samples.

Table 17: Probability of detecting ≥ 1 organism for various sample volumes (100 mL to 100 m^3) and concentrations (0 to 100 organisms m^{-3}). Gray boxes indicate probabilities of detection ≥ 0.95.

	True concentration (organisms per m^3)						
Sample volume, m^3	**0**	**0.001**	**0.01**	**0.1**	**1**	**10**	**100**
0.0001 (100 mL)	0	<0.001	<0.001	<0.001	<0.001	0.001	0.01
0.001 (1 L)	0	<0.001	<0.001	<0.001	0.001	0.01	0.095
0.01 (10 L)	0	<0.001	<0.001	0.001	0.01	0.095	0.632
0.1 (100 L)	0	<0.001	0.001	0.01	0.095	0.632	>0.99
1	0	0.001	0.01	0.095	0.632	>0.99	>0.99
5	0	0.005	0.049	0.393	>0.99	>0.99	>0.99
10	0	0.010	0.095	0.632	>0.99	>0.99	>0.99
25	0	0.025	0.221	0.918	>0.99	>0.99	>0.99
50	0	0.049	0.393	>0.99	>0.99	>0.99	>0.99
100	0	0.095	0.632	>0.99	>0.99	>0.99	>0.99
300	0	0.259	0.950	>0.99	>0.99	>0.99	>0.99

This analysis demonstrates that even when 0 organisms are detected in a sample, the true concentration may be large. A discharge standard of "zero detectable organisms" may appear very protective; however, the true degree of protection depends on the sample volume. From the Poisson distribution, the upper possible concentration (UPC, upper 95% confidence interval) of organisms can be estimated based on the number of organisms in a sample. We calculated the UPC (Appendix C) when zero organisms were detected in sample volumes ranging from 100 mL to 100 m^3 (Table 18). In this report, we primarily focus on confidence intervals from 2-tailed sampling probabilities, but in the case of zero detects, the lower estimate is always zero which is not very informative. For this reason, confidence intervals based on 1-tailed sampling probabilities may be preferred when there are zero detects. If zero organisms are detected in 1 m^3 of ballast, the true concentration could be as high as 3.7 organisms m^{-3}. Given the inherent challenges of sampling ballast water, especially on board a ship, a more realistic sample volume may be around 1 L. For a 1 L sample, the upper concentration could be >3,500 organisms m^{-3} even with zero detects.

Table 18: Upper possible concentration (UPC) of organisms based on one and two tailed 95% exact confidence intervals when zero organisms are detected in a range of sample volumes.

	Upper possible concentration, org m^{-3}	
Sample volume, m^3	**one-tailed**	**two-tailed**
0.0001 m^3 (100 mL)	29,960	36,890
0.001 m^3 (1 L)	2,996	3,689
0.01 m^3 (10 L)	299.6	368.9
0.1 m^3 (100 L)	29.96	36.89
0.5 m^3 (500 L)	5.992	7.378
1 m^3	2.996	3.689
10 m^3	0.300	0.369
100 m^3	0.030	0.037

In an ideal world, we would always detect ballast water with concentrations of organisms that exceed the discharge standard. In reality, this is not possible with current methodologies. The probability of detecting an exceedance depends on: 1) the volume of ballast that is sampled; 2) the stringency of the discharge standard; and, 3) the magnitude of the exceedance. To demonstrate the relationship among these variables, we estimated the likelihood of detecting an exceedance for a discharge standard of <0.01 organism m^{-3} when the sample volume ranged from 1-50 m^3 and the true concentration ranged from 0.01 to 1 organism m^{-3}. For each combination, we simulated 10,000 random samples (*rpois* function in R statistical program; R Development Core Team, 2008) and calculated the percentage of samples that were correctly identified as exceeding the discharge standard (Figure 11). Ideally, none of the samples would pass inspection because in all cases the concentration of organisms exceeds the discharge standard. However, as the concentration approaches the discharge standard, increasingly large volumes of ballast must be tested to confidently detect an exceedance. When the true concentration of organisms is 0.75 m^{-3} (75x the proposed U.S. Coast Guard Phase II standard) approximately 4 m^3 of ballast water must be sampled to detect this exceedance 95% of the time (Figure 11A). When the true concentration of organisms is 0.1 m^{-3} (10x the standard) approximately 30 m^3 of ballast water must be sampled (Figure 11B). For perspective on the magnitude of these sample volumes, a Volkswagen Transporter bus has a volume of about 14 m^3.

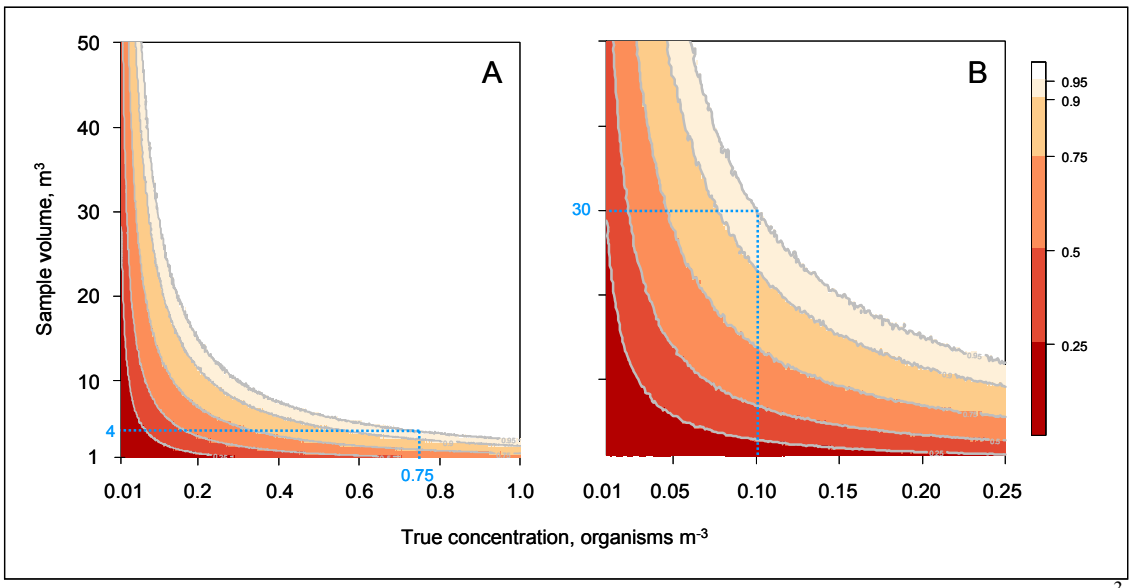

Figure 11: Probability of detecting an exceedance for sample volumes between 1 and 50 m^3 and a discharge standard of 0.01 organisms m^{-3}. The true concentration ranges from (A) 0.01 to 1 organisms m^{-3} or, (B) 0.01 to 0.25 organisms m^{-3}, all of which fail to comply with the discharge standard. The legend describes the proportion of samples in which the exceedance is detected: white regions of plot indicate a >95% probability of detecting the exceedance; the darkest red regions indicate a <25% probability of detecting the exceedance. See text for information about specific examples identified by the blue lines.

The examples thus far have been theoretical because we begin with a known concentration that exceeds the discharge standard and we calculate the probability of detecting the exceedance given a specific sample volume. These examples are useful because they demonstrate the power and limitations of specific sampling protocols. They are less useful from the perspective of actual sampling, because in reality the true concentration is unknown and must be estimated using sampling techniques. Ultimately, the goal of sampling is to determine whether a ballast discharge exceeds or meets the discharge standard with some pre-established degree of confidence. Two obvious verdicts of sampling are: 1) *Statistically Non-compliant*: The number of organisms in the sample is large enough that the true concentration likely exceeds the discharge standard; 2) *Statistically Compliant*: The number of organisms in the sample is small enough that the true concentration likely meets the discharge standard. However, there is also a third, *indeterminate* outcome due to the inherent stochasticity of sampling. For these samples, it is statistically unclear whether the discharge is statistically compliant or not. A random sample of ballast discharge may have a concentration that exceeds the discharge standard, but the true concentration may actually be less than the discharge standard. For example, if the true concentration of the discharged ballast water is 7 organisms m^{-3} and a volume of 1 m^3 is sampled, about 17% of samples will have 10 or more organisms, and will appear to exceed the current IMO standard of $\leq$ 10 organisms m^{-3}. The possibility of getting an indeterminate result increases as the sample volume decreases and as the desired level of certainty increases.

We calculated the total number of organisms that must be observed in a range of sample volumes to determine – based on two-tailed 95% confidence intervals – whether the true concentration is statistically compliant or non-compliant with a discharge standard of either <0.01 or <10 organisms m^{-3} (Figure 12). For a very stringent discharge standard, such as the <0.01 organisms m^{-3} proposed by the U.S. Coast Guard, only a few organisms must be observed before a ship's discharge is statistically non-compliant with the standard. If a single organism is detected in a sample volume of $\leq$2 m^3 then we can be fairly confident that the standard has been exceeded (Figure 12A, red region) given that the *lower* probable concentration, based on the 95% confidence interval (0.0125 to 2.786 organisms m^{-3}), is greater than the standard. On the other hand, very large amounts of water must be sampled before we can be equally confident that a ship's discharge is statistically compliant with the standard. For a discharge standard of <0.01 organisms m^{-3}, approximately 370 m^3 of ballast water must be sampled, with zero detects, before a ship's discharge is statistically compliant with the standard (Figure 12A, green region). Another way to think about this is that the probability of detecting 0 organisms in a 370 m^3 sample must be <2.5% (based on two-tailed test). Of course, the discovery of a single organism in a 370 m^3 sample does not suggest the true concentration exceeds the standard, in fact the ballast discharge in question is more likely to comply with the standard than not. However, based on this result we can not distinguish within our desired confidence whether the sample complies with the discharge standard given that the *upper* probable concentration, based on the 95% confidence interval (0.000068 to 0.015 organisms m^{-3}), exceeds the standard (Figure 12A, white region). For the IMO discharge standard of <10 organisms m^{-3} (Figure 12B) nearly 0.4 m^3 of ballast discharge must be sampled before it's possible for ballast discharge to be statistically compliant with the standard.

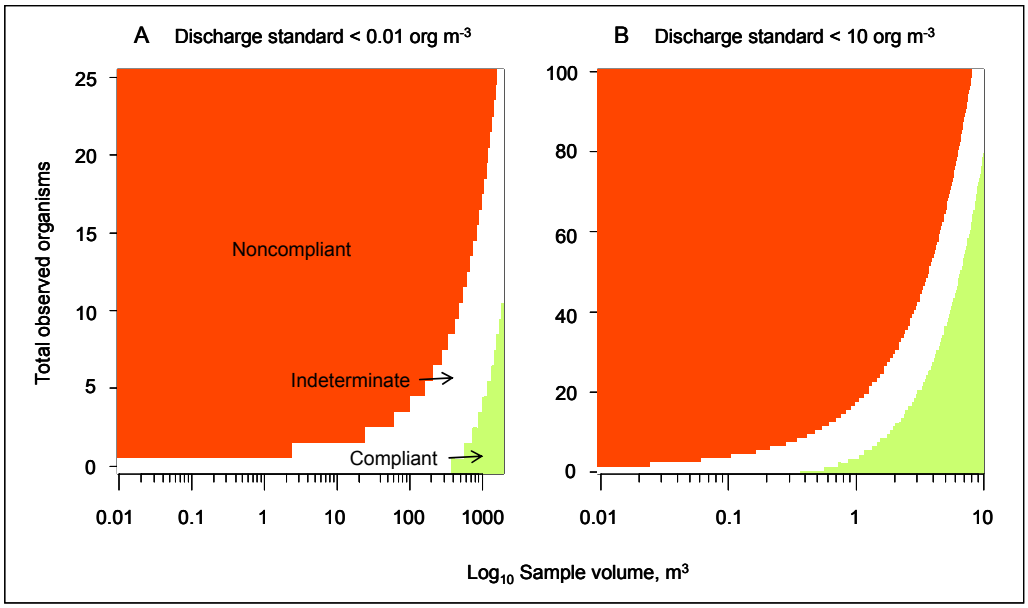

Figure 12: Determining whether ballast water discharge exceeds or meets a discharge standard of <0.01 (A) and <10 (B) organisms m^{-3} (note: axes have different scales). Red regions indicate total organism counts that would be statistically non-compliant with the standard. Green regions indicate total organism counts that would be statistically compliant with the standard. White regions indicate indeterminate results that are not statistically compliant or non-complaint with the discharge standard based on two-tailed 95% confidence intervals around sample counts.

Aggregated Populations:

Sampling aggregated populations, also known as clumped or contagious populations, is more complicated than sampling randomly distributed populations. One of the defining characteristics of aggregated populations is that the sample variance is greater than the mean ($\sigma^2 > \mu$, recall that for Poisson distributions $\sigma^2 = \mu$). As variance increases, the true concentration becomes increasingly difficult to accurately estimate because the number of organisms in a random sample becomes increasingly unpredictable. Consequently, aggregated populations must be sampled more intensively to estimate concentration confidently.

Aggregation results from many different ecological and physical processes, making it difficult to apply a single probability distribution to the diverse array of possible patterns of aggregation. Although many distributions have been used to model aggregated populations, the negative binomial is probably the most useful of these models (Elliott, 1971). Like the Poisson distribution, the negative binomial can be used to predict the probability of observing a specific number of organisms in a sample. Unlike the Poisson distribution, the negative binomial is defined by two parameters, the mean (μ) and the dispersion (θ, also called the size parameter). The dispersion parameter is related to the spatial distribution of organisms in their environment. More aggregated populations have smaller dispersion parameters. As the dispersion factor approaches positive infinity, the negative binomial approximates the Poisson distribution. The dispersion parameter of the negative binomial is related to both the mean and the variance (Bolker, 2008). An approximate estimate of this relationship is: $\theta = \mu^2/(\sigma^2 - \mu)$.

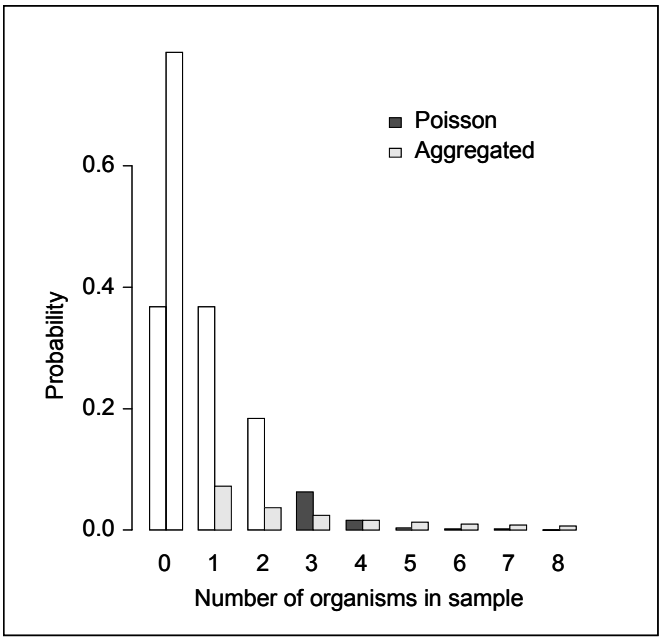

Figure 13: Comparison of sample probabilities from a randomly distributed (Poisson) population vs. an aggregated population with a dispersion parameter of 0.1 (negative binomial) for a sample volume of 1 m^3 and concentration of 1 organism m^{-3}. The probability that a sample will contain 0 organisms is greater for the aggregated population than for the Poisson distributed population.

As mentioned, one of the general challenges of sampling at low concentrations is the fact that a large number of samples will contain zero organisms. This problem can be compounded when organisms are aggregated. For example, if a 1 m^3 sample of ballast is taken from a randomly distributed population with a true concentration of 1 organism m^{-3}, about 37% of the samples will contain 0 organisms. In contrast, for an aggregated population with a dispersion parameter of 0.1, about 79% of samples will contain 0 organisms (Figure 13). Conversely, the probability of obtaining samples with large numbers of organisms, relative to the true concentration, also increases. For the randomly distributed population, the probability of a sample unit containing > 3 organisms is 1.9%, whereas, for the aggregated population, the probability is 8.3%. If large sample volumes are not taken from aggregated populations, then estimates of concentration are likely to be much lower or higher than the true concentration.

There are several ways to determine whether a population is aggregated, all of which require multiple sample units from a population to estimate both parameters of the negative binomial distribution (see Elliott, 1971). This is complicated by the fact that estimates of aggregation depend upon the scale of the aggregation pattern relative to the size of the sampling unit (Figure 14). One pattern of aggregation occurs when organisms form clumps that are randomly distributed throughout the environment. In this case, the population can be highly aggregated but if the sample volume is relatively small, such that most sample units contain 0 or 1 organisms, then the population will appear randomly distributed or only slightly aggregated. As the volume of the sample unit increases, the variation in the number of organisms will increase relative to the mean, peaking at the point when the sample volume is about equal to the volume of a single cluster. As sample volume increases beyond this point, the variance will decline relative to the mean because a sample unit will include several clusters. Given these and other issues, the

Taylor power law (Taylor, 1961) is an alternative to the negative binomial for modeling aggregated populations that may be applicable for a wider range of distributions than the negative binomial (Elliott, 1971; Downing et al., 1987).

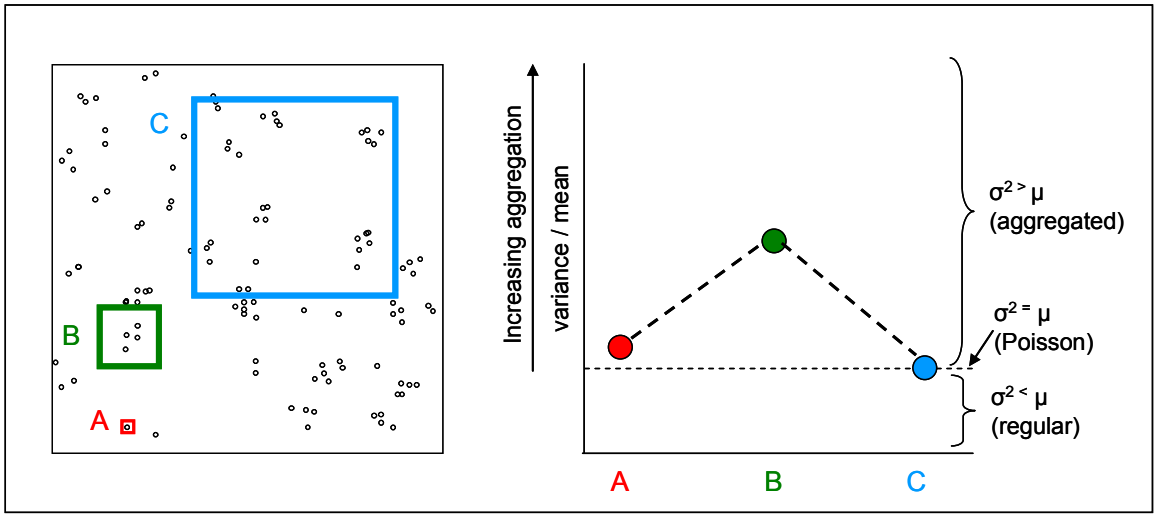

Figure 14: Theoretical example of how the apparent aggregation in the population will differ based on the scale of aggregation relative to the size of the sample unit.

Recommendations/Conclusions:
Instituting standardized sampling protocols is a critical component of implementing ballast discharge standards.

The degree of statistical certainty desired for ballast testing may differ according to the situation. For stringent discharge standards, such as the <0.01 standard proposed by the U.S. Coast Guard, a large quantity of water must be sampled to know with 95% confidence that the true concentration of organisms is less than the discharge standard (Figure 12A, counts in green region are acceptable, counts in red regions are unacceptable, and because counts in white regions are ambiguous they may be classified as unacceptable if a high degree of confidence is desired). This level of certainty is important when testing the performance of ballast treatment systems; however, it may be less critical during compliance monitoring, especially if the primary goals are to detect gross failures or to generate compliance records for individual ships so as to flag those that appear to have poor compliance. For compliance monitoring, one approach may be to employ a three class sampling criteria, such that ships with organism counts in the green region of Figure 12 are acceptable, counts in the red region are unacceptable, and counts in the white region are marginally acceptable.

In situations where indeterminate results are classified as acceptable or marginally acceptable it is critical to ensure that the upper possible concentration of organisms is reasonable from a regulatory perspective. For samples with counts that fall in the intermediate category, the possible concentration of organisms could be quite high depending on the sample volume. This can be true even if zero organisms are detected during sampling (Tables 17 and 18). If zero organisms are detected in a 1 L sample, the upper possible concentration could be nearly as high as 3,000 organisms m^{-3} based on the upper 1-tailed 95% confidence interval. Given this, for

ballast discharge standards of "zero detectable organisms" the sample volume must be large enough to ensure that the detection limit is ecologically protective.

Given the current challenges of ship-board testing and the stringency of the current and proposed discharge standards, it will be difficult to sample large enough volumes of ballast discharge to detect ballast discharges that do not meet the standard, even if the concentration of organisms is 1-3 orders of magnitude greater than the discharge standard. Consequently, the quality control to assure that ballast treatment systems are designed to adequately control the introduction of nonindigenous species may be best achieved primarily through rigorous type-approval of ballast water treatment systems in controlled testing facilities, rather than from after-the-fact compliance ship-board sampling.

Despite the limitations of compliance monitoring in the field this technique may still play an important role in the regulation of ballast discharge standards. This type of testing can detect gross exceedences of the discharge standard, which can be used to identify treatment system failures and problematic ships. Until new sampling technologies are developed, however, ship board testing using existing sampling methods are likely to be inadequate for accurately distinguishing among concentrations in the range of 0.001 to 10 organisms m^{-3}. However, it would be useful to institute a global repository of compliance test results for individual ships through the IMO in order to increase the probability of detecting troublesome patterns. For a discharge standard of <10 organisms m^{-3}, a single 1 m^3 sample containing 15 organisms does not necessarily indicate a ship's treatment system is failing to meet the discharge standard (95% CI: 8.4 to 24.8 organisms m^{-3}). If, however, the same pattern is observed at subsequent discharge events, there is mounting evidence that the treatment system may not be adequately reducing the concentration of organisms in the ballast discharge.

Aggregation may be a significant source of error in many sampling protocols, and estimating the extent of aggregation could be an important aspect of accurately estimating the concentration of organisms in ballast discharge. The extent that a population is aggregated must be determined empirically by taking many replicate samples. The problem is mitigated in the land-based testing facility in Key West, Florida (Lemieux et al., 2008) by continuously sampling throughout the ballast tank. This is achieved by removing all the water from the ballast tank while continuously diverting a relatively small portion of it for testing. This approach is currently not practical for ship-board testing.

In these analyses, we assume the goal of discharge standards is to directly regulate the concentration of organisms in ballast discharges using "average based sampling". However, if "maximum instantaneous" discharge standards are used instead then additional statistical factors must be considered because the results will be very sensitive to the sample number and volume. For example, if an IMO discharge standard of <10 organisms m^{-3} is enforced using instantaneous sampling, then a ship discharging ballast with a true concentration of 5 organisms m^{-3} will fail about 3.2% of the time based on a single sample of 1 m^3. However, as the number of samples increases the probability of a false failure increases, assuming failure is defined as one or more of the 1 m^3 samples having a concentration of ≥10 organisms m^{-3}. If five 1 m^3 samples are taken from a ballast discharge event, about 15% of the ships will have at least one sample with ≥10 organisms m^{-3}.

XI. COMPARISON OF APPROACHES

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It is difficult to compare the approaches that have been used to determine the risk of ballast-associated invasions because they do not use a consistent set of input values for ballast water discharges, organism concentrations, or historical invasion rates. Nonetheless, to assist in seeing the "forest from the trees", we present a comparison in Table 19. This comparison summarizes attributes related both to the scientific rigor of the approaches and to their applicability to risk management decisions, in particular as they relate to the development of a national discharge standard. A table can not capture all the nuances or reasoning behind our assessments, and the reader is referred back to the individual sections for more detailed information. Definitions for the attributes in Table 19 are:

Current implementation generates quantitative standards: Does the approach as reviewed in this document actually generate quantitative organism-based discharge standards? A particular approach may not generate quantitative standards, at least as currently implemented, because it is not based on organism concentrations, because the data are not currently available, or because the approach is inherently unsuitable to generate an actual estimate of risk (e.g., it is a relative evaluation among treatments).

Range in uncertainty in standard: What is the apparent range in uncertainty in the discharge standard? This is an estimate of the range in the standard itself, and does not attempt to capture all the potential sources of uncertainty of the input parameters going into the approach. Because the actual organism concentration generated from the zero detectable organism approach is dependent upon the sampling protocol, we compared the upper possible organism concentration from the 95% confidence interval (one-tailed test) for 1 liter sample to a 10 m^3 sample (Table 18). Note that this type of uncertainty would also apply to other approaches.

Key data needs for generation of quantitative standards: These are the most important types of data required to generate a national discharge standard via the identified approach. This is not necessarily a complete list of the data required.

Assumes linear dose response: Does the approach that the invasion rate from ballast discharges is directly proportional to the propagule supply? Note that approaches based on historical invasion rates may actually incorporate Allee effects if the invasion success of historical invaders was dependent upon them exceeding some critical population threshold.

Incorporates invasion risk from multiple species in a discharge: Does the approach assess the risk of invasion from all the species contained within a ballast discharge or does it inherently assume that all the discharged individuals are of a single species?

Incorporates invasion risk from multiple ship discharges: Does the approach assess the risk of establishment from discharges of the same species from multiple ships discharging within the same waterbody?

Based on historical invasion rates: Does the approach directly use historical invasion rates to generate a standard? The uncertainty in these methods will depend, in part, upon the accuracy of these historical invasion rates as well as the extent to which past invasion rates are predictive of future rates.

Based on population dynamics: Does the approach directly use population dynamics, such as growth rates, in predicting invasion risk? The uncertainty in these methods will depend, in part, upon the accuracy of these population vital rates as well as whether they represent the breadth of taxa found in ballast water.

Applicable to all taxa and guilds: Is the standard applicable to all taxonomic groups and guilds? For example, is the standard applicable for holoplanktonic species, such as calanoid copepods, as well as benthic species with a pelagic larval stage or nektonic species, such as fish?

Separates risk assessment from risk management: Does the approach separate the scientific assessment of invasion risk from the risk management decisions, or are the two intermingled? Intermingling the two makes it difficult to evaluate the decision making process rigorously or to determine what new information is required to improve the predictions.

Published in peer-reviewed scientific literature: Has the approach been published in the scientific peer-reviewed literature? Most government reports undergo independent peer review – nonetheless, publication in the peer-reviewed literature indicates some level of acceptance by the broader scientific community. There is an extensive literature on PVA models in general, but the application to ballast water discharges in the DPEIS (USCG, 2008) has not been published in the scientific literature. In addition to the specific Drake et al. (2005) paper on the application of reaction-diffusion models to ballast water, there is an extensive literature on this type of model. Individual experimental studies are generally published in the scientific literature.

Recommended for national standard development: This is the authors' evaluation of whether the approach is potentially suitable for the development of national discharge standards. It is meant to promote further technical review, and we recognize that other evaluations are possible based on the different weighting of the factors. The key factors used in our evaluation include an assessment of the scientific rigor of the approach, the apparent uncertainty in the standards, and whether the current implementation of the approach, or some foreseeable modification, could generate organism-based standards. We also considered whether the approach applies to the full suite of taxa and guilds and can address the combined risk from discharges from multiple ships.

Table 19: Comparison of approaches to generate national organism-based discharge standards for >50 micron organisms in ballast discharges. Assessment is based on current implementation; potential modifications are identified when appropriate. "Reality check" is used to denote that the approach could be used to help evaluate whether predictions from other approaches fall within a realistic range. "Recommend for national standard development" is our assessment of whether the approach should be considered for generating quantitative organism-based discharge standards at the national level. See the text for explanations of the attributes.

Approach / Attribute	**Expert Opinion / Management Consensuses**	**Zero Detectable Organisms**	**Natural Invasion Rate**	**Reaction – Diffusion**	**Population Viability Analysis**	**Per Capita Invasion Probabilities**	**Experimental**
Current implementation generates quantitative standards	Yes	Yes	Yes (prelim. for CA)	No (volume based)	No (relative comparison)	Yes	No
Apparent range of uncertainty in standard	10,000 fold (range of conc. proposed in IMO negotiations – 0.01 to 100 org m^{-3})	Uncertain since detectable conc. in samples have not yet been defined - could be as much as 10,000 fold (upper possible conc. w/1L vs. 10 m^3 sample)	100-fold (3 experts) or 10,000-fold (our analysis)	About 200 fold (approx range in "max. safe release volumes")	<2 fold (w/12 spp. in ballast) to 10,000 fold (multiple voyages – our analysis)	6-fold (among coasts) or 12-fold (w/Great Lakes)	NA
Key data needs for generation of quantitative standards	Unknown since decision process not transparent	Development of statistically rigorous sampling protocol	Natural invasion rates in range of ecoregions	Instantaneous population growth rates for a range of taxa	Instantaneous population growth rates & instantaneous variance of the population growth rate for a range of taxa	None	Extensive experimentation w/range of taxa
Assumes linear dose response	Unknown since decision process not transparent	No (assumes a single individual can become established)	Yes	No (can incorporate Allee effects)	No (can incorporate Allee effects)	Yes	NA (can be used to determine nature of a dose response)

Approach / Attribute	Expert Opinion / Management Consensuses	Zero Detectable Organisms	Natural Invasion Rate	Reaction – Diffusion	Population Viability Analysis	Per Capita Invasion Probabilities	Experimental
Incorporates invasion risk from multiple species in a discharge	Yes?	Yes	Yes	No?	Yes	Yes	No
Incorporates invasion risk from multiple ship discharges	Yes?	Yes	Yes	No	No (modify to incorporate multiple ships?)	Yes	No
Based on historical invasion rates	No	No	Yes	No	No	Yes	No
Based on population dynamics	No	No	No	Yes	Yes	No	Yes
Applicable to all taxa and guilds	Yes?	Yes	Yes? (depends on taxa included in analysis)	No (limited to short-lived holoplanktonic species)	Yes? (depends upon which species the pop. data can be obtained)	Yes	No (limited to taxa adaptable to experiments)
Separates risk assessment from risk management	No	No?	No	Yes	Yes	Yes	Yes
Published in peer-reviewed scientific literature	No	No	No	Yes (extensive literature on reaction-diffusion models)	No (extensive literature on PVA models)	No (in process)	Yes (individual experiments)
Recommended for national standard development	No (use as "reality check")	No	No (possible use as "reality check")	No (use as "reality check" for holoplanktonic species)	Yes (if sufficient pop. data available for predictions of actual vs. relative risk)	Yes	No (use as "reality check" and test assumptions)

XII. REFERENCES

Allen, A.P. and Gillooly, J.F. 2006. Assessing latitudinal gradients in speciation rates and biodiversity at the global scale. Ecological Letters 9: 947–954.

Andersen, M. 2005. Potential applications of population viability analysis to risk assessments for invasive species. Human and Ecological Risk Assessment 11:1083-1095.

Bailey, S.A., Velez-Espino, L.A., Johannsson, O.E., Koops, M.A., and Wiley, C.J. 2009. Estimating establishment probabilities of *Cladocera* introduced at low density: an evaluation of the proposed ballast water discharge standards. Canadian Journal of Fisheries and Aquatic Sciences 66:261-276.

Banas, N.S, Hickey, B.M., and MacCready, P. 2004. Dynamics of Willapa Bay, Washington: A highly unsteady, partially mixed estuary. Journal of Physical Oceanography 34:2413-2427.

Beissinger, S.R., and McCullough, D.R. (eds.) 2002. *Population Viability Analysis*. University of Chicago Press, Chicago, IL. 577 pp.

Behrens Yamada, S., Dumbauld, B.R., Kalin, A., Hunt, C.E., Figlar-Barnes, R., and Randall, A. 2005. Growth and persistence of a recent invader *Carcinus maenas* in estuaries of the northeastern Pacific. Biological Invasions 7:309-321.

Berec, L., Angulo, E., and Courchamp, F. 2006. Multiple Allee effects and population management. Trends in Ecology and Evolution 22:185-191.

Blueweiss, L., Fox, H., Kudzma, V., Nakashima, D., Peters, R., and Sams, S. 1978. Relationships between body size and some life history parameters. Oecologia 37:257-272.

Bolker, B.M. 2008. *Ecological Models and Data in R*. Princeton University Press, Princeton, NJ. 408 pp.

Box, G.E.P. and Draper, N.R. 1987. *Empirical Model-Building and Response Surfaces*. John Wiley and Sons, New York, NY. 424 pp.

Boyd, M.J., Mulligan, T.J., and Shaughnessy, F.J. 2002. Non-Indigenous Marine Species of Humboldt Bay, California: A Report to the California Department of Fish and Game. 118 pp. (http://dscholar.humboldt.edu:8080/dspace/bitstream/2148/463/1/non_indigenous_marine_boyd_etal_2002.pdf)

Britton-Simmons, K.H. and Abbott, K.C. 2008. Short and long term effects of disturbance and propagule pressure on a biological invasion. Journal of Ecology 96:68-77.

California Department of Fish and Game. 2009. California Aquatic Non-Native Organism Database (CANOD). (http://www.dfg.ca.gov/ospr/about/science/misp.html)

California State Lands Commission. 2009. Performance Standards for the Discharge of Ballast Water for Vessels Operating in California Waters. (http://www.slc.ca.gov/Regulations/Documents/Article_4.7.pdf)

Carlton, J.T. 1996. Pattern, process, and prediction in marine invasion ecology. Biological Conservation. 78:97-106.

Carlton, J. T. 2001. *Introduced Species in U.S. Coastal Waters: Environmental Impacts and Management Priorities*. Pew Oceans Commission, Arlington, VA. 36 pp.

Carlton, J.T. (ed.). 2007. *The Light and Smith Manual: Intertidal Invertebrates from Central California to Oregon.* 4th Edition. University of California Press, Berkeley, CA. 1001 pp.

Carlton, J.T. 2009. Deep Invasion Ecology and the Assembly of Communities in Historical Time. In: Rilov, G. and Crooks, J.A. (eds.) *Biological Invasions in Marine Ecosystems: Ecologial, Management, and Geographic Perspectives (Ecological Studies)*. Springer-Verlag, Berlin. pp. 13-56.

Carlton, J.T. and Cohen, A.N. 2003. Episodic global dispersal in shallow water marine organisms: the case history of the European shore crabs *Carcinus maenas* and *C. aestuarii*. Journal of Biogeography 30:1809-1820.

Carlton, J.T. and Geller, J.B. 1993. Ecological roulette: the global transport of nonindigenous marine organisms. Science 261:78-82.

Carlton, J.T., Reid, D. and van Leeuwen, H. 1995. The role of shipping in the introduction of nonindigenous aquatic organisms to the coastal waters of the United States (other than the Great Lakes) and an analysis of control options. US Coast Guard. Technical Report No. CG-D11-95, Washington, DC. 373 pp.

Chapman, P.M., Fairbrother, A., and Brown, D. 1998. A critical evaluation of safety (uncertainty) factors for ecological risk assessment. Environmental Toxicology and Chemistry 17:99-108.

Choi, K.-H., Kimmerer, W., Smith, G., Ruiz, G.M., and Lion, K. 2005. Post-exchange zooplankton in ballast water of ships entering the San Francisco Estuary. Journal of Plankton Research 27:707-714.

Chonsuvivatwong, V. 2008. epicalc: Epidemiological calculator. R package version 2.8.0.1. http://CRAN.R-project.org/

Clark, G.F. and Johnston, E.L. 2005. Manipulating larval supply in the field: a controlled study of marine invasibility. Marine Ecology Progress Series 298:9-19.

Cohen, A.N., Berry, H.D., Mills, C.E., Milne, D., Britton-Simmons, K., Wonham, M.J., Secord, D.L., Barkas, J.A., Bingham, B., Bookheim, B., Byers, J.E., Chapman, J.W., Cordell, J.R., Dumbauld, B., Fukuyama, A., Harris, L.H., Kohn, A.J., Li, K., Mumford, T.F., Radashevsky, V., Sewell, A.T., and Welch., K. 2001. Report of the Washington State

Exotics Expedition 2000: A Rapid Assessment Survey of Exotic Species in the Shallow Waters of Elliot Bay, Totten and Eld Inlets, and Willapa Bay. Nearshore Habitat Program, Washington State Department of Natural Resources, Olympia WA. 39 pp. plus appendices.

Cohen, A.N. 2005. Guide to the Exotic Species of San Francisco Bay. San Francisco Estuary Institute, Oakland, CA (www.exoticsguide.org)

Cohen, A.N. and Carlton, J.T. 1995. Nonindigenous Aquatic Species a United States Estuary: A Case Study of the Biological Invasions of San Francisco Bay and Delta. Report to U.S. Fish and Wildlife Service. 246 pp. plus appendices. (http://nsgl.gso.uri.edu/conn/connt95002/connt95002full.pdf)

Cohen, A.N. and Carlton, J.T. 1998. Accelerating invasion rate in a highly invaded estuary. Science 279:555-558.

Collin, R. 2003. Phylogenetic relationships among calyptraeid gastropods and their implications for the biogeography of marine speciation. Systematic Biology 52:618–640.

Colautti, R.I., Grigorovich, I.A. and MacIsaac, H.J. 2006. Propagule pressure: a null model for biological invasions. Biological Invasions 8:1023–1037.

Cordell, J.R., Lawrence, D.J., Ferm, N.C., Tear, L.M., Smith, S.S., and Herwig, R.P. 2009. Factors influencing densities of non-indigenous species in the ballast water of ships arriving at ports in Puget Sound, Washington, United States. Aquatic Conservation: Marine and Freshwater Ecosystems 19:322-343.

Costello, C.J. and Solow, A.R. 2003. On the pattern of discovery of introduced species. Proceedings of the National Academy of Sciences 100:3321–3323.

Coulson, T., Mace, G.M., Hudson, E., and Possingham, H. 2001. The use and abuse of population viability analysis. Trends in Ecology and Evolution 16:219-221.

Courchamp, F., Clutton-Brock, T., and Grenfell, B. 1999. Inverse density dependence and the Allee effect. Trends in Ecology and Evolution 14:405-410.

Daniels, R.A. and Peteet, D. 1998. Fish scale evidence for rapid post-glacial colonization of an Atlantic coastal pond. Global Ecology and Biogeography Letters 7:467-476.

de Queiroz, A. 2005. The resurrection of oceanic dispersal in historical biogeography. Trends in Ecology and Evolution 20:68-73.

Dennis, B., Munholland, P.L., and Scott, J.M. 1991. Estimation of growth and extinction parameters for endangered species. Ecological Monographs 61:115-143.

deRivera, C.E., Ruiz, G.M., Crooks, J.A., Wasson, K., Longhart, S.I., Fofonoff, P., Steves, B.P., Rumrill, S.S., Brancato, M.S., Pegau, W.S., Bulthuis, D.A., Preisler, R.K., Schoch, G.C., Bowlby, E., DeVogelaere, A., Crawford, M.K., Gittings, S.R., Hines, A.H., Takata, L., Larson, K., Huber, T., Leyman, A.M., Collinetti, E., Pasco, T., Shull, S., Anderson, M., and Powell, S. 2005. Broad-scale nonindigenous species monitoring along the West Coast in national marine sanctuaries and national estuarine research reserves. Report to the National Fish & Wildlife Foundation. 72 pp. plus appendices. (http://cain.nbii.gov/portal/community/Communities/Geographic_Perspectives/Pacific_Northwest/Regional_Planning_Documents/Invasive_Species/).

Desmond, J.S., Deutschman, D.H., and Zedler, J.B. 2002. Spatial and temporal variation in estuarine fish and invertebrate assemblages: analysis of an 11-year data set. Estuaries 25:552-569.

Dobbs, F.C. and Rogerson, A. 2005. Ridding ships' ballast water of microorganisms. Environmental Science and Technology 39: 259A-264A.

Dobroski, N., Scianni, C., Gehringer, D., and Falkner, M. 2009a. 2009 Assessment of the Efficacy, Availability and Environmental Impacts of Ballast Water Treatment Systems for Use in California Waters. Produced for the California State Legislature. 76 pp. plus appendices (http://www.slc.ca.gov/Spec_Pub/MFD/Ballast_Water/Documents/2009CSLCTechReportFinal.pdf)

Dobroski, N., Scianni, C., Takata, L., and Falkner, M. 2009b. October 2009 Update: Ballast Water Treatment Technologies for Use in California Waters. Produced for the California State Legislature. 19 pp. (http://www.slc.ca.gov/Spec_Pub/MFD/Ballast_Water/Documents/2009CSLC_TechUpdate_final.pdf)

Downing, J.A., Perusse, M., and Frenette, Y. 1987. Effect of interreplicate variance on zooplankton sampling design and data analysis. Limnology and Oceanography 32:673-680.

Drake, J.M. 2004. Allee effects and the risk of biological invasion. Risk Analysis 24:795-802.

Drake, J.M. 2006. Extinction times in experimental populations. Ecology 87:2215–2220.

Drake, J.M. and Bossenbroek, J.M. 2004. The potential distribution of zebra mussels in the United States. Bioscience 54:931-941.

Drake, J.M. and Griffen, B.D. 2009. Speed of expansion and extinction in experimental populations. Ecology Letters 12:772-778.

Drake, J.M. and Lodge, D.M. 2004. Effects of environmental variation on extinction and establishment. Ecology Letters 7:26–30.

Drake, J.M., Costello, C., and Lodge, D.M. 2005. When did the discovery rate for invasive species in the North American Great Lakes accelerate? Bioscience 55:4.

Drake, J.M., Lodge, D.M., and Lewis, M. 2005. Theory and preliminary analysis of species invasions from ballast water: Controlling discharge volume and location. American Midland Naturalist 154:459–470.

Drake, L.A., Doblin, M.A., and Dobbs, F. 2007. Potential microbial bioinvasions via ships' ballast water, sediment, and biofilm. Marine Pollution Bulletin 55:333-341.

Drury, K.L.S., Drake, J.M., Lodge, D.M. and Dwyer, G. 2007. Immigration events dispersed in space and time: Factors affecting invasion success. Ecological Modelling 206:63-78.

Duggan, I.C., Bailey, S.A., Colautti, R.I., Gray, D.K., Makarewicz, J.C., and MacIsaac, H.J. 2003. Biological invasions in Lake Ontario: past, present and future. In: Munawar, M. (ed.) *State of Lake Ontario: Past, Present and Future.* Ecovision World Monograph Series, Aquatic Ecosystem Health & Management Society, New Delhi. pp. 541-558.

Eckert, G.L. 2003. Effects of planktonic period on marine population fluctuations. Ecology 84:372-383. (Supplemental data available in Ecological Archives E084-009-A1 at http://esapubs.org/archive/archive_E.htm).

Eckert, G.L. 2009. A synthesis of variability in nearshore Alaskan marine populations. Environmental Monitoring and Assessment 155:593-606.

Eldridge, L.G. 2006. Numbers of Hawaiian Species for 2003 – 2005. Bishop Museum Occasional Papers 88:62-79.

Elliot, J.M. 1971. *Some methods for the statistical analysis of samples of benthic invertebrates.* Freshwater Biological Association Scientific Publication No. 25.

Falkner, M., Takata, L., and Gilmore, S. 2006. California State Lands Commission Report on Performance Standards for Ballast Water Discharges in California Waters. Report produced for California State Legislature. Appendix 5 (Memo on a Natural Invasion Rate Standard) and Appendix 6 (Addendum to the Memo on a Natural Invasion Rate Standard) in Appendix A (Majority Report and Recommendations [sic] of the California Advisory Panel on Ballast Water Performance Standards. (http://www.slc.ca.gov/Spec_Pub/MFD/Ballast_Water/Documents/Appendix_A.pdf)

Faria, J.C. 2009. Resources of Tinn-R GUI/Editor for R Environment. UESC, Ilheus, Brazil.

Fenchel, T. 2005. Cosmopolitan microbes and their 'cryptic' species. Aquatic Microbial Ecology 41:49-54.

Fieberg, J. and Ellner, S.P. 2000. When is it meaningful to estimate an extinction probability? Ecology 81:2040-2047.

Finlay, B.J. 2002. Global dispersal of free-living microbial eukaryote species. Science 296:1061-1063.

Fofonoff, P.W., Ruiz, G.M., Steves, B., and Carlton, J.T. 2003a. In ships or on ships? Mechanisms of transfer and invasion for non-native species to the coasts of North America. In: Ruiz, G.M. and Carlton, J.T (eds.) *Invasive Species: Vectors and Management Strategies*. .Island Press, Washington, DC. pp. 152-182

Fofonoff, P.W., Ruiz, G.M., Steves, B., Hines, A.H., and Carlton, J.T. 2003b. National Exotic Marine and Estuarine Species Information System (NEMESIS). (http://invasions.si.edu/nemesis/overview.html)

Galil, B.S. and Zenetos, A. 2002. A sea change: Exotics in the eastern Mediterranean Sea. In:Leppäkoski, E., Gollasch, S., and Olenin, S. (eds.) *Invasive Aquatic Species of Europe: Distribution, Impacts and Management*. Kluwer Academic Publishers, Dordrecht, The Netherlands. pp. 325-336.

Gascoigne, J. and Lipcius, R.N. 2004. Allee effects in marine systems. Marine Ecology Progress Series 269:49-59.

Gollasch, S. 2006. A new ballast water sampling device for sampling organisms above 50 micron. Aquatic Invasions 1:46-50.

Gollasch, S., David, M., Voigt, M., Dragsund, E., Hewitt, C., and Fukuyo, Y. 2007. Critical review of the IMO international convention on the management of ships' ballast water and sediments. Harmful Algae 6: 585–600.

Gray, D.K., Johengen, T.H., Reid, D.F., and MacIsaac, H.J. 2007. Efficacy of open-ocean ballast water exchange as a means of preventing invertebrate invasions between freshwater ports. Limnology and Oceanography 52:2386-2397.

Gregg, M., Rigby, G., and Hallegraeff, G.M. 2009. Review of two decades of progress in the development of management options for reducing or eradicating phytoplankton, zooplankton and bacteria in ship's ballast water. Aquatic Invasions 4:521-565.

Griffen, B.D. and Drake, J.M. 2008. A review of extinction in experimental populations. Journal of Animal Ecology 77:1274-1287.

Grosholz, E. D. 1996. Contrasting rates of spread for introduced species in terrestrial and marine systems. Ecology 77:1680-1686.

Herborg, L.M., Jerde, C.L., Lodge, D.M., Ruiz, G.M., and MacIsacc, H.J. 2007. Predicting invasion risk using measures of introduction effort and environmental niche models. Ecological Applications 17: 663-674.

Hewitt, C.L, Gollasch, S., and Minchin, D. 2009. The Vessel as a Vector – Biofouling, Ballast Water, and Sediments. In: Rilov, G. and Crooks, J.A. (eds.) *Biological Invasions in Marine Ecosystems: Ecologial, Management, and Geographic Perspectives (Ecological Studies).* Springer-Verlag, Berlin. pp: 117-131.

Hickey, B.M., Pietrafesa, L.J., Jay, D.A., and Boicourt, W.C. 1998. The Columbia River Plume Study: Subtidal variability in the velocity and salinity fields. Journal of Geophysical Research 103:10,339-10,368.

Holeck, K.T., Mills, E.L., MacIsaac, H.J., Dochoda, M.R., Colautti, R.I., and Ricciardi, A. 2004. Bridging troubled waters: Biological invasions, transoceanic shipping, and the Laurentian Great Lakes. BioScience 54:919-929.

Holmes, E.E. 2004. Beyond theory to application and evaluation: Diffusion approximations for population viability analysis. Ecological Applications 14:1272–1293.

Holmes, E.E., Fagan, W.F., Rango, J.J., Folarin, A., Sorensen, J.A., Lippe, J.E., and McIntyre, N.E. 2005. Cross Validation of Quasi-extinction Risks from Real Time Series: An Examination of Diffusion Approximation Methods. NOAA Technical Memorandum NMFS-NWFSC-67. 37 pp. (www.nwfsc.noaa.gov/assets/25/6256_08092005_121845_CrossValidationTM67Final.pdf)

Hoof, R. 2008. Oregon Task Force Report on Shipping Transport of Aquatic Invasive Species.

Hutchinson, G.E. 1965. *The Ecological Theater and the Evolutionary Play*. Yale University Press, New Haven, CT. 139 pp.

IMO. 2004a. Adoption of the Final Act and Any Instruments, Recommendations and Resolutions Resulting from the Work of the Conference. BWM/CONF/36, International Maritime Organization, London. (http://www.epa.gov/owow/invasive_species/BWM-Treaty_36.pdf)

IMO. 2004b. Consideration of the Draft International Convention for the Control and Management of Ships' Ballast Water and Sediments. Ballast Water Discharge Standards - Regulation D-2. BWM/CONF/14. International Maritime Organization, London.

Jarvis, B. 2000. Sampling for microbiological analysis. In Lund, B.M., Baird-Parker, T.C., and Gould, G.W. (eds.) *The Microbiological Safety and Quality of Food Vol. 1.* Aspen Publishers, Gaithersburg, MD. pp. 1691-1732.

Johnston, E.L., Piola, R.F., and Clark, G.F. 2008. Chapter 7. The Role of Propagule Pressure in Invasion Success. In: Rilov, G. and Crooks, J.A. (eds.) *Biological Invasions in Marine Ecosystems: Ecological, Management, and Geographic Perspectives (Ecological Studies).* Springer-Verlag, Berlin. pp:133-151.

Jossi, J.W., John, A.W.G., and Sameoto, D. 2003. Continuous plankton recorder sampling off the east coast of North America: history and status. Progress in Oceanography 58:313–325.

Kinlan, B.P. and Hastings, A. 2005. Rates of population spread and geographic range expansion: What exotic species tell us. In: Sax, D.F., Stachowicz, J.J., and Gaines, S.D. (eds.) *Species Invasions: Insights into Ecology, Evolution, and Biogeography*. Sinauer Associates, Sunderland, MA. pp. 381-419.

Knowlton, N. 1993. Sibling species in the sea. Annual Review of Ecology and Systematics 24:189-216.

Kolar, C.S. and Lodge, D.M. 2001. Progress in invasion biology: predicting invaders. Trends in Ecology and Evolution 16:199-204.

Kramer, A.M., Dennis, B., Liebhold, A.M., and Drake, J.M. 2009. The evidence for Allee effects. Population Ecology 51:341-354.

Kuhnert, P.M., Hayes, K., Martin, T.G., and McBride, M.F. 2009. Expert opinion in statistical models. In: Anderssen, R.S., Braddock, R.D., and Newham L.T.H. (eds.) 18th World IMACS Congress and MODSIM09 International Congress on Modelling and Simulation. Modelling and Simulation Society of Australia and New Zealand and International Association for Mathematics and Computers in Simulation, July 2009. pp. 4262-4268. (http://www.mssanz.org.au/modsim09/J2/kuhnert_J2.pdf)

Lavoie, D.M., Smith, L.D., and Ruiz, G.M. 1999. The potential for intracoastal transfer of non-indigenous species in the ballast water of ships. Estuarine, Coastal and Shelf Science 48:551–564.

Lee II, H. and Chapman, J.W. 2001. Nonindigenous Species - An Emerging Issue For The EPA. Volume 2: A Landscape In Transition: Effects Of Invasive Species On Ecosystems,Human Health, and EPA Goals. U.S. EPA report by the ORD Regional Science Program. 54 pp. (http://www.epa.gov/owow/invasive_species/workshop/nisvol2.pdf)

Lee II, H., Thompson, B., and Lowe, S. 2003. Estuarine and scalar patterns of invasion in the soft-bottom benthic communities of the San Francisco Estuary. Biological Invasions 5:85-102.

Lee II, H. and Reusser, D.A. 2006. Pacific Coast Ecosystem Information System (*PCEIS*) V. 1.0. United States Environmental Protection Agency and United States Geological Survey (Microsoft Access 2003 database).

Lee II, H., Reusser, D.A., Lamberson, J.O. and Nelson, W.G. 2006. Off the beaten track: Invasion of minimally exposed estuaries in the Columbian Biogeographic Province. 14th Intern. Conf. Aquatic Invasive Species. Key Biscayne, FL, May 14-19 (http://www.icais.org/pdf/Abstracts_2006.pdf)

Lemieux, E.J., Robbins, S., Burns, K., Ratcliff, S., and Herring, P. 2008. Evaluation of representative sampling for rare populations using microbeads. U.S. Coast Guard Research and Development Center. Report No. CG-D-03-09.

Leslie, H.M. 2005. Positive intraspecific effects trump negative effects in high-density barnacle aggregations. Ecology 86:2716–2725.

Lessios, H.A., and Robertson, D.R. 2006. Crossing the impassable: genetic connections in 20 reef fishes across the eastern Pacific barrier. Proceedings of the Royal Society, Series B 273:2201–2208.

Lessios, H.A., Kessing, B.D. and Robertson, D.R. 1998. Massive gene flow across the world's most potent marine biogeographic barrier. Proceedings of the Royal Society, Series B 265:583-588.

Levings, C.D., Cordell, J.R., Ong, S., and Piercey, G.E. 2004. The origin and identity of invertebrate organisms being transported to Canada's Pacific coast by ballast water. Canadian Journal of Fisheries and Aquatic Sciences 61:1–11.

Lewis, M.A. and Kareiva, P. 1993. Allee dynamics and the spread of invading organisms. Theoretical Population Biology 43:141–158.

Locke, A., Reid, D.M., van Leeuwen, H.C., Sprules, W.G., and Carlton, J.T. 1993. Ballast water exchange as a means of controlling dispersal of freshwater organisms by ships. Canadian Journal of Fisheries and Aquatic Sciences 50:2086-2093.

Lockwood, J.L., Cassey, P., and Blackburn, T. 2005. The role of propagule pressure in explaining species invasions. Trends in Ecology and Evolution 20:223-228.

Lodge, D.M., Williams, S., MacIsaac, H.J., Hayes, K.R., Leung, B., Reichard, S., Mack, R.N., Moyle, P.B., Smith, M., Andow, D.A., Carlton, J.T., and McMichael, A. 2006. Biological invasions: Recommendations for U.S. policy and management. Ecological Applications 16:2035-2054.

Ludwig, D. 1999. Is it meaningful to estimate a probability of extinction? Ecology 80:298-310.

Lloyd's Register. 2008. Ballast Water Treatment Technology – Current Status. 32 pp. (http://www.lr.org/NR/rdonlyres/6B8B67B8-C135-4E55-B5B1-4BB6A4A10632/85364/BWT021009.pdf)

McMichael, A.J. and Bouma, M.J. 2000. Global Changes, Invasive Species, and Human Health. In: Mooney, H.A. and Hobbs, R.J. (eds.) *Invasive Species in a Changing World*. Island Press, Washington, DC. pp. 191-210.

MEPC. 2003a. Harmful Aquatic organisms in Ballast Water: Summary of an International Workshop on Ballast Water Discharge Standards. MEPC 49/INF.31, Marine Environment Protection Committee, International Maritime Organization, London. (http://www.sjofartsverket.se/upload/5290/49-INF31.pdf)

MEPC. 2003b. Comments on Draft Regulation E-2 Concentrations of Organisms Delivered in Ships' Ballast Water in the Absence of Any Treatment: Establishing a Baseline for Consideration of Treatment Efficacy. MEPC 49/2/21, Marine Environment Protection Committee, International Maritime Organization, London.

MEPC. 2003c. Harmful Aquatic Organisms in Ballast Water. MEPC 49/2/14, Marine Environment Protection Committee, International Maritime Organization, London. (http://www.sjofartsverket.se/upload/5290/49-2-14.pdf)

MEPC. 2008a. Resolution MEPC.174(58). Guidelines for Approval of Ballast Water Management Systems (G8). MEPC 58/23. Annex 4. Marine Environment Protection Committee, International Maritime Organization, London. (http://www.bsh.de/de/Meeresdaten/Umweltschutz/Ballastwasser/MEPC_125_%2853%29.pdf)

MEPC. 2008b. Resolution MEPC.169(57). Procedure for Approval of Ballast Water Management Systems That Make Use of Active Substances (G9). Marine Environment Protection Committee, International Maritime Organization, London.

MEPC. 2008c. Guidelines For Ballast Water Sampling (G2). Annex 3. Resolution MEPC.173(58). MEPC 58/23. Adopted on 10 October 2008. Marine Environment Protection Committee, International Maritime Organization, London.

McCollin, T., Shanks, A.M., and Dunn, J. 2007. The efficiency of regional ballast water exchange: Changes in phytoplankton abundance and diversity. Harmful Algae 6:531-546.

Miller, A.W., Lion, K., Minton, M.S., and Ruiz, G.M. 2007. Status and Trends of Ballast Water Management in the United States. Third Biennial Report of the National Ballast Information Clearinghouse. (January 2004 to December 2005). Smithsonian Environmental Research Center. Report Submitted to the United States Coast Guard. 62 pp. (http://invasions.si.edu/nbic/reports/NBIC_Biennial_Report3_2004-05.pdf)

Minton, M.S., Verling, E., Miller, A.W., and Ruiz, G.M. 2005. Reducing propagule supply and coastal invasions via ships: effects of emerging strategies. Frontiers in Ecology and the Environment 3:304-308.

Morris, W.F. and Doak, D.F. 2002. *Quantitative Conservation Biology: Theory and Practice of Population Viability Analysis*. Sinauer Associates, Sunderland, MA. 480 pp.

Murphy, K.R., Ritz, D., and Hewitt, C.L. 2002. Heterogeneous zooplankton distribution in a ship's ballast tanks. Journal of Plankton Research 24:729-734.

National Research Council. 1996. *Stemming the Tide: Controlling Introductions of Nonindigenous Species by Ships' Ballast Water*. National Research Council. National Academic Press, Washington, DC. 141 pp.

Nilsson, P.A., Turesson, H., and Brönmark, C. 2006. Friends and foes in foraging: intraspecific interactions act on foraging-cycle stages. Behaviour 143:733-745.

Okubo, A. and Levin, S.A. 2002. *Diffusion and Ecological Problems: Modern Perspectives*. Springer-Verlag, New York, NY. 492 pp.

Pershing, A.J., Greene, C.H., Planque, B., and Fromentin, J-M. 2004. The influences of climate variability on North Atlantic zooplankton populations. In: Stenseth, N.C., Ottersen, G., Hurrell, J.W., and Belgrano, A. (eds.) *Marine Ecosystems and Climate Variation: The North Atlantic: A Comparative Perspective.* Oxford University Press, Oxford. pp. 59-69.

Peterson, A.T. and Vieglais, D.A. 2001. Predicting species invasions using ecological niche modeling: New approaches from bioinformatics attack a pressing problem. Bioscience 51:363-371.

Phillips, S. 2005 (updated 2006). Ballast Water Issue Paper. Report for the Pacific States Marine Fisheries Commission. Portland, OR. 34 pp. (http://www.aquaticnuisance.org/wordpress/wp-content/uploads/2009/01/ballast_water_issue_paper.pdf)

Pimentel, D., Zuniga, R., and Morrison, D. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecological Economics 52:273-288.

Piola, R.F. and Johnston, E.L. 2009. Comparing differential tolerance of native and non-indigenous marine species to metal pollution using novel assay techniques. Environmental Pollution 157:2853–2864.

Price, J.P. and Clague, D.A. 2002. How old is the Hawaiian biota? Geology and phylogeny suggest recent divergence. Proceedings of the Royal Society, Series B 269:2429-2435.

R Development Core Team. 2008. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, (http://www.R-project.org).

Raaymakers, S. 2002. 1st International Ballast Water Treatment Standards Workshop, IMO London, 28-30 March 2001: Workshop Report. GloBallast Monograph Series No. 4. IMO London. (http://globallast.imo.org/monograph%204%20standards%20workshop.pdf)

Reid, D.M. and Carlton, J.T. 1997. Shipping Study 1-A: A Study of the Introduction of Aquatic Nuisance Species by Vessels Entering the Great Lakes and Canadian Waters Adjacent to the United States. Final Report to U.S. Dept. of Transportation and U.S. Coast Guard. Report No. CG-D-17-97. 86 pp.

Reusser, D.A. and Lee II, H. 2008. Predictions for an invaded world: a strategy to predict the distribution of native and non-indigenous species at multiple scales. ICES Journal of Marine Science 65:742–745.

Ricciardi, A. 2006. Patterns of invasion in the Laurentian Great Lakes in relation to changes in vector activity. Diversity and Distributions 12:425-433.

Robertson, D.R., Grove, J.S. and McCosker, J.E. 2004. Tropical transpacific shore fishes. Pacific Science 58:507-565.

Ruiz, G.M. and Carton, J.T. 2003. Chapter 18: Invasion Vectors: A Conceptual Framework for Management. In: Ruiz, G.M. and J.T. Carlton (eds.) *Invasive Species: Vectors and Management Strategies.* Island Press, Washington, DC. pp. 459-504.

Ruiz, G.M. and Reid, D.F. 2007. Current state of understanding about the effectiveness of ballast water exchange (BWE) in reducing aquatic nonindigenous species (ANS) introductions to the Great Lakes Basin and Chesapeake Bay, USA: synthesis and analysis of existing information. NOAA Technical Memorandum GLERL-142. NOAA Great Lakes Environmental Research Laboratory, Ann Arbor, MI. 127 pp. ftp://ftp.glerl.noaa.gov/publications/tech_reports/glerl-142/tm-142.pdf

Ruiz, G.M., Fofonoff, P.W., Carlton, J.T., Wonham, M.J., and Hines, A.H. 2000. Invasion of coastal marine communities in North America: Apparent patterns, processes, and biases. Annual Review of Ecology and Systematics 31:481-531.

Ruiz, G.M., Carlton, J.T., Grosholz, E.D. and Hines, A.H. 1997. Global invasions of marine and estuarine habitats by nonindigenous species: Mechanisms, extent, and consequences. American Zoologist, 37:621-632.

Sakai, A.K., Allendorf, F.W., Holt, J.S., Lodge, D.M., Molofsky, J., With, K.A., Baughman, S., Cabin, R.J., Cohen, J.E., Ellstrand, N.C., McCauley, D.E., O'Neil, P., Parker, I.M., Thompson, J.N., and Weller, S.G. 2001. The population biology of invasive species. Annual Review of Ecology and Systematics 32:305–332.

Sandin, S.A., Smith, J.E., DeMartini, E.E., Dinsdale, E.A., Donner, S.D.,Friedlander, A.M., Konotchick, T., Malay, M., Maragos, J.E., Obura, D., Pantos, O.,Paulay, G., Richie, M., Rohwer, F., Schroeder, R.E., Walsh, S., Jackson, J.B.C., Knowlton, N., and Sala, E. 2008. Baselines and degradation of coral reefs in the northern Line Islands. PLoS ONE 3: e1548. doi:10.1371/journal.pone.0001548. (http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0001548)

Scheltema, R.S. 1986. Long-distance dispersal by planktonic larvae of shoal-water benthic invertebrates among central Pacific islands. Bulletin of Marine Science 39:241-256.

Scheltema, R.S. 1988. Initial evidence for the transport of teleplanic larvae of benthic invertebrates across the east Pacific barrier. Biological Bulletin 174:145-152.

Scheltema, R. S., and I. P. Williams. 1983. Long-distance dispersal of planktonic larvae and biogeography and evolution of some Polynesian and western Pacific mollusks. Bulletin of Marine Science 33:545–565.

Simberloff, D. 1989. Which Insect Introductions Succeed and Which Fail? In: Drake, J.A., Mooney, H.A., di Castri, F., Groves, R.H., Kruger, F.J., Rejmanek, M., and Williamson, M. (eds.) *Biological Invasions: a Global Perspective*. John Wiley and Sons, NewYork, NY. pp. 61-75.

Simberloff, D. 2009. The role of propagule pressure in biological invasions. Annual Review of Ecology Evolution and Systematics 40:81–102.

Simkanin, C., Davidson, I., Falkner, M., Sytsma, M., and Ruiz, G. 2009. Intra-coastal ballast water flux and the potential for secondary spread of non-native species on the US West Coast. Marine Pollution Bulletin 58:366–374.

Sexton, J.P., McIntyre, P.J., Angert, A.L., and Rice, K.J. 2009. Evolution and ecology of species range limits. Annual Review of Ecology Evolution and Systematics 40:415–36.

Stephens, P.A. and Sutherland, W.J. 1999. Consequences of the Allee effect for behaviour, ecology and conservation. Trends in Ecology and Evolution 14:401–405.

Taylor, L. R. 1961. Aggregation, variance and the mean. Nature 189: 732-735.

Taylor, C.M. and Hastings, A. 2005. Allee effects in biological invasions. Ecology Letters 8:895-908.

Taylor,W.D. and Shuter, B.J. 1981. Body size, genome size, and intrinsic rate of increase in ciliated protozoa. American Naturalist 118:160-172.

Thiel, M. and Gutow, L. 2005. The ecology of rafting in the marine environment. II. The rafting organisms and community. Oceanography and Marine Biology-An Annual Review 43:279-418.

USCG. 2002a. Ballast water treatment standards – East Coast and West Coast workshop summaries. Produced by Battelle for U.S. Department of Transportation/U.S. Coast Guard, Final Report on Contract No. DTCG39-00-D-R00019, March 2002. [From "Report and Recommendation of the California Advisory Panel on Ballast Water Performance Standard"]

USCG. 2002b. Standards for Living Organisms in Ship's Ballast Water Discharged in U. S. Waters. U.S. Federal Register 67(42):9632 (March 4, 2002). (http://frwebgate.access.gpo.gov/cgi-bin/getdoc.cgi?dbname=2002_register&docid=02-5187-filed.pdf)

USCG. 2008. Ballast Water Discharge Standard - Draft Programmatic Environmental Impact Statement. Prepared by the United States Coast Guard, USCG-2001-10486. 221 pp.

USCG. 2009. Proposed Ballast Water Discharge Standard Rulemaking. Fact sheet produced for U.S. Coast Guard Office of Operating and Environmental Standards. (http://www.safinah.co.uk/uploads/assets//PDFs/Ballast_Water_Discharge_Standard.pdf). The proposed rulemaking can be found at: http://www.regulations.gov. Enter docket number USCG-2001-10486 in the Search box.

U.S. EPA. no date. Water Permitting 101. Office of Wastewater Management - Water Permitting. (http://www.epa.gov/npdes/pubs/101pape.pdf)

U.S. EPA. 1986. Ambient Water Quality Criteria for Bacteria – 1986. U.S. Environmental Protection Agency, Office of Water. EPA440/5-84-002. 18 pp. (http://www.epa.gov/waterscience/beaches/files/1986crit.pdf)

U.S. EPA. 1996. U.S. EPA's NPDES Permit Writers' Manual. U.S. Environmental Protection Agency, Office of Water. EPA-833-B-96-003. 292 pp. (http://www.epa.gov/npdes/pubs/owm0243.pdf)

U.S. EPA. 1999. Appendix A, Clean Ballast: Nature of Discharge in "Phase I Final Rule and Technical Development Document of Uniform National Discharge Standards (UNDS)". EPA-842-R-99-001.

U.S. EPA. 2003. Bacterial Water Quality Standards for Recreational Waters (Freshwater and Marine Waters) - Status Report. U.S. Environmental Protection Agency, Office of Water. EPA-823-R-03-008. 9 pp. plus appendix.

U.S. EPA. 2004a. An Examination Of EPA Risk Assessment Principles and Practices. Staff Paper Prepared for the U.S. Environmental Protection Agency by members of the Risk Assessment Task Force. EPA/100/B-04/001. 180 pp. (http://www.epa.gov/osa/pdfs/ratf-final.pdf)

U.S. EPA. 2004b. Overview of the Ecological Risk Assessment Process in the Office of Pesticide Programs, U.S. Environmental Protection Agency - Endangered and Threatened Species Effects Determinations. Office of Prevention, Pesticides and Toxic Substances Office of Pesticide Programs Washington, DC. 92 pp. (http://www.epa.gov/espp/consultation/ecorisk-overview.pdf)

U.S. EPA. 2008a. 2008 Final issuance of National Pollutant Discharge Elimination System (NPDES) Vessel General Permit (VGP) for Discharges Incidental to the Normal Operation of Vessels Fact Sheet. (www.epa.gov/npdes/vessels)

U.S. EPA. 2008b. Vessel General Permit. Finalized December 19, 2008. (www.epa.gov/npdes/vessels)

Verling, E., Ruiz, G.M., Smith, L.D., Galil, B., Miller, A.W., and Murphy, K.R. 2005. Supply-side invasion ecology: characterizing propagule pressure in coastal ecosystems. Proceedings of the Royal Society, Series B 272:1249–1257.

Vermeij, G.J. 1991. When biotas meet: Understanding biotic interchange. Science 253:1099-1104.

Vermeij, G.J. 2005. From Europe to America: Pliocene to recent trans-Atlantic expansion of cold-water North Atlantic molluscs. Proceedings of the Royal Society, Series B 272:2545–2550.

Wares, J.P. 2001. Biogeography of Asterias: North Atlantic climate change and speciation. Biological Bulletin 201: 95–103.

Wonham, M.J. and Carlton, J.T. 2005. Trends in marine biological invasions at local and regional scales: the Northeast Pacific Ocean as a model system. Biological Invasions 7:369–392.

Wright, D.A. 2007. Logistics of shipboard and dockside testing of ballast water treatment systems in the United States. In: Gherardi, F. (ed.) *Biological Invaders in Inland Waters: Profiles, Distribution, and Threats.* Springer, The Netherlands. pp. 657-668.

Zongguo, H. 2001. *Marine Species and Their Distribution in China's Seas*. Krieger Publishing Co., Malabar, FL. 599 pp.

Appendix A: Overview of Human Health Microbial Standards

Henry Lee II

Overview:
It is beyond the scope of this document and the expertise of the author to critically review the microbial human health discharge standards proposed by IMO or other entities (see Table 1), and the reader is referred to water quality criteria documents and websites for additional information (e.g., U.S. EPA, 1986, 2003; http://www.epa.gov/beaches/) as well as to reviews on microbes in ballast water (e.g., Dobbs and Rogerson, 2005; Drake et al., 2007). One approach taken by the IMO and the USCG is to use indicator organisms, specifically *Escherichia coli* and intestinal enterococci (Table 1). *E. coli* is usually non-pathogenic but is considered an indicator of fecal contamination. *Enterococcus* is a genus of bacteria that is a sub-group of fecal streptococci, and is also an indicator of fecal contamination. EPA's 1986 guidance (U.S. EPA, 1986) listed both *E. coli* and enterococci as indicators in freshwater but only enterococci for marine waters since it survives longer than *E. coli* in marine waters. The IMO and USCG, however, do not differentiate between fresh and marine waters in their standards.

The genesis of the proposed IMO and USCG Phase I microbial standards is not well defined. They are both set at about twice the criteria for steady state geometric mean densities for "Bathing (Full Body Contact) Recreational Waters" in the 1986 "Ambient Water Quality Criteria for Bacteria" document. These values are based on multiple samples: "generally not less than 5 samples equally spaced over a 30-day period". The USCG Phase II standards and the Wisconsin and interim California standards are equal to the freshwater standards in the 1986 criterion document.

Both the IMO and the USCG also list standards for *Vibrio cholerae* (serotypes O1 and O139) as does California in its interim standards (Table 1). *Vibrio cholerae* is the pathogen causing cholera, and as noted by the MEPC (2003c), "Some cholera epidemics appear to be directly associated with ballast water. One example is an epidemic that began simultaneously at three separate ports in Peru in 1991, sweeping across South America, affecting more than a million people and killing more than ten thousand by 1994. This strain had previously been reported only in Bangladesh." It is not clear to the current authors how the particular standards were derived by the IMO or USCG. It is interesting to note that in a presentation by Professor Rob Bragg ("Understanding Cholera - A Review"; http://www.ufs.ac.za/apps/congress/documents/05/Presentations/107-Prof%20R%20Bragg.ppt) he stated that it took about one million bacteria to start an infection in a healthy person. However, it is not possible to compare this value with the ballast standards because they are given in colony forming units" (cfu). It is also interesting to note that the U.S. EPA recently removed *Vibrio cholerae* from its final Contaminant Candidate List 3 (CCL 3) for drinking water (http://www.epa.gov/ogwdw/ccl/pdfs/ccl3_docs/fs_cc3_final.pdf) because of the low incidence of cholera in the United States.

Recommendation/Conclusions:
As a first step, we suggest that a clearer rationale for the microbial standards be developed. Is the purpose of the standards to protect bathers or is it to protect drinking water? Or are the

standards considered surrogates to help protect against the transport of animal diseases, such as viral hemorrhagic septicemia virus (VHS; see http://biology.usgs.gov/faer/vhs.html)? These are all laudable objectives but the standards, and the indicators, are likely to differ depending upon the most important objective(s).

Consideration should be given to the design of sampling protocols both during land/ship based verification testing and during compliance monitoring. As detailed in the 1986 "Ambient Water Quality Criteria for Bacteria", there are different standards for long-term means and individual samples.

Appendix B: Calculation of Coastal Per Capita Invasion Probabilities

Deborah A. Reusser

Statistical Analysis Using R:
Analysis was done using the statistical program R (R Development Core Team 2008) because it is widely available and free. The scripts were developed in the text editor Tinn-R (Faria 2009). The R script below reads foreign ballast water discharge values for ship discharges for each coast in the United States. The calcPCIP function runs a simulation 10,000 times. For each run, a random organism concentration is selected for each ship based on estimates of Minton et al. (2005) sample data. The function then calculates the high, median and low quantiles of PCIP values for each coast. A histogram of the PCIP values is generated for each coast and written to a png file. Code is also provided that uses the PCIP values to generate contour plots indicating the number of invaders per year given organism concentrations and total amount of ballast water discharged. The code to generate contour plots is given, based on a safety factor of 1. If the safety factor is changed, the text locations will need to be modified to plot correctly on the contour plot. Ballast water discharge data are required, along with an organism density file and historical invasion rate to run this code.

Load the library files needed
```
>library(Hmisc)
>library(MASS)
> library(RColorBrewer)
>library(fields)
```

Identify the column definitions for reading in the file
```
>col.defs<-c(rep("numeric",2))
```

Read in the density Values from the Minton graph
```
>ballastDF <- read.csv("DensityVals.csv", colClasses=col.defs)
```

Create the MeanData table from the density values (N=354)
```
>MeanData <- rep(ballastDF$Density, ballastDF$NumShips)
```

The density values data is a table of the number of ships with organism concentrations of a certain value. The MeanData table contains 354 values with approximate organism concentrations extracted from the table in Minton et al. (2005).

Indentify the columns and read the ballast water file
```
>col.defs <- c(rep("character", 5),"numeric", rep("character",2), rep("numeric",2))
>allBallast<-read.csv("coastforiegnballast.csv", colClasses = col.defs)
```

Identify the columns and read the number of invaders per coast in
```
>col.defs <- c("character", "numeric")
>ballastInvaders<-read.csv("Ballast_Invaders.csv", colClasses = col.defs)
```

Create a summary table containing the sums for each coast

```
>ballastSums<-tapply(allBallast$DISCHARGE, allBallast$Coast, sum)
>bwSumsdf <- data.frame(ballastSums)
>bwSumsdf$coast<-row.names(bwSumsdf)
>bwSumsdf$annualForeign <- bwSumsdf$ballastSums/3
```

Function CalcPCIP runs 10,000 simulations randomly assigning an organism concentration to each discharge event, summing the total organism concentrations for the run and calculating the PCIP for each run. After all runs are completed, a histogram of the PCIP values is written to a png file and the 2.5, .5, 97.5 quantile values are calculated for the set of PCIP values generated.

```
>calcPCIP <-function(bInfo, bData) {
  #Define a dataframe to contain the calculated values
  >RandRun=data.frame(MeanConc=rep(NA,10000), TotalProp=rep(NA, 10000),
PCIP=rep(NA,10000))

  #Run the calculations 10,000 times to get a normal distribution of per capita probabilities
   >for (i in 1:10000) {
      # Get a random array of concentrations for all
      >Conc <- sample(MeanData, size=bInfo$shipCount, replace=TRUE)
      # Calculate the mean concentration for this run and store it
      >RandRun$MeanConc[i] <- mean(Conc)
      # Calculate the number of organisms for each ship for this run
      >Prop<- round(Conc*bData$DISCHARGE,0)
      # Calculate the total organism inoculation from all ships for this run and store it
      >RandRun$TotalProp[i] <- sum(Prop,na.rm=TRUE)
      # Calcuate the annual per capita probability
      >RandRun$PCIP[i] <- bInfo$TotBWInvaders/(RandRun$TotalProp[i]/3)
   >}

# Create a file name and write out the data generated by the Random Run
> csvFile <- paste(bInfo$Coast, "RanRun", ".csv", sep="")
>write.csv(RandRun, file = csvFile, append = FALSE, na = "NA", row.names = TRUE)

#Calculate the lower, median and upper bound of the annual per capita invasion probability
>tmp <- quantile(RandRun$PCIP, probs=c(0.025,.5, 0.975))
>bInfo$medianPCIP <- tmp[2]
>bInfo$hbPCIP <- tmp[3]
>bInfo$lbPCIP <- tmp[1]

# Create a histogram of all calculated annual PCIPs, write the graphic to a png file
# Create the name of the file to be written
>pngFile <- paste(bInfo$Coast, ".png",sep="")
# Open the png file for writing
>png(pngFile)
```

```
# Create a title for the Historgram based on the name of the coast being processed
 >hTitle <- paste("Histogram of",  bInfo$Coast, "Coast Annual\nPer Capita Invasion
Probabilities")
>hist(RandRun$PCIP, font=2, font.lab=2,main=hTitle, xlab="Per Capita Invasion Probabilites")
# add lines for the lower, median and upper quantile PCIP values on the histogram
>abline(v=bInfo$lbPCIP, col="red")
>abline(v=bInfo$hbPCIP, col="red")
>abline(v=bInfo$medianPCIP, col="blue")
# Close the png file
>dev.off()
# Return the dataframe of information for the coast to the calling routine
>return(bInfo)
}
## END FUNCTION
Create a unique list of Coasts in allBallast
>coastlst<-unique(allBallast$Coast)
>allBallastLst <- unique(allBallast$Coast)
>recCount <- length(coastlst)

Create a dataframe to hold the information calculated for each coast
>CoastInfo=data.frame(CoastName=rep(NA,recCount), shipCount=rep(NA, recCount),
TotFB=rep(NA,recCount),TotAnnFB=rep(NA,recCount),TotBWInvaders=rep(NA,recCount),lb
PCIP=rep(NA,recCount), medianPCIP=rep(NA,recCount), hbPCIP=rep(NA,recCount))

Loop  through all the coasts calling the PCIP function
>for(j in 1: length(coastlst)){

    ## Get the name of the current coast ##
    >CoastInfo$CoastName[j] <- coastlst[j]
    ## Get the records for the current coast ##
    >CoastData <- allBallast[allBallast$Coast %in% CoastInfo$CoastName[j],]
    ## Get the count of the number of records for the current coast ##
    >CoastInfo$shipCount[j] <-length(CoastData$Coast)
    ## Get only the records that have foreign ballast discharge ##
    >FBCoastData <- CoastData[CoastData$DISCHARGE > 0,]
    ## Calculate the total foreign ballast
    >CoastInfo$TotFB[j] <- sum(FBCoastData$DISCHARGE)
    ## Calculate the total annual foreign ballast
    >CoastInfo$TotAnnFB[j] <- sum(FBCoastData$DISCHARGE)/3
    ## Store the ballast water invaders per year for a coast
    >CoastInfo$TotBWInvaders[j] <- ballastInvaders$invpyr[ballastInvaders$Coast %in%
CoastInfo$CoastName[j]]
    ## Calculate the PCIP values for the Coast
    >CoastInfo[j,] <- calcPCIP(CoastInfo[j,], CoastData)

  >}
```

Write out the results for each coast to a CSV file

```
>write.csv(CoastInfo, file = "RegionalPCIP.csv", append = FALSE, na = "NA", row.names =
TRUE)

# Build a vector of values for 3D plot- Organism Concentrations 0 - 1000
>conc<-c(seq(0.0001, 0.001, by = 0.00001),
      seq(0.0011, 0.01, by = 0.0001),
      seq(0.011, 0.1, by=0.001),
      seq(0.11, 1, by = 0.01),
      seq(1.1, 100, by = .1),
      seq(101, 1000, by=1))

# Build a vector of discharge values from 0 to 30,000,000
>discharge<-seq(0,30000000, length=6001)

# Get the stored value for the upper quantile for West Coast
>probinv<- CoastInfo$hbPCIP[3]

# Set the safety factor
>safetyFactor<- 1

#Create a matrix to contain the number of invaders given a concentration and discharge
>num_invaders=matrix(data=NA, nrow=6001, ncol=2251, byrow="T", dimnames=NULL)

# Fill the matrix looping through each concentration and discharge value
>for (i in 1:6001) {
  >for (j in 1:2251) {
    >num_invaders[i,j]=probinv*discharge[i]*conc[j]*safetyFactor
 > }
>}

# Make a plot of the probabillity Matrix
#  Set the Breaks for the Plot
>brk <- c(0,0.0001, 0.001, 0.01, 0.1,1, 10)

#Create a color pallette of Red Yellow Green with six different colors
>myPal<-brewer.pal(6,"RdYlGn")

# Identify the png the plot will  be written to
>png("WCRegionalPCIPJan2010.png")
>par(xaxs="i", family="serif")
>iTitle <-  paste("Predicted Number of Invaders Per Year \n Given Per Capita Invasion
Probability of", format(probinv,scientific = TRUE, digits=4), " \n West Coast")

# Make the plot
```

```
>image.plot(x=discharge, y=log(conc,10),z=num_invaders, axes=F, breaks=brk, font.lab=2,
col=rev(myPal),lab.breaks=names(brk),
 xlab="Foreign Ballast Water Discharge in Million m3 Per Year", ylab="Concentration
(organism/m3)", main=iTitle, add=FALSE, legend.shrink=100)
#label the axes
>axis(1, at=c(0, 1000000, 5000000,10000000,15000000,20000000,25000000, 30000000), labels
= c(0, '1', '5','10','15','20','25','30'),font=2, las=1)
>axis (2, at = c(-4,-3, -2, -1, 0, 1, 2, 3), labels = c('0.0001','0.001', '0.01', '0.1', '1', '10', '100',
'1000'), font=2, las=1)

# Label the plot with the number of NIS per year for each color
>text(27900000,-2.3, "# NIS < 1 per 10,000 years", cex=1, col="black", font=2, adj=c(1,0))
>text(27900000,-.4, "# NIS < 1 per 1000 years", cex=1, col="black", font=2, adj=c(1,0.5))
>text(27900000,.55, "# NIS < 1 per 100 years", cex=1, col="black", font=2,adj=c(1,0.5))
>text(27900000,1.5, "# NIS < 1 per 10 years", cex=1, col="black", font=2, adj=c(1,0))
>text(27900000,2.50, "# NIS < 1 per year", cex=1, col="black", font=2, adj=c(1,0))
>dev.off()
```

Appendix C: R Statistical Tools to Develop/Evaluate Ballast Water Sampling Protocols

Melanie Frazier

Statistical Tools Using R:

Here we describe some tools that can be used to develop and evaluate sampling protocols. For these examples we use the statistical program R (R Development Core Team 2008) because it is widely available, free, and the preferred program for analysis of many researchers. We also suggest working with a data editor such as Tinn-R (Faria 2009). We do not attempt to provide an overview of R; however, some excellent introductory materials can be found at http://cran.r-project.org by following the "Contributed" link. Input and output from R are represented with `courier` font, and input is preceded by ">". The symbol "`<-`" indicates the assignment of a variable name to a variable. R will not read input preceded by "#", which is for user documentation.

Random distributions

To calculate the confidence interval around a concentration based on the results from sampling, the *ci.poisson* function from the *epicalc* package (Chonsuvivatwong 2008) can be used. This function calculates the possible range of concentrations based on the desired confidence interval (alpha = significance level = 1 – CI/100), the total number of observed organisms (events), and the total sample volume (person.time). For example, if 0 organisms are observed in a 0.1 m^3 sample volume, the true concentration may be as high as 36.89 organisms m^{-3} based on the two-tailed 95% confidence interval:

```
> ci.poisson(0, 0.1, alpha=0.05)
 events person.time incidence se exact.lower95ci exact.upper95ci
      0         0.1         0  0                0           36.89
```

If 10 organisms are observed in a 1m^3 sample volume, the concentration is estimated to be between 4.8 and 18.4 organisms based on the 95% confidence interval:

```
> ci.poisson(10, 1, alpha=0.05)
 events person.time incidence se exact.lower95ci  exact.upper95ci
 10     1           10        3.162     4.79                18.4
```

The Poisson distribution can be further explored using dpois, ppois, qpois, and rpois functions. These functions allow lots of flexibility for evaluating and developing sampling protocols. Poisson distributions are described by a single parameter, λ, which equals both the mean and the variance ($\lambda = \mu = \sigma^2$). As the mean of a Poisson distribution increases, the variance also increases. For ballast water analyses, λ represents the concentration of organisms in the ballast water. For more information about these functions, type the function name preceded by a "?" (i.e., ?dpois) into the R console. For the following examples, we assume a sample volume of 1 m^3.

The dpois function is the probability density function for a Poisson distribution. It calculates the probability of obtaining a specific number of organisms in a sample unit based on λ. For example, if a ballast tank contains 1 organism m^{-3} the probability of a 1 m^3 sample volume containing zero organisms is 36.8%:

```
> dpois(0, lambda=1)
     0.3678794
```

A plot of the probability distribution can be created (see Fig. 9):

```
> counts <- c(0,1,2,3,4,5,6)
> poissonDist <- dpois(counts, lambda=1)
> barplot(poissonDist, ylab="Probability", xlab="Count of
organisms in sample", names.arg=counts)
```

The ppois function is the cumulative distribution function. This is used to calculate the probability of a sample containing ≤ a specified number of organisms. The probability of a 1 m^3 sample volume containing ≤ 3 organisms when the concentration is 1 organism m^{-3} is 98.1%:

```
> ppois(3, lambda=1)
     0.9810118
```

The probability that a sample will contain >3 organisms is 1.9%:

```
> 1 - ppois(3, lambda=1)
     0.01898816
```

or, alternatively:

```
> ppois(3, lambda=1, lower.tail=FALSE)
     0.01898816
```

The qpois function is the quantile function and returns the number of organisms predicted to be in a sample for a given quantile of data (the inverse of ppois). For example, if the concentration of organisms in ballast is 10 m^{-3}, about 95% of 1 m^3 samples will contain 15 or fewer organisms.

```
> qpois(0.950, lambda=10)
     15
```

The rpois function generates random values from a Poisson distribution with a specified lambda. To obtain ten 1 m^3 random samples from a population with concentration 1 organism m^3:

```
> rpois(10, lambda=1)
     1 0 3 1 2 1 0 0 3 2
```

<u>Aggregated distributions</u>

One way to determine whether a population is aggregated is to compare the observed distribution of sample data with an expected distribution derived from the mean and variance of the sample data. A chi-square test can then be used to compare the observed and expected values (this and other methods are described in Jarvis, 2000). Negative binomial distributions are described by two parameters, μ (mean) and θ (dispersion factor, referred to as "size" in R). These parameters can be estimated with maximum likelihood techniques using the fitdistr function from the MASS

package from replicate samples taken from a population. Once the parameters have been estimated, the probability distribution for the negative binomial can be used to develop sampling protocols for aggregated populations. The negative binomial functions in R are: dnbinom, pnbinom, qnbinom, and rnbinom.