

Aquatic Invaders

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BALLAST

Evaluation of Different Biocides for Potential Use in Treating Overseas Unballasted Vessels Entering the Great Lakes

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Note: Much of the material presented in this manuscript is available from citations in the reference section.

Introduction

The North American Great Lakes are arguably one of the world's greatest natural resources. Containing more than 5,500 cubic miles of freshwater and possessing 10,000 miles of coastline, the Great Lakes constitute a unique freshwater habitat that provides water and generates jobs for tens of millions of U.S. and Canadian citizens. Despite the immense size, the Great Lakes are extremely vulnerable to human activities. Over the decades, the ecosystem has succumbed to anthropogenic pressures associated with over-fishing, eutrophication, and urbanization. Perhaps one of the most profound anthropogenic effects, however, has been from the introduction of aquatic nonindigenous species. Although

more than a dozen of these species have been introduced intentionally, most often for recreational enhancement and as biological controls for species such as the invading alewife, some of the more problematic ones are due to unintentional introductions. Two of the most notorious unintentional invaders include the sea lamprey, which was first sighted in the upper Great Lakes the early 1900s, and the zebra mussel, which was first discovered in Lake St. Clair in 1988 (Herbert et al. 1989)

Although much progress has been made in thwarting additional introductions, the Great Lakes remain vulnerable to releases of unwanted aquatic nonindigenous species through the ballast water of unballasted vessels. To date, more than 170 aquatic nonindigenous species have been identified in the Great Lakes (Mills et al. 1993; Ricciardi 2001; Grigorovich et al. 2003). Although several vectors are responsible for the introduction of these species, transoceanic shipping has been the primary source of nonindigenous species into the Great Lakes in the last four decades (Mills et al. 1993; Ricciardi 2001), associated with approximately one new nonindigenous species established per year since 1970 (Grigorovich et al. 2003). Since the mid-1980s, approximately half of new invaders have originated from the Ponto-Caspian region of Europe (i.e., Caspian, Azov, and Black Seas: Ricciardi and MacIsaac 2000; Ricciardi 2001). Of these invaders, it is estimated that more than 70% were introduced through ballast water (Holeck et al. 2004).

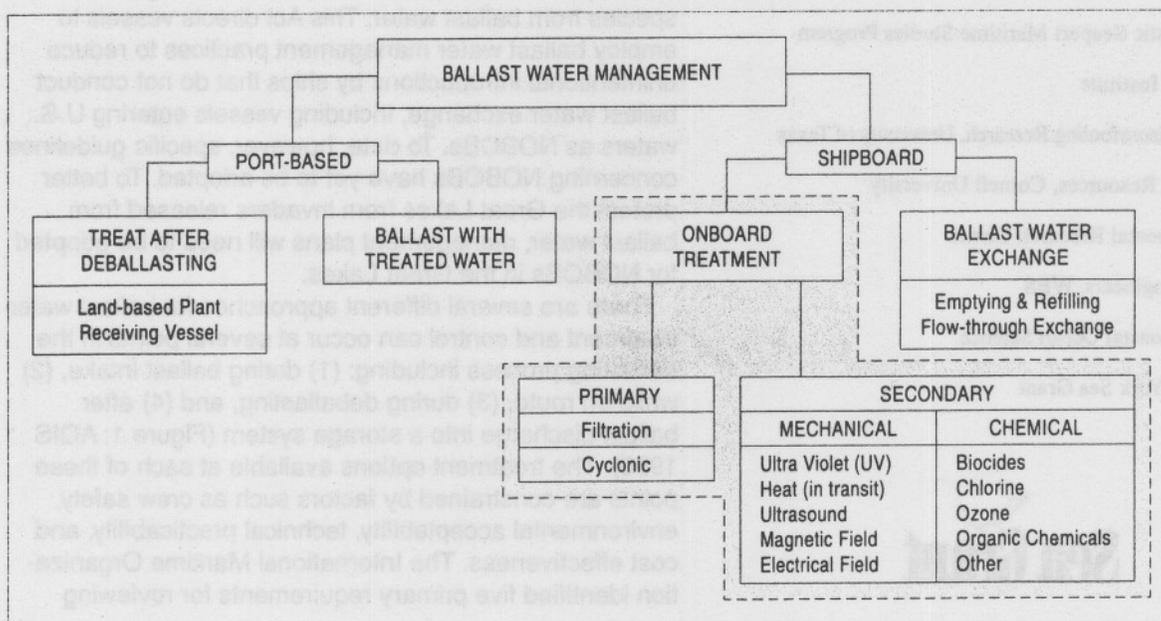


Figure 1. Schematic for different types of ballast water treatment options. From Glosten-Herbert Hyde Marine 2002.

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Ballast Water Vector

Prior to the early 1990s, vessels with ballast on board (BOBs) were considered the primary source of aquatic nonindigenous species. Risk from this vector, however, was reduced with enactment of the Nonindigenous Aquatic Nuisance Prevention and Control Act of 1993 (US Coast Guard 1993). Under the requirements of this act, and through the regulations of the United States Coast Guard (in charge of implementing the act: 33 CFR Part 151), all ships entering the Great Lakes that carry pumpable ballast must follow one of three courses of action: (1) conduct an exchange of ballast water in the open ocean, 200 miles from land and in a depth of 2000 meters, to provide a final ballast water salinity of at least 30 parts per thousand; (2) retain the ballast during transit throughout the Great Lakes; or (3) conduct an alternative, environmentally-sound method of ballast water management (only with prior approval from the Coast Guard).

Although these regulations reduced the risk of potential invaders, the Great Lakes remain vulnerable to ballast water releases from no ballast on board vessels (NOBOBs), which constitute the majority (up to 90%) of overseas vessel traffic (Colautti et al. 2003). Technically classified as unballasted, NOBOBs contain residual amounts of unpumpable sediment and water in their tanks. Viable organisms found in this residual material can be released during reballasting operations, in which NOBOBs take on water in exchange for the cargo unloaded in the lower lakes and then generally deballast this additional water when they take on outbound cargo in the upper lakes. It is through this final deballasting operation that nonnative organisms transported into the Great Lakes can be released.

To address gaps in existing legislation, the United States National Invasive Species Act (16 U.S.C. 4701-4710, 4711, 4722, 4751 *et seq.*, §§1101-1104, 1202, 1203) amended the Nonindigenous Aquatic Nuisance Prevention and Control Act by adding regulations to further prevent the spread of aquatic nonindigenous species from ballast water. This Act directs vessels to employ ballast water management practices to reduce unintentional introductions by ships that do not conduct ballast water exchange, including vessels entering U.S. waters as NOBOBs. To date, however, specific guidelines concerning NOBOBs have yet to be adopted. To better protect the Great Lakes from invaders released from ballast water, management plans will need to be adopted for NOBOBs in the Great Lakes.

There are several different approaches for ballast water treatment and control can occur at several points in the ballasting process including: (1) during ballast intake, (2) while en route, (3) during deballasting, and (4) after ballast discharge into a storage system (Figure 1: AQIS 1993). The treatment options available at each of these points are constrained by factors such as crew safety, environmental acceptability, technical practicability, and cost effectiveness. The International Maritime Organization identified five primary requirements for reviewing

treatment technologies, including: 1) Safety consideration for the ship and crew; 2) Environmental acceptability, in terms of not causing greater environmental risks than those they solve; 3) Practicability issues, such as compatibility with ship design and operations; 4) Cost effectiveness of technologies; and 5) Biological effectiveness of treatments.

Currently, the only management practice that has been adopted by several countries (i.e., the United States, Australia, New Zealand, Chile, and Israel; USCG 2003) is ballast-water exchange for BOB vessels. A limited number of ports have also adopted site-specific treatment guidelines, such as Scapa Flow, Scotland which requires most vessels to discharge ballast at a shore reception facility, and Buenos Aires, Argentina, which permits in-tank treatment of ballast water using chlorine (USCG 2003). In order to reduce the release of nonindigenous species from ballast water, additional treatment technologies will need to be adopted. Some of the more promising ship-board treatment approaches include filtration, thermal treatment, UV light treatment, ozone treatment, deoxygenation, and chemical biocide application. Any viable treatment approach will need to meet new IMO (2004) standards for ballast water discharge.

This article summarizes data generated from four years of biocide studies (Sano et al. 2003, 2004, 2005). The overall objective of these studies was to develop a framework for comparing the potential effectiveness of different biocides in treating residuals (water and sediment) from unballasted vessels. The use of biocides to treat ballast water has been identified by several reports as a potentially viable management option (e.g., AQIS 1993, NRC 1996). Appropriate biocides for ballast water treatment include any inorganic or organic substance (or mixture thereof) that inhibits the growth, or causes the death, of a variety of organisms. The use of biocides to treat ballast water may offer several potential advantages: Biocide treatment can be readily adapted into both current and future vessel designs. Most biocides can also effectively eliminate a wide range of organisms at relatively low concentrations. The efficacy of biocides, however, may vary dramatically depending on treatment conditions. For example, in tanks with high sediment loads or a large amount of organic material and reactive compounds, oxidizing biocides may dissipate rapidly, thereby reducing effectiveness. In addition, the possible advantages of biocide use need to be weighed against prominent disadvantages, particularly potential risks to crew and ship safety and environmental impacts from the release of active residuals into receiving waters.

Several different biocides are currently being considered for ballast water treatment, including ozone, sodium hypochlorite, peracetic acid, SeaKleen®, and glutaraldehyde. The primary focus of the studies reported here was on the biocide glutaraldehyde, although certain data were also collected for hypochlorite (the active form of chlorine) and SeaKleen® (a vitamin K derivative). The general methodology of these studies and highlights from the

results are presented in this overview, which concludes with a discussion of the results in the context of utility for ballast water treatment.

Materials and Methods

To evaluate the potential efficacy of biocides in a ballast tank application, several types of laboratory experiments were conducted to evaluate organism mortality rates at different biocide concentrations. A general description of the experimental approaches is provided and additional details on methodology can be found in the appropriate citations (Sano et al. 2003, 2004, 2005).

Chemical compounds

Four different compounds were used to evaluate biocide efficacy: Glutaraldehyde, Disinfekt 1000® (glutaraldehyde with a surfactant adjuvant), sodium hypochlorite, and SeaKleen® (menadione and menadione metabisulfite 2:8).

Glutaraldehyde (1,5-pentanedial, CAS Registry No. 111-30-8) is a five-carbon dialdehyde that is widely used for disinfection in biomedical applications. In aqueous solution, glutaraldehyde exists as a monomer, but polymerizes readily under more alkaline conditions. The biocidal properties of glutaraldehyde are attributed primarily to the reaction of its aldehyde moiety with the amino groups in proteins to form a Schiff base (Peters and Richards 1977). These nucleophilic reactions (with the nitrogen group as the nucleophile) are enhanced by the low steric hindrance of glutaraldehyde: because glutaraldehyde is aliphatic, it can interact with both primary and secondary amine groups, thereby enhancing its biocidal properties (Simons et al. 2000). Disinfekt 1000® (Diversified Nutri-Agri Technologies, Inc., Gainesville, GA) contains glutaraldehyde as the active ingredient (20%) in combination with a proprietary surfactant adjuvant (79%), which enhances biocidal activity.

Toxicity of hypochlorite is generally attributed to the formation of hypochlorous acid. In aqueous solution, hypochlorite reacts with water to form hypochlorous acid (HOCl), which can then dissociate to produce hydrochloric acid (HCl) and nascent oxygen ($[O]$). Hypochlorous acid is known to be able to penetrate through cell walls and to react rapidly with the sulfhydryl groups of cysteine thereby destroying cellular proteins (Pereira et al. 1973). In addition, hypochlorite reacts readily with nitrogen-containing compounds (primarily ammonia) to form various types of chloramines. These latter compounds are also highly toxic and readily interfere with certain enzymatic reactions in addition to altering binding of oxygen with hemoglobin (which makes them particularly toxic to fish: Farrell et al. 2001).

SeaKleen® (Vitamir Inc., Memphis, TN) is a commercially developed product that consists predominately of menadione (2-methyl-1,4-naphthoquinone, vitamin K₃), although some formulations contain a certain percentage of sodium bisulfite (Cutler et al. 2003). Menadione is a member of the quinone family and differs from vitamins K,

and K_2 in that it must be synthetically manufactured. Quinones are the oxidation products of phenols and toxicity is due primarily to metabolic by-products. Metabolism of menadione generates formation of reactive oxygen species such as hydrogen peroxide (H_2O_2) and superoxide ion (O_2^-) and can generate reactive hydroxyl radical ($OH\cdot$). Oxidative damage associated with this process includes macromolecular damage, disruption of calcium homeostasis, depletion of cellular thiol levels, and increases in lipid peroxidation and cell death (Malorni et al. 1993; Tzeng et al. 1995; Chiou and Tzeng 2000).

Acute toxicity bioassays

Water-only exposures

For most acute water-only toxicity experiments, four different freshwater macroinvertebrates were tested: the amphipod *Hyalella azteca*, the oligochaete *Lumbriculus variegatus*, the cladoceran *Daphnia magna*, and the zebra mussel *Dreissena polymorpha*. For SeaKleen®, however, only *L. variegatus* and *H. azteca* were tested. In addition to these species, experiments using *Artemia* spp. cysts (a representative resting stage) were also conducted using NaOCl and Disinfekt 1000®.

The efficacy of the four biocides under water-only exposures was assessed using 24-h static bioassays (except for *D. polymorpha*, which extended to 48 h). Experiments were conducted in accordance with ASTM (1998a) and USEPA (1993) protocols. *H. azteca*, *D. polymorpha*, and *D. magna* were tested in filtered river water, while *L. variegatus* was tested in filtered well water. In addition, *L. variegatus* was acclimated to 10°C for tests at a lower exposure temperature. All other water-only experiments were conducted in the dark at $\pm 1^\circ\text{C}$ of the culture temperature. After the 24-hr exposure period, organism status was determined in terms of alive/dead or mobile/immobile.

The protocol for *D. polymorpha*, however, differed slightly: Prior to exposure, mussels were placed in glass Petri dishes and allowed to attach to the substrate. Organism status (alive/dead) was assessed both at 24 and again at 48 hrs. Status was determined based on response of the mussels to touch of a blunt probe. If mussels were closed, the probe was gently inserted in the gape and if no adductor muscle activity was noted, the organism was considered dead (Fisher et al. 1999).

Water samples for analyzing biocide concentrations were collected at both the start and the end of the experiment. Glutaraldehyde concentrations were determined with a spectrophotometric assay using 3-methyl-2-benzothiazolinone hydrazone hydrochloride (Sawicki et al. 1962; Pakulski and Benner 1992). Chlorine content was measured using the VVR Water Analysis System from CHEMetrics, Inc. (Calverton, VA, USA) as free chlorine. No analytical method was available for SeaKleen® so reported concentrations are nominal.

Additional bioassays using the cysts of the brine shrimp, *Artemia*, were also performed, based on the methods outlined in USEPA (1993). The base solution for the

exposures was synthetic seawater (i.e., Instant Ocean®) and the exposure period lasted for 24 h, during which the cysts were maintained in the dark at 27°C. At the end of the exposure phase, cysts were transferred to replacement vessels containing untreated synthetic seawater and were maintained for an additional 48 h grow-out period. During this grow-out segment, cysts were kept at 27°C with a 16 h:8 h light:dark photoperiod and organisms were observed daily to evaluate hatching success.

Sediment-water exposures

Sediment-water bioassays were conducted in a manner similar to water-only exposures, however only *L. variegatus* and *H. azteca* were tested. Different sediment:water ratios were used to evaluate sediment effects on efficacy. Experiments were initiated by adding test organisms to Erlenmeyer flasks containing a sediment-water matrix. Flasks were then placed on an orbital shaker, to simulate mixing that might occur in ballast tanks, and maintained in the dark for 24 hours. At the end of the experiment, the contents of the flasks were sieved to retain organisms and organism status was assessed.

Three different sediment types were used for sediment-water exposures: Terwilliger's Pond (TP) sediment (South Bass Island, Lake Erie); Lake Michigan (LM) sediment (off of Muskegon, MI); and Gallup Park (GP) sediment (from Geddes Pond, Ann Arbor, MI). The organic carbon content of the sediment was determined by CHN analysis after removing carbonates: Terwilliger's Pond sediment ($6.5 \pm 0.3\%$ OC on a dry weight basis), Gallup Park sediment ($2.6 \pm 0.4\%$), and Lake Michigan sediment ($0.49 \pm 0.05\%$). Other physical characteristics (such as particle size distribution) were not assessed.

Ballast tank simulation experiments

Potential biocide efficacy was further evaluated using sediment and water residuals from actual NOBOB tanks. These toxicity tests were performed using glutaraldehyde, Disinfekt 1000®, and hypochlorite. The protocol for the simulation experiments differed from that for the other sediment-water experiments as these bioassays assessed the efficacy at a single concentration and under more realistic exposure conditions. Ballast tank sediments were collected from ships of convenience using sterile scoops and spatulas and generally taken from the longitudinal shell frames of the tanks that tend to trap sediments (Bailey et al. 2003). The simulation experiments were initiated with a 1:2 ratio of ballast sediments and prefiltered riverine water in 20-L carboys. The experiments started by adding 30 individuals of *L. variegatus* and *H. azteca* each to the carboys. The test vessels were then placed on an orbital shaker to simulate ship movement. Exposures were conducted for 24 h in the dark at 21°C. After the 24-h treatment period, the toxicant solutions were drained from the carboys, and the remaining sediment mixture was diluted with approximately 20 L of prefiltered riverine water to simulate re-ballasting. A 10

day grow-out period was then initiated at 21°C, with a 16 h:8 h light regimen and with aeration, to prevent oxygen depletion. Organism mortality was assessed at the end of the grow-out period by sieving the carboy contents and determining organism status.

Chronic toxicity bioassays

The potential for chronic toxicity associated with biocide treatment was also evaluated for glutaraldehyde. Three different bioassays were used to evaluate effect-level concentrations for chronic exposures: 96-hour algal growth bioassays using the unicellular Chlorophyceae, *Pseudokirchneriella subcapitata*; 8-day reproduction and growth experiments using the cladoceran, *Ceriodaphnia dubia*; and an embryo-larval survival and growth experiment using wild steelhead trout (*Oncorhynchus mykiss*).

To evaluate potential effects on algal populations, 96-hour growth experiments using *P. subcapitata* (formerly *Selenastrum capricornutum*; Hindak 1990) were conducted according to ASTM (1998b) and USEPA (1994) standards. Flasks were inoculated with an initial population of algal cells and then placed on an orbital shaker held at 25°C with a 16 h:8 h light:dark cycle. Samples for estimating algal density were collected at 0, 72, and 96 hours after test initiation. Cell density was estimated both by spectrophotometric analysis (Geis et al. 2000) and confirmed with manual cell counts using a hemacytometer (Bright-Line counting chamber, Hausser Scientific, Horsham, PA, USA).

Reproduction and growth effects of glutaraldehyde were evaluated for *C. dubia* using a static renewal 3-brood reproduction bioassay (ASTM 1998b). Test solutions were renewed daily and glutaraldehyde concentrations were measured before and after solution renewal. Test organisms were fed daily and observations were made daily on the status of the female daphnids and on the number of neonates. The toxicity test was terminated once at least 60% of the control organisms had produced three broods. At the end of the experiment, surviving females were gently blotted, dried for 48 hours and weighed.

To evaluate the potential sensitivity of early life stages of fish to glutaraldehyde, a static renewal embryo-larval bioassay was developed from Canaria et al. (1999) and ASTM (1998c). Experiments were initiated with newly fertilized embryos and were conducted at 11°C (\pm 2°C). Glutaraldehyde solutions and control water were changed approximately daily and concentrations of glutaraldehyde were measured both prior to and after renewing test solutions. Daily observations were made on embryo condition (alive/dead/deformed) and on hatching time. Embryos were maintained in the dark until one week after 50% of the control group hatched from the chorion (i.e., became alevins or sac-fry). After the majority of embryos hatched, they were transferred to aquaria and maintained under low-intensity light with a 16 h light:8 h dark photoperiod. At the end of the exposure period, fry length and weight were both measured. The total exposure period for the experiment was 62 days.

Data Analysis

Acute data were analyzed by estimating the 24 h (or 48 h) LC_{50} (50% lethal concentration) and LC_{90} (90% lethal concentration) values and associated 95% confidence intervals with logit analysis using SYSTAT Version 8.0 (SPSS, Chicago, IL, USA, 1998). For the *Artemia* bioassays, Abbott's Correction (Abbott 1925) was used to normalize the toxicant exposure data to control values prior to logit analysis. For the chronic data, threshold concentrations including the NOEC (no observed effect concentration) and the LOEC (lowest observed effect concentration) were estimated using traditional hypothesis testing techniques. Point estimates for the chronic toxicity data were estimated using either probit analysis or the Spearman-Kärber method (Finney, 1978). A linear interpolation method was used to estimate inhibition concentrations (IC) for algal growth, reproduction rates, and hatch-out rates, using ICPIN software (Version 2.0 (USEPA, Duluth, MN, USA)). One-way ANOVA was used to assess differences in dry weights for the *C. dubia* and embryo-larval experiments (using SYSTAT Version 10 (SPSS, Inc., Chicago, IL, USA)).

Results and Discussion

Water-only acute exposures

Organism sensitivity to the biocides varied across compounds (Figure 2). In general, SeaKleen® and hypochlorite proved effective at low concentrations against most organisms. The limited toxicity data set for SeaKleen® suggest efficacy at comparable concentrations as hypochlorite for the tested amphipod and oligochaete species. In terms of hypochlorite, most organisms were quite sensitive to low concentrations in water-only exposures, with predicted 90% lethal concentrations (LC_{90}) generally less than 5 mg L⁻¹. The two notable exceptions, however, are the zebra mussel and *Artemia* cysts. Substantially higher hypochlorite concentrations were required to elicit comparable toxicity in these two species. Zebra mussels, in particular, are known to be quite resistant to compounds such as hypochlorite, as the mussels can clamp their shells and effectively avoid lethal exposures.

Glutaraldehyde and Disinfekt 1000® proved less effective against the tested organisms in water-only exposures with two exceptions: *Artemia* cysts and zebra mussels. In these cases, Disinfekt-1000® was actually more effective than sodium hypochlorite. Glutaraldehyde (and to a lesser extent Disinfekt-1000®) also demonstrated strong interspecific variations in efficacy. The majority of the tested organisms had 24-h LC_{90} values of less than 100 mg L⁻¹. Surprisingly, however, the amphipod *H. azteca* was relatively insensitive to glutaraldehyde with a 24-h LC_{90} of 550 mg L⁻¹.

Results from the 24-h bioassays using glutaraldehyde also indicate the potential for variations in sensitivity of different life stages. Both *C. dubia* neonates and adults demonstrated similar sensitivity to glutaraldehyde as reflected in similar acute lethal concentrations. In con-

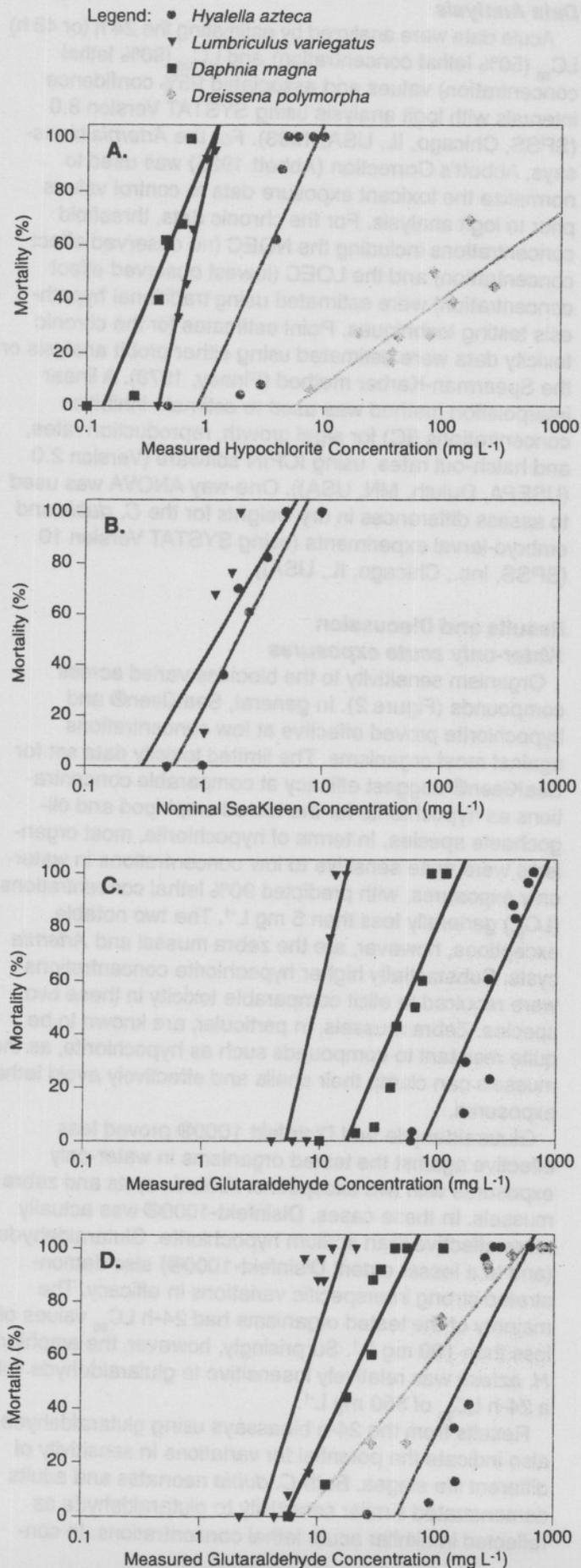


Figure 2 (left). Concentration-response curves of the four chemical biocides tested under water-only exposure conditions. Only a representative group of the tested species is illustrated and provides for comparison across compounds. A) Sodium hypochlorite; B) SeaKleen®; C) Glutaraldehyde; D) Disinfekt-1000®. Note that the concentrations of Disinfekt-1000® are reported in terms of glutaraldehyde, since this is the active ingredient that was measured.

trast, neonates of *D. magna* were substantially more sensitive than adults to glutaraldehyde. Neonates had a 24 h LC₅₀ (50% lethal concentration) value of 14 mg L⁻¹, while adults were 4 times less sensitive than neonates, with a 24 h LC₅₀ value of 56 mg L⁻¹.

Experiments conducted at 10°C indicate that decreases in ambient water temperature may act to decrease effectiveness, at least for oligochaete populations. For both hypochlorite and Disinfekt 1000®, higher concentrations were required to achieve the same mortality rates when water temperatures were lowered, indicated the compounds were less toxic at this lower temperature. For hypochlorite, the decrease in temperature caused the LC₅₀ value to increase by a factor of 1.4 and the LC₉₀ value to increase by a factor of 1.6. For Disinfekt 1000®, the LC₅₀ value increased by a factor of 2.5 at the lower temperature and the LC₉₀ value increased by a factor of 2.4.

Sediment-water exposures

The addition of sediment to exposure chambers dramatically altered efficacy. For hypochlorite, a substantial decrease in efficacy occurred for both *H. azteca* and *L. variegatus* (Table 1). For the amphipod, *H. azteca*, the LC₅₀ value was 43 mg L⁻¹ for a 1:8 sediment-water ratio and increased to 67 mg L⁻¹ for a 1:4 sediment-water ratio. For the oligochaete, *L. variegatus*, the 24 h LC₅₀ in a 1:8 sediment-water exposure using sediments from Gallup Park was 42 mg L⁻¹, compared to 0.70 mg L⁻¹ for water-only exposures. These estimates changed dramatically when the sediment-water ratio was elevated to 1:4, with the LC₉₀ value increasing to 2904 mg L⁻¹.

For glutaraldehyde, the effect of added sediment was slightly different than that observed for hypochlorite. For *H. azteca*, the lethal concentration values in sediment-water exposures did not significantly differ and were comparable to those derived from the water-only exposures. For *L. variegatus*, however, the 24 h LC₉₀ values were substantially higher for all sediment-water ratios tested e.g., 1:4 ratio (Table 1). Further, the actual sediment-water ratio was related to biocide efficacy: an increasing ratio of sediment-to-water (less water, more sediment) resulted in a substantial increase in the 24 h LC values (i.e., decreased efficacy).

The source and associated organic carbon content of sediments also affected toxicity of the biocides to *L. variegatus*. For hypochlorite, the sediment with the highest organic carbon content (e.g., from Terwilliger's Pond) resulted in a dramatic decrease in biocide efficacy. The LC₅₀ values for *L. variegatus* bioassays in Gallup Park and Lake Michigan sediments were similar (110 mg L⁻¹ and 75 mg L⁻¹, respectively). However, the LC₅₀ value for *L. variegatus* using Terwilliger's Pond sediments was

Organism	Glut ¹	Glut + Sed ²	Disinfekt	Disinfekt+Sed	Chlorine	Chlorine+Sed	SeaKleen	SeaKleen+Sed
<i>Hyalella azteca</i>	550	563	272	496	4.7	106	2.5	3.5
<i>Lubriculus variegatus</i>	13	134	7.6	248	0.7	2904	1.8	88
<i>Daphnia magna</i>	102	NT ³	33	NT	0.7	NT	NT	NT
<i>Dreissena polymorpha</i>	NT	NT	50	NT	130	NT	NT	NT
<i>Artemia</i> cysts	NT	NT	353	NT	53	NT	NT	NT

Key: ¹ glutaraldehyde; ² sediment; ³ Not Tested

Table 1. Acute toxicity of potential biocides. Values are expressed as the 90% lethal concentration (i.e., LC₉₀) in mg L⁻¹. The length of exposures was generally 24 hours, except for *Dreissena polymorpha* (which had a 48 hr exposure) and *Artemia* cysts (which had a 24 hr exposure but with cumulative mortality reported at 72 hrs).

substantially higher at 1014 mg L⁻¹. These results suggest that hypochlorite reacted with the organic material in the sediments. In addition, in the sediment-water exposures, 100% mortality was not obtained at the tested concentrations using hypochlorite. These results suggest that although hypochlorite may be effective in water-only exposures, efficacy will likely be dramatically reduced in the presence of sediments and also in waters with a high natural dissolved organic carbon content.

Similar to hypochlorite, both sediment source and sediment-water ratio affected glutaraldehyde and Disinfekt 1000® toxicity; however this effect was largely limited to *L. variegatus*. For *H. azteca*, lethal concentration (LC) values using Gallup Park sediment at a 1:8 sediment-water ratio were similar to those from water-only exposures. In contrast, the LC values at a 1:4 and 1:2 sediment-water were higher than the value at a 1:8 ratio, indicating minimal effect of sediments on toxicity to *H. azteca*. For *L. variegatus*, the impact of sediment was more profound, but not as dramatic as seen with hypochlorite. For example, the LC₅₀ value at a 1:8 sediment-water ratio was 39 mg L⁻¹, while the value at a 1:2 sediment-water ratio was 236 mg L⁻¹. This added amount of sediment resulted in a 6-fold increase in the LC₅₀ values.

The organic carbon content of the sediments additionally impacted Disinfekt 1000® efficacy against *L. variegatus*. The sediment with the lowest organic content (Lake Michigan sediment) was associated with the lowest LC₅₀ and LC₉₀ values (59 mg L⁻¹ and 75 mg L⁻¹, respectively). The LC values for the sediment with the highest organic carbon content (Terwilliger's Pond sediments) were substantially larger: The LC₅₀ value was 157 mg L⁻¹, and the LC₉₀ value was 443 mg L⁻¹.

Ballast tank simulation experiments

The results from the ballast tank simulation experiments were not entirely consistent with those from the sediment-water bioassays. For hypochlorite, mortality rates were higher than predicted based on the sediment-water exposures. The lowest concentration used in these experiments, 1000 mg L⁻¹, was lower than the predicted 24-h LC₉₀ for this compound, and yet elicited 100% mortality in both test *H. azteca* and *L. variegatus* (Table 2). It is unknown from these results whether a lower concentration of hypochlorite would be as effective as the 1000 mg L⁻¹ concentration employed in this experiment. This discrepancy in the results is likely due to slight

differences in experimental design: Although the ballast tank simulation experiments were conducted at a 1:4 sediment-water ratio, the sediment layer in these exposures was substantially less than that used in the sediment-water bioassay.

The results for glutaraldehyde were relatively consistent with the predictions from the sediment-water exposures: The average survival rate of *L. variegatus* at the tested concentration of 500 mg L⁻¹ was 5.6% (SD 5%), while the average survival rates for *H. azteca* were zero in all of the exposures. For Disinfekt 1000®, 100% mortality was observed for both *H. azteca* and *L. variegatus* at the tested

Experiment	Nominal Conc. (mg L ⁻¹)	Mortality (%)	
		<i>H. azteca</i>	<i>L. variegatus</i>
Hypochlorite 1	0 A	33.3	0
	0 B	20	0
	2000 A	100	100
	2000 B	100	100
Hypochlorite 2	0 A	20	0
	0 B	10	0
	1000 A	100	100
	1000 B	100	100
Glutaraldehyde 1	0 A	13.3	0
	0 B	13.3	0
	500 A	100	100
	500 B	100	100
Glutaraldehyde 2	0 A	33.3	0
	0 B	20	0
	500 A	100	93
	500 B	100	90
Disinfekt 1000® 1	0 A	23.3	0
	0 B	30	0
	250 A	100	100
	250 B	100	100
Disinfekt 1000® 2	0 A	3.3	0
	0 B	13.3	0
	250 A	100	100
	250 B	100	100

Table 2. Summary of ballast tank simulation experiments employing sodium hypochlorite, Disinfekt-1000® and glutaraldehyde. Each concentration had two replicates, denoted by the A, B label. The experiments were performed twice, using different sediment sources.

Table 3. Estimated effect concentrations for the three test organisms exposed to glutaraldehyde. Endpoints represent exposures of 96 h for *Pseudokirchneriella subcapitata* and 8 days for *Ceriodaphnia dubia*. Embryo-larval exposures for *Oncorhynchus mykiss* lasted a total of 62 days, with the embryo period lasting for 35 days. All values are presented as mg glutaraldehyde L⁻¹. NC indicates that endpoints were not calculable based on these data (e.g., there was no statistically significant response for that endpoint at the concentrations tested).

Species	Endpoint	IC ₂₅ , LC ₂₅ (95% C.I.)	IC ₅₀ , LC ₅₀ (95% C.I.)	NOEC	LOEC
<i>P. subcapitata</i> (exp. #1)	cell growth	0.6 (0.12-1.10)	1.0 (0.44-1.29)	0.7	1.4
<i>P. subcapitata</i> (exp. #2)	cell growth	1.3 (1.05-1.54)	1.8 (1.59-1.90)	1.3	2.1
<i>C. dubia</i>	survival	N.C.	4.7 (3.92-5.17)	2.4	4.9
	reproduction	3.5 (2.91-4.97)	4.7 (3.85-5.95)	2.4	4.9
	growth	N.C.	N.C.	4.9	N.C.
<i>O. mykiss</i>	survival (embryos)	N.C.	N.C.	13.6	N.C.
	hatch-out rate	1.5 (1.32-1.55)	1.82 (1.73-1.89)	1.3	2.5
	survival (larvae)	N.C.	N.C.	1	N.C.
	growth (larvae)	N.C.	N.C.	1	N.C.

dose of 250 mg L⁻¹. Similar to hypochlorite, this efficacy for Disinfekt-1000® is higher than what was predicted for the sediment-water exposures, indicating that experimental design may have augmented exposures and thus efficacy in the ballast tank simulation experiments.

Chronic toxicity tests

Chronic toxicity bioassays are reported for glutaraldehyde only. Results from experiments using the alga, *P. subcapitata*, indicate relatively high sensitivity to glutaraldehyde based on the no-observable and lowest-observable effect concentrations. The NOEC (no-observable-effect-concentrations) for the two experiments were 0.7 mg L⁻¹ and 1.3 mg L⁻¹ for the first and second experiments, respectively. The estimated inhibition concentrations were close in values (Table 3), indicating a steep concentration-response curve for glutaraldehyde. At all effect-level concentrations, the cells of *P. subcapitata* appeared enlarged and bloated compared to controls.

Adult survival of *C. dubia* was adversely affected by glutaraldehyde exposure at the concentrations tested. The estimated LC₅₀ for adults was 4.7. The NOEC under these exposure conditions was 2.4 mg L⁻¹, and the LOEC was 4.9 mg L⁻¹. In terms of reproduction effects, the estimated inhibition concentrations were close together, with an IC₂₅ of 3.5 mg L⁻¹, and an IC₅₀ of 4.7 mg L⁻¹. There were no significant differences between treatments up to 4.9 mg L⁻¹ and the controls for several reproduction parameters including days to first brood, average number of young per brood, and average total number of young per adult.

Survival rates of *O. mykiss* embryos, up until the controls began to hatch on day 25, were comparable for all concentrations tested including the controls. Although most of the embryos survived the initial embryonic period, the majority of organisms at the 2.5 mg L⁻¹ treatment level and higher were not able to hatch from the embryo stage into the sac-fry stage. Even at concentrations as low as 1.3 mg L⁻¹ embryos had difficulty emerging from the chorion see Figure 3. This effect manifested itself primarily as an extended hatching period, during which sac-fry remained in a "partially hatched" condition. The average

time for all viable embryos to complete hatching ranged from 3.25 days (1 standard deviation: 1.26) for the controls to 15.5 days (1 standard deviation: 3.87) for the 1.3 mg L⁻¹ treatment group. The overall hatching rate for this group was also lower than that for the controls.

After the 10-day post-hatch period (up to day 35), only 3% of the surviving embryos treated at 2.5 mg L⁻¹ had successfully emerged from the chorion and none of the embryos at the higher concentrations had survived. In many cases, the embryos treated at 2.5 mg L⁻¹ and higher clouded up and disintegrated over time (particularly towards the end of the hatching period, day 35). In other cases, the embryos appeared viable until the chorion first started to break open, after which the embryos quickly clouded up, with no viable sac-fry emerging.

The survival rates of larval fish were estimated separately due to the large effect of glutaraldehyde exposure on hatching success and due to differences in measured glutaraldehyde concentrations over the experimental period. Larval fish were followed for 27 days after hatching, through the alevin and the fry stages. At the end of this period, there was no significant difference between survival in the controls and the two remaining treatments (0.4 mg L⁻¹ and 1.0 mg L⁻¹).

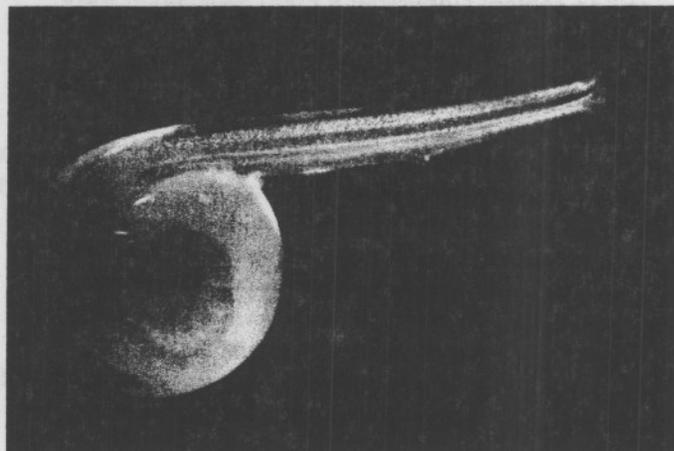


Figure 3. Picture of an "imprisoned" larva of *O. mykiss*. This larva was exposed to a concentration of 2.5 mg L⁻¹ glutaraldehyde and the image was taken 10 days after 60% of the control organisms successfully hatched from the chorion.

Conclusions

The results from these laboratory studies indicate that biocides can likely be used effectively to eliminate numerous aquatic organisms from ballast water. Of the biocides tested, hypochlorite and SeaKleen® appear to be most effective under water-only conditions. Hypochlorite was, however, much less effective in these experiments against a representative resting stage (*Artemia* cysts) and adult zebra mussels. Hypochlorite efficacy was also dramatically reduced in the presence of sediments. Under these conditions, hypochlorite concentrations exceeding 50 to 100 mg L⁻¹ would be necessary to eliminate benthic invertebrates such as amphipods, but would likely still be ineffective against truly benthic organisms and resting stages that burrow and hide in the sediments. Ballast water treatment requiring these concentrations of hypochlorite, however, is not feasible due to corrosion and environmental risks. For example, treatment of ballast water to 10 mg L⁻¹ hypochlorite has been found to acidify water, reducing pH values to 5 (compared to control waters with a pH of 7: Vianna da Silva and da Costa Fernandes 2003). This drop in pH poses an unacceptable corrosion risk to ballast tanks. In addition, treatment of ballast water with higher concentration of hypochlorite will produce unacceptable environmental risks due primarily to the formation of trihalomethanes, which are formed through the reaction of chlorine with organic matter. Trihalomethanes are of particular environmental concern since they are possible carcinogens that are persistent and accumulate in adipose tissue of organisms (Jenner et al. 1997). Both ship-based and laboratory-based experiments indicate that significant amounts of trihalomethanes may be formed due to chlorination of ballast water, particularly in waters taken from eutrophic regions with high organic loads (Vianna da Silva and da Costa Fernandes 2003). Because most vessels tend to reballast in port and harbor areas, the probability of taking on water with high organic load is high and the associated risk to the environment needs to be evaluated prior to large-scale application of hypochlorite for ballast water treatment.

In contrast to hypochlorite, the efficacy of SeaKleen® in laboratory bioassays did not appear as dramatically impacted in the presence of sediments. This biocide was not as extensively tested as the other compounds, however, since experiments were limited to *H. azteca* and *L. variegatus* and only one sediment-water ratio. Recent additional tests using Cladoceran ehippia have produced an LC₉₀ of 8.7 mg L⁻¹ (David Raikow, Unpublished Data Great Lakes Environmental Research Laboratory). This relatively low lethal concentration value suggests that even more resistant resting stages may be susceptible to SeaKleen®, indicating this biocide may be a viable option for ballast water treatment. A more rigorous evaluation of SeaKleen® application requires additional studies to evaluate degradation rates and potential environmental risks.

Finally, glutaraldehyde and Disinfekt-1000® also proved effective against tested organisms, but with some significant interspecific variations in sensitivity. The lethal con-

centrations for Disinfekt-1000® were lower than those for glutaraldehyde, likely due to the addition of a surfactant adjuvant which should enhance biocidal efficacy. Notably, Disinfekt-1000® was significantly more effective against the zebra mussel than hypochlorite. The efficacy of both compounds, however, was reduced in the presence of sediments, but only for the oligochaete, *L. variegatus*. These results generally indicate that for glutaraldehyde and Disinfekt-1000®, the sediment provide a protective refugia for truly benthic species such as *L. variegatus*. A major advantage to both biocides compared to hypochlorite is that they are less reactive with organic material and are likely to persist longer in a ballast tank environment than oxidizing compounds such as hypochlorite. In addition, glutaraldehyde poses little risk to structural materials found within ballast tanks.

One of the major issues in identifying candidate biocides for use in preventing invasions is the potential impact of biocide residuals in receiving waters when discharged from the ballast tank. The results from chronic toxicity bioassays using glutaraldehyde indicate that longer-term ambient concentrations of 1 mg L⁻¹ may pose significant risks to algal populations and fish embryos. However, given the significant amount of dilution and degradation associated with reballasting operations, the probability of maintaining these ambient environmental concentrations are low (Larissa Sano, data in press). In addition, the spatial and temporal scales of releases are small given the isolated nature of NOBOB deballasting and the relatively rapid biodegradation of glutaraldehyde (Landrum et al. 2003). These factors combined greatly reduce the potential ecological risks of glutaraldehyde, Disinfekt-1000® and comparable biocides.

The primary goal of this research was to evaluate the potential effectiveness of several different biocides for treating NOBOBs and to evaluate the potential environmental effects of the release of active residual of glutaraldehyde. Because all of the results are based on laboratory bioassays, the interpretation of *in situ* efficacy is limited. A more thorough assessment of the actual effectiveness of these different biocides requires careful evaluation using field studies. In addition, only two criteria associated with biocide treatment were evaluated (efficacy and environmental impacts). Several other equally important considerations must also be evaluated in order to assess the actual practicability and acceptability of biocide, including the potential risks to crew and ship safety and economic costs.

Concluding Remarks

The decision to utilize biocides to treat ballast tanks (whether ballasted or unballasted) remains a delicate and politically-sensitive issue. Due to the long and unfortunate history of environmental problems associated with chemical contaminants, it is necessary to proceed with caution in developing management practices based on chemical disinfection. However, like all other management approaches, biocide treatment of ballast water

deserves a rigorous assessment prior to final management decisions. This is particularly true due to the enormous ecological risks associated with continued release of nonindigenous species into new environments. These invasion events are characterized by high uncertainty and can result in considerable ecological and economic impacts to receiving environments. Like many other risk-based decisions, low probability events that have catastrophic results should be given serious consideration and high management priorities.

As long as NOBOBs remain unregulated, the Great Lakes remain vulnerable to future invasions caused by release of ballast water. Although it is regrettably difficult to predict which organism might become the next harmful invader, there is ample evidence both that viable organisms are being released into the Great Lakes from ballast water (Bailey et al. 2003; Johengen et al. 2005) and that there remain several "high" risk organisms that are likely to be successful invaders into the Great Lakes (Kolar and Lodge 2001; Grigorovich et al. 2003). These two factors alone should provide the impetus to rapidly implement ballast water management practices for NOBOBs that will help reduce the risk of future invasive species introductions.

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Investigation of the Need and Options for an Exotic Species Barrier on the Champlain Canal

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The first step in protecting a body of water from exotic species invasions is to determine the sources and vectors of current and potential invaders. Current work in Lake Champlain is focused on preventing further invasions by a combination of legislation, public education, and research on probable routes of future invasion. This research indicates that the Champlain Canal appears to present the highest risk for new invasions.

Lake Champlain is a 193 km long, narrow lake bounded by Vermont on the east, New York on the south and west, and Quebec on the north (Figure 1). The lake flows from

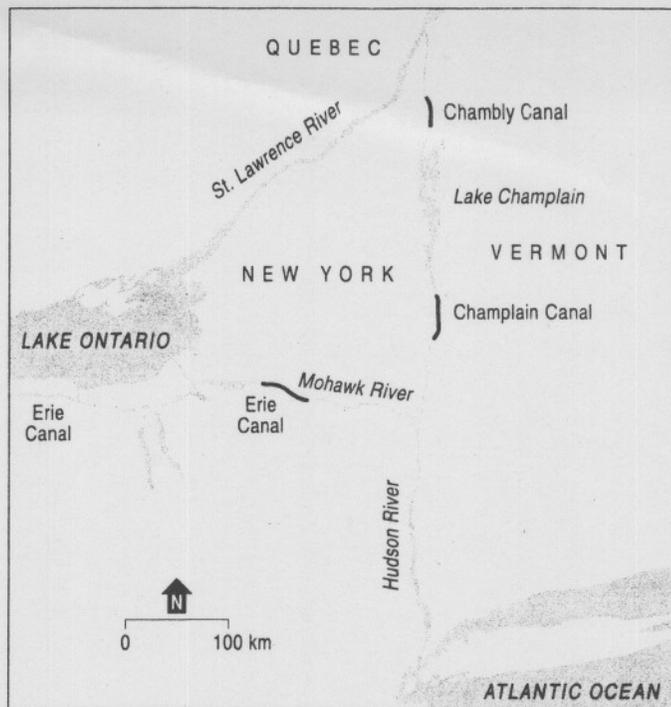


Figure 1. Lake Champlain and its connections to the Great Lakes, St. Lawrence, and Hudson River drainage.

Whitehall in the south to its outlet, the Richelieu River, at the north. Habitats in the lake vary from shallow, eutrophic bays to the oligotrophic main lake. Compared with the Great Lakes and adjacent waterways, Lake Champlain has received few invasions of exotic species; an estimated 47 species are established in the lake, compared with 160 in the Great Lakes, 87 in the St. Lawrence River, and 113 in the Hudson River (Mills et al. 1993, 1996, de Lafontaine and Costan 2002, Strayer 2005). Lake Champlain has been relatively protected from invasions by the absence of modern commercial traffic; no vessels containing ballast water from outside North America have access to the lake because of the restricted size of the waterways into the lake. Mills et al. (1996) estimated that shipping was responsible for 33% of the invasions into the Great Lakes, and 13% of invasions into the Hudson River; de Lafontaine and Costan (2002) estimated that shipping had introduced 40% of the exotic species in the St. Lawrence River and Great Lakes. Only one species, flowering rush (*Butomus umbellatus*), appears to have entered Lake Champlain in solid ballast.

Vectors of invasions into Lake Champlain include fish stocking (rainbow trout, brown trout, common carp), accidental release from culture (goldfish, tench, big-ear radix), bait bucket releases (rudd, rusty crayfish), deliberate but unauthorized releases (alewife, purple looste-ribe), and canals (zebra mussel, white perch, blueback herring, water chestnut and others; Figure 2). Most of these vectors have already been addressed to some extent. Stocking of non-indigenous fish species is now discouraged as a fisheries management tool. The adop-

continued on p. 14

