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Design and Preliminary Use of a Commercial Filter Skid to Capture Organisms ≥ 50 µm in Minimum Dimension (Nominally Zooplankton) for Evaluating Ships' Ballast Water Management Systems at Land-Based Test Facilities

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1 Introduction

In 2004, the International Maritime Organization adopted the International Convention for the Control and Management of Ships' Ballast Water and Sediments, which establishes standards for ballast water discharge and the performance of ballast water management systems (IMO, 2004). Among the criteria for ballast water discharge is the density of organisms ≥ 50 µm (nominally zooplankton), which is set at < 10 viable organisms m^{-3}. This numerical standard, intended to "prevent, minimize and ultimately eliminate the risks to the environment, human health, property and resources arising from the transfer of harmful aquatic organisms and pathogens", is also proposed by the US Coast Guard (2009).

Because the probability of finding a living zooplankter in a water sample meeting the proposed discharge standard follows a Poisson distribution (Lemieux et al., 2008b), the key test statistic is the number of organisms counted. With respect to land-based and shipboard testing of ballast water management systems, accurate enumeration of organisms in ballast tanks depends on several factors: the volume of water sampled, the volume of sample analyzed (e.g., the entire sample or a sub-sample), the level of desired precision, and the relevant discharge standard (e.g., < 10 viable organisms m^{-3} or < 0.1 m^{-3}). Previous work at the Naval Research Laboratory in Key West (NRLKW) has shown for a 3 m^3 zooplankton sample treated by a ballast water management system and concentrated to a volume of 1 l, only a 20 ml subsample of the concentrate could be evaluated before zooplankton in the subsample died because they were held in artificial conditions in the laboratory (i.e., concentrated and gently aerated in a flask; Lemieux et al., 2008a). If only 20 ml of a concentrated sample can be evaluated, the required sample volume is large. For example, to know with 95% confidence a ballast tank contains < 10 living zooplankters m^{-3}, a sample volume of 60 m^3 would need to be concentrated to 1 l and a 20 ml subsample examined (Lemieux et al., in review). Several factors affect the statistics; if a larger volume of subsample (or the entire concentrated sample) could be analyzed, the sample volume concentrated to 1 l would decrease accordingly. Nonetheless, given the work at NRLKW and other test facilities, it appears large sample volumes—at least 10 m^3–will need to be concentrated and evaluated.

The large volumes result in two challenges: adverse effects on organisms filtered through a fine mesh must be minimized, and the size of the individual nets or filters must be manageable (the latter is especially relevant for shipboard testing). Traditionally, marine scientists capture organisms by filtering water through a plankton net. Alternatively, a set of filter bags enclosed in individual housings—a configuration commonly used in water-treatment facilities—can be employed. An argument can be made that filter bags are superior to a plankton net for ballast water testing because filter assemblies do not require a mechanism to lift them from a tank to collect samples. Additionally, the filter bags are enclosed in a housing, so they are less vulnerable to inclement weather than plankton nets.

2 Objectives

The objectives of this work were to (1) develop and validate a procedure for retaining organisms ≥ 50 µm in minimum dimension (nominally zooplankton) in large volumes of water used for testing ballast water management systems, (2) design and build the equipment to do so, and (3) validate the equipment. Importantly, zooplankton needed to be kept alive and minimally affected by filtering and handling, since the ballast water discharge standard prescribes the number of *viable* (living) organisms (IMO, 2005).

3 Experimental Approach

All laboratory and field experiments were conducted at the Ballast Water Treatment Test Facility (BWTTF) at NRLKW. An initial field experiment compared the recovery of zooplankton proxies (red, 50 µm diameter microbeads) between a plankton net and two filter bags enclosed in individual filter housings. After microbeads were added to the net or filter bag, ambient seawater was pumped through them, and the filtrand of each was examined for the presence of microbeads (see Appendix 1 for experimental details). Although NRLKW is surrounded by oligotrophic seawater with relatively low levels of plankton, debris, or colored dissolved organic matter, the samples were clouded with material so it was difficult to see the microbeads. Recovery efficiency of the microbeads was very low (Appendix 1; 25 and 28% from the plankton net and filter bags, respectively).

To address this outcome, trials using microbeads were conducted in the laboratory using filter bags or sieves. The initial laboratory trials also yielded low microbead recovery efficiencies, so a number of improvements was made to the experimental approach (e.g., as described in sections 3.2 and 3.3.3 below). Next, field trials were conducted using fresh water and filter bags.

Considering the initial field trial with a plankton net—and taking into account previous experiences at NRLKW—it was concluded using plankton nets to process the large volumes of water required to evaluate treated water at land-based testing facilities would not be feasible. Nets are cumbersome, prone to tearing, open to the atmosphere and thus vulnerable to wind and weather, and difficult to configure in a flow-through sampling apparatus suitable for large volumes. In response, engineers at NRLKW designed and built a filter skid containing multiple filter bags, each enclosed in a stainless steel housing. Here, we describe preliminary trials to evaluate the retention efficiency of filter bags using microbeads, the design and construction of the filter skid, and initial validation trials.

3.1 Ballast Water Treatment Test Facility (BWTTF) Description

As part of the United States Environmental Protection Agency‘s Environmental Technology Verification (ETV) program and in partnership with the U.S. Coast Guard, a BWTTF was established at NRLKW, where research is conducted to provide technical guidance for the ETV‘s Generic Protocol for the Verification of Ballast Water Treatment Technologies (Lemieux et al.,

in review). The BWTTF is located on Fleming Key at Trumbo Point Annex, Naval Air Station Key West, FL. As part of the Naval Research Laboratory's Center for Corrosion Science and Engineering, it functions as a scientific test platform for the standardized assessment of technologies designed to reduce or eliminate aquatic nuisance species in shipboard ballast water.

If land-based test facilities incorporate a standard set of challenge conditions, then comparable and defensible results are generated. To that end, the BWTTF is designed to conduct experiments and develop methods to inform the ETV verification protocol and thereby has a fully instrumented seawater storage and transfer system that replicates the volumes, flows, and pressures typical of ballast water systems on marine vessels. The BWTTF pumps can provide flow rates up to 5110 l min^{-1} (1350 gpm), and on-site tanks include a 382 m^3 (101,000 gal) ballast tank, a 151 m^3 (40,000 gal) ballast test tank, and a 394 m^3 (104,000 gal) discharge tank. The facility provides for the injection of specified test organisms as well as the means to monitor test conditions, conduct sampling, and analyze samples in the laboratory. All water from experiments is treated prior to final discharge to remove all added challenge components and any by-products of any treatment systems tested. To accommodate yet undefined and unidentified technologies, the system can be reconfigured to accommodate treatment systems at uplift, in-tank, or discharge locations in the flow path.

Control of all systems and instrumentation is provided by the Honeywell Plantscape Industrial Control and Automation system (Honeywell, Morris Township, NJ), which has been customized by engineers at NRLKW to provide BWTTF-specific data acquisition, operational control, and system monitoring with alarm and interlock functionality. The industrial control hardware consists of standalone control panels with programmable logic controllers, relays, and analog-to-digital converters connected to a redundant computer server system to log all actions and data. A computer in a control room adjacent to the ballast tanks with five displays provides the primary operator interface to control of the BWTTF. This control system provides a series of graphical control screens from which the operator can select and view the overall system with key parameters displayed.

3.2 Microbeads

The proxies used for zooplankton were red, ChromoSphere-T NIST Certified microspheres (Microgenics Corporation, Fremont, CA). Depending on the trial, one or two sizes of microbeads were used: with a nominal diameter of 49 µm (CV = 7.8%), 96 µm (CV = 7.8%) or 150-µm ± 3.6 µm (CV = 6%). All microbeads were composed of a cross-linked polystyrene divinylbenzene copolymer. Microbeads were counted in 15 ml Bogorov counting chambers or 1-ml Sedgewick Rafter counting chambers using an Olympus SZH10 dissecting microscope, a Nikon AZ100 Multizoom microscope, or a Nikon Eclipse TS100 inverted microscope.

Usually, microbeads were counted the day before an experiment and placed in a beaker with Type II water (5-µm filtered and treated by reverse osmosis and deionization). The contents of the beaker were poured into the plankton net, filter bag, or sieve with care to minimize splashing and loss of microbeads. Initial trials used 600 microbeads to approximate a discharge volume of 60 m^3 meeting the IMO zooplankton discharge standard (10 zooplankton m^{-3} x 60 m^3 = 600

microbeads); subsequent trials typically used 100 or 200 microbeads (which allowed more trials to be run, as less time was spent counting microbeads). In all cases, the beaker containing the microbeads was rinsed 3-5 times with Type II water, and the beaker and wash water were examined for microbeads that may have adhered to the beaker wall.

At the end of laboratory trials or field runs, the sieve or filter bags were rinsed with Type II water or artificial seawater (Instant Ocean®; Aquarium Systems, Inc., Mentor OH) into beakers. Subsamples of approximately 15 ml were pipetted into Bogorov counting chambers. Two researchers counted subsamples until the entire volume of each beaker was counted. Additionally, surfaces that touched the sample were examined for microbeads: beakers, pipet tips, and funnels.

For each Bogorov chamber analyzed, counts were made by a researcher until two subsequent counts agreed. In most experiments, two researchers examined at least one of the Bogorov Chambers to ensure counts were the same. If the final count differed between the researchers, which was rare (approximately 20% of the time), it differed by one or two microbeads. Because it was assumed microbeads were much more likely to be undercounted than over counted, the highest number was used in the final tally.

3.2.1 Microbead Disintegration

When the initial recovery rates were low in field and laboratory trials, it seemed unlikely, but possible, the microbeads dissolved in seawater or Type II water. An assay was conducted: in separate wells of a 12-well plate, 10 50-µm microbeads and 10 150 µm microbeads were dispensed into 5 ml of filtered seawater (0.22 µm), and ten microbeads of each size class were dispensed into 5 ml of Type II water. The four wells were covered and placed on a laboratory bench for 7 days, and then microbeads in each well were counted.

3.2.2 Method of Microbead Counting

The microbead recovery efficiency was low in initial trials using 50 µm diameter microbeads in field and laboratory experiments, which was surprising, as the microbeads had been accounted for in all possible places: the filtrate, filtrant (material retained in the filter bag), residue in the beaker that initially held the microbeads, and residue in the pipette used to dispense samples. Given this unbalance, it seemed possible the method for initially counting microbeads to add to filter bags, sieves, or plankton nets was somehow undercounting them. Until this point, the microbeads had been counted 'dry', that is, a sterile pipette tip was dipped into the bottle containing the microbeads to deliver a pile of microbeads to a gridded Sedgewick Rafter (SR) counting chamber, which did not have water on it. After the microbeads were counted, the SR was gently rinsed with Type II water from a squirt bottle into a beaker, and the beaker‘s contents were poured into a filter bag or sieve for an experiment. To ensure the SR did not have any remaining microbeads, it was placed on a Kim wipe and examined under the microscope. Perhaps rinsing the dry microbeads into a beaker caused them to be dispersed into the air rather than the beaker.

Addressing this hypothesis, recovery efficiency of 100 50-µm microbeads was determined in 5 trials with _dry‘ counting and 4 trials with _wet‘ counting. In the latter method, a pipette tip was dipped into the bottle containing the microbeads and tapped into a drop of Type II water on a SR slide to remove the microbeads from the pipette tip. The water did not touch the edge of the slide, nor was a cover slip used. After the microbeads were dispensed (dry or wet) into the SR, they were counted and rinsed from the SR with a squirt bottle containing Type II water into a beaker. To determine that 100 microbeads were, in fact, delivered to the beaker, its contents were pipetted into Bogorov chambers and counted. When the entire sample was counted, the pipette and beaker were examined for any residual microbeads. For each trial, the name of the scientist counting the microbeads was recorded to determine if the poor recovery was attributable to a given researcher.

3.2.3 Intercalibration

To ensure all microbeads were counted, an intercalibration exercise was conducted in which two observers counted the same 15 ml sample dispensed in a Bogorov counting tray. Four trays (each with a different sample from the filter bag) were counted by each observer, and then each tray was recounted by each observer. An additional 10 trays (each with a different sample from the filter bag) were counted by both observers, for a total of 18. Although the seawater used for the experiment had been filtered, debris (sediment and plankton) was present in the samples. Thus, extra care was required to find the microbeads, and each tray took approximately 45 min to examine.

To determine the difference between observers the mean of the observers‘ counts for a single tray was determined, and the percent difference for each observer‘s count was calculated as follows:

Equation 1. Calculation to determine percent difference between microbead counts.

$$\frac{|\text{Mean of Counts - Observer 1 Count}|}{\text{Mean of Counts} \times 100}$$

Because there were two observers, the percent difference was the same for each observer for each tray counted.

In a separate trial, one observer dispensed 15 ml of sample from the first filter bag and counted it five times. Each time, she shook the tray before counting to determine if the movement of debris and microbeads resulted in a different microbead count.

3.3 Filter Bags

In laboratory trials using microbeads, nylon filter bags (18 cm [7”] in diameter at the top and 41 cm [16”] long) with various mesh sizes (100 µm, 50 µm, 25 µm, or 10 µm) were used singly (Universal Filters, Inc., Asbury Park, NJ). In some cases, sieves (20 cm [8”] in diameter) with 25 µm and 10 µm nylon mesh were used in lieu of filter bags to test recovery in a very straightforward way (i.e., from a flat surface free of seams and dimples found in filter bags).

In field trials, filter bags 18 cm (7”) in diameter and 41 cm (16”) long were placed in filter housings arranged in series. A variety of mesh sizes was used: 25 µm, 35 µm, 50 µm, 100 µm. Each filter bag was enclosed in a fiberglass filter housing (Hayward Flow Control Systems, Clemmons, NC) containing a polypropylene filter basket (Figure 1). Flow and pressure were monitored using a paddle wheel flow sensor downstream of the second housing and with three pressure sensors (one before and after the first housing and one after the second housing; all sensors were manufactured by GF Signet, El Monte, CA). Flow was controlled manually using a 5 cm (2”) diaphragm valve at the bottom of the right-hand filter housing (Figure 1, black handle). Ambient seawater was taken up by a 30-hp, horizontal centrifugal pump, passed through a 15 cm (6”), polyvinyl chloride (PVC) line, passed through a manifold, and delivered to the Hayward units by a 5 cm (2”), reinforced PVC plastic hose. Freshwater was added to a 3-m3 storage tank using a hose and pumped to the Hayward units through a 5 cm (2”), reinforced PVC plastic hose.

Figure 1. Two 41 cm long filter bags in Hayward housing arranged in series; water entered the system via the green hose at the top of the left-hand housing.

Early field trials employed filter bags with 25-µm mesh. Later trials used filter bags with 35 µm mesh, because it better approximated the mesh size used by researchers at test facilities in the US and abroad (37 µm) and was available in commercially manufactured filter bags. To capture organisms in the ≥ 50 µm size class, the IMO Convention (2004) states the hypotenuse of the

mesh must be no longer than 50 µm; that requirement is met using 35 µm mesh (hypotenuse = 49.5 µm).

When microbeads were used as proxies for zooplankton, immediately prior to the trial, they were rinsed into a filter bag placed in the second filter housing in series (downstream of a housing with a filter bag used as a prefilter). The cover of the second filter housing was removed, and the housing had been filled with freshwater. After the cover was secured on the filter housing and the air bled from the housing using a valve on its cover, water was pumped through the system. The filter bags were retrieved and the microbeads counted from the filter bag to which the microbeads had been added and from the filter bag downstream of it. Often, a filter bag was used as a prefilter upstream of the filter bag containing the microbeads to remove plankton, sediment, and detritus.

3.3.1 Filter Bags Field Trials—Water Flow Rate

To determine the appropriate mesh size for filter bags arranged in series and the appropriate water flow rate through them, five trials were run. The flow rates varied (25 gpm, 125 gpm, 150 gpm), as did the filter bags‘ mesh sizes (25 µm, 50 µm, 100 µm). The differential pressure between filter housings was monitored, and whether the filter bags clogged was noted.

3.3.2 Filter Bags—Laboratory and Field Validation with Microbeads

In laboratory trials, microbeads were counted using a Sedgewick Rafter counting chamber in the wet or dry manner, rinsed into a beaker with Type II water, and the beaker‘s contents gently rinsed into a filter bag or onto a sieve. The material retained on the filter bag or sieve was rinsed with Type II water into a beaker, and its contents were transferred with a serological pipet into Bogorov counting chambers. The recovery efficiency of 50 µm microbeads was low in the first laboratory (and field) trials, so 150 µm and 100 µm microbeads were added to experiments because their large size rendered them easier to find, thus they served as quasi-positive controls. The following parameters were recorded to account for any biases: type of filter bag (plastic-topped vs. felt-topped), the glue used to seal the seams, how the seams were glued (e.g., once on the inside and once on the outside), the method of counting, and the filter bag number (to record the number of uses).

After initial field trials with ambient seawater yielded low microbead recovery efficiencies, freshwater was used exclusively in the field. One 25-µm filter bag was placed in each of three Hayward filter housings arranged in series (with the pressure and flow sensors described above). A filter bag served as a prefilter to remove debris from the water; a known number of microbeads in a beaker was rinsed with Type II water into the second filter bag in series. After approximately 3785 liters (1000 gal) was pumped through the system at a flow rate of 25 gpm, the second and third filter bags were removed, rinsed with Type II water, and the material retained in the bags was rinsed into a beaker, pipette into Bogorov counting chambers, and examined for microbeads. The filter bags‘ seams used in field trials were sealed with 3M® 5200: 25 µm filter bags were sealed twice on the inside. To reduce handling time, the 35 µm

filter bags were sealed once on the inside with twice as much sealant typically used for the 25 µm filter bags; a laboratory trial showed 100% recovery of 50 µm microbeads.

In laboratory and field trials, the filter bags‘ seams, the beakers, and pipettes were examined for residual microbeads.

3.3.3 Filter Bags—Seams

After initial experiments showed a low recovery efficiency of microbeads, it was discovered that the holes at the seams were much greater than the nominal mesh size, and it was hypothesized the microbeads passed through the filter bags via the holes. A survey of filter bag manufacturers showed bags with heat-welded seams were available, which would ameliorate the problem, but bags were available only in microfilament or polypropylene felt material. Neither was appropriate, as organisms could not be retrieved from these surfaces.

Closing off the bags‘ seams with a marine sealant was a viable solution, as long as the cured sealant was malleable, not tacky (so it would not trap zooplankton), and non-toxic to marine organisms over the short time they would be sequestered in the filter bags during sampling (approximately 2 h). Seven sealants were evaluated: Marine Goop® (Eclectic Products, Inc., Eugene, OR), INSTANT Adhesive (GC™ Electronics, Rockford, IL), Weld·On PVC 717™ Plastic Pipe Cement (IPS Corporation, Gardena, CA), 2-part epoxy (John C. Dolph Company, Monmouth Junction, NJ), white 3M™ Marine Fast Cure 5200 Adhesive Sealant (3M, St. Paul, MN), and Elmer‘s® No-Wrinke Rubber Cement (Elmer‘s Products, Inc., Columbus, OH).

Using a-41 cm (16”) long filter bag with 10 µm mesh, each adhesive was applied in approximately 5-cm long sections the outside and inside of the bags, and the adhesives cured for 36 h (longer than any of the recommended curing times).

3.3.4 Filter Bags—Toxicity of Sealant on the Seams

To test if zooplankton were killed by brief exposure to the filter bags‘ sealant (3M™ 5200, chosen after the trial described in section 3.3.3), bioassay experiments were conducted with brine shrimp *Artemia franciscana* (previously used at NRLKW as a standard test organism) and subsequently with ambient zooplankton (copepods). Cysts of *A. franciscana* were purchased from a vendor (Brine Shrimp Direct, Ogden, UT) and incubated in 5-µm-filtered, aerated artificial seawater (salinity = 36) for 24 h in 25°C with a 12:12 light:dark cycle under fluorescent bulbs (72 µM Einsteins m^{-2} s^{-1}). Hatched nauplii approximately 12 h old and 300 - 400 µm long were removed from the culture in 1 ml aliquots and dispensed into each of eight Petri dishes (47 mm diameter) with 10 ml of 0.22 µm-filtered seawater. Next, the dishes were examined using a dissecting microscope to ensure all *A. franciscana* were living (determined by movement), which was the case. Four Petri dishes served as controls, and to the remaining four, a strip (approximately 4 cm x 1 cm) of 25 µm, nylon filter bag with a bead of sealant (approximately 3 cm x 0.5 cm) that had been cured for 24 h was added.

Petri dishes were arranged haphazardly on a shelf in the incubator (described above) for 2 h, the estimated amount of time zooplankton would be sequestered in a filter bag during collection. After the incubation, the number of dead *A. franciscana* was counted. If an organism was not moving, it was gently prodded with a probe, and if it did not move within 10 s, it was scored as dead. Following the tally, all samples were fixed with Lugol's iodine solution, the total number of *A. franciscana* counted, and the number of living organisms determined by subtraction.

The trial was repeated with ambient copepods, which were concentrated from a seawater hose onto a 31-µm sieve. Copepods of all-life history stages (≥ 50 µm in minimum dimension) were removed from the sample individually using a pipet. To each of eight Petri dishes (47 mm diameter) containing 10 ml of 0.22 µm filtered seawater, 20 copepods were added. Four dishes served as controls, and strips of mesh with sealant were added to the other four Petri dishes (treatment). Dishes were examined to ensure all copepods were living (they were), and dishes were arranged haphazardly on a shelf in the incubator and incubated for 2 h at the settings from the previous trial. Following incubation, the number of dead copepods was counted and preserved as above. Statistics were calculated with SigmaPlot® v. 11 (Systat Software Inc., San Jose, CA).

3.4 Filter Skid

3.4.1 Filter Skid Requirements

The filter skid was designed with several considerations in mind: first, it was desirable to construct an apparatus using common, commercially available products so the skid could be replicated easily at other test facilities and did not require special fabrication techniques. Second, the prototype was developed to eliminate large, cumbersome plankton nets currently used by test facilities, so it needed to have nets in self-contained filter housings. Third, the apparatus was designed so it might be accommodated in future shipboard sampling programs, e.g., a small footprint was required. Because the skid would be used with seawater, the materials needed to withstand a corrosive environment (e.g., stainless steel or PVC). Both the cost and availability of corrosion-resistant metals were considered. Grade 316 stainless steel was chosen for the filter housings, and PVC was chosen for the piping.

3.4.2 Toxic Effects of Stainless Steel Filter Housings

A bioassay test was conducted to determine if the brine shrimp *Artemia franciscana* or ambient zooplankton would die from exposure to stainless steel filter housing. Cysts of *A. franciscana* were hatched as in section 3.3.4, and 3 1-ml subsamples of 12-h old Nauplii (300 - 400 µm long) were removed from the culture and evaluated qualitatively using a dissecting microscope. If a nauplius was not moving, it was gently prodded with a probe, and if it did not move within 10 s, it was scored as dead. All nauplii (70 - 77 per subsample) were living. Two ml aliquots of the culture were dispensed into each of five 1000 ml beakers containing 800 ml of 0.45 µm-filtered seawater.

Ambient zooplankton (primarily copepods) were concentrated from a seawater hose onto a 37-µm sieve. The filtrand was suspended in 0.45 µm-filtered seawater. A 1 ml sample was removed and zooplankton (≥ 50 µm in minimum dimension) were qualitatively assessed: 1 was dead, 6-10 were living. Four-ml aliquots of the sample were placed into each of the five beakers.

Two beakers served as controls, with only seawater, *A. franciscana*, and ambient zooplankton. To the remaining three beakers, 10 washers (grade 316 stainless steel, 1.9 mm thick, and 38.1 mm in diameter with a hole in the center 15.8 mm in diameter) were added. Their surface area (SA) in the beaker was calculated to equal the SA:volume ratio of water in a filter skid housing.

Beakers were arranged haphazardly on a shelf and covered with a black tray in the incubator for 2.5 h, the estimated amount of time zooplankton would be sequestered in a stainless steel filter housing during collection. After the incubation, the washers were removed from the treatment beakers using forceps, and the contents of each beaker were gently poured through a 37 µm sieve, the filtrand rinsed with filtered seawater into a beaker, and the entire volume transferred to a Petri dish. The number of dead *A. franciscana* and zooplankton were counted. Following the tally, all samples were fixed with Lugol‘s iodine solution, the total number of organisms counted, and the number of living organisms determined by subtraction.

3.4.3 Comparison of Zooplankton in the Ballast Tank vs. the Filter Skid

In a preliminary experiment to determine if collecting zooplankton (copepods) using the filter skid killed them, the percentage of living, ambient copepods was quantified after they were pumped into a ballast tank at NRLKW, and that number was compared to the percentage of living zooplankton collected in the filter bags in the skid as the tank was drained. The ballast tank was filled with 219 m^3 (57,775 gal) of ambient seawater at a flow rate of 3834 l min^{-1} (1013 gpm), and immediately afterwards, a vertical plankton tow was taken in the tank using a 25 µm mesh, 0.75 m mouth diameter, nylon plankton net. Originally, a quantitative sample was to be collected, but when the Niskin bottle used to collect the sample broke, a qualitative plankton tow was taken instead. The net was rinsed with ambient seawater and its contents suspended in filtered seawater (0.22 µm) and diluted 10x with filtered seawater. After the container was sealed and gently inverted 3x, 2 subsamples were removed and evaluated in Bogorov counting chambers. The number of dead copepods (nauplii; copepodites and adults) was counted, the samples fixed in Lugol‘s iodine solution, and the number of living copepods determined by subtraction.

The water was held in the ballast tank for two hours (as the net tow was analyzed) and then drained at flow rate of 3834 l min^{-1} (1013 gpm), with 65 m^3 (17226 gal) diverted to flow through the filter skid at a rate of 454 l min^{-1} (120 gpm), with 114 l min^{-1} (30 gpm) through each of the 8 housings containing filter bags (all flows are approximate averages). The first two filter housings in the filter skid were empty (except for the metal liners with 3 mm holes); the 8 housings downstream each held a 25-µm filter bag with the seams glued twice on the inside with 3M® 5200 sealant. After the drain operation, filter bags were removed from the housings, rinsed with ambient seawater, and their filtrands consolidated into a 2 l graduated cylinder. The cylinder was gently inverted 5x, and, using 10 ml serological pipettes, three researchers each

immediately and simultaneously took a 10 ml sample from the center of the cylinder and dispensed their samples into a 50 ml centrifuge tube. The inversion was repeated, and the researchers took a 5-ml sample and placed it into their centrifuge tube, bringing each tube‘s volume to 15 ml. This process was undertaken to ensure subsamples were representative of the sample in the 2 l graduated cylinder. Due to the high concentrations of plankton, sediment, and debris, it was necessary to dilute the samples 10x using filtered seawater before they were analyzed in the same manner as the samples from the plankton tow.

3.4.4 Effect of Crowding on Ambient Zooplankton

The effect of crowding during sampling was evaluated by comparing (1) a sample simulating a parcel of water 60 m^3 meeting the zooplankton discharge standard (10 living organisms m^{-3}) and concentrated during sampling to a volume of 1 l (= 600 zooplankters l^{-1}) to (2) a control sample with a much lower concentration of zooplankton (10 l^{-1}). Six hundred or ten copepods (*Acartia tonsa*, purchased from LiveCopepods.com, Seattle, WA; salinity = 33.5), were manually counted and added to a beaker containing 1 l of artificial seawater (salinity = 34). Beakers were placed in an incubator set at 25°C and illuminated by fluorescent bulbs (72 µM Einsteins m^{-2} s^{-1}), gently aerated, and incubated for 4 h. Previous work at NRLKW has shown die-off occurs in samples held longer than six h (Riley et al., 2006), so the zooplankton handling time and incubation time was a total of 6 h. After incubation, the copepods were recovered from the beakers using a 50-µm sieve. The dead *A. tonsa* were counted. Next, the samples were fixed with Lugol‘s iodine solution, the total number of copepods counted, and the number of living copepods was determined by subtraction. Because the sample handling time was too long to prepare and analyze replicate samples in a single day, the experiment was repeated the following day. In that trial, only 572 *Acartia tonsa* were available, so 28 brine shrimp (*Artemia franciscana*) were added to bring the treatment beaker‘s total zooplankton count to 600. Because no *A. franciscana* were added to the control sample, the survival was determined only for *A. tonsa* in both treatments.

4 Results

4.1 Microbeads

4.1.1 Microbead Disintegration

The counts of microbeads (10 50 µm microbeads and 10 150 µm microbeads) at the start of the trial and 7 days later were the same in both the filtered seawater and the Type II water (data not shown). Therefore, microbeads did not disintegrate on the time scales used for trials.

4.1.2 Method of Microbead Counting

The nine trials comparing ‗dry‘ to ‗wet‘ counting of microbeads showed the wet method to be superior (Figure 2). In only one of five trials using the dry method, microbead recovery was ≥ 90%; however, in all four trials using the wet method, microbead recovery was ≥ 90%. No bias

was attributed to any researchers, as all three researchers achieved both good and poor recovery. Henceforth, microbeads were counted only in the wet manner.

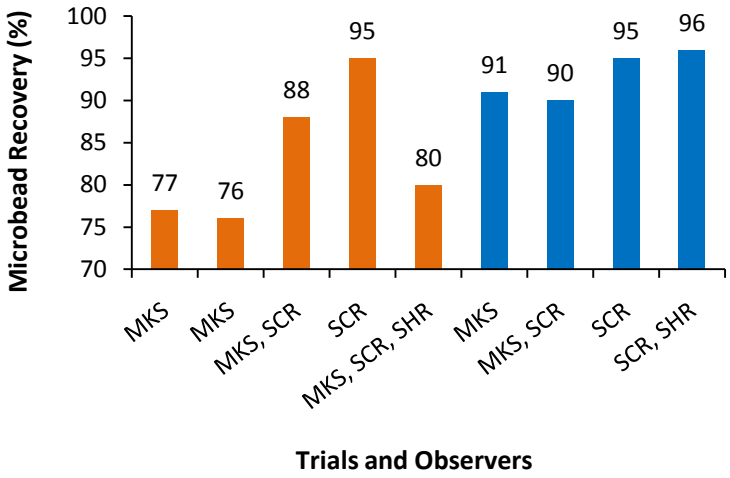

Figure 2. Recovery after microbeads were counted using the _dry‘ and _wet‘ methods. Orange bars represent dry trials, and blue bars represent wet trials; numbers above bars indicate the percent microbead recovery; letters on the x-axis labels indicate the researcher‘s initials.

4.1.3 Intercalibration

When 18 Bogorov trays with a seawater sample were counted by two observers to determine the difference from the mean of the counts, the grand mean was 12% (SD = 14%; data not shown). When one observer counted the same tray with a seawater sample five times, the number of microbeads counted varied over three-fold: 5, 5, 4, 2, 7 microbeads.

The intercalibation showed good agreement between observers, but because different counts resulted when a sample with seawater (and corresponding debris) was counted repeatedly, it was necessary to conduct validation trials—in both the laboratory and the field—using freshwater, so microbeads would be visible.

4.2 Filter Bags

4.2.1 Filter Bag Field Trials—Water Flow Rate

The filter bags clogged immediately in the first trials, which were conducted at relatively high flow rates (e.g., 150 gpm; Appendix 2). Subsequent trials showed a flow rate of 25 gpm allowed sustained flow with low pressure differentials across filter bags having relatively small mesh sizes (50 µm or 25 µm). In subsequent trials, the flow rate was set at 25 gpm. With the

Hayward units, a 5 cm (2”) hose supplied water to the system; at 25 gpm, water flowing through the pipe would travel at 0.78 m s^{-1} (1.7 mph).

4.2.2 Filter Bags—Field and Laboratory Validation Using Microbeads

Recovery efficiencies of 50 µm microbeads in the laboratory ranged from 41% - 84% when the microbeads were counted in the wet fashion and the filter bags‘ seams were unglued or glued insufficiently with Marine Goop® (Figure 3). Usually, no microbeads were found in the filtrate; on one occasion, 7 microbeads were found (100 were added to the filter bag; recovery efficiencies exclude microbeads found in the filtrate). Recovery of the 50-µm microbeads generally improved after the microbeads were counted in the wet fashion and the seams were glued more thoroughly. When 3M 5200® was used, recovery efficiency of the smallest microbeads ranged from 82% - 110% (mean = 94%). The larger two size classes showed excellent recovery efficiencies, at least 95%, with one exception (87%, Trial 21). A laboratory trial using 50 µm microbeads in a 50 µm filter bag with seams glued twice on the inside with 3M 5200® sealant yielded a 20% recovery efficiency (data excluded from Figure 3).

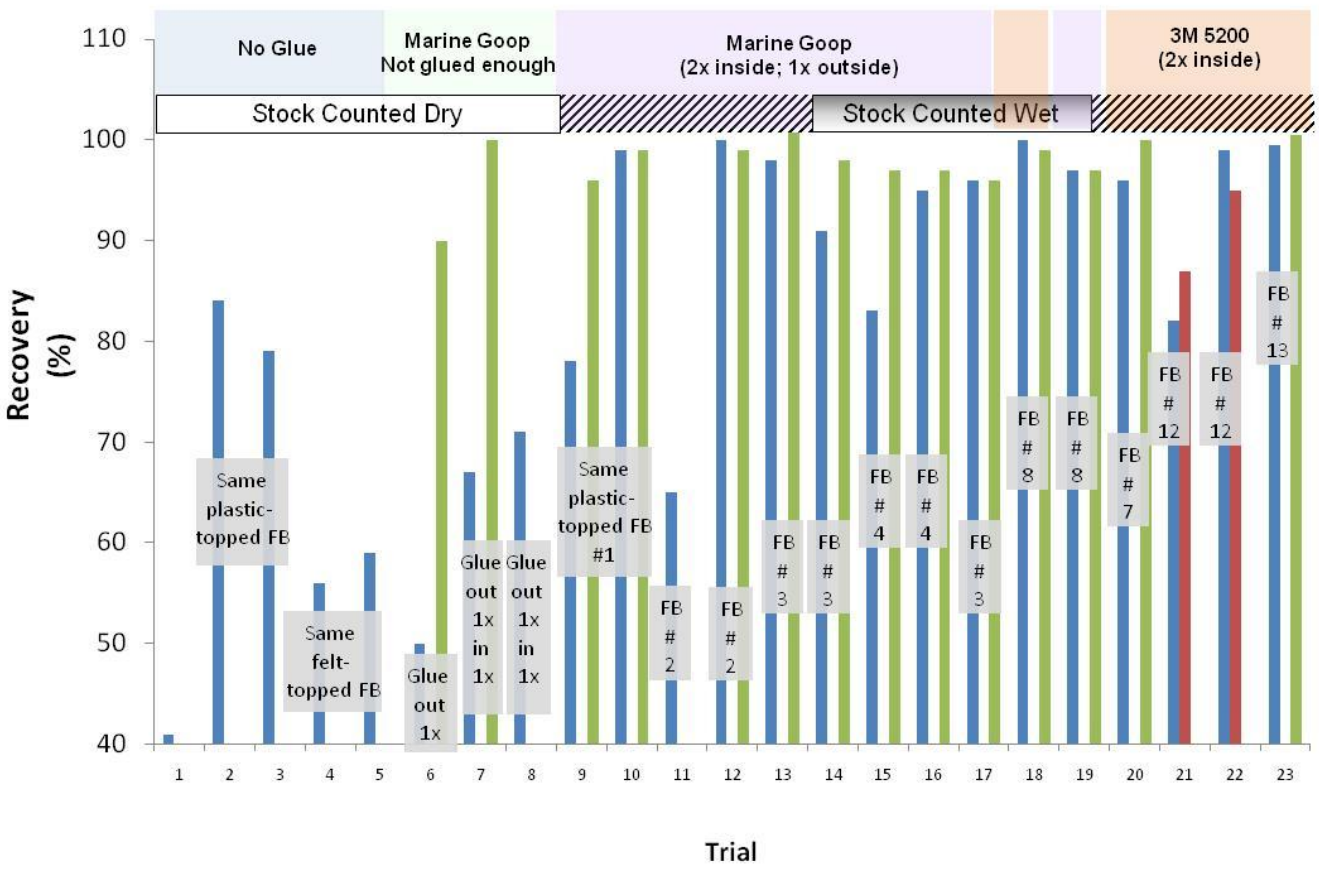

Figure 3. Recovery efficiency of microbeads in laboratory trials using 25 µm filter bags (FB). Blue, red, and green bars represent 50 µm, 100 µm, and 150 µm microbeads, respectively.

Although the tank that acted as a freshwater reservoir for field trials was cleaned beforehand, a small amount of green algae from the tank and the piping in the system was collected and concentrated in the filter bags (Figure 4, Trial 1; all trials except the first one had a 25-µm filter bag placed upstream to filter material from the water). During sample analysis, removing algae from one Bogorov counting chamber with many microbeads and re-counting it led to a 20% increase in the number of microbeads recovered (Trial 7). The little bit of algae in the samples, it seems, obscured the 50 µm microbeads, making recoveries appear to be low even though microbeads were likely captured in the filter bags. The tank was rigorously scrubbed to remove algae, and the experiment was repeated with 200 50 µm and 200 150 µm microbeads. The results were better: 93% and 100% recovery efficiency (50 µm and 150 µm microbeads, respectively); there was noticeably less algae in the sample than in previous experiments. Subsequent trials with very clean tanks showed recovery efficiencies > 87% of 50 µm microbeads. Recovery efficiency of larger (100 µm and 150 µm) microbeads prior to trial 7 ranged from 74% to 101% (the latter represents a counting error or a microbead leftover from a previous trial, although filter bags were examined for residual microbeads on a dissecting microscope prior to each trial). Following Trial 7, efficiencies were > 99%. In all trials, nearly all of the microbeads were recovered from the filter bag to which they had been added; in one instance, 13 microbeads were found in the filter bag downstream of it.

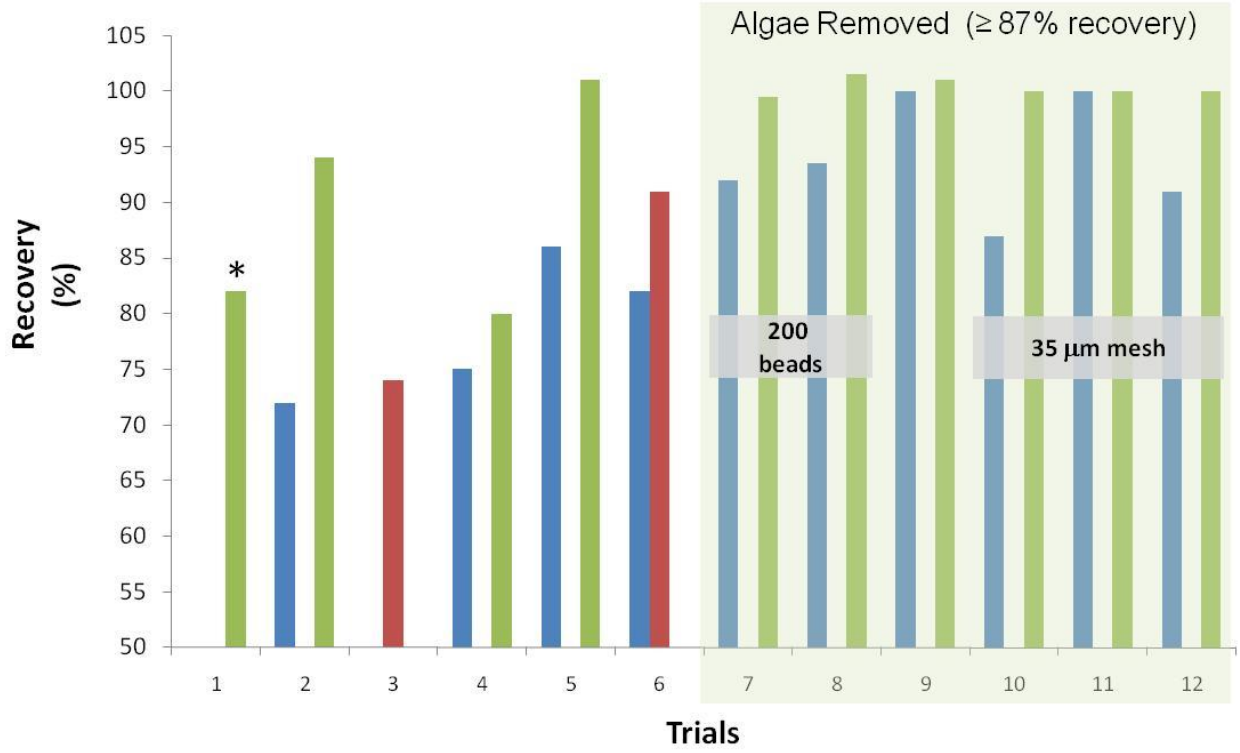

Figure 4. Recovery efficiency of microbeads in field trials. Unless noted, filter bags‘ mesh was 25 µm. Blue, red, and green bars represent 50 µm, 100 µm, and 150 µm microbeads, respectively. * = a notable amount of green algae was collected in filter bags.

Because microbead recovery efficiencies increased after improvements were made to the protocol for trials in the laboratory (i.e., counting using the wet method and sealing the filter bags‘ seams) and the field (i.e., removing algae from the reservoirs used to hold freshwater), the data collected after improvements were compiled to give a true picture of microbead recovery (Figure 5). With the exception of the laboratory trial using 50 µm microbeads in a 50-µm filter bag, the average recovery efficiency was ≥ 89%.

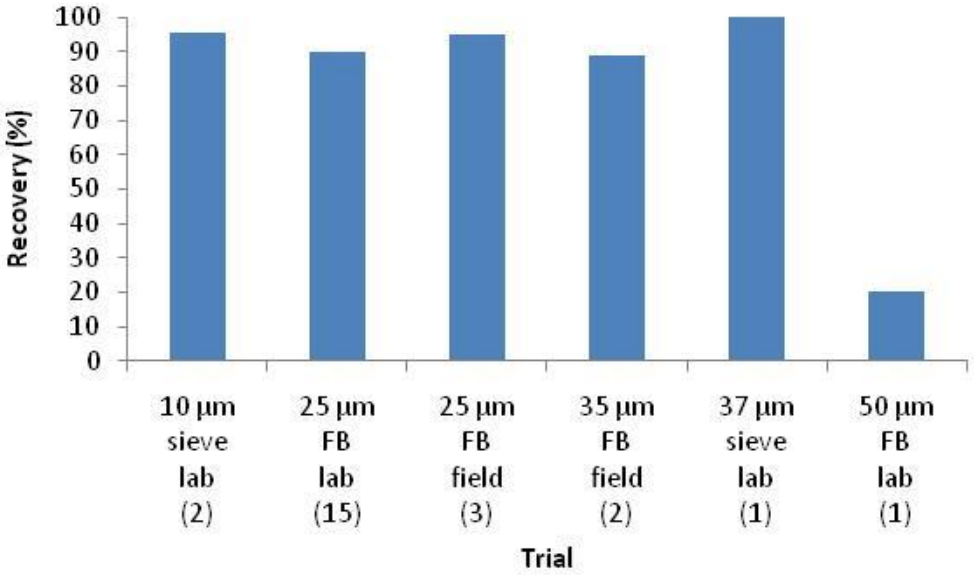

Figure 5. Recovery efficiency of 50 µm microbeads in laboratory and field trials conducted after improvements were made to the microbead protocol. Numbers in parentheses represent replicates; FB = filter bag.

4.2.3 Filter Bags—Seams

Of the seven sealants examined, Marine Goop® appeared best for sealing the filter bags‘ seams because it was the most pliable but not sticky. After being used in field tests, however, the clear sealant began to flake apart, allowing microbeads to become trapped in the sealant and potentially uncovering the relatively large holes along the bags‘ seams. 3M™ 5200 was re-evaluated, and after using it in a field trial and noticing no breakdown, it was used in all subsequent experiments. Like Marine Goop, it was also pliable but not sticky, and it was white, so red microbeads were visible along the seams.

4.2.4 Filter Bags—Toxicity of Sealant on the Seams

There was no apparent, negative effect of the 3M™ 5200 sealant on *Artemia franciscana* or ambient copepods. In both trials, in all replicates, the percentage of living organisms was at least

90% (Appendix 3). In the *Artemia franciscana* experiment, because the data were not distributed normally, a Mann-Whitney Rank Sum Tests was used, and no significant difference was found ($p = 0.49$). In the copepod experiment, a t-test showed no significant difference between treatment and control groups ($p = 0.54$). When all of the *A. franciscana* results (from both control and treatment groups) were averaged into a grand mean and compared to the grand mean of the copepod results, the numbers were nearly identical (98.3% and 98.1%, respectively).

4.3 Filter Skid

4.3.1 Filter Skid Design

After investigating commercially available filtration devices, the Eaton Topline™ Type II housing (Eaton Filtration, LLC, Iselin, NJ) was chosen. To capture zooplankton effectively in large volumes of water, the filter skid was designed with 10 cylindrical filter housings, each containing a metal filter basket with holes 3 mm (0.12") in diameter (Figure 6; schematic in Appendix 4 and operations manual in Appendix 5). As water entered the skid, it was split to flow into one of two prefilters, which contained no filter bags, but the metal filter baskets served as prefilters to collect large organisms, sediment, and rust flakes from the ballast tank. Following each prefilter, water flow was split again, into two filter housings, thus evenly distributing the water flow between the housings and reducing the filtration load for each filter by a factor of four. Each of the four housings was followed by another filter housing, which was intended to capture anything organisms that may have gone through the first set of housings. All filter housings, excluding the prefilters, contained a filter bag designed to capture all organisms greater than 50-µm minimum dimension in each experiment. A key feature of the Eaton design is the tight seal among the filter bag, housing, and housing cover.

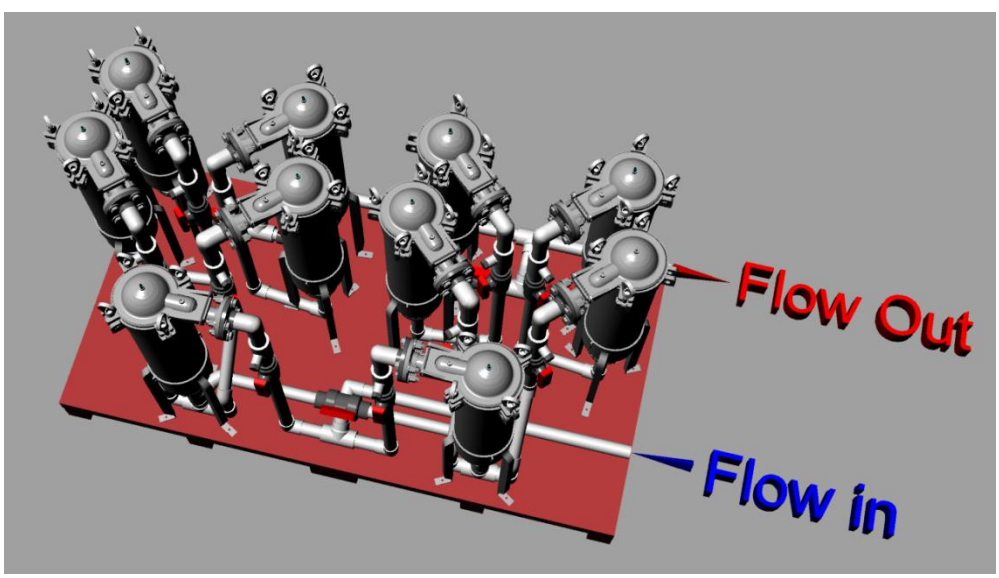

Figure 6. Filter skid design for flow-through sampling of discharged ballast water.

4.3.1.1 Filter Housings

The cylindrical Eaton Topline™ bag filter housings, constructed of corrosion-resistant 316 stainless steel, had dimensions of 18 cm (7”) diameter x 91 cm (36”) long (Figure 7; drawing created using SolidWorks 2010 3D Design Software, Concord, MA). The height and orientation of the housings could be varied using their support legs.

Figure 7. Eaton Topline™ filter housing.

Water entered the side of the housing through a 5 cm (2”) flanged connection and flowed over the top of the filter bag, sealing it in place. This design resulted in a minimal volume of unfiltered liquid (i.e., between the housing‘s lid and the top of the filter bag) and provided optimum sealing of the filter bag so a minimal amount of water bypassed the filter bag. Although each of the Eaton Topline™ filter housings are manufactured for a maximum flow rate of 681 l m^{-1} (180 gpm) and 10,500 g cm^{-2} (150 pounds per square inch, psi) maximum pressure, the filter skid was designed so each of the housings would realize approximately 95 l min^{-1} (25 gpm) during normal operation (see section 3.3.1 for rationale). Additionally, the manufacturer‘s operation instructions indicated low-flow conditions lead to good filtration results. By splitting the flow four ways, it was possible to slow velocity through the skid by a factor of four.

A main component of the filter skid is the filter bags that were snapped into place in the eight filter housings after the two prefilters. The material of the filter bags was chosen to (1) mimic the filtration efficiency of plankton nets, which are commonly used by test facilities, and (2) capture organisms and allow them to be gently removed from the filter bag surface without increasing mortality. Felt, microfiber, and monofilament meshes were considered, and nylon monofilament filter bags best met the criteria (Eaton part number BNMO35P2P, Figure 8; the filter bag‘s polypropylene ring is snapped into place at the top of the filter housing. The 3 mm holes in the metal liner are visible through the filter bag‘s mesh).

Figure 8. Nylon monofilament mesh filter bag secured in an Eaton Topline™ filter housing.

4.3.1.2 Piping, Valves, and Diaphragm Pump

All piping between the filter housings was 5 cm (2”) diameter, Schedule 40, PVC (Spears® Manufacturing Company, Sylmar, CA), with glued fittings (Figure 9). Piping was connected to the filter housings using 5 cm (2”) diameter 68 kg (150 lb) Van Stone flanges with ethylene propylene diene Monomer (M-class) rubber (EPDM) gaskets.

Figure 9. Plan view of the prototype filter skid at NRLKW. Blue arrow shows water entering the filter skid; yellow arrow shows water exiting the skid.

To allow filter housings to be individually isolated, 5 cm (2”) diameter valves were fitted to the inlet and outlet of each filter housing (Spears® Manufacturing Company, model Compact 2000 ball valves). Because all isolation valves were left in the 100% open position during operation, they did not add shear forces to the organisms during sampling.

At the base of every filter housing, a threaded 1.3 cm (0.5”) diameter PVC ball valve (Spears® Manufacturing Company) was installed to serve as a drain valve. The valves remained closed during filtration operations but were opened at the end of a run to drain the filter housings from the bottom. In this manner, filter bags were removed from the housings without the bottoms of the filter bags floating upwards and potentially losing part of the sample.

A threaded, 0.64 cm (0.25”) diameter PVC ball valve was installed atop of each filter housing, and it was used to manually bleed air trapped within the housings prior to use. This important safety feature prevented air from being trapped within filter housings and potentially building to several times greater than atmospheric pressure. This feature allowed the system operator to know when the filter housings were full of water (i.e., water exits the valves), thereby ensuring the filter bags were wet prior to sampling.

A Yamada NDP-80 8 cm (3”) diaphragm pump (Yamada® America, Inc., Arlington Heights, IL) was installed to draw a sample from the discharge line exiting the ballast tank into the filter skid (Figure 10). The pump was needed because the discharge line was under suction (as water was pumped to a discharge tank for filtration prior to discharge into ambient waters); therefore, to collect a representative sample of the line, a diaphragm pump was been installed. It was constructed of polypropylene with an EPDM rubber diaphragm in contact with the water passing through the pump. Previous testing by engineers at NRLKW on various pump types on organism mortality has shown diaphragm pumps to be the superior choice (Riley et al., 2009). Although it was capable of flowing at a rate of 220 gpm, the pump was set at 100 gpm and operated by compressed air supplied from a portable Sullair® 185SCFM air compressor (Michigan City, IN). A time-averaged sample was removed from the discharge line from the ballast tank to the line into the filter skid using a tee; an isokinetic sample port was not necessary, as the sample volume was greater than 10% of the tank volume (i.e., 60 m^3 of 200 m^3; Richard et al., 2008).

Figure 10. Yamada NDP-80 Air diaphragm pump as installed.

4.3.1.3 Pressure Drop Calculations vs. Flow Rate

Maintaining a low pressure drop across a filter bag is critical because it reduces the chances organisms are killed during sampling, and it enhances filtration efficiency and increases the service life of the filter bag, thus reducing the operating cost of the system. During the design phase, the filter skid was analyzed to determine where the bulk of the friction losses occurred. Data for the filter housings and filter bags were available from the manufacturer (Figure 11). The analysis was completed using data from 25 µm filter bags, since data for 35 µm nylon monofilament filter bags were unavailable. As expected, the pressure drop increased as the flow rate increased. At a flow rate of 95 l min^{-1} (25 gpm), the pressure drop across an individual housing with a 25 µm nylon monofilament bag was slight, approximately 35 g cm^{-2} (0.5 psi). It is anticipated the pressure drop would be a bit smaller using a 35-µm filter bag.

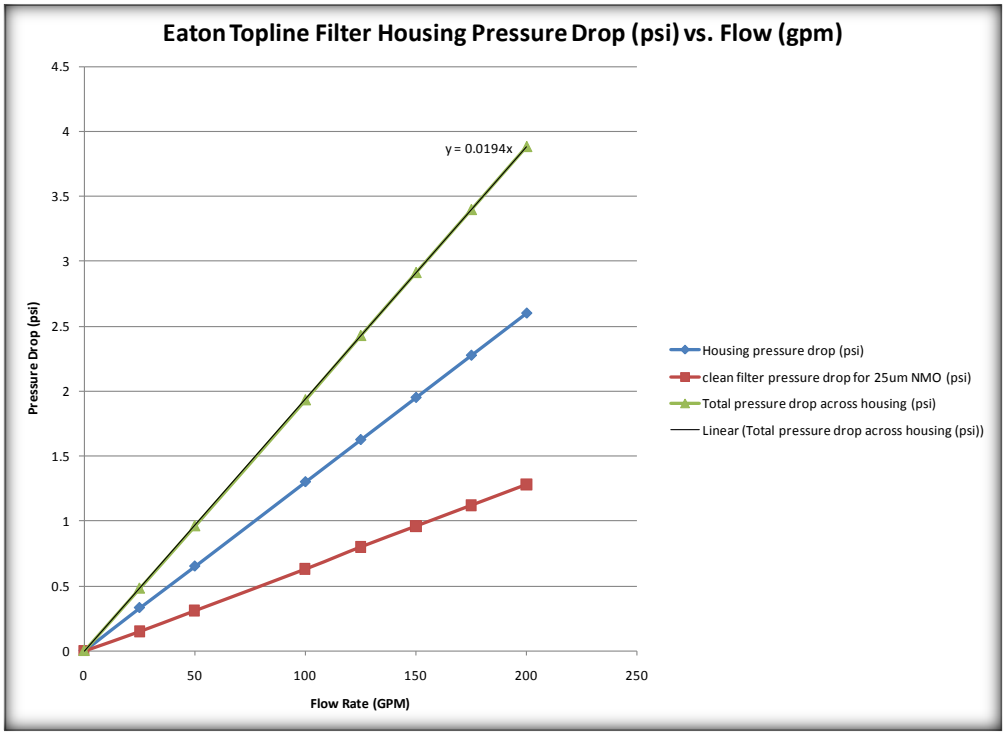

Figure 11. Pressure drop of filter skid vs. flow rate (figure from Eaton Filtration, LLC).

Because the skid was designed with a small footprint, there were several elbows and fittings, as well as flow direction changes, which contributed to the pressure drop across the skid. When all pressure changes were calculated, the pressure drop across the entire skid was 631 g cm^{-2} (9 psi) at 379 l min^{-1} (100 gpm; calculations not shown).

4.3.1.4 Flow Rate, Flow Control, and Volume Measurement through the Filter Skid

The flow rate was monitored using a GF Signet 2551 Magmeter, which was situated on the inlet line to the skid using a saddle fitting. The total volume sampled was calculated from a totalizer installed downstream of the skid that used the electrical signal generated by the Magmeter to calculate the total volume by multiplying the flow rate by time and summing it over the entire test run Because the sampling was a continuous operation, rather than a batch process, the totalizer was used to sum the flow rate. Flow was controlled through the filter skid using a linear, pneumatic-actuated, 7 cm (3”) diameter diaphragm valve. In turn, valve position was controlled using Proportional, Integral, Derivative (PID) feedback control via Honeywell controls logic based on the Magmeter reading to maintain a flow of 100 gpm through the skid.

4.3.1.5 Cost and Weight of the Filter Skid

When the cost of the materials and labor to build the filter skid was tabulated, the total cost of the prototype was $19,851, and it took approximately 60 hours to construct it (Appendix 6).

The skid was analyzed to determine its weight with and without water. Using the respective weight of each of the skid‘s components from the manufacturer‘s literature, the weight of the skid without water was estimated to be 228 kg (503 lb); when seawater is contained in all of the housings and the piping system, the total weight of the skid was an estimated 556 kg (1225 lb).

4.3.2 Filtration Area of Filter Skid vs. Plankton Net

The effective surface area of the filter skid was compared to that of typical plankton net. The effective surface area of a single filter bag within the filter skid was 0.43 m^2 (calculations not shown). Because the sample flow was split into four filter housings in parallel, the effective surface area is four times the surface area of one housing, or 1.70 m^2. The surface area could easily be increased by increasing the number of filter housings arranged in parallel.

In the past, a Sea-Gear Model 9000 Plankton Net with a mouth opening of 60 cm and a length to mouth ratio of 3:1 was used at NRLKW (Sea-Gear Corporation, Melbourne, FL). Its surface area was 1.72 m^2, nearly identical to the filter skid (calculations not shown).

4.3.3 Flow Velocity through the Filter Skid vs. Plankton Net

The filter skid was designed to obtain discharge water samples at a volumetric water flow rate of 100 gpm, and the flow through the skid was plumbed to split into a set of pre-filters at a volumetric flow rate of 50 gpm. After the two pre-filters, the flow split again to flow into a set of four filter housings that contained 35µm filter bags to capture organisms ≥ 50 µm. The volumetric flow rate to these four housings was 25 gpm. The flow velocity of the water as it flows through the pre-filters and filters was calculated and compared to velocities encountered in theoretical, horizontal plankton tows using plankton nets.

4.3.3.1 Flow Velocity through Two Prefilters (No Filter Bags)

Water was supplied to the filter housings through a 5 cm (2”) inner diameter PVC pipe. The flow velocity of the water entering the pre-filter housings is 1.55 m s^{-1} (3.5 mph) (Appendix 7, Equation 1).

As the water enters the filter housing, the flow velocity is reduced because the cross sectional area increases from the 5 cm (2”) pipe to the 18 cm (7”) housing (Figure 12; model created using SolidWorks). Correspondingly, the flow velocity decreases from 1.55 m s^{-1} (3.47 mph) to 0.51 m s^{-1} (1.13 mph; Appendix 7 Equation 2). As the water leaves the housing, the flow velocity returns to 1.55 m s^{-1} (3.47 mph).

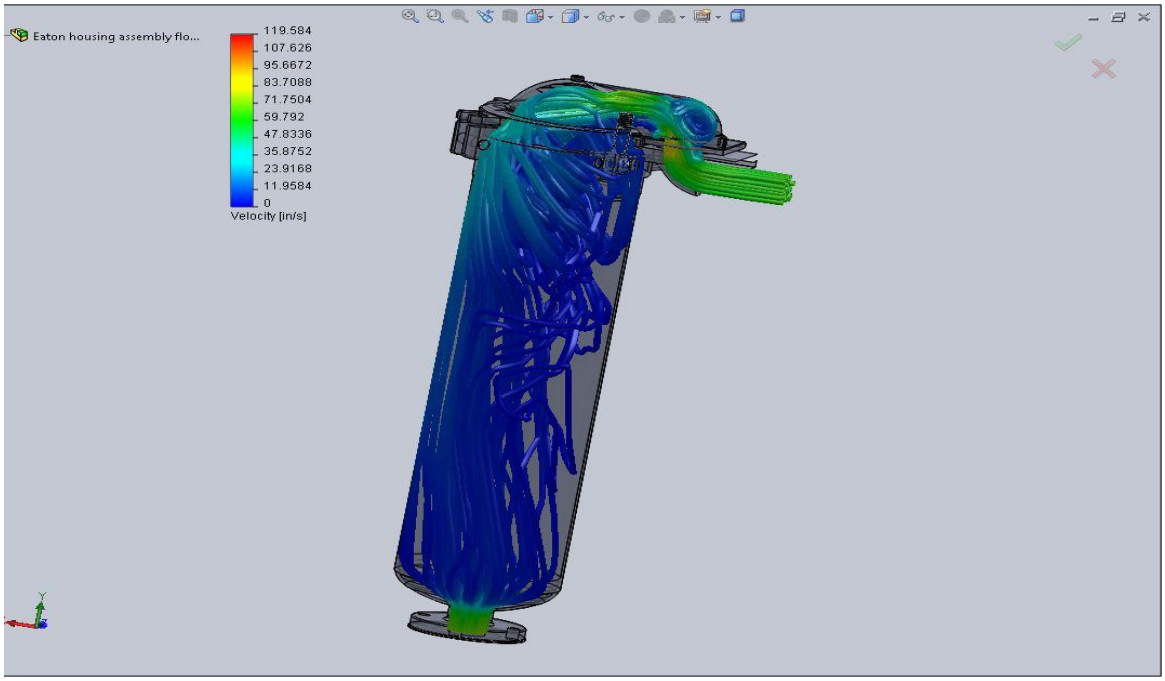

Figure 12. Flow velocity model for water flow through an Eaton Topline™ Filter Housing at 50 gpm, entering though the top of the housing and exiting at the bottom.

4.3.3.2 Flow Velocity through Housings with Filter Bags

Downstream of the pre-filters, the water flow split to enter the next four housings, which contained filter bags. The volumetric flow rate to each of these filter housings is 25 gpm. The flow velocity entering these filters is 0.77 m s^{-1} (1.7 mph; Appendix 7, Equation).

As seen in the first set of pre-filters, as the water enters into the housing, the flow velocity decreases because the cross sectional area increases from 5 cm to 18 cm (2" to 7", Figure 13). Here, the flow velocity slows from 0.77 m s^{-1} (1.72 mph) to 0.25 m s^{-1} (0.56 mph; the velocity of the water when contacting the filter bags; Appendix 7, Equation 4). As the water leaves the housing, it returns to 0.77 m s^{-1}.

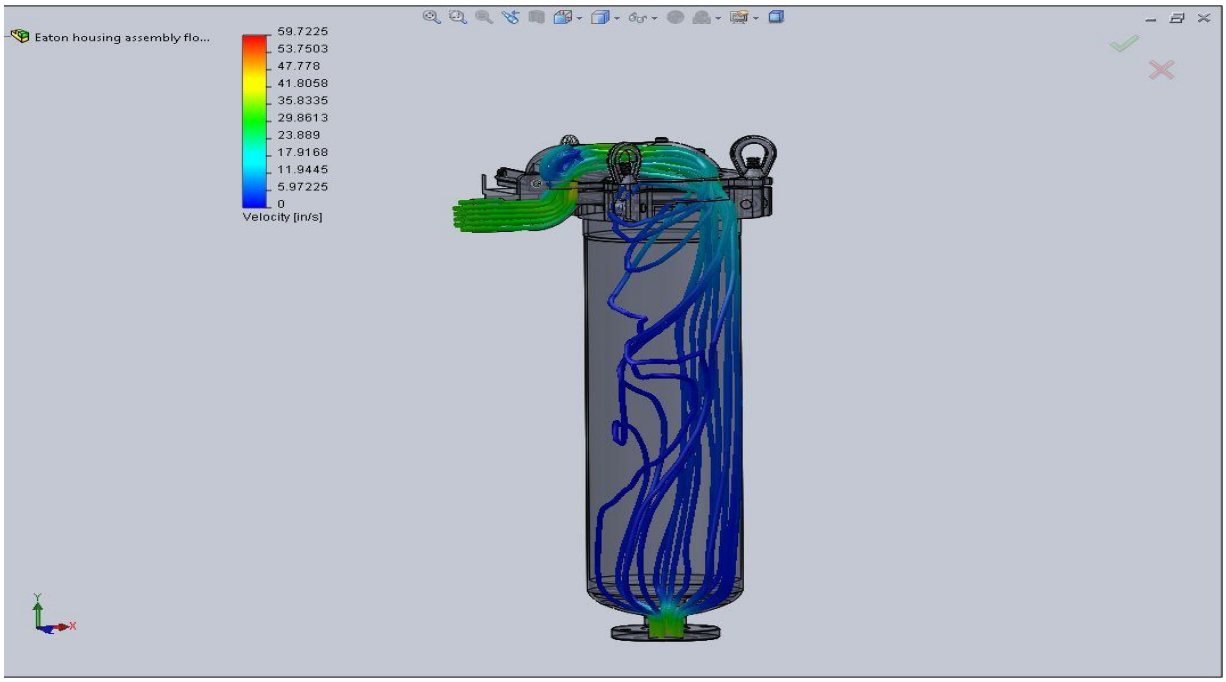

Figure 13. Flow velocity model illustration for water flow through an Eaton Topline™ Filter Housing at 25 gpm, entering though the top of the housing and exiting at the bottom.

4.3.3.3 Flow Velocity through a Plankton Net

The flow velocity of water entering a plankton net as it is towed behind a boat was calculated. For this example, it was assumed the velocity of the boat during sampling was 0.51 - 3.1 m s^{-1} (1 - 6 kts; e.g., Aron, 1965), the mouth diameter of the net was 1 m, and the exit diameter near the cod end was 10 cm. Because of its conical shape, the net tends to concentrate organisms strained from the water sample in the cod end and uses surface filtration to separate organisms and particles ≥ 35 µm. The entrance velocities of the water entering the plankton net for speeds 1 kt, 2 kts, and 6 kts are 0.51 m s^{-1}, 1.03 m s^{-1}, and 3.1 m s^{-1}, respectively. The velocity of water entering the filtration housings in the filter skid (0.77 m s^{-1}, Appendix 7, Equation) is similar to a horizontal plankton tow between 0.51 m s^{-1} - 1.03 m s^{-1} (1 - 2 kts).

4.3.4 Toxic Effects of Stainless Steel Filter Housings

Stainless steel did not induce mortality on 12-hour old *Artemia franciscana* nauplii or ambient zooplankton (≥ 50µm) over a 2.5 h exposure time, as there was no significant difference between the percentage of living organism in the control and treatment groups (Appendix 8; t-test p = 0.59 for *A. franciscana*, p = 0.33 for zooplankton). Although the grand mean from all *A. franciscana* measurements (both control and treatment groups) was greater than the grand mean

from all ambient zooplankton (99.1% vs. 87.8%), there was no statistical difference between them (Mann-Whitney Rank Sum Test, $p = 0.15$).

4.3.5 Comparison of Zooplankton in the Ballast Tank vs. the Filter Skid

A sample collected using a plankton net tow from a ballast tank holding 200 m^3 of ambient seawater was compared to a time-averaged, 60-m^3 sample collected as the tank was drained. The percentage of living zooplankton was nearly identical (Appendix 9). The mean percentage of living copepod nauplii was 98.3% and 97.8% (plankton tow and filter skid, respectively), and for copepod adults and copepodites, it was 84.2% and 83.7% (plankton tow and filter skid, respectively).

4.3.6 Effect of Crowding on Ambient Zooplankton

When approximately 600 copepods (*Acartia tonsa*) or 10 copepods were added to 1 l of artificial seawater and incubated for 4h, the percentage of living copepods was > 90% in each treatment for both trials: 98.0% and 100% (600, 10 *A. tonsa*) and 97.5% and 90% (600, 10 *A.tonsa*; data not shown).

5 Discussion

A filter skid was successfully designed to sample large volumes of water to determine if its zooplankton concentration meets a ballast water discharge standard of < 10 organisms m^{-3}. Using off-the-shelf components, it was built to meet the requirements of having a low water-flow rate, self-contained nets, and a small footprint. Calculations of the filtration surface area and flow velocity show the skid is comparable to a standard plankton net, and the number of filter housings in the skid could be increased to allow more surface area. The trade off, of course, is a greater handling time of the sample, as more filter bags would require rinsing.

Initial trials using zooplankton proxies (microbeads 50 µm in diameter) to validate filter bags‘ efficiencies revealed issues that were addressed: the method for counting microbeads was improved, the filter bags‘ seams were sealed to prevent microbeads from slipping through the holes, and the small amount of algae found in holding tanks used in freshwater field trials was removed to ensure all microbeads were visible within samples. The latter point illustrates the importance of employing fluorescent stains or movement or both to quantify zooplankton. Otherwise, zooplankton may be undercounted when small organisms are obscured by dead organisms, sediment, and debris in samples. Although not addressed in this study, the same is true of the protist (≥ 10 µm and < 50 µm) size class. In the end, microbeads approximating the lower end of the zooplankton size class could be recovered from filter bags in field and laboratory trials with good efficiency, ≥ 87%.

To address concerns that the sealant used to fuse the filter bags‘ seams and the stainless steel of the filter housings may be toxic to plankton as they are sequestered (albeit for a short time) in the

filter bags within the housings, short-term toxicity tests were conducted. Both toxicity tests exposed the test organism *Artemia franciscana* and ambient zooplankters to the potential toxicant. No immediate or apparent negative effect on the crustaceans was evident. In each instance, no significant difference between treatment or control groups was detected.

Validating nets‘ or filters‘ retention efficiencies is, as far as we know, an uncommon practice, both in oceanographic research in general, and in ballast water treatment testing specifically. Regarding the latter case, it seems especially relevant given the potential for fines to be levied when ballast water management systems exceed a discharge standard. Microbeads are not a perfect proxy for living organisms, which may be squeezed through a net more readily than polystyrene spheres. The microbeads do, however, represent a good metric, as they can be purchased at the lower size class (e.g., with a diameter of 50 µm) and have no spines or setae to cling to a net or filter. A logical—and necessary—next step is to compare retention efficiencies of natural aquatic communities between the filter skid and plankton nets. Those trials are underway at NRLKW.

6 Acknowledgements

William _BJ‘ Kinee and Barron Stringham helped construct the filter skid and run field trials, and we are grateful for their assistance. We appreciate and acknowledge the support of the US Coast Guard Environmental Standards Division (CG-5224) for funding this research.

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Appendix 1

Data from initial field trials conducted at NRLKW comparing microbead recovery efficiency between a plankton net and filter bags arranged in series.

Type of Filtration	**Parameter**			
	Mesh size	**Dimensions**	**Flow rate (gpm)**	**Number of 50 µm-diameter microbeads recovered[a]**
Plankton net	35 µm net with two filter bags in series as prefilters: 50 µm (first in series) and 25 µm (second)[b]	99 cm (39”) diameter at the top and 162 cm (64”) long	100	150/600 (25%)
Filter bags (2 in series)	100 µm (first) and 25 µm mesh (second)	18 cm (7”) diameter at the top and 41 (16”) cm long	25	168/600 (28%)

[a]The 600 microbeads, in Type II water in a beaker, were gently poured into the cod cup of the plankton net or the first filter bag in series. Afterwards, the beaker was examined for residual microbeads, and in both cases, 0 were found.

[b]A previous trial conducted with ambient seawater pumped through the plankton net with no prefilters in place yielded a sample so loaded with debris and sediment that it would have taken days to analyze all of it. In the 8% of sample analyzed, 1 microbead (of 600 added) was found. All subsequent trials used prefilters with ambient seawater (as was the case for data in this table) or freshwater.

Appendix 2

Results of preliminary trials to determine the appropriate flow rate through the filter bags to allow maximum flow and minimum clogging.

Mesh in first bag in series (µm)*	**Mesh in second bag in series (µm)**	**Flow rate ($l\ min^{-1}$) (gpm)**	**Duration of the run (min)**	**Outcome**
25	25	568 (150)	9	The bags clogged and water flow nearly stopped almost immediately; a total of 87 gal (330 l) flowed
50	50	474 (125)	46	The bags clogged, pressure differential between filter housings increased (P1 = 5.3 psi, P2 = 19.1, P3 = 59.4 at the end of the run), and the flow rate slowed to 62.1 gpm; 3312 gal (12,550 l) flowed
100	25	95 (25)	64	The pressure differential remained slight (P1 = 64.8 psi, P2 = 63.9, P3 = 64.3 at 53 min); 1485 gal (5627 l) flowed with little clogging of bags
50	25	95 (25)	80	The pressure differential remained slight (P1 = 65.2 psi, P2 = 63.9, P3 = 64.3 at 70 min); 1460 gal (5533 l) flowed with little clogging of bags
25	25	95 (25)	72	The pressure differential remained slight (P1 = 64.5, P2 = 63.2, P3 = 63.3); 1833 gal (6946 l) flowed with little clogging of bags

*All experiments were conducted using Hayward Filtration units with 41-cm long filter bags.

Appendix 3

Data from toxicity tests evaluating the effect of 3M™ 5200 sealant on brine shrimp *Artemia franciscana* (top table) and ambient zooplankton (bottom table) after two hours of exposure time.

Replicate	**Live Af**	**Total Af**	**% living Af**
C1*	78	79	98.7
C2	60	60	100.0
C3	43	43	100.0
C4	48	48	100.0
T1	49	54	90.7
T2	56	56	100.0
T3	50	50	100.0
T4	66	68	97.1

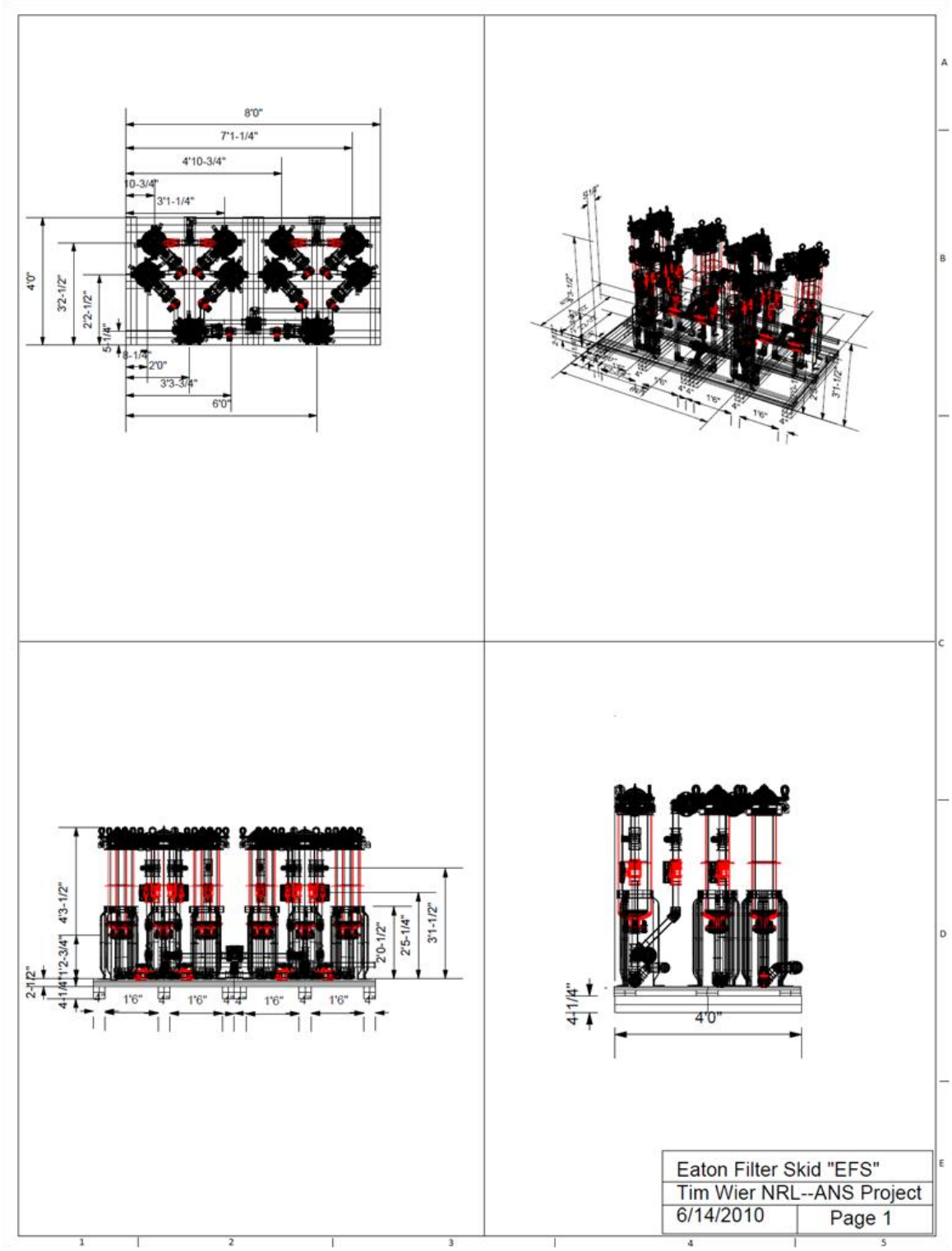
*C = control group, T = treatment group, Af = *Artemia franciscana*, cultured brine shrimp.

Replicate	**Live copepods (≥ 50 µm)**	**Total copepods (≥ 50 µm)**	**% living copepods**
C1*	20	20	100.0
C2	19	20	95.0
C3	19	20	95.0
C4	20	20	100.0
T1	20	20	100.0
T2	19	20	95.0
T3	19	19	100.0
T4	20	20	100.0

*C = control group, T = treatment group

Appendix 4

Eaton Filter Skid drawing with dimensions.



Appendix 5

Eaton Filter Skid operations manual.

These instructions are specific to operations using the Eaton Filter Skid (EFS, shown in Figure 1) and performed at the Naval Research Laboratory ballast water treatment test facility (BWTTF) in Key West, Florida. The procedures typically used in trials with the EFS are broadly categorized into preparation, operation, and shutdown.

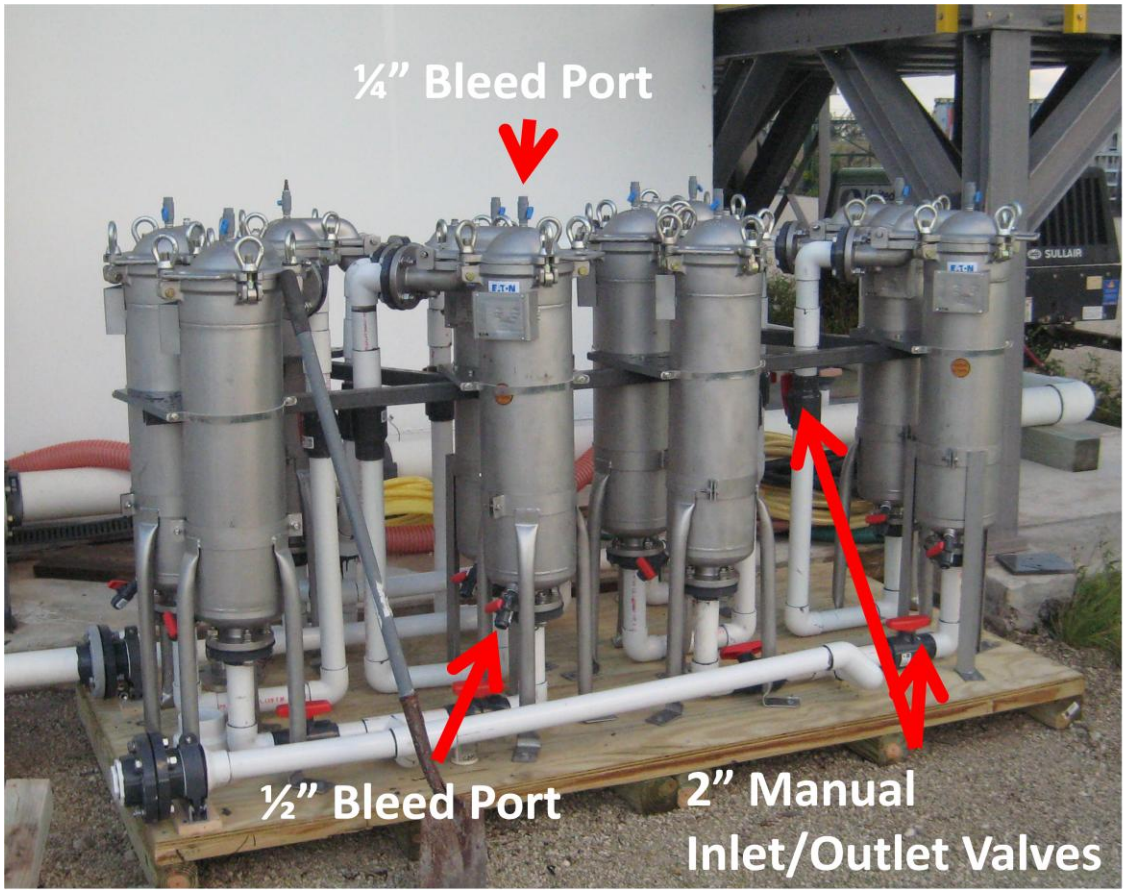


Figure 1. Eaton Filter Skid showing air bleed and drain ports and housing manual inlet and outlet ball valves.

Only a trained system operator shall perform operations specified in this document; improper setup or operation can jeopardize personnel safety or damage equipment.

Preparation

1. Verify there is sufficient capacity in the discharge tank to receive the entire volume of water contained in the ballast tank.
2. Isolate the EFS by closing the manual 3" butterfly inlet and outlet valves (Figure 2, Valves 510 and 404, respectively), which are upstream and downstream of the EFS.

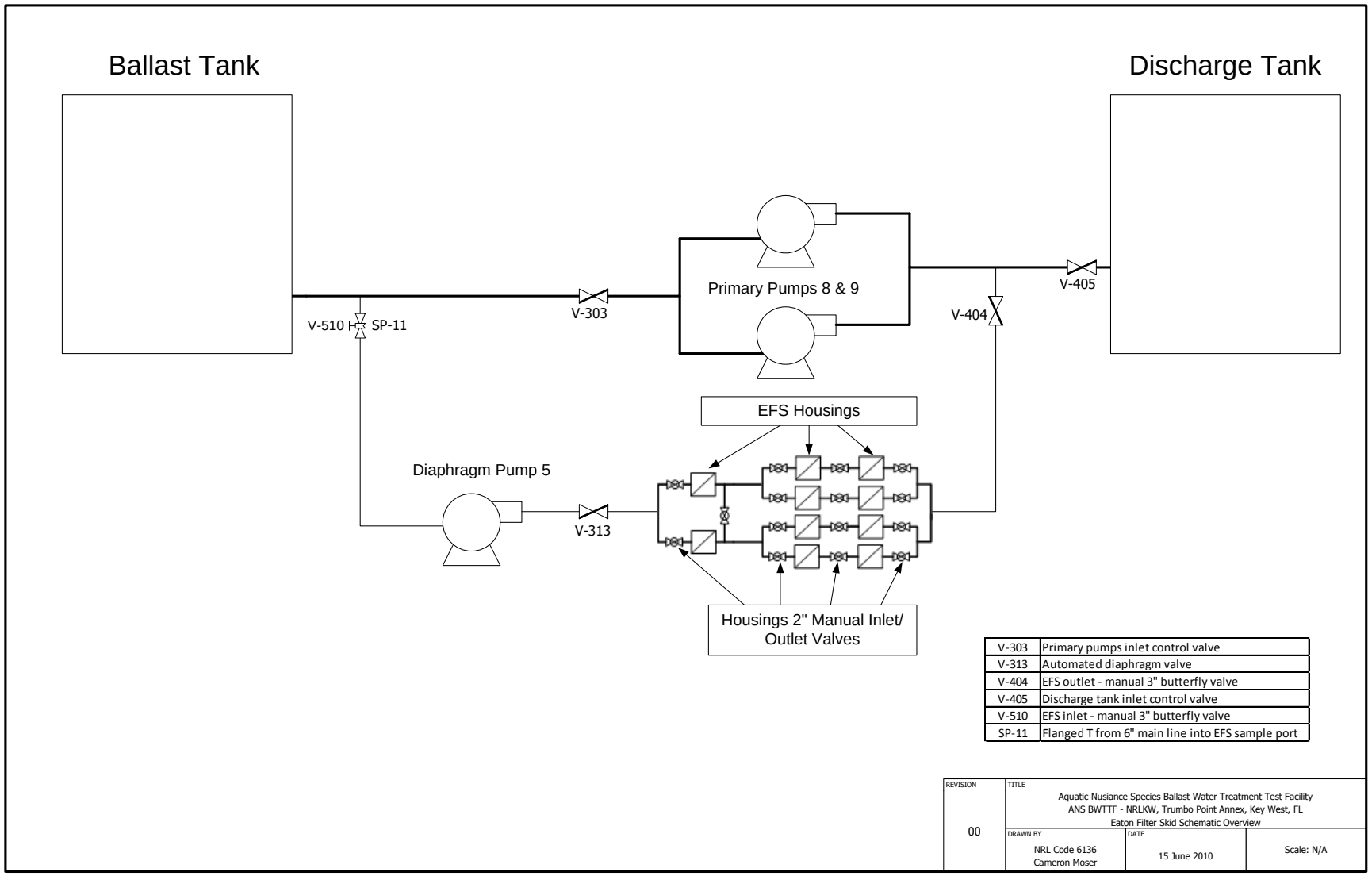


Figure 2. Eaton Filter Skid piping and instrumentation diagram.

3. Close 1/2” drain port valves on each of the individual Eaton Topline™ filter housings at their posterior ends (Figure 1 and Figure 3, balloon 5).

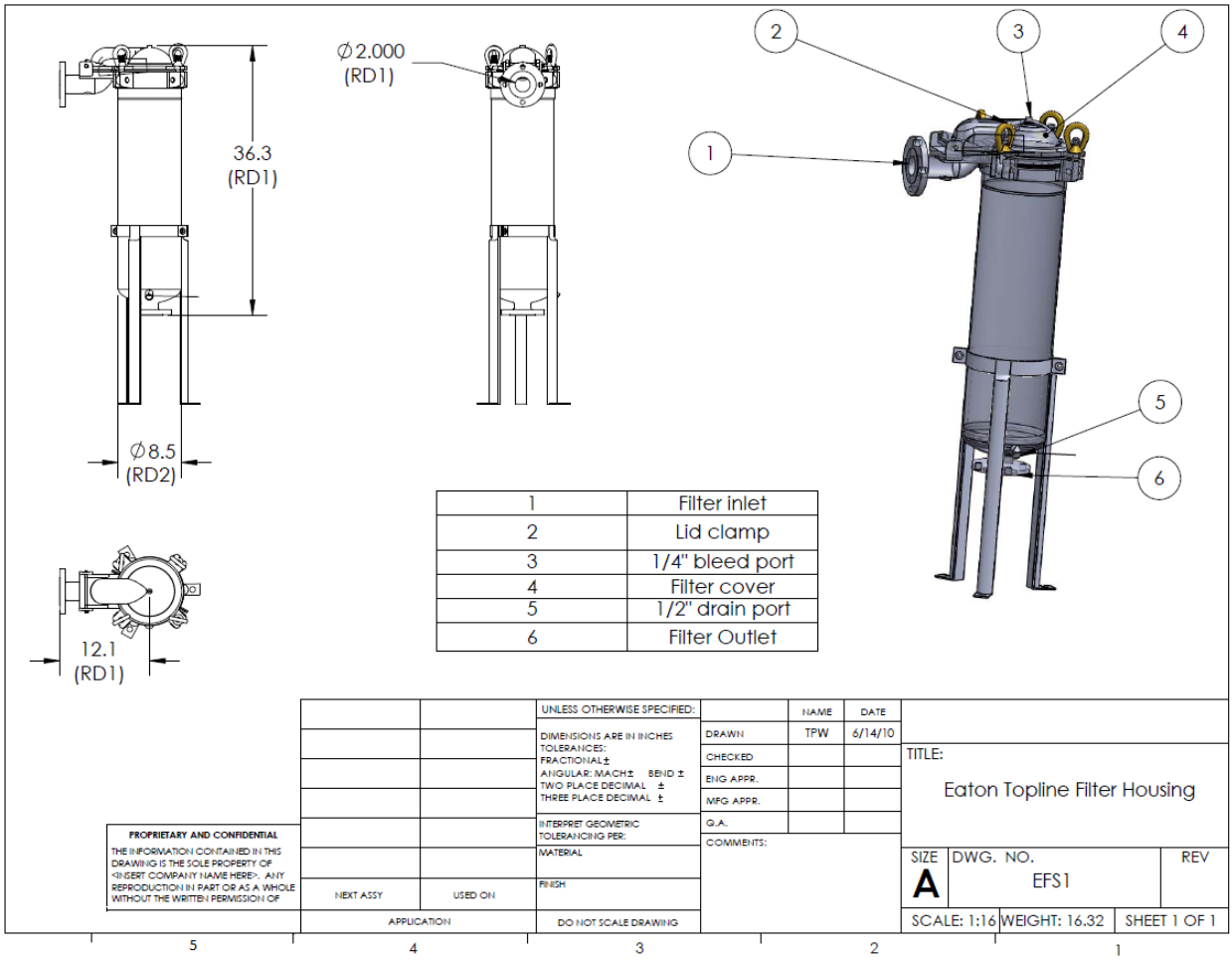


Figure 3. Eaton Topline™ Filter Housing used in the Eaton Filter Skid.

4. Open each housing by unscrewing the four lid clamps at the anterior end and lifting the hinged lid by its handle (Figure 1 or Figure 3, balloon 2).
5. Install a filter bag in each of the filter housings (Figure 4). The filter bag will slide into the housing, and the top plastic ring of the filter bag should seat into the top of the housing. Take care when installing the filter bags to prevent their ripping on sharp edges during installation.
6. Fill each of the housings from the top using a hose with running seawater. Filter the seawater by placing the end of the hose in a 25-µm mesh filter bag and directing the water that passes through the filter bag into all filter housings.
7. Close each of the individual filter housings by ensuring O-rings are in the proper position, which is determined by ensuring each O-ring lies flat in its designated circular slot (Figure 4). Shut the lid and re-tighten the four lid clamps.

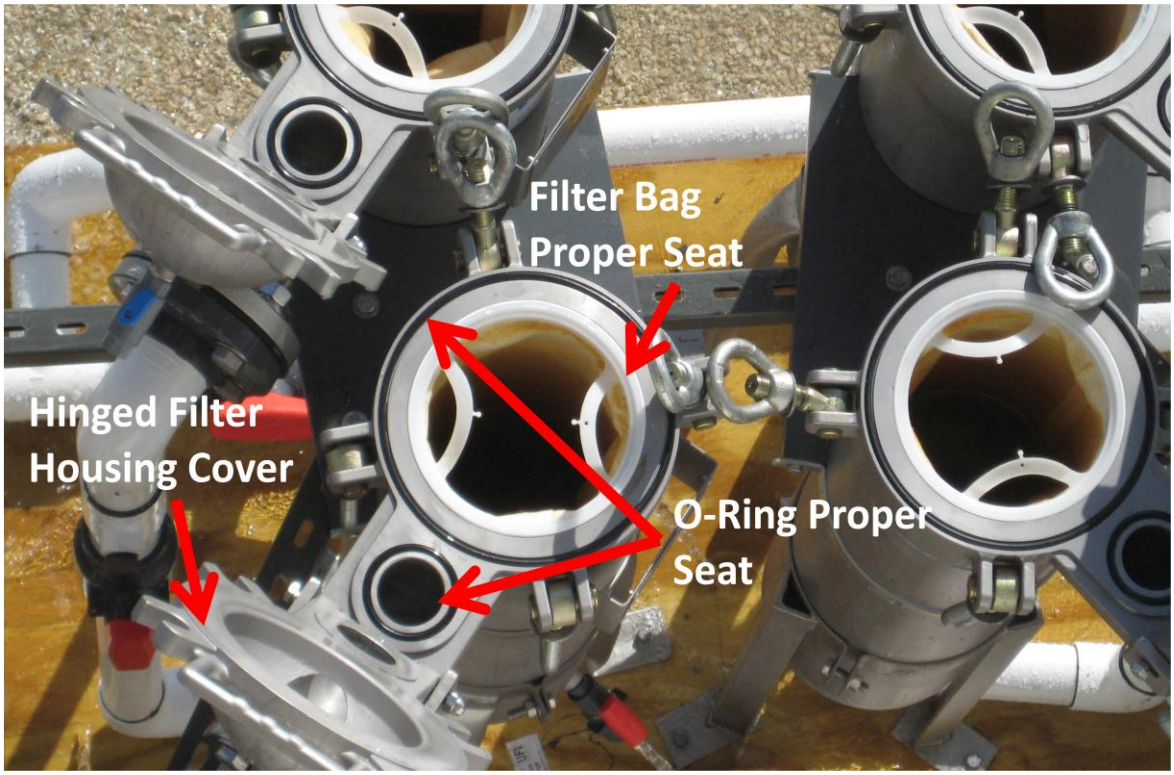


Figure 4. Filter housing with cover open and filter bag installed; O-rings are properly seated.

8. Open ¼" bleed port valves at the anterior end of each housing (Figure 3, balloon 3).
9. Open all manual and control valves to create a flow path from the ballast tank to the discharge tank with an exception of the EFS and discharge tank inlets, which are to remain *closed*.
10. The EFS draws flow from sample port 11(Figure 2, SP-11) which connects to the main 6" ballast tank discharge line by a T fitting. Slowly open the EFS inlet valve to approximately 50% until water under head pressure from the ballast tank generates a steady stream flowing from the open ¼" filter housing bleed ports, indicating a full prime is achieved.
    a. At this point, if a housing lid is not properly sealed, a leak (due to a loose lid clamp or unaligned O-ring) will be apparent. This situation must be remedied by closing the EFS inlet valve, isolating the leaking housings at their individual inlets and outlets by closing 2" valves (Figures 1 and 2), and then correcting the issue.
    b. Re-open housing inlet and outlet valves then repeat step 8.
11. Throttle back the manual EFS inlet valve until the flow in step 10 is reduced to a minimum, then close all bleed port valves.
12. Open the EFS inlet valve completely. Pressure from the ballast tank will equalize throughout the system upstream of the discharge tank inlet valve such that opening it would invoke flow, i.e., this valve should be the last obstacle preventing head pressure from the ballast tank from causing a gravity-induced flow into the discharge tank.
13. Check the fuel level for portable air compressor to ensure there is an ample amount for the desired run time.

14. Hook up the ¾" air hose from the diaphragm pump to the portable air compressor. The ¾" air hose has Chicago-type twist-lock fittings. These fittings should align and mate correctly.
15. The diaphragm pump, powered from high-pressure air generated by the compressor, feeds into and is controlled binarily using an electrically actuated solenoid valve located toward the top end of the pump. Ensure the power plug from the solenoid valve is plugged into a power receptacle near the diaphragm pump.
16. Start the compressor and then open the corresponding manual valves that feed air to the diaphragm pump (Figure 2, Diaphragm Pump).
17. At the BWTTF in Key West, a Honeywell Plantscape Control and Automation system is integrated such that all switches, sensors, and machinery send inputs and receive outputs from a programmable logic controller that can be monitored, operated, and controlled from a human-machine interface (HMI) in a centralized control room. Using an identical or similar system is required to enter proper parameters for desired pressure and ballast tank discharge and EFS sampling flow set points into control operation input fields in the HMI. Depending on the location of the controlling flow meter, the set point should be adjusted to account for sampling flow to achieve total desired flow, e.g., if the primary pumps are controlled using a flow meter upstream from where sampling flow rejoins main flow; the set point should be the total designed flow minus the sampling flow.

Operation

1. Partially open the inlet control valve to the discharge tank (Figure 2, Valve 405) using the HMI; verify that flow is initialized from the ballast tank to the discharge tank.

   Note: If little or no flow observed, an issue needs further investigation by the system operator. Causes may include valves set in an improper position, an inoperable flow meter, or a higher level of water in the discharge tank than the ballast tank.
2. Begin the diaphragm pump and sampling automation from the HMI by clicking ‗Start' under the appropriate control operations. Pumps and valves will actuate to achieve desired set points.
3. Continually monitor EFS pressure and flow to ensure filter bags do not clog; pressure should not exceed 30 pounds per square inch.

Shutdown

1. As head pressure from the ballast tank decreases, the primary pumps (Figure 2, Pumps 8 and 9) will start to overpower the suction from the diaphragm pump drawing water from the sample port (Figure 2, SP-11). This situation must be prevented by closely monitoring EFS flow as the ballast tank level becomes low (relative to the total flow and tank dimensions, i.e., approximately 10 minutes remaining at the current flow rate and remaining volume) until it suddenly begins to decrease dramatically. At this point:
   a. Shut down the primary pumps (Figure 2).
   b. Close the primary pumps' inlet control valve (Figure 2, Valve 303) to isolate all flow through EFS.
   c. Open the control valve to the discharge tank inlet to 100% (Figure 2, Valve 405).
   d. Normal flow through EFS should be restored and stable.

2. Depending on tank dimensions, flow will continue for a period until it again begins to decrease drastically. At this point:
    a. Close the automated diaphragm valve (Figure 2, Valve 313) at the outlet of the diaphragm pump.
    b. Immediately shut down the diaphragm pump.
    c. Immediately isolate EFS from the system by closing the six 2" ball valves at the farthest upstream and downstream filter inlets and outlets (Figure 5).

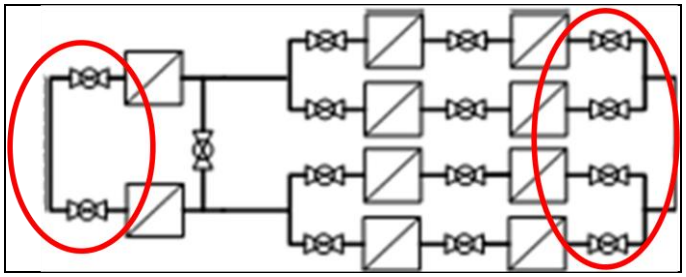


Figure 5. Ball valves (in red circles) closed immediately after the diaphragm pump is shut down.

3. Isolate the remaining housings by closing all 2" ball valves.
4. Release pressure at the posterior end 1/2" drain valves such that flow channels through the filters and out through the drain valves.
5. Once pressure releases, open the ¼" bleed valves at the top of the housings. The remaining water will drain onto the ground until no more water exits the housings.
6. Open the lid of each housing by unscrewing lid clamps and lifting at the handle.

The filter bags are ready for extraction.

Appendix 6

Eaton Filter Skid bill of materials and calculation of weight.

	Equipment	Quantity	Cost	Total Cost
Filter Housing	Eaton 316SS Topline™ Housing	10	$1,707.00	$17,070.00
Filter Bags	Filter Specialist Incorporated 35µm size 2 NMO 35 with plastic snap rings	10	$20.43	$204.30
PVC Piping and Fittings	2" Spears van stone flange socket style	28	$7.59	$212.52
	PVC/EPDM COMPACT B VLV 2" S	14	$50.51	$707.14
	2" schedule 40 socket Tees	10	$1.50	$15.00
	2" x 2" x 3/4" reducing TEE Soc x Soc x FIPT schedule 40	20	$2.50	$49.95
	2" Soc x Soc schedule 40 ELL	30	$1.22	$36.45
	3/4" plug	20	$0.52	$10.35
	PVC/EPDM COMPACT B VLV 1/2" S	12	$14.18	$170.10
	3" Spears van stone flange socket style	10	$12.95	$129.54
	3" Soc x Soc schedule 40 ELL	10	$4.42	$44.18
	Low-Pressure PVC Ball Valve 1/2" NPT Female, White (Same as 4876K11)	10	$5.96	$59.60
	Thk-Wall Dk Gray PVC Thrd-One-End Pipe Nipple 1/2" Pipe Size, 2" Length, Schedule 80	20	$1.21	$24.20

	Equipment	Quantity	Cost	Total Cost
Wood Skid	4x8 treated plywood 3/4"	2	$32.00	$64.00
	2x6 treated 8' board	3	$5.50	$16.50
	4x4 treated 8' board	2	$9.50	$19.00
	Wood Screws	40	$0.10	$4.00
Stainless hardware	5/8-11x3" 316 stainless steel hex cap screw partially threaded	100	$4.32	$432.00
	5/8-11 316 Stainless steel hex cap nut	22	$8.00	$176.00
	Type 316 SS Type A SAE Flat Washer 5/8" Screw Size, 1-5/16" OD, .07"-.13" Thick, Packs of 10	20	$8.54	$170.80
	Type 316 Stainless Steel Split Lock Washer 5/8" Screw Size, 1.08" OD, .15" min Thick, Packs of 10	12	$5.37	$64.44
	Type 316 Stainless Steel Hex Head Cap Screw 5/8"-11 Thread, 3-1/4" Length, Packs of 1	10	$4.69	$46.90
Cam-Lock Fittings	Aluminum Cam-and-Groove Hose Coupling Plug, PFA Adapter, 3 Coupling Size, 3" Pipe Size	2	$62.30	$124.60
Cost of Equipment Components				**$19,851.57**
Labor	Hours to build skid per design drawing	80	$150.00	$12,000.00
Total Discharge Skid Cost				**$19,851.57**

Appendix 7

Calculations of flow velocity in the filter skid and during a hypothetical plankton tow.

Equation 1. Flow velocity calculation for 50 gpm in a 5 cm (2") diameter pipe.

$$Flow\ Velocity\ \left(\frac{ft}{\sec}\right) = \frac{Volumetric\ Flow\ Rate}{Cross\ Sectional\ Area} = \frac{(50\ (\frac{gal}{min}) * 0.133\ (\frac{ft^3}{gal}) * 144(\frac{in^2}{ft^2})}{\left(60\left(\frac{sec}{min}\right) * \pi * \left(\frac{2in}{2}\right)^2\right)}$$

$$= 5.08\left(\frac{ft}{sec}\right) * 0.3048\ \left(\frac{m}{ft}\right) = \mathbf{1.55}\ \left(\frac{\boldsymbol{m}}{\boldsymbol{sec}}\right)$$

Equation 2. Flow velocity calculation for 50 gpm in an 18 cm (7") diameter filter housing.

$$Flow\ Velocity\ \left(\frac{ft}{\sec}\right) = \frac{Volumetric\ Flow\ Rate}{Cross\ Sectional\ Area} = \frac{(50\ (\frac{gal}{min}) * 0.133\ (\frac{ft^3}{gal}) * 144(\frac{in^2}{ft^2})}{\left(60\left(\frac{sec}{min}\right) * \pi * \left(\frac{3.5in}{2}\right)^2\right)}$$

$$= 1.66\left(\frac{ft}{sec}\right) * 0.3048\ \left(\frac{m}{ft}\right) = \mathbf{0.51}\ \left(\frac{\boldsymbol{m}}{\boldsymbol{sec}}\right)$$

Equation 3. Flow velocity calculation for 25-gpm flow in a 5 cm (2") diameter pipe.

$$Flow\ Velocity\ \left(\frac{ft}{\sec}\right) = \frac{Volumetric\ Flow\ Rate}{Cross\ Sectional\ Area} = \frac{(25\ (\frac{gal}{min}) * 0.133\ (\frac{ft^3}{gal}) * 144(\frac{in^2}{ft^2})}{(60(\frac{sec}{min}) * \pi * (\frac{2in}{2})^2)}$$

$$= 2.54\left(\frac{ft}{sec}\right) * 0.3048\ \left(\frac{m}{ft}\right) = 0.77\ (\frac{m}{sec})$$

Equation 4. Flow velocity calculation for 25-gpm flow in an 18 cm (7") diameter filter housing.

$$Flow\ Velocity\ \left(\frac{ft}{\sec}\right) = \frac{Volumetric\ Flow\ Rate}{Cross\ Sectional\ Area} = \frac{(25\ (\frac{gal}{min}) * 0.133\ (\frac{ft^3}{gal}) * 144(\frac{in^2}{ft^2})}{(60(\frac{sec}{min}) * \pi * (\frac{3.5in}{2})^2)}$$

$$= .83\left(\frac{ft}{sec}\right) * 0.3048\ \left(\frac{m}{ft}\right) = \mathbf{0.25}\ (\frac{\boldsymbol{m}}{\boldsymbol{sec}})$$

Appendix 8

Results of toxicity tests evaluating the effect of stainless steel on brine shrimp *Artemia franciscana* and ambient zooplankton for 2.5 h of exposure.

Replicate	Live Af	Total Af	% living Af	Live zoopl (≥ 50 µm)	Total zoopl (≥ 50 µm)	% living zoopl
C1*	264	265	99.6	34	38	89.5
C2	262	267	98.1	15	20	75.0
T1	353	354	99.7	34	38	89.5
T2	229	232	98.7	28	33	84.9
T3	348	350	99.4	30	30	100

* C = control group, T = treatment group, Af = *Artemia franciscana*; zoopl = ambient zooplankton ≥ 50 µm.

Appendix 9

Results of the comparison between living copepods in the ballast tank and the filter skid.
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Replicate	Live nauplii	Total nauplii	% living nauplii	Live A + C	Total A + C	% living A + C
PT1*	83	86	96.5	39	43	90.7
PT2	104	104	100	28	36	77.8
FS1	293	299	98	79	97	81.4
FS2	279	286	97.6	98	114	86

* PT = plankton tow; FS = filter skid; A + C = adult and copepodite stages. All copepods were ≥ 50 µm in minimum dimension.