

Effect of a ballast water treatment system on survivorship of natural populations of marine plankton

T. F. Sutherland^{1,*}, C. D. Levings¹, C. C. Elliott¹, W. W. Hesse²

¹Fisheries and Oceans Canada, Science Branch, West Vancouver Laboratory, 4160 Marine Drive, West Vancouver, British Columbia V7V 1N6, Canada

²Velox Technology Inc., Ste 910, 602 12th Ave SW, Calgary, Alberta T2J 1R3, Canada

ABSTRACT: A field experiment was carried out to determine the influence of a 2-stage ballast water treatment system on the survivorship of natural populations of plankton. This Integrated Cyclone-UV Treatment System (ITS) was designed and constructed by Velox Technology Inc. and consisted of 2 treatment phases: (1) the cyclonic pre-treatment phase, (2) the ultraviolet-radiation phase (UV-C). The ITS was deployed on the Vancouver Port Authority dock, British Columbia on April 11, 1999. Sea-water samples were collected from ports located along the treatment stages of the ITS and analyzed for plankton survivorship. The sampling stages were defined as Pre-Intake, Pre-Cyclone, Post-Cyclone, Post-Solids, and Post-UV-on and Post-UV-off. The survivorship of planktonic invertebrates was assessed immediately through direct observations, while phytoplankton survivorship was assessed through incubation grow-out experiments. With respect to zooplankton, live copepods were observed in the Pre-Intake and Pre-Cyclone samples, while dead or moribund copepods were observed in samples collected from both early and late stages of the ITS. Statistical comparisons were carried out on phytoplankton growth parameters such as starting concentration, lag phase, growth rate, and relative abundance generated during the incubation experiment. *Chaetoceros gracile* appeared to be the most sensitive organism to the ITS as it exhibited a 4 d lag phase prior to growth. The starting concentration, growth rate, and relative abundance of this species observed in the Post UV-on samples were significantly lower than those observed in the Pre-Intake samples (control). In addition, the auxospores formed by *Skeletonema costatum* during the incubation experiment were observed in all treatment samples with the exception of those exposed to the Post-UV-on stage of the ITS. A second phytoplankton incubation experiment was carried out using the original samples following a 3 mo storage period in dark, cold conditions (4°C). The results of this experiment revealed that the phytoplankton population in the UV-treated samples was not capable of growth, while those in the remaining treatments exhibited growth. Thus, future studies assessing the effect of the ITS on phytoplankton survivorship should incorporate increases in the intensity and exposure period of ultraviolet radiation followed by a dark, cold-storage period, thereby reducing the chance of photorepair.

KEY WORDS: Ballast water · Treatment · Cyclonic pre-treatment · Ultraviolet radiation · Plankton · Exotic species

Resale or republication not permitted without written consent of the publisher

INTRODUCTION

The introduction of harmful phytoplankton and exotic invertebrate species can occur through transport vectors such as ship ballast water and sediment (Carl-

ton & Geller 1993). Dinoflagellates responsible for producing paralytic shellfish poisoning (PSP) have been introduced to Australia from Asia (Hallegraeff & Bolch 1991). Toxic phytoplankton pose a serious threat to human health and may potentially threaten natural and cultivated shellfish and fish resources important to local economies. In addition, the establishment of

*E-mail: sutherlandt@pac.dfo-mpo.gc.ca

introduced species could create shifts in natural populations within a defined ecosystem. Numerous introductions of non-native species have occurred within Canada, particularly in the Great Lakes and St. Lawrence region, leading to considerable environmental and socio-economic impacts (Mills et al. 1993).

Shipping activities along the Canadian coastline are intense, with the majority of incoming ships laden with ballast from foreign origins. Ballast water is taken on by vessels for stability, prior to a trans-oceanic journey. Since ballast water tonnage ranges between several hundred to 100 000 metric tonnes, the potential exists for the transfer of considerable amounts of viable organisms over time (Gauthier & Steel 1996). Ballast water treatment methods need to be developed to aid in the mediation of ballast water introductions of non-indigenous species into coastal waters worldwide. Although offshore ballast water exchange methods are presently employed by commercial ships entering North American ports, this practice has been assessed with an efficiency value of 65% (Locke et al. 1993) and can pose hazardous situations and onboard safety problems in the event of ship exposure to offshore storms.

The Integrated Cyclone-UV Treatment System (ITS) offers an alternative method to mid-ocean ballast exchange and minimizes the introduction of foreign species, while providing a 'flow-through', on-line processing of large flows of incoming seawater to ballast tanks. A containerized ITS (prototype system) was built by Velox Technology Inc. as a research prototype unit designed to process between one-half to one-sixth of the flow capacities of the proposed Velox ballast water product line. The ITS was primarily designed for research and demonstration purposes.

The objective of this study was to determine the effect of the ITS on the survivorship of natural plankton populations. The ITS serves 2 main functions: (1) to pre-treat and improve water clarity of incoming seawater and separate sediments and biota from a seawater source using cyclonic technology; (2) to sterilize the clear seawater fraction resulting from the cyclonic pre-treatment stage using ultraviolet radiation (UV-C) technology. This processed clear-water fraction of seawater provides a sterilized source of water to be used as ballast required by ships for stability during trans-oceanic journeys.

MATERIALS AND METHODS

Description of Integrated Cyclone-UV Treatment System (ITS). Fig. 1 shows a basic schematic diagram of the ITS outlining the 2 treatment stages: the pre-treatment module (cyclonic separation) and the ultraviolet (UV-C) sterilization chamber. The portable ship-

ping container which houses the ITS has approximate size dimensions of 2.4 m × 3 m × 12.2 m and weighs approximately 8.5 t. Flow into the ITS was provided by external electric submersible pumps deployed at an optimal water depth and linked to the container by temporary steel piping and flexible hoses. Upon entering the container system, seawater immediately passes through an inlet pipeline to 2 distribution headers connected to individual banks of separators, which form the pre-treatment module. The flowing water is then subjected to cyclonic action within the individual separators through the generation of centrifugal force. This force propels larger particles (clays, sediments, and larger biota) outwardly to form the resulting 'reject' stream (Post-Solids), leaving a 'clear-water' fraction within the central core of each separator apparatus (Post-Cyclone). Each separator contains 2 separate fluid outlets (1 reject stream, and 1 clear water stream) where the individual streams are captured and directed into individual piping headers located at the end of the separator module.

After the cyclonic pre-treatment stage, the remaining Post-Cyclone stream may potentially contain harmful bacteria, viruses, and microalgae. This Post-Cyclone stream is then routed to the ultraviolet radiation chamber for final sterilization. This chamber contains approximately 20 germicidal lamps (UV-C light at 253.7 nm) and delivers a total output of 2.5 kW. In a shipboard application, the sterilized Post-Cyclone fraction (90% of inlet flows) would be sent to onboard ballast tanks, while the reject stream fraction would be discharged overboard and returned to the harbour of origin. The ITS process is monitored and automatically controlled by a Programmable Logic Controller (PLC), whose instrumentation provide a real-time display of time-series data of pressure, inlet and outlet flows flowing through the system, along with other pertinent process data.

Methods. The ITS was deployed on the Vancouver Port Authority Dock located in Vancouver harbour, British Columbia, on April 11, 1999. Sampling ports were installed and located during ITS fabrication, at various points along the piping system to allow for the collection of seawater samples passing through specific process locations defined as Pre-Cyclone, Post-Cyclone, Post-Solids, and Post-UV (activated and deactivated) treatments (Fig. 1). The submersible pump intake was located approximately 20 m offshore at a water depth of approximately 3 m. Tests examining the effect of the ITS on natural populations of planktonic invertebrates were carried out on April 13, 1999, while a test examining the effect of the ITS on phytoplankton was carried out on April 14.

Invertebrate experiment: Seawater was flushed through the ITS for 30 min at a flow rate of approximately 312 to 350 m³ h⁻¹. Three replicate seawater sam-

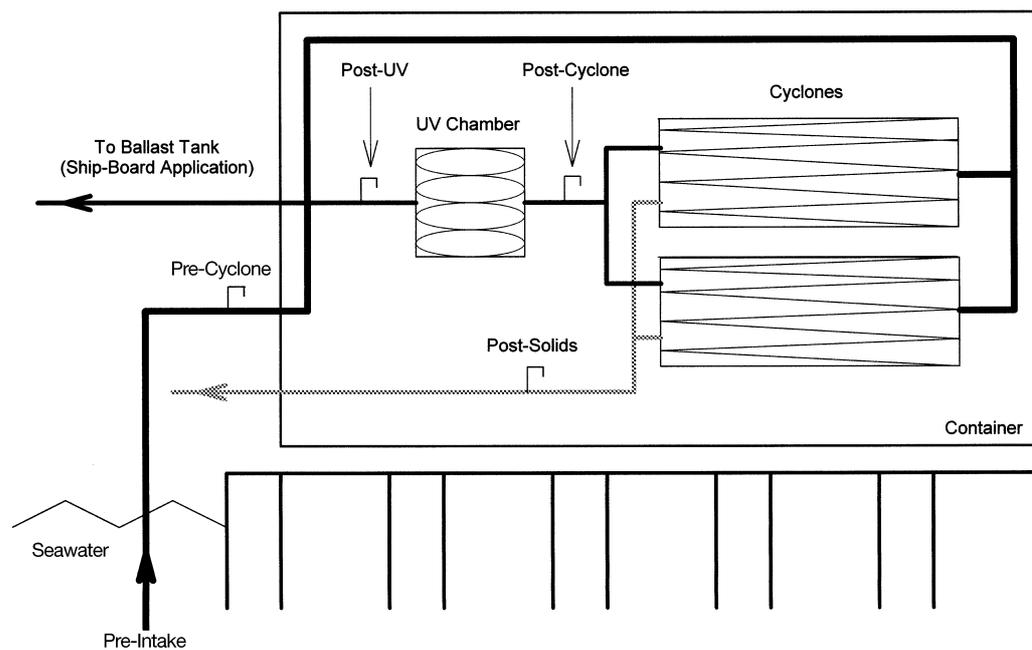


Fig. 1. Schematic diagram in cross-section of Integrated Cyclone-UV Treatment System (ITS) outlining sample collection ports

ples (500 ml each) were collected simultaneously from each of the ITS sample ports, Pre-Cyclone, Post-Cyclone, Post-Solids, and Post-UV-on, while the UV-C lamps were activated and illuminated. Replicate seawater samples ($n = 3$) were also collected from a location beside the pump intake using a weighted bucket and rope, and labelled 'Pre-Intake'. In order to test for the effect of ultraviolet light on phytoplankton survivorship, replicate samples were then collected from the Post-UV-off port after the UV-C lamps had been turned off, and seawater was allowed to flush through the system for 15 min. Thus, the seawater samples collected within the vicinity of the pump intake, Pre-Intake, served as a control for the ITS, while seawater samples collected during the Post-UV-off phase served as a control for the ultraviolet light treatment (Post-UV-on phase). A total of 18 samples were collected. A second series of replicate samples (18 bottles) was then collected from the ITS in a similar fashion as described above, although with the ultraviolet lamps initially turned off.

Seawater samples were transported in a cooler back to the West Vancouver Laboratory (Department of Fisheries and Oceans) and analyzed for the survivorship of zooplankton and invertebrate larvae. Each sample was concentrated through a $37 \mu\text{m}$ sieve, transferred to a scored petri plate ($9 \text{ cm} \times 9 \text{ cm}$) using filtered seawater, and viewed under a Leitz microscope using $250\times$ magnification. Zooplankton and invertebrate larvae were enumerated and their mortality determined. A poker was used to prod the plankton to assess their survivorship based on their activity response to physical stimulus.

Phytoplankton experiment: An incubation experiment was set up to determine the growth or mortality of various phytoplankton groups collected during the ITS experiment. The system was flushed with seawater for approximately 30 min at a flow rate between 312 and $350 \text{ m}^3 \text{ h}^{-1}$ with the UV-C lamps turned on. Replicate seawater samples ($n = 3$) were collected from (1) a location beside the pump intake, and (2) from each of the ITS sample ports in a fashion similar to that described in the previous subsection. In addition, replicate samples were collected from the post-ultraviolet port 15 min after the UV-C lamps had been turned off. Samples were then similarly transported to the laboratory in a cooler.

All glassware used in this experiment was acid-washed with 10% HCl, rinsed with distilled water 3 times, and autoclaved for 20 min at 20 psi. From each 500 ml sample, 50 ml of sample suspension were transferred to individual 250 ml erlenmeyer flasks. HESNW growth medium (Harrison et al. 1980) was added to each flask to make up a total volume of 200 ml. The resulting cultures were exposed to $100 \text{ quanta m}^{-2} \text{ s}^{-1}$ on a light:dark cycle of 12:12 h. Subsamples (5 ml) were collected every day during exponential growth and then every 2 d during the stationary phase of growth. Subsamples were preserved with Lugol's solution and stored in scintillation vials. An aliquot from each subsample was settled in a settling chamber and viewed under an inverted microscope. Phytoplankton groups were enumerated according to the Utermöhl method (Lund et al. 1958) and converted to cells l^{-1} .

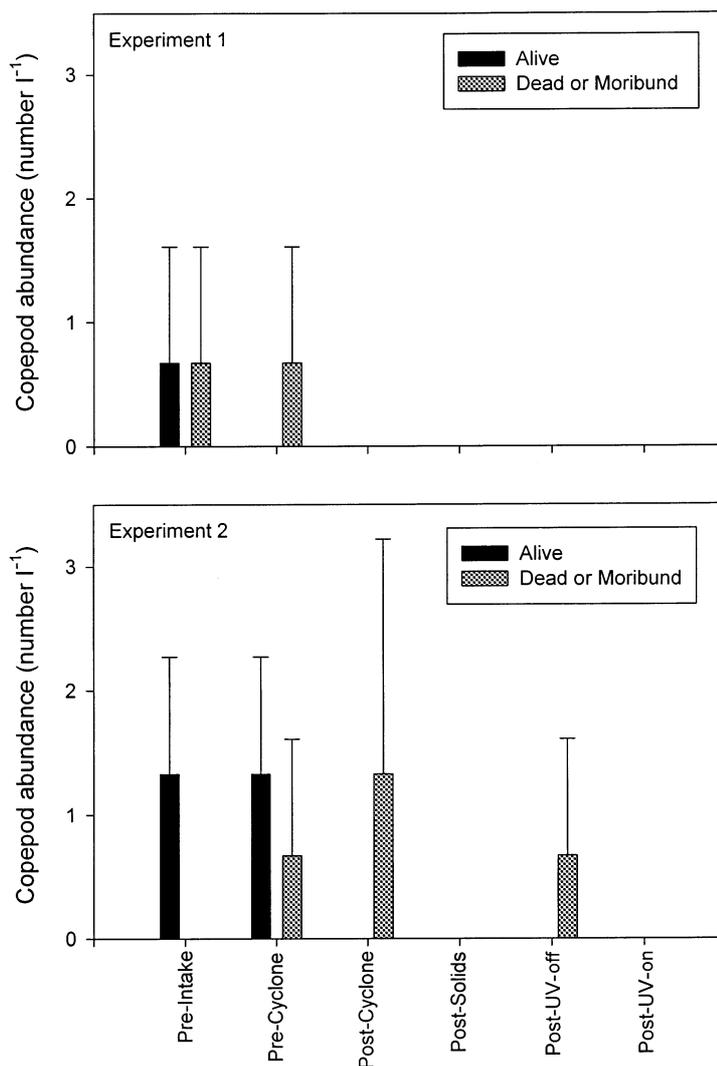


Fig. 2. Abundance of living and dead copepods observed in seawater samples collected from various stages located along the ITS. Error bars = ± 1 SD

Combined UV-darkness incubation experiment: A second incubation experiment was carried out to determine the combined effect of ITS treatment and dark storage on the survivorship of phytoplankton. Dark storage may minimize the potential for photorepair mechanisms to take place and mimic ballast water tank conditions. The original samples collected on April 14, 1999, were stored in dark and chilled conditions (4°C) for approximately 3 mo. The replicate samples were then incubated in conditions similar to those described above in the phytoplankton experiment (excluding the addition of nutrient growth medium). Subsamples were collected periodically (every 3 to 4 d) and analyzed for chlorophyll concentration.

Statistical analysis: Statistical tests carried out on the phytoplankton data sets included ANOVA and post-

hoc Tukey tests ($p = 0.05$). These tests are designed to determine (1) a statistical difference within a series of mean values, and (2) statistical pairwise comparisons of individual mean values making up each data series. Specifically, the statistical tests were used to compare mean values within data sets characterized by phytoplankton starting concentrations, growth rates, relative abundance, and auxospore concentrations (*Skeletonema costatum*).

RESULTS

Invertebrate survivorship

Live copepods were observed in the ambient seawater samples collected from a location beside the pump intake of the ITS during Expts 1 and 2 (Fig. 2). In addition, live copepods were observed at the Pre-Cyclone stage of Expt 2. Dead or moribund copepods were observed at the early treatment (pre-treatment) stages of the ITS of Expt 1, and at both the early and late treatment stages of the ITS during Expt 2. No live copepods were observed downstream of the Pre-Cyclone collection port in either experiment. The low abundance and patchy distribution of this zooplankton explains why copepods were not observed at every treatment stage. The collection of a larger volume of seawater for each replicate sample is necessary to reduce the relatively large variance associated with the low mean values observed in these 2 experiments.

Phytoplankton survivorship

We focused the results of the phytoplankton incubation experiment on the growth characteristics of 3 dominant phytoplankton taxa, *Skeletonema costatum*, *Thalassiosira* sp., and *Chaetoceros gracile*, observed in the seawater samples. Firstly, incubation measurements including the starting concentration, lag phase, growth rate, and relative abundance were examined to determine the effect of the ITS on the survivorship of these dominant phytoplankton groups. Secondly, the mean auxospore concentrations produced through sexual reproductive mechanisms by the phytoplankton, *S. costatum*, were compared between control and treatment subsamples collected during the incubation experiment. Finally, size parameters such as cell width, chain length, and chain volume, of the 3 dominant phytoplankton species were outlined.

Starting concentrations of phytoplankton

The starting concentration was defined as the initial abundance of phytoplankton observed at the onset of the incubation experiment following the ITS experiment. In general, the lowest concentrations of the 3 dominant phytoplankton species were observed in the Post-UV-on samples, while the highest concentrations were observed in the Post-Solids samples. Fig. 3 shows the starting concentrations observed on Day 1 of the incubation experiment for *Skeletonema costatum*, *Thalassiosira* sp., and *Chaetoceros gracile*, respectively. Replicate 1 of the Post-Solids samples contained large amounts of orange flocculant material, primarily rust flakes, while Replicates 2 and 3 did not. The lack of rust material in the second and third replicates suggests that an accumulation of solid material occurred during the initial system startup prior to the 15 min flushing period. In addition, phytoplankton growth did not take place in the rust-laden, replicate sample and, thus, was eliminated from the data analysis.

Statistical comparisons (ANOVA) were carried out on the mean starting concentrations of each phytoplankton species observed in the Pre-Intake, Post-UV-off, and post UV-on samples. The Pre-Intake samples provide a control for the Velox pre-treatment stage, while the Post-UV-off samples provide a control for the effect of ultraviolet light (Post-UV-on). The results of the pairwise comparison test (Tukey test) revealed that the mean starting concentration of *Skeletonema costatum* observed in the Pre-Intake and Post-UV-on samples were significantly different than that observed in the Post-UV-off samples ($p = 0.006$). In addition, a statistically significant difference ($p = 0.05$) was found between the mean starting concentrations of *Chaetoceros gracile* observed in the Post UV-off and Post-UV-on samples. The mean starting concentration of *S. costatum* and *C. gracile* observed in the Post-UV-on samples were lower than that observed in the Post-UV-off

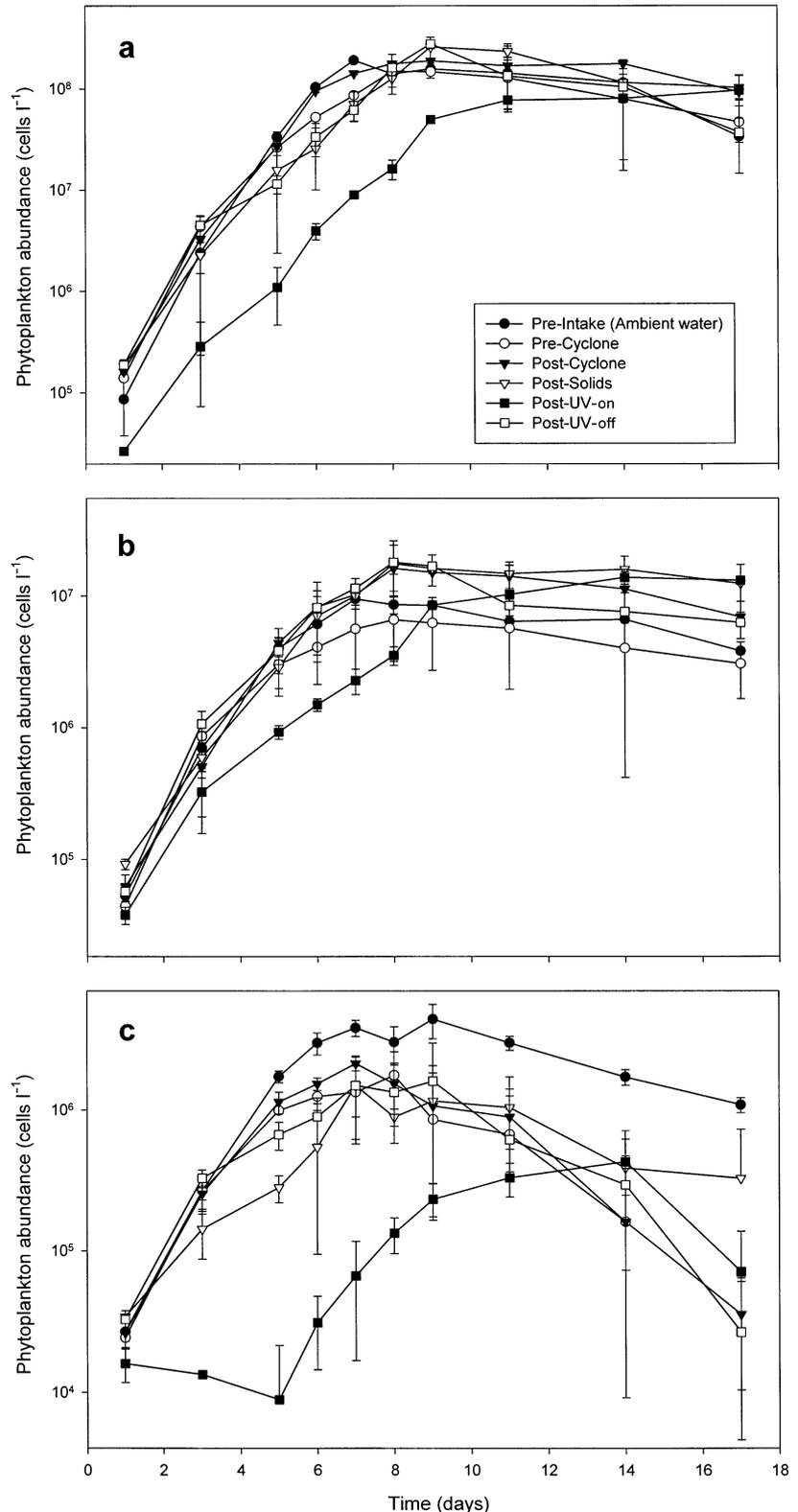


Fig. 3. (a) *Skeletonema costatum*, (b) *Thalassiosira* sp., and (c) *Chaetoceros gracile*. Growth curves generated during incubation experiments based on seawater samples collected from various treatment and control stages along ITS. Error bars = ± 1 SD

samples by a factor of 7 and 2, respectively (Table 1). The lower mean concentration observed for Pre-Intake samples relative to that observed for Post-UV-off samples is probably due to the patchy occurrence of phytoplankton in natural ecosystems. These statistical findings suggest that ultraviolet light exposure had a direct effect on the abundance of the latter 2 phytoplankton species. However, no statistically significant difference ($p = 0.05$) was found between the observed mean starting concentrations of control and treatment for *Thalassiosira* sp.

Lag phase, growth rate, and relative abundance of phytoplankton

Data on lag phase, growth rate, and relative abundance of phytoplankton generated during the incubation experiment are shown in Fig. 3a (*Skeletonema costatum*), Fig. 3b (*Thalassiosira* sp.), and Fig. 3c (*Chaetoceros gracile*). Lag phase is defined as the period of no growth followed by exponential increases in cell abundances (exponential growth). *C. gracile* exhibited a lag phase of approximately 4 d preceding growth in all replicate samples of the Post-UV-on treatment. Since a lag phase was not observed during the growth

Table 1. *Skeletonema costatum* and *Chaetoceros gracile*. Multiplication factor (MF) of starting concentrations to post-treatment concentrations. Data from statistically significant comparisons are listed

Phytoplankton group	Stage comparison	MF
<i>S. costatum</i>	Post-UV-off: Post-UV-on	7.1
<i>C. gracile</i>	Post-UV-off: Post-UV-on	2.1

cycles of this phytoplankton species collected from the remaining control and treatment sample ports, it is suggested that the ultraviolet light treatment influenced the continuance or onset of growth by *C. gracile*. *S. costatum* and *Thalassiosira* sp. did not exhibit lag phases preceding growth.

Growth rate was calculated from the exponential portion of the growth curve determined as the steepest overall linear slope beyond or beneath which the slope deviated significantly. Growth rate was defined as the differential natural-logarithm of cell concentration standardized by time. Conversely, relative abundance was defined as the difference between the maximum and minimum cell concentration of the exponential portion of the growth curve standardized by the maximum cell

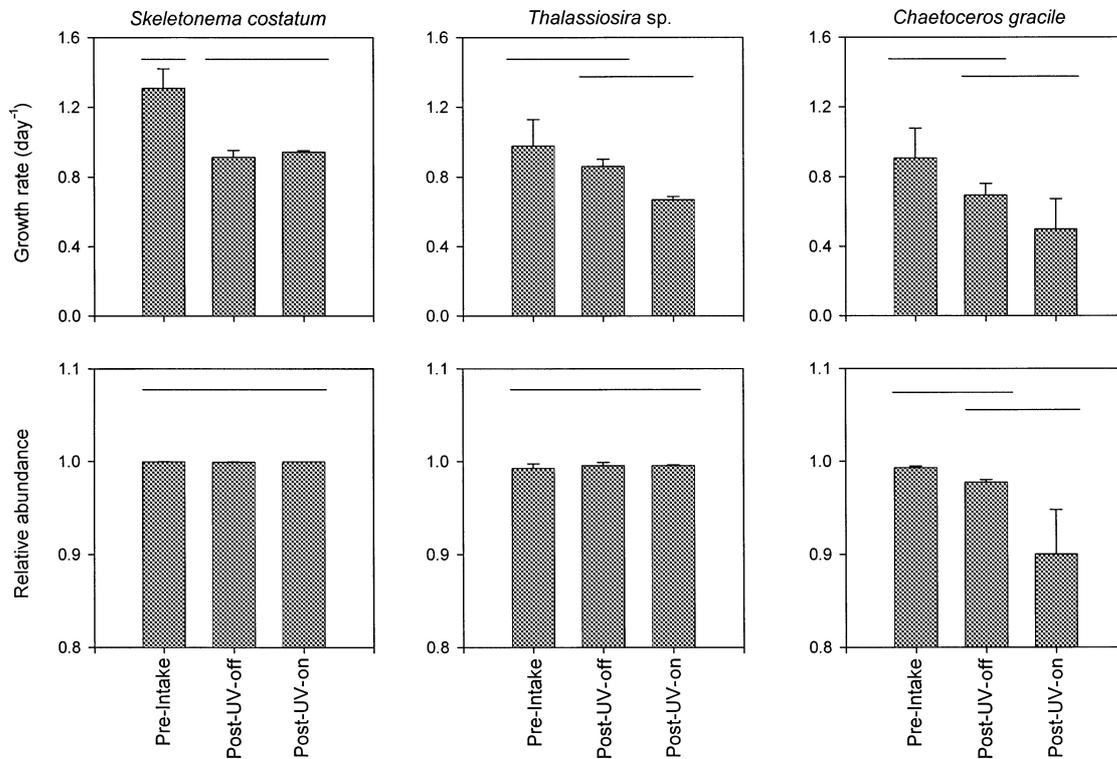


Fig. 4. Growth rate and relative abundance of 3 dominant groups of phytoplankton observed at 3 stages located along ITS. Lines above histograms connect treatment groups exhibiting no significant difference (ANOVA and Tukey tests). Error bars = ± 1 SD

Table 2. *Skeletonema costatum*, *Thalassiosira* sp. and *Chaetoceros gracile*. Multiplication factor (MF) of growth rates for control in post-treatment samples. Data from statistically significant comparisons are listed

Phytoplankton group	Stage comparison	MF
<i>S. costatum</i>	Pre-Intake: Post-UV-on	1.4
<i>Thalassiosira</i> sp.	Pre-Intake: Post-UV-on	1.5
<i>C. gracile</i>	Pre-Intake: Post-UV-on	1.8

Table 3. *Chaetoceros gracile*. Multiplication factor (MF) of relative abundance in control and post-treatment samples. Data from statistically significant comparisons are listed

Phytoplankton group	Stage comparison	MF
<i>C. gracile</i>	Pre-Intake: Post-UV-on	1.1

concentration. Fig. 4 shows the growth rate and relative abundance of phytoplankton (*Skeletonema costatum*, *Thalassiosira* sp., and *Chaetoceros gracile*) observed for all control and treatment samples collected from the ITS. In general, the growth rates of phytoplankton observed in the Post-UV-on samples tended to be significantly lower than those observed in the control Pre-Intake samples (Table 2). The relative abundance of *C. gracile* observed in the Post-UV-on samples was significantly

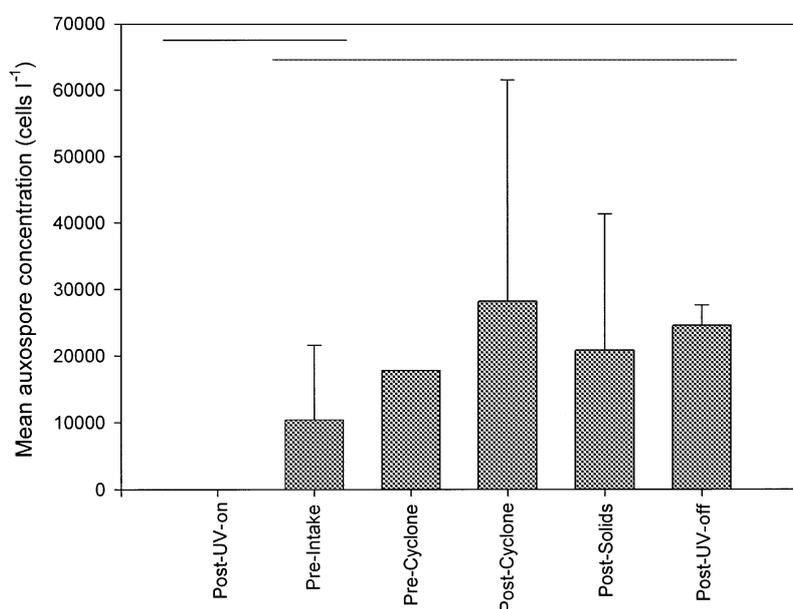


Fig. 5. *Skeletonema costatum*. Mean auxospore concentration observed during the incubation experiment (between Days 3 and 7) at various stages of Velox ballast water management system. Lines above histograms connect treatment groups exhibiting no significant difference (ANOVA and Tukey tests). Error bars = ± 1 SD

lower than those observed in the Pre-Intake samples (Fig. 4). Tables 2 & 3 outline the multiplication factors that quantify the reductions in growth rates and relative abundances observed between treatment and control samples exhibiting statistical differences.

Formation of auxospores

Auxospores of *Skeletonema costatum* were not observed in cultures inoculated from the seawater samples collected from the Post-UV-on port, while auxospores were observed in cultures inoculated from the seawater samples collected from the remaining treatment ports of the ITS and ambient seawater samples (Fig. 5). The statistical results of the ANOVA test reveal a statistically significant difference ($p = 0.05$) in the mean auxospore concentrations between incubation treatments. The mean auxospore concentrations were calculated by averaging the replicate auxospore concentrations observed for each treatment between Days 3 and 7 of the incubation experiment. The results of the post-hoc Tukey test show that the mean auxospore concentration observed for the Post-UV-on incubation was significantly different than those observed for the remaining treatment incubations. Although no auxospores were observed in the Post-UV-on incubation, no statistical difference ($p = 0.098$) in the mean auxospore concentration was found between the Post-UV-on and the Pre-Intake incubations. This finding was probably due to the relatively low concentration and variance of auxospores observed in the Pre-Intake incubations. Direct observations of presence or absence of auxospore cells may be more appropriate than the use of statistical comparisons when assessing the effect of the various ITS stages of treatment on the reproductive potential of phytoplankton.

Combined UV-darkness incubation experiment

Fig. 6 shows that phytoplankton were capable of growth after 3 mo storage in dark and chilled conditions, with the exception of those phytoplankton exposed to the UV treatment. The UV-treated chlorophyll concentrations fell near to the detection level of the fluorometer and thus exhibited much variability.

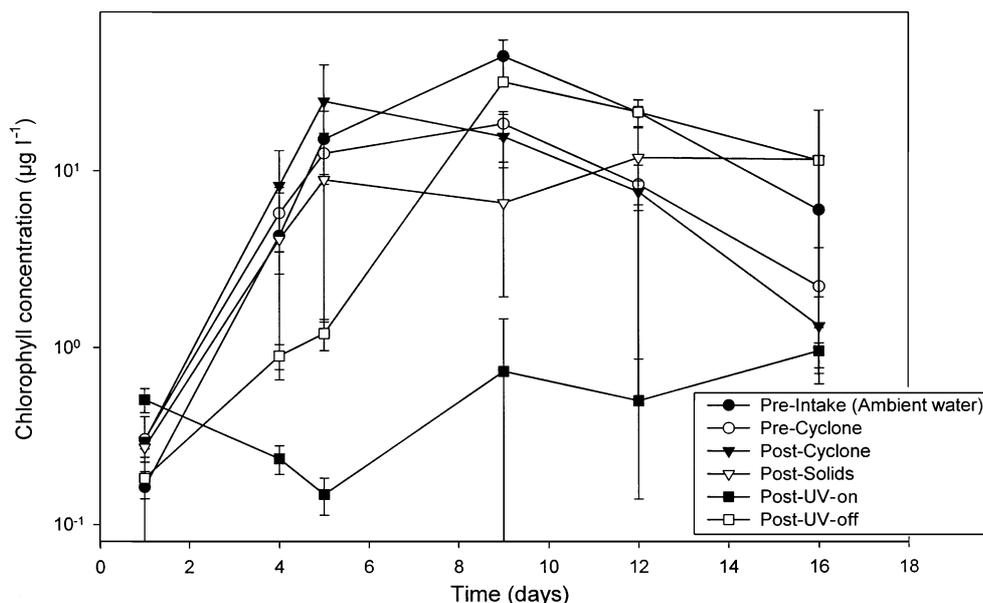


Fig. 6. Growth curves of phytoplankton generated during incubation experiment designed to determine combined effect of UV-treatment and dark storage on survivorship of phytoplankton. Error bars = ± 1 SD

DISCUSSION

The results of this study show that the ultraviolet radiation-treatment stage of the ITS influenced the abundance and growth characteristics of the natural populations of phytoplankton present in the seawater source. In general, decreases in both the abundance and growth rates of diatoms were observed following UV exposure. Specifically, a lag period was established prior to the onset of growth of the diatom *Chaetoceros gracile* during the incubation experiment following treatment. A previous treatment study carried out by Jelmers (1999) showed that an integrated hydrocyclone/UV-treatment system, designed to treat ballast water, also had an impact on the survivorship of phytoplankton. In this case, the dinoflagellate *Prorocentrum minimum* and the prymnesiomonad *Tetraselmis* sp. exhibited mortality levels as high as 84.7 and 87.6%, respectively, following UV exposure.

Chaetoceros gracile appeared to be the most sensitive of the phytoplankton groups to the influence of the ITS. The lag phase exhibited by *C. gracile* in the UV-treated samples indicates that an interruption in the continuance or onset of growth occurred relative to the remaining control and treatment stages (Fig. 3c). The onset of growth by this species following the 4 d lag period suggests that some form of recovery took place to carry out vegetative growth (mitosis). These observations are supported by Gieskes & Buma (1997), who observed that the marine diatom *Cyclotella* sp. was capable of repairing UVB-induced DNA damage following exposure to visible light (photorepair). Since *Skeletonema costatum* and *Thalassiosira* sp. did not exhibit lag phases prior to growth, these phytoplank-

ton groups may have also undergone a similar photorepair process of DNA material during the period following sample collection but prior to the start of the incubation experiment (Fig. 3a,b). The lack of growth of UV-treated phytoplankton observed in the combined UV-darkness incubation experiment suggests that dark storage of UV-treated seawater will minimize the recovery of phytoplankton populations (Fig. 6).

Many investigators have found that photoreactivation or photorepair of damaged DNA may not result in a complete recovery of viruses or micro-organisms (Stolarek & Karcz 1987, Nielson et al. 1995, Weinbauer & Suttle 1996, Gieskes & Buma 1997, Nilawatie et al. 1997, Wilhelm et al. 1998). These observations are consistent with the results of the incubation experiment carried out in the present study, which demonstrated decreased growth rates of phytoplankton exposed to ultraviolet light relative to those of the control samples (Pre-Intake). The lower relative abundance of UV(C)-treated phytoplankton relative to control populations also indicates that a complete recovery of normal cell functions did not take place. It should be noted that the optimal nutrient and light conditions of the incubation experiment would likely enhance the survivorship of phytoplankton relative to those stored in a ballast tank or discharged to coastal waters.

The ultraviolet treatment stage of the ITS appeared to be 100% effective in eliminating the ability of the phytoplankton, *Skeletonema costatum*, to form auxospore cells generated by sexual reproductive processes (Fig. 5). Typically, auxospore formation in diatom populations is triggered by a cell size reduction that takes place during successive vegetative (mitotic) growth (Drebes 1977, Perez-Martinez et al. 1992), as

well as changes in environmental conditions (Schultz & Trainer 1970, Davis et al. 1973, Ambrust et al. 1990, Eilertsen et al. 1995). The small-sized diatom cells observed in this study, along with the altered incubation conditions, may have been responsible for inducing auxospore formation in *S. costatum*. The formation of large-sized auxospore cells is necessary to restore normal physiological processes of phytoplankton cells, since the ratio of surface area:volume of diatom cells is altered during vegetative growth. Other investigators have observed that the greatest impact of ultraviolet light delivered by germicidal lamps was the loss of the ability of microbes and viruses to reproduce themselves (Harm 1980).

The results of the invertebrate experiment indicate an impact of the ITS on the survivorship of copepods. However, higher concentrations of copepods in the source seawater are required to discern statistical statements towards the survivorship of copepods subjected to the various treatment levels of the ITS. In order to achieve higher concentrations of invertebrates in source waters required for statistical testing, follow-up experiments will include inoculating the ITS with dense invertebrate cultures prior to the exposure experiment.

The cyclonic separation phase of the ITS appeared to clarify the clear-water fraction prior to the ultraviolet sterilization phase. The Post-Solids fraction of seawater contained higher concentrations of larger particles, debris, and dense aggregations of small-diameter phytoplankton cells. The turbulence created by the cyclonic pre-treatment may have acted to increase the encounter rate of particles and enhance aggregation processes. Microalgal aggregates have been suggested to form through collisions of individual phytoplankton cells induced by turbulent shear (Jackson 1990). The high densities of biological material observed in this study may have increased the 'stickiness' factor of particles, further enhancing the flocculation process (Kjørboe et al. 1990). These aggregations will act to scavenge small-diameter cells that may normally flow through the Post-Cyclone fraction. The results of this study reveal a tendency for the Post-Solids fraction to have a higher concentration of the small-sized phytoplankton relative to those in the remaining fractions.

Future studies examining the impact of ultraviolet light on plankton survivorship should consider (1) variations in ultraviolet light dosages (photoperiod and intensity), and (2) variations in the darkness period following ultraviolet light exposure. The flow rate within the ITS can be varied to alter the photoperiod of ultraviolet light exposure, while the intensity of ultraviolet light can be enhanced by increasing the number of operating germicide lamps (UV-C) within the ultraviolet

radiation chamber. The dark and cold environment of a typical ballast tank would provide conditions conducive for minimizing or prohibiting DNA repair, depending on location (temperature) and journey time (darkness period) of the traveling ship. Certain bacteria have been shown to undergo photorepair of UV-damaged DNA upon exposure to higher temperatures (Wood et al. 1997). If a mandatory darkness period is required to minimize survival following exposure to ultraviolet light, it would be necessary to maintain a dark environment following the treatment of ballast water and preceding discharge or de-ballast processes upon arrival at port. A matrix-style testing approach incorporating a range of oceanographic conditions (turbidity and salinity) and species responses will enable the application of this system worldwide, increasing its global value.

Acknowledgements. The success of this project was due to the collaborative effort of many people. We would like to thank Drs R. Devlin and I. Birtwell for providing environmental chambers to carry out the incubation experiment, and Earl MacIsaac for providing an inverted microscope for phytoplankton enumeration (DFO). Alan Martin (Lorax Environmental Services) drafted the schematic diagram of the Velox Ballast Water Management System. The ultraviolet radiation chamber was supplied by Halvor Nilsen of Tech-Trade A/S while dock facilities were supplied by the Vancouver Port Authority. This project was funded by Velox Technology Inc. and Fisheries and Oceans Canada.

LITERATURE CITED

- Ambrust EV, Chisholm SW, Olson RJ (1990) Role of light and the cell cycle on the induction of spermatogenesis in a centric diatom. *J Phycol* 26:470-478
- Carlton JT, Geller JB (1993) Ecological roulette: the global transport of nonindigenous marine organisms. *Science* 261:78-82
- Davis CO, Harrison PJ, Dugdale RC (1973) Continuous culture of marine diatoms under silicate limitation. I. Synchronized life cycle of *Skeletonema costatum*. *J Phycol* 9: 175-180
- Drebes G (1977) Sexuality. In: Werner D (ed) *The biology of diatoms*. University of California Press, Berkeley, p 250-283
- Eilertsen HC, Sandberg S, Tollefsen H (1995) Photoperiodic control of diatom spore growth: a theory to explain the onset of phytoplankton blooms. *Mar Ecol Prog Ser* 116: 303-307
- Gauthier D, Steel DA (1996) A synopsis of the situation regarding the introduction of nonindigenous species by ship-transported ballast water in Canada and selected countries. *MS Rep Fish Aquat Sci Can* 2380:vi+157
- Gieskes WWC, Buma AGJ (1997) UV damage to plant life in a photobiologically dynamic environment: the case of marine phytoplankton. *Plant Ecol* 128(1-2):16-25
- Hallegraeff GM, Bolch CJ (1991) Transport of toxic dinoflagellate cysts via ship's ballast water. *Mar Pollut Bull* 22: 27-30
- Harm W (1980) *Biological effects of UV radiation*, Cambridge University Press, Cambridge

- Harrison PJ, Waters RE, Taylor FJR (1980) A broad spectrum artificial medium for coastal and open ocean phytoplankton. *J Phycol* 16:28–35
- Jackson GA (1990) A model of the formation of marine algal flocs by physical coagulation processes. *Deep-Sea Res* 37: 1197–1211
- Jelmers A (1999) Testing the effectiveness of an integrated hydrocyclone/UV-treatment system for ballast water. Consultancy report, Institute of Marine Research, Austevoll Aquaculture Research Station, Norway, p 1–22
- Kjørboe T, Anderson KP, Dam HG (1990) Coagulation efficiency and aggregate formation in marine phytoplankton. *Mar Biol* 107:235–245
- Locke A, Reid DM, van Leeuwen HC, Sprules WG, Carlton JT (1993) Ballast water exchange as a means of controlling dispersal of freshwater organisms by ships. *Can J Fish Aquat Sci* 50:2086–2093
- Lund JWG, Kipling C, Le Cren ED (1958) The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. *Hydrobiologia* 11: 143–170
- Mills EL, Leach JH, Carlton JT, Secor CL (1993) Exotic species in the Great Lakes: a history of biotic crises and anthropogenic introductions. *J Great Lakes Res* 19:1–54
- Nielsen T, Bjoern LO, Ekelund NGA (1995) Impact of natural and artificial UVB radiation on motility and growth rate of marine dinoflagellates. *J Photochem Photobiol* 27(1): 73–79
- Nilawati J, Greenberg BM, Smith REH (1997) Influence of ultraviolet radiation on growth and photosynthesis of two cold ocean diatoms. *J Phycol* 33(2):215–224
- Perez-Martinez C, Cruz-Pizarro L, Sanchez-Castillo P (1992) Auxosporulation in *Cyclotella ocellata* (Bacillariophyceae) under natural and experimental conditions. *J Phycol* 28: 608–615
- Schultz ME, Trainer FR (1970) Production of male gametes and auxospores in a polymorphic clone of the centric diatom *Cyclotella*. *Can J Bot* 48:947–951
- Stolarek J, Karcz W (1987) Effect of UV-C radiation on membrane potential and electric conductance in internodal cells of *Nitellopsis obtusa*. *Physiol Plant* 70:473–478
- Weinbauer MG, Suttle C (1996) Potential significance of lysogeny to bacteriophage production and bacterial mortality in coastal waters of the Gulf of Mexico. *Appl Environ Microbiol* 62:4374–4380
- Wilhelm SW, Weinbauer MG, Suttle CA, Pledger RJ, Mitchell DL (1998) Measurements of DNA damage and photoreactivation imply that most viruses in marine surface waters are infective. *Aquat Microb Ecol* 14:215–222
- Wood ER, Ghane F, Grogan DW (1997) Genetic responses of the thermophilic archaeon *Sulfolobus acidocaldarius* to short-wavelength UV light. *J Bacteriol* 179:5693–5698

Editorial responsibility: Otto Kinne (Editor),
Oldendorf/Luhe, Germany

Submitted: November 24, 1999; Accepted: June 8, 2000
Proofs received from author(s): January 8, 2001