

AN ABSTRACT OF THE THESIS OF

Nathan Anthony Krzyaniak for the degree of Master of Science in Radiation Health Physics presented on August 21, 2013.

Title: Quantification of Dry Concentration Factor for Cs-134 in Marine Diatom *Thalassiosira weissflogii*.

Abstract approved:

Kathryn A. Higley

The Fukushima Daiichi nuclear disaster of 2011 made it apparent that releases of fission products to marine environments is a very real possibility. Additional data on the movement of material through marine environments can be used by radioecology personnel to assist with both ecosystem protection as well as environmental cleanup planning. Batches of the marine diatom *Thalassiosira weissflogii* were grown in a laboratory environment for the purpose of quantifying the dry concentration factor for ^{134}Cs . Samples were grown in f/2 enriched seawater under 12 hour light/dark cycles at 22°C, spiked with approximately 200 Bq/ml of ^{134}Cs and uptake was monitored over a twelve day period. It was expected that uptake would have been slightly higher due to the higher temperature and resulting higher growth rates, but measurement indicated that there was negligible uptake over the monitored period. These results are in close agreement with the published ICRP and IAEA values for ^{134}Cs despite the different growth conditions and it appears as though ^{134}Cs uptake is not a metabolically-driven process in the phytoplankton studied.

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Quantification of Dry Concentration Factor for Cs-134 in Marine Diatom *Thalassiosira Weissflogii*

by
Nathan Anthony Krzyaniak

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Nathan Anthony Krzyaniak, Author

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The views expressed in this thesis are those of the author and do not reflect the official policy of position of the United States Air Force, Department of Defense, or the U.S. Government.

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1.0 BACKGROUND

1.1 OBJECTIVE

The objective of this study was to study the uptake of cesium-134 in *Thalassiosira weissflogii*, a species of radially symmetrical coastal diatom, over a twelve day period and attempt to quantify uptake in the form of a concentration factor. Secondary objectives included evaluation of the impact of temperature on uptake of cesium-134 as well as the viability of using glass fiber filters to measure cesium retention on phytoplankton.

1.2 FUKUSHIMA DAIICHI DISASTER

On 11 March 2011 the Great East Japan earthquake, the fifth highest magnitude earthquake ever recorded worldwide, occurred in the Pacific Ocean 130 km east of Japan (United States Geological Survey, 2011). The resulting tsunami struck and overcame the seawalls at the Fukushima Daiichi nuclear power plant situated on the east coast of Japan. The earthquake knocked out offsite power to the plant, and the tsunami eventually knocked out the backup generators located below ground. Core coolant systems ran on battery power for approximately three days following this prior to failure (Government of Japan, 2011). The water level inside the reactors later dropped below the top of the fuel rods, allowing the decay heat to damage the zirconium cladding causing a large amount of hydrogen gas to escape. This hydrogen gathered inside the containment buildings and resulted in three hydrogen explosions on the 12th, 14th, and 15th of March 2011 (Government of Japan, 2011).

The earthquake, tsunami, and hydrogen explosions at the Fukushima Daiichi nuclear power plant lead to both accidental and deliberate discharges of radioactive material to the environment. These discharges included the noble gas inventory of the reactor cores, as well as large amounts of radioactive iodine and cesium. Tokyo Electric Power Company (TEPCO) estimated as of 24 May 2012 that the airborne releases included approximately 5×10^{17} Bq of noble gas, 5×10^{17} Bq of iodine-131, and 1×10^{16} Bq each of cesium-134 and cesium-137; and that the aqueous releases included approximately 1.1×10^{16} Bq of iodine-131 and 3.5×10^{15} Bq each of cesium-134 and cesium-137 (Tokyo Electric Power Company, 2012).

Stohl et al estimated that 78% of the airborne releases were deposited over the Pacific Ocean (2012). Assuming homogenous distribution of the radionuclides listed above, this nets approximately

1.13×10^{16} Bq of cesium-134 or cesium-137 or 2.26×10^{16} Bq of total radioactive cesium that was deposited or released directly into the Pacific Ocean. The release from Chernobyl in 1986 is used as a fairly well-known frame of reference as the largest unintentional release from a nuclear power plant in history. A 1990 estimate of the Chernobyl source term placed the Fukushima aqueous cesium release at 17-41% of the total cesium released during the Chernobyl disaster, but the majority of the Chernobyl release was deposited on the land (IAEA TECDOC1240, 2001). Deposition of radioactive material on water did occur following the Chernobyl release, but at a much smaller scale.

1.3 IMPLICATIONS OF RADIONUCLIDE RELEASE TO THE ENVIRONMENT

Transport of radionuclides following any intentional or unintentional release to the environment is of interest to various stakeholders along the whole spectrum of emergency response: prevention, protection, mitigation, response, and recovery (US Department of Homeland Security, 2013). Transport parameters give planners an idea of what organisms are at risk, what crops are unsafe to eat, what habitable zones will take longer to recover, and can aid with the eventual cleanup.

1.4 BIOACCUMULATION PARAMETERS

The International Atomic Energy Agency (IAEA) has compiled tables of concentration factors (CF) that can be used to model the transport of nearly all radionuclides from the environment into various organisms (IAEA Technical Report Series 422, 2004). The concentration factor for an organism describes the concentration of a radionuclide one could expect to find based upon the concentration found in the organism's environment. For example, if the chlorine CF was 1,000 for some generic plant living in soil that contains 10 Bq/kg of chlorine-36, one would expect to find 10,000 Bq/kg of chlorine-36 in the organism once equilibrium was reached. The annual limit of intake for ingestion of chlorine-36 is 60 MBq, so for this example intake would be restricted to 6,000 kg prior to surpassing an occupational dose limit (Eckerman, Wolbarst, and Richardson, 1988). This plant may still be deemed safe to eat, or in restricted amounts. Depending on land-use scenarios and license conditions the area may need to be decontaminated.

The CF is defined as the equilibrium activity per unit mass of a nuclide in an organism divided by the activity per unit mass of a nuclide in the environment (soil, water, air) and describes the ratio of specific activity in the organism to the specific activity in the environment. The units generally work out to be

dimensionless (Bq/kg/Bq/kg), but it can also be defined by volume of the organism rather than mass (kg/L) or by volume of water (L/kg) for waterborne organisms. The IAEA Technical Report 422 contains tables of CF values for types of organisms and most nuclides, as well as references to the primary study that determined the CF. The table does include data gaps where experimental or in situ data was unavailable. Where data gaps exist it is sometimes possible and appropriate to make assumptions about the chemical behavior of the nuclide and use a chemical analog, e.g. K^+ in place of Cs^+ values, if K uptake is better understood.

1.5 LITERATURE REVIEW

The uptake of radioactive materials in marine primary producers such as plankton may be an important route of material concentrating up the food chain. The plankton community is an extremely large and diverse grouping of organisms with many subdivisions and unique features, but the roles they play in the food chain are all similar and vital. In general plankton are any organisms that exist in the water column and are incapable of controlling their own motion- wind, currents, and tides dictate their motion (Lalli and Parsons, 1997). Phytoplankton represents the typical base of the food chain in open water ecosystems, and typically live near the surface because they require light. They are the only class of plankton capable of undergoing photosynthesis, the conversion of sunlight and carbon dioxide into usable organic compounds. Zooplankton on the other hand consume phytoplankton for sustenance, and are incapable of photosynthesizing on their own. Fish and crustacean larvae may begin their lives as zooplankton prior to maturation into the adult organism, but that is typically not the case for phytoplankton; phytoplankton remain simple organisms that are used as foodstuffs for slightly larger organisms. A third subgroup of plankton is the bacterioplankton- the waterborne bacteria that drifts freely in the water column. These bacteria fill similar positions in the food web as phytoplankton, and can be responsible for breaking down organic matter in the water column, performing photosynthesis, and can also be preyed upon by zooplankton.

These three types of organisms represent the lowest trophic levels in the ocean, but not necessarily the smallest organism size. Phytoplankton may be of special importance because it is produced in bulk for aquaculture or commercial fish farming, much like those that exist in Japanese waters.

The IAEA published value for cesium uptake in marine phytoplankton is 20, and is based upon a single laboratory study by Heldal, Stupakoff, and Fisher (2001). In situ studies have been performed in various bodies of water around the world on other classes of plankton (Tateda and Koyanagi, 1994), but the literature is mostly lacking for phytoplankton. Additionally, the Heldal et al. (2001) study was performed under laboratory conditions at relatively low temperatures. There is evidence to suggest that more metabolically active population could be obtained at higher temperatures to help determine if cesium uptake is a metabolically-driven process (Halac, Villafañe and Helbling, 2010).

It is estimated from past conclusions that that uptake of cesium in phytoplankton will be low due to high concentrations of potassium in seawater and in phytoplankton, mitigating the impact of added cesium plumes to the environment (Heldal et al, 2001). The concentration of cesium used in this experiment, while relatively high in specific activity, was approximately six to eight orders of magnitude lower than existing concentrations of potassium (see Appendix A).

Higher up the food chain in some large fish an inverse relationship between cesium CF and K^+ concentration has been demonstrated in freshwater environments (Vanderploeg, Parzyck, Wilcox, Kercher, and Kaye, 1975). Potassium ion concentration in seawater is generally around 400 ppm and around 2 ppm in freshwater environments. Consequently, it is reasonable to assume that CFs for Cs-134 are significantly lower in marine environments than CFs in freshwater environments.

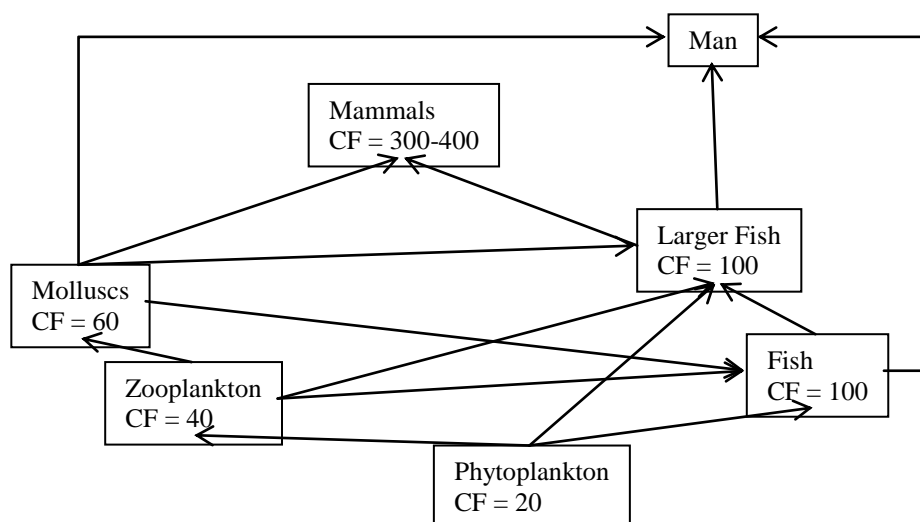
1.6 SIGNIFICANCE

Cesium uptake at the lowest trophic levels is of significance because larger organisms primarily obtain their cesium from their food. At the lowest levels it tends to be taken from the environment via the same processes as potassium uptake, but is removed from organisms at a much lower rate. This leads to cesium concentrations biomagnifying at the higher trophic levels in marine environments- that is to say that the predator will have higher concentrations of cesium in its body than the prey. Freshwater studies at the big US sites for cesium releases to the environment (Hanford, Oak Ridge, and Savannah River) show that this ratio $[A_{\text{predator}}]/[A_{\text{prey}}]$ is around 3 (Whicker et al, 2006). The magnitude of biomagnification between each trophic level depends on many factors including, but not limited to, diet, potassium concentrations, and dissolved solids in the water.

Larger organisms take up very little cesium directly from the marine environment, and therefore the precedent for cesium concentrations in larger fish tends to be set by initial uptake by the lowest trophic levels. In freshwater environments it has been shown that fish whose diets consist primarily of plankton generally have lower cesium concentrations than those whose diet consists of benthic organisms (Whicker et al, 2006). This finding, coupled with the much higher potassium concentrations in the marine environment, implies that uptake of cesium through the consumption of phytoplankton is likely not the most significant route of concentrating cesium after a release to the sea. Consumption of phytoplankton may still play a significant role in concentrating cesium in some discrete open-water food webs. In situ data is typically the preferred method for calculating concentration factors, but it can be difficult to isolate phytoplankton from seawater in situ without picking up other unwanted organisms and particulates.

Figure 1 represents a very simplified open water food web with the corresponding IAEA CF values for cesium. Open ocean food webs can be extremely complex, and potentially very tall prior to reaching man. With biomagnification of cesium uptake being such a concern in aquatic environments the listed CFs for the higher trophic levels do not necessarily tell the whole story. For example, some species of whales consume vast quantities of plankton directly from the environment (e.g. baleen whales), while others may consume larger fish or other mammals (e.g. orcas consuming seals). Both species of whale will be heavily impacted by the trophic level of their prey more so than their own CF value. Additional data sets can be used to increase confidence in environmental transport modeling.

Figure 1: Sample Generic Open Water Food Web



The purpose of this work was to study the uptake of cesium-134 in *Thalassiosira weissflogii*, a species of radially symmetrical diatom, over a twelve day period and determine an equilibrium CF in laboratory conditions. Cultures were grown in f/2 (Guillard and Ryther, 1962 and Guillard, 1975, see Appendix A) media and maintained at 22°C and 35‰ salinity. The salinity used was similar to previous work, but growth rates of this phytoplankton species should have been substantially higher at 22°C rather than 12°C. Uptake of metals in phytoplankton can be heavily influenced by the nutrient concentrations present in local seawater (Wang and Dei, 2001), but f/2 enrichment is a very standardized mixture that is easy to replicate. This temperature was selected to closely mimic the standard incubation temperature used by the Oregon State University (OSU) College of Earth, Ocean, and Atmospheric Sciences (CEOAS) incubators from which the plankton cultures were procured, and the species was selected for its ability to thrive in diverse living conditions.

2.0 EXPERIMENTAL DESIGN

2.1 MATERIALS

Cultures of the coastal diatom *Thalassiosira weissflogii* (University of British Columbia strain 709) were provided by Dr. Ricardo Letelier of the Oregon State University CEOAS. F/2 media was produced according to the Guillard methodology (see Appendix A) using sterile filtered seawater collected one mile offshore on the Newport Hydrographic Line in Newport, Oregon by Katie Watkins-Brandt of OSU. $^{133}\text{CsCl}$ (Lot A0292563, product number 192810100) was purchased from Acros Organics and converted via neutron activation in the OSU reactor by Dr. Scott Menn to ^{134}Cs approximately five months prior to use (see Appendix B).

2.2 METHODS

A plexiglass basin approximately 0.6m wide, 1.2m long, and 0.3m tall was held within a rigid wood frame. The wood frame was contained within an approximately 1.5m diameter inflatable wading/children's swimming pool that acted as a tertiary containment for radioactive materials. The plexiglass basin acted as the secondary containment, and the 0.5L glass flasks containing cultures were the primary containment for radioactive material. Ten 40-watt Philips F40T12 CW fluorescent light bulbs were suspended around the basin and operated on 12 hour light/dark cycles: light from 0600-1800 each day, dark from 1800-0600 each day. Lighting was asymmetrical, but flasks within the basin were randomly re-distributed on a daily basis in an attempt to reduce the impact of this asymmetry on growth rates. See Figure2 below.

Flasks were held down within the basin using 500 g weight rings. The basin was filled to ~40% volume with regular tap water, and this water temperature was monitored on a 24-hour basis. A Neslab Coolflow CFT-33 Refrigerated Recirculator was set with hoses in a closed circuit running through the water in the basin (See Figure2). This served to regulate the temperature of the water within the basin and therefore the 0.5L glass flasks contained in the basin. The Neslab was set such that the water temperature was a constant $22\pm 1^\circ\text{C}$ for the entire experiment duration, obtained at around 18°C on the Neslab control knob. All glassware and plastic ware used was soaked in Alconox for 48 hours, scrubbed with Alconox, and then autoclaved for 20 minutes prior to use.

Figure 2.



Figure 2: Plexiglass basin contained in wood frame, placed within inflatable wading pool with lights on. 0.5L glass flasks are empty in this photograph. Photograph brightness increased by 30%.

Cultures of the coastal diatom *Thalassiosira weissflogii* were grown in glass flasks at 24°C under a 12 hour light:12 hour dark cycle and maintained in artificial f/2 media (Guillard and Ryther, 1962 and Guillard, 1975). Cultures used in this experiment were in the exponential growth phase. While these cultures were not axenic, sterile techniques were used throughout the process to minimize any contamination. Two 2L clear polycarbonate bottles were autoclaved for 20 minutes and allowed to rest for one hour in the 24°C culture incubator prior to being filled with f/2 media. A 40 ml dense sample of *T. Weiss.* was added to one of these bottles, and both were allowed to incubate for an additional 120 hours. The one hour incubation of the empty bottle was an attempt to minimize shocking the culture with a drastic change in temperature, and to allow the f/2 media to reach the incubation temperature. The 120 hours of additional incubation was to allow the culture to grow to fill its new 2L bottle from its previous 50 ml

plastic centrifuge tube. It was estimated that this amount of *Thalassiosira weissflogii* f/2 media would take roughly 8-12 days to reach carrying capacity¹, and it was assumed that more nutrients and metals such as cesium would be concentrated by a metabolically active population rather than a stagnant/mature one. This assumption was made because phytoplankton metabolism refers to the act of accruing nutrients from seawater for conversion to other useful organic compounds.

Day zero began once the 120 hours of incubation was complete so that the cultures were along the exponential growth curve but not at carrying capacity. At this point the 2L bottle containing culture was split into four autoclaved 0.5L glass Erlenmeyer flasks, with approximately 350 ml in each flask. The 2L bottle containing only f/2 media was split into four separate autoclaved 0.5L glass Erlenmeyer flasks with approximately 350 ml in each. An air gap was left at the top of each flask to allow adequate surface area for the organisms to exchange gas with the outside environment. Leaving too small of an air gap and therefore smaller surface area may lead to an initial stifling of growth rates that increased as the experiment progressed. Conversely, leaving too large of an air gap may have led to a much larger relative change in surface area as the experiment progressed and drastic changes in gas exchange and metabolism rates. Too large of an air gap also may lead to a larger surface area of the media in contact with the flask surfaces relative to the volume of media, possibly increasing the impact of adsorption onto the glass. Additionally, past experience from CEOAS personnel growing the same species of phytoplankton in the same flask size indicated that approximately 350 ml was the maximum those flasks should be filled, depending upon how much water would be used over the duration of the experiment².

Two flasks containing f/2 media and plankton and two containing just f/2 media were spiked with 0.9 ml of distilled water containing initially 78.8 kBq/ml of Cs-134 (as cesium chloride) to a total activity of approximately 190Bq/ml after two months of decay between quantification and spiking. Spike solution activity was not verified at the end of the experiment. With the filtration and counting scheme described below this specific activity yielded high counting rates and detector dead time under 5%. The Cs-134 spike

¹ Katie Watkins-Brandt, private communication

² Marnie-Jo Zirbel, personal communication

solution was created by irradiating approximately 640 grams of Cs-133 chloride at 1000 kW in a sealed poly vial for 2.2 hours in the Lazy Susan loop in the Oregon State University reactor. This irradiation configuration yielded approximately 500 μCi of Cs-134 that was diluted into approximately 40 ml of distilled water. The diluted solution was quantified by Dr. Scott Menn of Oregon State University in a well counter approximately three months prior to use and provided the 78.8 kBq/ml value that was used above (see Appendix B).

Figure 3 displays a simplified flowchart of the sampling, filtration, and counting scheme used in this experiment, and a more detailed description follows.

Figure 3.

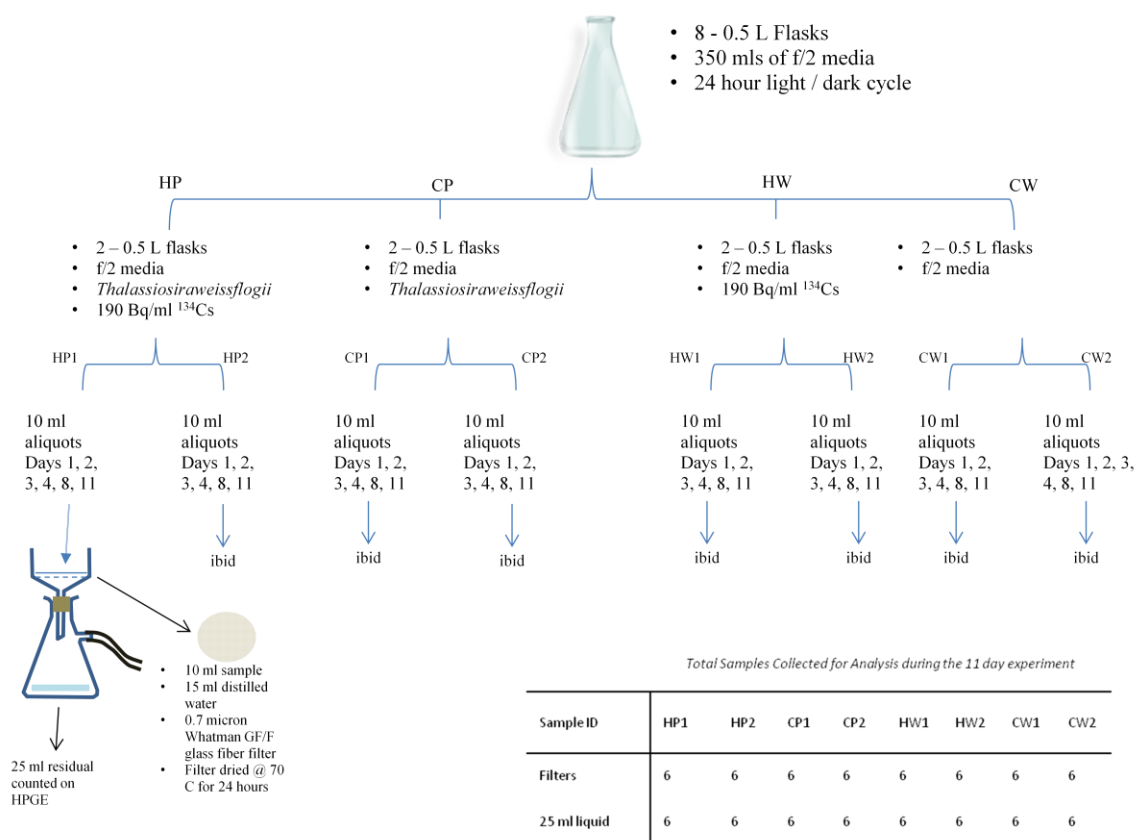


Figure 3: This figure shows a simplified flowchart of the growth, sampling, filtration, and counting scheme used.

There were a total of eight 0.5 L Erlenmeyer flasks used: two containing just f/2 media (CW1 and CW2); two containing f/2 media with *Thalassiosira weissflogii* (CP1 and CP2); two containing f/2 media

and approximately 190Bq/ml of cesium-134 (HW1 and HW2); and two containing f/2 media, approximately 190Bq/ml of cesium-134, and *Thalassiosira weissflogii* (HP1 and HP2). The pH of the flask contents was monitored before and after the addition of cesium to ensure there was no change. All flasks were agitated daily for approximately 30 seconds by swirling in alternating directions, and positioning within the plexiglass basin was randomized daily to reduce the impact of uneven lighting on the growth rates.

On days one, two, three, four, eight, and eleven, 10ml samples were pulled from each flask using a 5 ml Eppendorf pipette. Past work by Heldal et al performed measurements over a ten day period. Ten ml was used so that the experiment could go on for longer if needed while still maintaining a good surface-area to volume ratio for gas exchange. Additionally, this filtration volume was selected at the same time mass estimates were completed. Ten ml of f/2 media containing phytoplankton should have yielded masses that were close to measurable by available equipment. Unfortunately, the relative uncertainty in weighing of samples was too high. Sampling was initially performed daily but since no change in activity was observed in the first few days the frequency was decreased.

A separate pipette was used for samples containing cesium than from those not containing cesium. Eight different pipette tips were used for each of the eight samples removed on each sampling day. Each 10 ml sample was pulled via vacuum filtration into a 125 ml vacuum flask through a 55 mm diameter plastic Buchner funnel containing a 55 mm diameter Whatman GF/F glass fiber filter. This filter has a 0.7 µm pore size that is sufficiently small to gather all *Thalassiosira weissflogii* contained in the growth media. All filters were dried in bulk at 70°C for 72 hours prior to counting and weighing.

An additional 15 ml of distilled water was then pulled through the filter into the same vacuum flask containing the 10 ml trial sample. This additional distilled water was pulled through to remove excess salt weight from the filter and suspended excess cesium not bound to the plankton. Without the rinse, filters that were saturated with f/2 media had a wet weight of approximately 2 g heavier. After drying they weighed approximately 60 mg heavier. This is approximately consistent with 35 grams of salt per 1000 grams of seawater (assuming 35‰ salinity average), and indicates that excess salt is left on the filter after

drying. Fifteen ml of distilled water was experimentally determined to be the adequate amount to remove all excess salt weight.

The 125 ml vacuum flask size was used because with a total volume of 25 ml being pulled into each vacuum flask selection they were less than 20% full, and the diameter was such that the water formed a thin disk on the bottom of the flask. The diameter of this liquid disk was slightly smaller than the diameter of the High-purity Germanium (HPGe) detector used for counting, and the similarity between the HPGe diameter and flask outer diameter made for easily reproducible counting geometry.

2.3 INSTRUMENTATION

The chlorophyll *a* content was measured using a 10AU laboratory fluorometer from Turner³. The fluorometer was calibrated to the appropriate wavelength for chlorophyll *a* for various experiments involving phytoplankton cultures and productivity by Katie Watkins-Brandt of CEOAS. Radioactivity was measured using the OSU Vertical #2 HPGe detector (Ortec GEM 30185P, s/n 33TP20814A) operating at 2000V and analyzed using GammaVision by Ortec. Masses were obtained using a Mettler AG285⁴ balance (I.D. number 1120462519, calibrated 3/28/2012).

2.4 INSTRUMENTATION USE

Vacuum flasks were corked and bagged using quart size Ziploc bags prior to counting to minimize contamination risks. They were counted within two hours of filtration on an HPGe detector for five minutes each. A ten minute background count was conducted to verify there were no unusual peaks or unwanted nuclides in use nearby. Filters were dried on a disposable aluminum sheet for 24 hours at 70°C based upon previous work and then bagged (2" x 3" poly zipper) prior to counting to minimize contamination risks (see Figure 4). No change in mass was noticed beyond four hours of drying time, but 24 hours was used for convenience. Filters were counted on the same detector as the vacuum flasks for the same duration with the same background scheme (5 minutes and 10 minutes, respectively), but several weeks later.

³<http://www.turnerdesigns.com/products/laboratory-fluorometer/10au-laboratory-fluorometer>

⁴http://us.mt.com/us/en/home/phased_out_products/others/AG285.html

Figure 4.

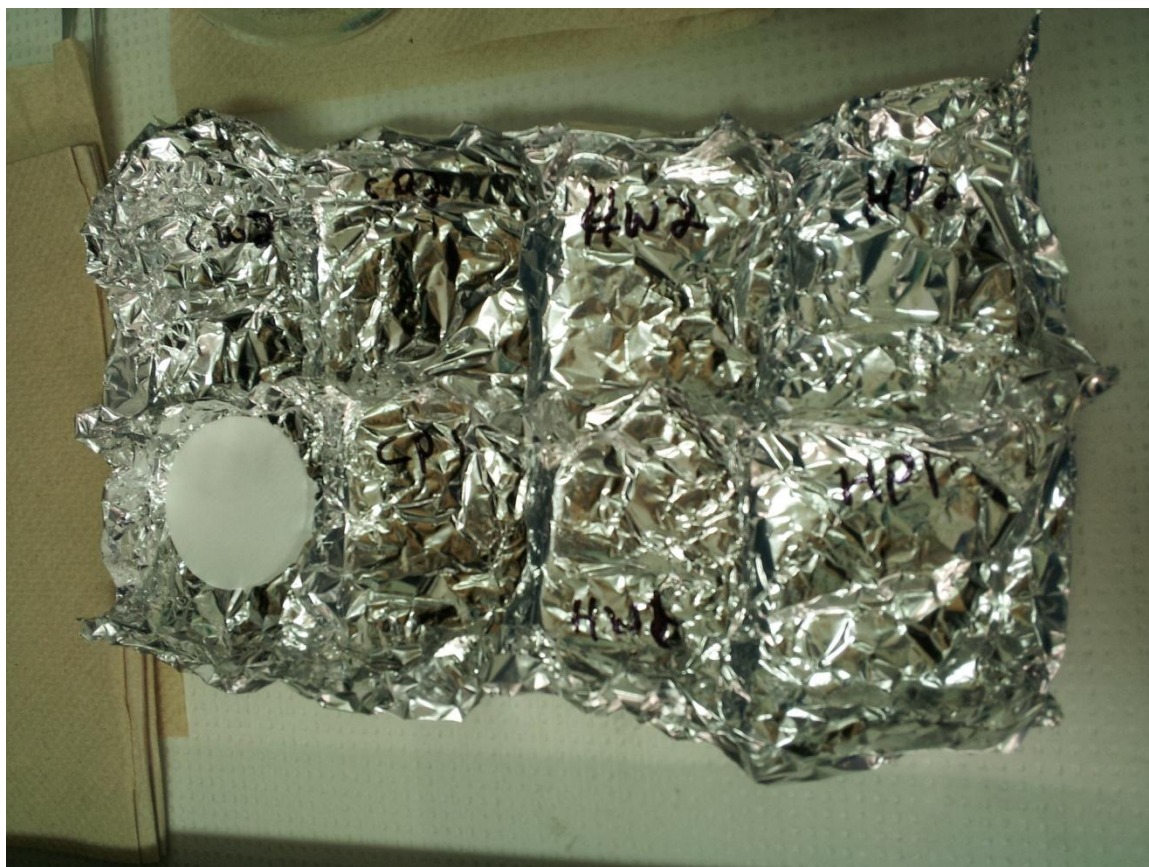


Figure 4: Disposable aluminum tray used for drying filters. The filters dried placed in the same direction as when filtration was performed- collected organic matter would be on the opposite side from the aluminum sheet.

The filter counting results were decay-corrected back to the date of filtration prior to comparison with the vacuum flask counting results. The vacuum flasks containing filtrate were counted within two hours of filtration, and no decay-correction was performed. Decay-correction factors are displayed in Table 1.

Table 1: Elapsed time between contamination and counting, and decayed fraction.

	Day 1	Day 2	Day 3	Day 4	Day 8	Day 11
Date/time of sampling	1/29/2013 1230 hrs	1/30/2013 1135 hrs	1/31/2013 1445 hrs	2/1/2013 1140 hrs	2/5/2013 1300 hrs	2/8/2013 1050 hrs
Hours since contaminated	23.94	46.58	72.75	94.67	191.00	260.83
Time of counting	3/21/2013 1200 hrs	3/21/2013 1200 hrs	3/21/2013 1200 hrs	3/21/2013 1200 hrs	4/16/2013 1500 hrs	4/16/2013 1500 hrs
Hours elapsed between filtration and counting	1223.00	1200.50	1173.25	1152.33	1682.00	1612.00
Decay fraction	0.9542	0.9550	0.9560	0.9568	0.9376	0.9401

This table represents the values that were used to decay-correct the measured activity of Cs-134 on the glass fiber filters back to the day of sampling.

The bagged filters were placed on top of a ~3 mm thick cardboard ring, with the inner diameter of the ring being slightly smaller than the outer diameter of the filter. See Figure 5 and 6 for counting geometry. This geometry was intended to replicate the geometry of a pre-made filter calibration source, but this calibration source was not used due to it containing mixed isotopes, and displaying a significantly lower counting rate than was obtained by trials in this experiment.

Figure 5.

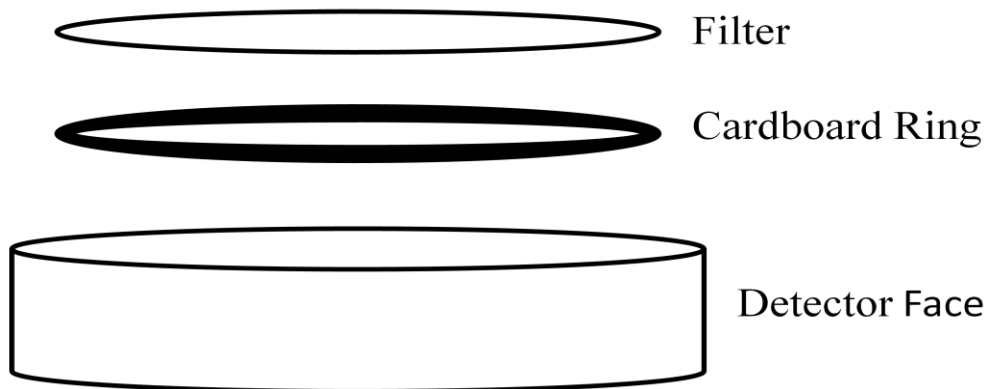


Figure 5: Filter counting geometry. The cardboard ring was centered directly on the detector face, and the bagged filter was centered on top of the ring.

Figure 6.



Figure 6: Vacuum flask sample counting geometry. The Ziploc bag was removed for visibility during this photograph.

A vacuum flask calibration source was created by adding 10 ml of f/2 media, 15 ml of distilled water, and 1718 Bq of Cs-134 (as cesium chloride) to a clean 125 ml vacuum flask. This flask was counted under the same geometry as the trial flasks as described above. This activity was selected because it was close to the maximum activity that should have been remaining in the trial vacuum flasks ($10 \text{ ml} \times (190 \text{ Bq/ml}) = 1900 \text{ Bq}$ of Cs-134). The calibration source was created and counted at a later date, with the

difference in activity between the calibration source and the trial vacuum flasks resulting solely from radioactive decay. Two filter calibration sources were created using clean, pre-dried GF/F filters and adding 1718 Bq of Cs-134 directly to one filter and 1375 Bq of Cs-134 to the other filter while they were in bags. This ensured that no cesium was lost to drying sheets, and a known amount was placed into the bag. The bags containing filters were placed on the cardboard ring and counted in the same fashion as the trial filters. The same style 2" x 3" poly bags were used for the calibration source as the trial filters.

Both the vacuum flasks and the filters were counted and calibrated using the 795.864 keV gamma emission. Cesium-134 emits eight gammas with a yield greater than 1% but the 795.864 keV gamma is a good mix of high energy and high yield (85.46%) (Frame, 2008), and was towards the higher energy end of the Cs-134 spectrum. The 795.864 keV gamma peak was past the bulk of the Compton continuum and background counts were extremely low at that level. An alternative that was well past the Compton continuum would have been the 1365.185 keV gamma at 3.017% yield, but this peak was not detectable in the filter spectra. See Appendix B for calibration information. Efficiency units were counts per five minutes per Bq, and both the filters and filtrate were counted for five minutes, eliminating a conversion to "per second" or "per minute" in the CF calculations and error propagation.

On days four and eleven a 10 ml sample was pulled from each of the Erlenmeyer flasks containing f/2 media and plankton (CP1 and CP2) and combined into a single 20 ml glass scintillation vial. This scintillation vial was transported to a different lab containing the Turner 10 AU fluorometer that had been warmed up prior to use. Chlorophyll *a* measurements can be used to determine population growth rate and indicate relative health and productivity of the population. For the purposes of this experiment the exact growth rate was not determined, but relative chlorophyll *a* content was determined to verify that the population of plankton was alive and metabolically active for the duration of the experiment.

An empty Turner cuvette was cleaned and inserted into the fluorometer and analyzed as a blank with the cap on the cuvette receptor. Two additional blanks were run containing f/2 media, and then the 20 ml of f/2 media and trial plankton was divided into 3 parts and poured into Turner cuvettes and each read separately, all in the same fashion. This same procedure was followed for both days four and eleven, but due to accidental spillage only two plankton samples were read on day four. The same cuvette was used

for all measurements, and cleaned in between each reading. The fluorometer reads in relative fluorescence units in real-time; no additional manipulation of the equipment was required beyond situating the cuvettes, capping the cuvette holder, and waiting a few seconds for the reading to stabilize. See Appendix C for results.

On day 4 one of the Erlenmeyer flasks containing f/2 media and *Thalassiosira weissflogii* without cesium-134 (CP2) was removed from the water bath, and 100 ml of the remaining volume was ran through a single GF/F filter via vacuum filtration using an Eppendorf pipette in 5 ml increments. A 25 ml amount of distilled water was then pulled through the same filter to reduce salt retention. The filtrate was discarded, and the filter was dried for 48 hours at 70°C and then weighed. The filter that had collected the organism was weighed after drying was complete on a Mettler AG285 balance and the resulting value was divided by ten to determine the mass of plankton in each of the daily 10 ml samples. The factors of salt retention on the filters and balance uncertainty combined to make the relative uncertainty in plankton mass statistically too large to accurately determine the plankton mass using only the 10 ml daily samples. It was also estimated that the plankton mass in a 10 ml sample might be below the limit of detection on the balance used (See Appendix B for calculation of plankton mass). Consequently, a value of 0.52 mg plankton per 10 ml sample of trial or control batch was used for all days of this experiment. The implication of using a single value for plankton mass for the duration of the experiment will be discussed later.

The 5ml Eppendorf pipettes were calibrated by placing a clean 250 ml beaker approximately half full with distilled water onto a balance. A 5 ml sample was removed from the beaker via Eppendorf pipette and then the balance was tared. The 5 ml was pipetted back into the beaker, and the mass difference was recorded. This process was repeated a total of thirty trials, with the pipette tip being exchanged every ten trials to compensate for potential randomness in pipette tip design. This mass of distilled water was converted to an equivalent mass of F/2 media and the error was propagated into the CF calculations. See Appendix D for results.

2.5 CONCENTRATION FACTOR CALCULATION

The concentration factors were calculated with the following formula derived from the IAEA technical report 422:

$$CF = \frac{\frac{A}{\frac{B \times M_{\text{plankton}}}{C}}}{\frac{D \times E}{C}};$$

where A is the average of two 5-minute counts of the filters from trial flasks containing plankton and cesium (HP1 and HP2) minus the average of two 5-minute counts of the filters of control flasks containing no plankton (HW1 and HW2), and was calculated as:

$$A(\text{counts}) = \frac{N_{HP1}(\text{counts}) + N_{HP2}(\text{counts})}{2} - \frac{N_{HW1}(\text{counts}) + N_{HW2}(\text{counts})}{2};$$

B is the filter efficiency calculated by adding a known amount of activity to two different filters and counting for five minutes under identical geometry:

$$B\left(\frac{\text{counts}}{\text{Bq}}\right) = \frac{\frac{N_{\text{Filter } 1}(\text{counts})}{\text{Activity}_{\text{Filter } 1}(\text{Bq})} + \frac{N_{\text{Filter } 2}(\text{counts})}{\text{Activity}_{\text{Filter } 2}(\text{Bq})}}{2};$$

M_{plankton} is the day 4 plankton mass contained in 100 ml of trial water divided by 10:

$$M_{\text{plankton}}(\text{grams}) = \frac{M_{\text{dried filter post-filtration}} - M_{\text{dried filter pre-filtration}}}{10}$$

C is the average of two 5-minute counts of filtrate from the trial flasks containing plankton and cesium (HP1 and HP2), and was calculated as:

$$C(\text{counts}) = \frac{N_{HP1}(\text{counts}) + N_{HP2}(\text{counts})}{2};$$

D is the filtrate efficiency calculated by adding a known amount of radioactivity to one vacuum flask and then filling to 25 ml with distilled water and counting for five minutes under identical geometry as the trial samples:

$$D\left(\frac{\text{counts}}{\text{Bq}}\right) = \frac{N(\text{counts})}{\text{Activity}(\text{Bq})};$$

and E is the average mass of 10 ml of seawater calculated by determining the average pipette volume over 30 trials (as described in section 2.4) multiplied by an average density of 1.025 g/ml for seawater:

$$E(\text{grams}) = \frac{\sum M_{\text{Distilled}}}{30} \times \frac{\rho_{\text{seawater}}}{\rho_{\text{distilled water}}}$$

2.6 ERROR ANALYSIS

Error analysis was performed using methods described in *An Introduction to Error Analysis* (Taylor, 1997). The deviations in the net filter and filtrate counts for both the trial and control groups were calculated as follows:

$$\sigma_{trial} = \sqrt{\left(\frac{1}{N}\right) \Sigma (x_i - x_{avg})^2}$$

$$\sigma_{control} = \sqrt{\left(\frac{1}{N}\right) \Sigma (x_i - x_{avg})^2}$$

Most filtration days resulted in two samples being procured, but due to cross-contamination and other error some samples had to be discarded, resulting in single filters being counted on some days. $1/N$ was used rather than $1/(N-1)$ for consistency, and to avoid dividing by 0.

The deviation in the adjusted net average counts was calculated as follows:

$$\sigma_{adjusted} = \sqrt{\sigma_{HP}^2 + \sigma_{HW}^2}$$

The deviation in the filter efficiency was calculated as follows:

$$\sigma_{eff, filter} = \sqrt{\frac{1}{N} \Sigma (counts \text{ per Bq} - average \text{ counts per Bq})^2}$$

The deviation in the plankton mass was assumed to be 1% based upon equipment used.

The deviation in the filtrate efficiency was calculated as follows:

$$\sigma_{eff, filtrate} = \frac{\sqrt{filtrate \text{ counts}}}{2000 \text{ Bq}}$$

The deviation in the filtrate mass was calculated as follows:

$$\sigma_{filtrate \text{ mass}} = 1.025 \frac{g}{ml} \times \sqrt{\frac{1}{N} \Sigma (x_i - x_{avg})^2}$$

If A is the average filter counts, B is the average filter efficiency, C is the filtrate counts, D is the average filtrate efficiency, and E is the average filtrate mass, the final error was calculated as follows:

$$\sigma_{CF, unadjusted} =$$

$$|CF| \times \sqrt{\left(\frac{\sigma_{HP, filter}}{A_{Unadjusted}}\right)^2 + \left(\frac{\sigma_{eff, filter}}{B}\right)^2 + (0.01)^2 + \left(\frac{\sigma_{HP, filtrate}}{C}\right)^2 + \left(\frac{\sigma_{eff, filtrate}}{D}\right)^2 + \left(\frac{\sigma_{filtrate \text{ mass}}}{E}\right)^2}$$

$$\sigma_{CF,adjusted} = |CF| \times \sqrt{\left(\frac{\sigma_{HP,filter}}{A_{adjusted}}\right)^2 + \left(\frac{\sigma_{eff,filter}}{B}\right)^2 + (0.01)^2 + \left(\frac{\sigma_{HP,filtrate}}{C}\right)^2 + \left(\frac{\sigma_{eff,filtrate}}{D}\right)^2 + \left(\frac{\sigma_{filtrate\ mass}}{E}\right)^2}$$

Assumptions that were made that could have had an un-quantified impact on the results of this experiment include, but are not limited to, the following: assuming a static plankton mass for the experiment's duration, and assuming that all four flasks containing plankton grew at the same rate; assuming that the cesium (stable and radioactive) did not impact the growth rates in the HP flasks; assuming that daily agitation was conducted in the exact same fashion for each flask and that samples were drawn from the same position in each flask; assuming that the counting geometry was identical for all samples on all days in the absence of a rigid sample holder; assuming that all glass fiber filters had the same geometry and that the counting efficiency was not impacted by any random differences in filter thickness; assuming that all vacuum flasks had the same geometry and that the counting efficiency was not impacted by any random differences in glass thickness; assuming that all plastic bags were the same thickness and had no impact on counting efficiency; sampling and counting the flasks in the same order on each day rather than randomly; and not adding stable cesium to a batch of phytoplankton as an additional control group. None of these factors were quantified, nor was a flat error percentage applied in the error propagation section to account for these potential sources of error.

3.0 RESULTS

The net counts obtained after five minutes of counting the 795.864 keV gamma peak of processed trial and control filters are displayed in Table 2. Sample 4 trial filter 2's data was discarded due to a loss of filter geometry. Sample 6 control filter 2's data was discarded due to that control batch becoming contaminated with a living organism between days 9 and 11 of the experiment.

Table 2: Net 5-minute counts of filter with and without *Thalassiosira weissflogii*.

		Day 1	Day 2	Day 3	Day 4	Day 8	Day 11
Hours since spike		23.94	46.58	72.75	94.67	191.00	260.83
With <i>Thalassiosira weissflogii</i>	HP1-F ¹	549	485	555	531	530	587
	HP 2-F	607	581	434	--	492	496
	Average	578	533	494.5	531	511	541.5
	Std. Dev.	29	48	60.5	23	19	45.5
Without <i>Thalassiosira weissflogii</i>	HW1-F	575	605	584	587	683	591
	HW2-F	643	625	555	616	629	--
	Average	609	615	569.5	601.5	656	591
	Std. Dev.	34	10	14.5	14.5	27	24.3
Net counts	Net counts	-31	-82	-75	-70.5	-145	-49.5
	Std. Dev.	44.37	49.0	62.2	27.2	33.0	51.6
1F=Filter values							
All values are rounded to one decimal place. Calculations were performed with un-rounded values.							

The net counts obtained after five minutes of counting the 795.864 keV gamma peak of the f/2 enriched seawater that passed through the glass fiber filters described above are displayed in Table 3.

Table 3: Net 5-minute counts of filtrate with and without *Thalassiosira weissflogii*.

		Day 1	Day 2	Day 3	Day 4	Day 8	Day 11
With <i>Thalassiosira weissflogii</i>	HP1-R ¹	10818	10668	10297	10285	10992	10630
	HP2-R	10854	10882	10780	11025	10718	11170
	Average	10836	10775	10538.5	10655	10855	10900
	Std. Dev	18	107	24.5	370	137	270
Without <i>Thalassiosira weissflogii</i>	HW-1R	11062	11038	11007	11244	10996	11076
	HW2-R	11138	11122	11224	11240	11132	
	Average	11100	11080	11115.5	11242	11064	11076
	Std. Dev	38	42	108.5	2	68	105.2
Filtrate and Control	Percent difference	2.4	2.8	5.5	5.5	1.9	1.6
1-R=25 ml residual liquid							
All values rounded to one decimal place. Calculations were performed on un-rounded values.							

The CF for ^{134}Cs calculated for the phytoplankton *Thalassiosira weissflogii* grown in F/2 media over an eleven day period was not significantly above zero. The CF was calculated using:

$$CF = \frac{\frac{A_{\text{Filter,HP}} - A_{\text{Filter,HW}}}{M_{\text{Filter}}}}{\frac{A_{\text{Filtrate,HP}}}{M_{\text{Filtrate}}}}$$

where A is the activity in Becquerels and M was the mass in grams. $A_{\text{Filter, HW}}$ refers to the batches containing F/2 media and ^{134}Cs in the absence of phytoplankton to correct for adsorption of cesium onto the filter. The final CF value is unitless. Pure F/2 media was counted on each sampling day to ensure that it contained no naturally-occurring ^{134}Cs , and the CF was assumed to be zero at time zero. Concentration factors were also calculated without removing the control groups in order to evaluate the order of magnitude difference from published values that this method can obtain, and results are summarized in Table 4. See Appendix E for tabulated raw data. There may have been some motion towards or away from equilibrium in the first 24 hours or in between sampling points, but no time-dependence was observed on the time step that was utilized by this experiment.

Table 4. Adjusted and Unadjusted Concentration Factors

	Day 1	Day 2	Day 3	Day 4	Day 8	Day 11	Average
Elapsed Time (hours)	23.9	46.6	72.8	94.7	191.0	260.8	
Unadj. CF	571±31	529±49	501±63	532±32	513±23	540±49	531±41
Adj. CF	ND	ND	ND	ND	ND	ND	ND

All CF values are rounded to the nearest whole number. Full values are kept in Appendix E.

Curiously, all trials containing phytoplankton that were filtered and counted showed less total activity between the filter and filtrate than those samples that contained no plankton. Differences ranged from 5.4% to 28.4% and averaged 14.5% for the filters, and were between 1.6% and 5.5% and averaged 3.3% for the filtrate. Differences represented approximately 1σ to 3σ . Results are summarized in Table 5, and full results are in Appendix E.

Table 5: Gross Activity and Activity Balance

	Day 1	Day 2	Day 3	Day 4	Day 8	Day 11	Average
Elapsed Time (hours)	23.9	46.6	72.8	94.7	191.0	260.8	
HW gross activity (Bq)	1869.6	1862.8	1868.6	1890.9	1862.3	1864.9	1871.3
HP gross activity (Bq)	1824.8	1811.0	1768.9	1768.6	1822.2	1832.2	1808.3
Difference (Bq)	44.8	51.8	99.7	122.3	40.1	32.7	63.0
HP as percent of HW	97.6	97.2	94.7	93.5	97.8	98.2	96.5
Error as percent	3.4	3.4	3.3	3.3	3.4	3.4	3.4

This table displays the gross total of filter and filtrate activity for batches containing F/2 media and ^{134}Cs , the trial represents batches with phytoplankton while control represents batches without phytoplankton. Each gross activity value is the average of two replicate batches in each class.

Figure 7: Activity Balance

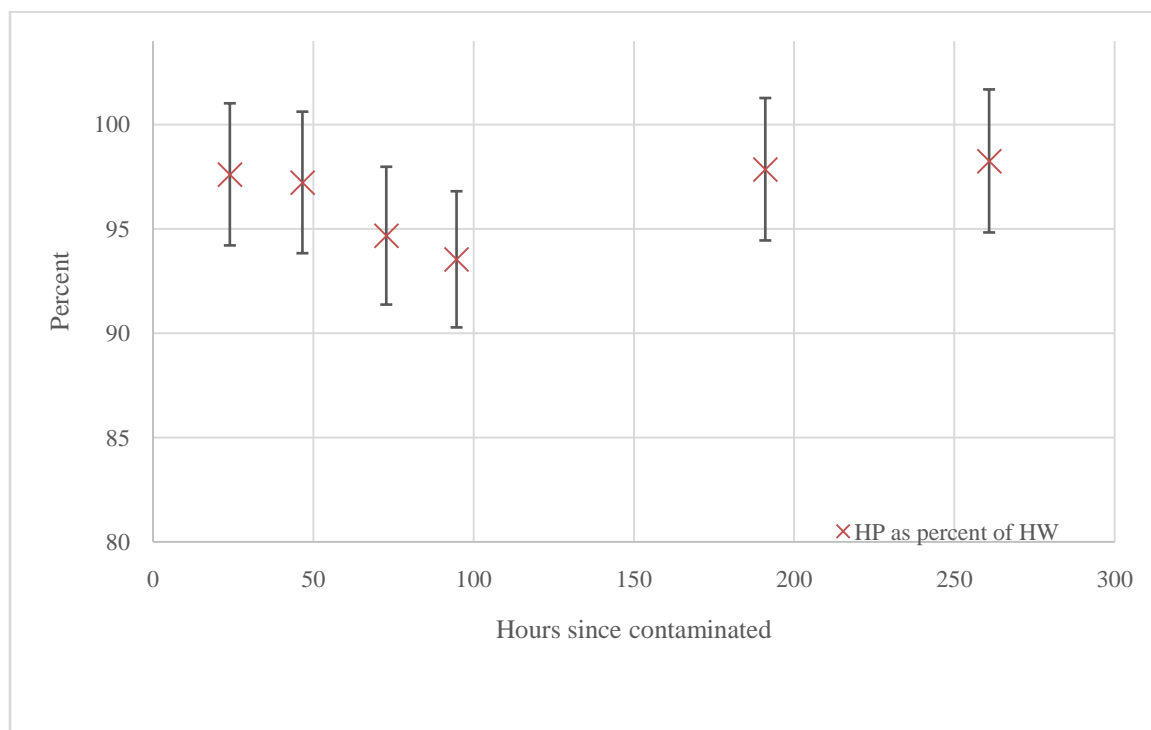


Figure 7: This figure displays the gross activity found in HP flasks as a percentage of the gross activity found in HW flasks. It utilizes data from Table 5, and includes 1σ error bars.

The calculated CFs obtained with this method were all below zero for all 6 days. A one-tailed paired t-test was conducted to determine if the calculated values were significantly below zero. To conduct this test it was assumed that equilibrium was reached prior to the first (day 1) measurement, and that all 6

measurements represent equilibrium measurements, and that the expectation value was 0. Using data pulled from Appendix G and the following formula:

$$t_{cal} = \frac{|\overline{CF} - 0|}{\overline{\sigma_{CF}}\sqrt{n}}$$

where $\overline{CF} = -75.6$, $\overline{\sigma_{CF}} = 44.7$, and $n = 6$, $t_{cal} = 4.14$. Since 4.14 exceeds the critical value for 5 degrees of freedom on a one-sided paired t-test of 2.02, the results obtained using this method are significantly below zero (Reeuwijk and Houba, 1998). This result indicates that Cs-134 was removed from the system being sampled; possible causes are presented in the next section.

4.0 DISCUSSION

Uptake of cesium and the calculated concentration factors for *Thalassiosira weissflogii* were not significantly above zero under the experimental conditions described above. Based upon past work this is not entirely surprising, despite the differences in experimental set up from past work that initially identified this result. It was expected that the plankton grown at 22°C would be very metabolically active, and therefore if uptake of cesium was an activity driven by metabolism the resulting uptake would be more significant than if the cultures were maintained at 12°C; however, this effect was not observed. Past work concluded that uptake was negligible in some species of phytoplankton, and this experiment did not refute that conclusion. It is possible that Cs does not follow K^+ uptake routes in the same fashion simply because it is a larger ion, and phytoplankton interior composition is very tightly regulated.

The net cesium counts from both filter and filtrate water in samples containing plankton was lower than the net cesium counts in samples without plankton. Differences were greatest in the filters, and averaged 14.5% while the water counts averaged 3.3%. If one conservatively estimated that the plankton built up on the filter was as thick as the filter itself and was positioned in between the cesium and the detector, one would expect only a ~0.3% reduction in counts (see Appendix A). The plankton did not occupy this great of a space however, and this estimated counting rate reduction is not on the same order of magnitude as the observed "missing" activity. It may have been useful to use the highly-loaded filter that was used to calculate the average plankton mass as shielding for a NIST-traceable source in a rigid geometry for comparison with a filter that only had f/2 media run through it; this could have ruled out self-shielding as an explanation.

Adsorption onto the sides of the flask should have been equal for both the control groups and the culture samples, and neither should have displayed preferential loss of activity. It is possible that the cesium loss was due to some mechanism of dead plankton material binding that was not shaken off the sides of the flask sufficiently during the daily agitation. If this were the case, it is expected that there would be a time-dependence to this activity as the population matured and died. However, no such trend was observed. Quantifying this mechanism was beyond the scope of this experiment.

While net filter counts displayed the largest percent disparity, net activity was generally within 2σ and the disparity could simply be due to the random nature of counting radioactivity. Lower net counts in trial batches than the control batches was merely a curious result in that it happened with all eight flasks on all sampling days. This method is capable of quantifying concentration factors down to approximately 111 using the specific activities described earlier (See Appendix A).

One possible explanation for lower net counts in flasks containing phytoplankton could be the sinking mechanism that diatom populations undergo as a regular stage in their life cycles. If a diatom population is under stress due to poor light, low nutrient availability, etc. some diatoms are able to control their own buoyancy to sink to lower levels in the water column. This allows the population to survive for extended periods of time while the surface conditions recover and become favorable again. While sinking is occurring the diatoms secrete additional mucus that scavenges minerals and particulates from the surrounding water, as well as forming aggregates with other diatoms, to increase sinking rate. Sinking rates can be as high as 7 cm per minute (Smetacek, 1985).

If active sinking was occurring in the phytoplankton population studied in this experiment it is possible that cesium was scavenged from the medium by mucus that is otherwise not present in the drifting population. This cesium would have been concentrated on the bottom of the growth flasks, and due to high sinking rates and a relatively small flask volume it is possible that the daily agitation of samples was not sufficient to dislodge this population of diatoms and cesium from the glass flask bottoms. Agitation during sample removal may have mitigated these effects.

Chlorophyll *a* content was increasing over the course of this experiment, but that does not rule out the possibility of sinking occurring. Sinking may occur as populations near their carrying capacity, and has been shown to occur in high productivity areas (Smetacek, 1985). It would be expected that the missing activity would have followed some type of time-dependant pattern if this mechanism was occurring at a constant rate for the experiment duration, and this was not observed. Total missing cesium would have increased as the experiment progressed, or only begin to show towards the later days of the experiment as the population approached carrying capacity. It is possible that sinking occurred only in the 24 hour period between spiking the batches with cesium and performing the first filtration and measurement, as a result of

gamma and beta doses to the population. Taking several measurements beginning immediately after spiking with cesium and then during the initial 24-hour period may have assisted in quantifying the missing activity; a time-dependence may have existed in the first 24-hours, but the population death, sinking, and reproduction rates may have equilibrated by the end of that period. Quantification of dose to cells and the population response to gamma and beta doses was beyond the scope of this experiment, but it may have been significant. The impact of this cell killing could have been reduced by lowering the specific activity used, and counting rates could have been maintained by increasing the volume analyzed.

Another possible, although unlikely, explanation for total activity counts being lower in samples containing plankton is that plankton material or byproduct material competed for adsorption sites on the glass fiber filters with cesium ions, slightly reducing the available amount of adsorption sites available for cesium adsorption. This explanation is unlikely since this would have led to higher filtrate counts from samples containing plankton, which was not observed. Direct counting of the trial and control media without filtration may have provided an indication on whether or not the activity discrepancy was related to filtration at all.

In past work by Heldal et al. (2001) the membrane filters were washed with NH_4COOH prior to use. The purpose was not explained in the published paper, however it is likely that it was to donate NH_4^+ to the filter. NH_4^+ may preferentially occupy sorption sites on the filters, preventing Cs^+ from binding to the filters during filtration. It is possible that a similar wash would have reduced or even completely eliminated Cs^+ sorption onto the glass fiber filters used in this experiment, thus allowing quantification of lower CFs.

This experiment assumed that the plankton mass was the same for all days of this experiment and was equal to the average plankton mass on day four only. Fluorometry results indicated that the population was still growing in some fashion, and this assumption of a static plankton density would have the effect of underestimating the CF prior to day four, and overestimating the CF after day four. However, the magnitude of this discrepancy cannot be calculated. Fluorometry results only indicate that chlorophyll *a* content was increasing, and while this is related to population health it is not directly related to population mass. Each organism can have differing amounts of chlorophyll *a*, and span a wide range of sizes and

chlorophyll *a* densities. A simple scaling by relative chlorophyll *a* content may have sufficed for a more accurate estimate had a positive CF been obtained- CF below zero is undefined though, so scaling an effective zero is not a quantity worth defining.

Additional replicates could have been grown and analyzed to reduce the uncertainty in the daily measurements. However, one replicate was sufficient to demonstrate that CF for this species of phytoplankton is likely low, and to show that something interesting was occurring regarding the "missing" activity that warrants further investigation. It is also possible that the "missing" activity phenomenon would have simply disappeared with more replicate samples.

5.0 CONCLUSIONS

Overall the results obtained are consistent with those obtained by Heldal et al. (2001) and it is unlikely that phytoplankton are a significant pathway for concentrating cesium further up the food chain. The selection of filters and the plankton mass calculation could be improved upon. Actual measurement of mass could be performed by filtering more of the flask contents on each day through a lighter-weight filter and using a more sensitive balance. For example, a membrane filter could be used. These weigh at most a few milligrams as opposed to the approximately ~180 for the GF/F filters used, which would drastically reduce the uncertainty when subtracting the filter weight. Membrane filters are not as rigid or easy to transport as GF/F filters and contamination control became a larger concern when membrane filters were tested. The methodology described in this project could be used to fill in blanks in the IAEA tables of concentration factors for other nuclides that were likely released in Fukushima, but no additional work on cesium uptake is recommended. It is recommended that the mucus secreted during the sinking process is analyzed for its uptake properties, and it is recommended that additional samples be taken very soon after contamination with radioactive materials in phytoplankton populations to assess the speed at which equilibrium is achieved.

For future work it may be of interest to quantify trace metals in both f/2 media as well as phytoplankton to have a better idea of the relative concentrations of the ions that are related to the isotope that is being studied.

It is recommended that the IAEA value of 20 for the cesium concentration factor in phytoplankton be used in the future when looking at concentration of cesium up the food chain since it will yield more conservative risk estimates in the absence of field data. When modeling the geographic transport of cesium bound in plankton, however, it is recommended to treat the concentration factor as zero. This is a relatively safe estimate to make since motion of plankton is regulated by the currents anyhow- they do not migrate seasonally like some larger marine life.

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APPENDICES

Appendix A: F/2 Media Composition

Table A1: The following ingredients are to be mixed into 1L of sterile filtered seawater:

Component	Stock Solution	Quantity	Molar Concentration in Final Medium
NaNO ₃	75 g/L dH ₂ O	1 mL	8.82 x 10 ⁻⁴ M
NaH ₂ PO ₄ H ₂ O	5 g/L dH ₂ O	1 mL	3.62 x 10 ⁻⁵ M
Na ₂ SiO ₃ 9H ₂ O	30 g/L dH ₂ O	1 mL	1.06 x 10 ⁻⁴ M
trace metal solution	(see recipe below)	1 mL	---
vitamin solution	(see recipe below)	0.5 mL	---

Table A2: The following trace metal solution components are to be mixed into 1L of distilled water:

Component	Primary Stock Solution	Quantity	Molar Concentration in Final Medium
FeCl ₃ 6H ₂ O	---	3.15 g	1.17 x 10 ⁻⁵ M
Na ₂ EDTA 2H ₂ O	---	4.36 g	1.17 x 10 ⁻⁵ M
CuSO ₄ 5H ₂ O	9.8 g/L dH ₂ O	1 mL	3.93 x 10 ⁻⁸ M
Na ₂ MoO ₄ 2H ₂ O	6.3 g/L dH ₂ O	1 mL	2.60 x 10 ⁻⁸ M
ZnSO ₄ 7H ₂ O	22.0 g/L dH ₂ O	1 mL	7.65 x 10 ⁻⁸ M
CoCl ₂ 6H ₂ O	10.0 g/L dH ₂ O	1 mL	4.20 x 10 ⁻⁸ M
MnCl ₂ 4H ₂ O	180.0 g/L dH ₂ O	1 mL	9.10 x 10 ⁻⁷ M

Table A3: The following vitamin solution components are to be mixed into 1L of distilled water:

Component	Primary Stock Solution	Quantity	Molar Concentration in Final Medium
Thiamine HCl (vit. B ₁)	---	200 mg	$2.96 \times 10^{-7} \text{ M}$
biotin (vit. H)	0.1 g/L dH ₂ O	10 mL	$2.05 \times 10^{-9} \text{ M}$
cyanocobalamin (vit. B ₁₂)	1.0 g/L dH ₂ O	1 mL	$3.69 \times 10^{-10} \text{ M}$

Appendix B: Gammavision Spectrum Report for 140 µl Sample of Cs-134

The following is the report produced by Dr. Scott Menn of OSU using his well counter to quantify the Cs-134 post-activation.

ORTEC g v - i (175) Wan32 G53W2.10 02-NOV-2012 13:13:20 Page 1
Oregon State University Spectrum name: Cs-134sampleNathan.An1

Sample description
Cs-134 140 uL

Spectrum Filename: P:\USER\Hp\Hp2012\HP 11.Nov12\Cs-134sampleNathan.A

Acquisition information

Start time: 02-Nov-2012 12:48:00
Live time: 600
Real time: 697
Dead time: 13.96 %
Detector ID: 1

Detector system

B125-01 MCB 129

Calibration

Filename: b125sh2-Aug2012.Clb
b125 shelf 2 calibration 10ks ROI 1274 KeV b125sh2-Aug2012

Energy Calibration

Created: 13-Aug-2012 10:01:16
Zero offset: 0.280 keV
Gain: 0.199 keV/channel
Quadratic: 1.808E-08 keV/channel^2

Efficiency Calibration

Created: 13-Aug-2012 10:15:18
Type: Polynomial
Uncertainty: 1.092 %
Coefficients: -0.511722 -4.746608 0.463098
-0.023935 -0.001576 0.000091

Library Files

Main analysis library: Cs-134.Lib
Library Match Width: 0.500

Analysis parameters

Analysis engine: Wan32 G53W2.10
Start channel: 50 (10.22keV)
Stop channel: 16000 (3186.07keV)
Peak rejection level: 20.000%
Peak search sensitivity: 3
Sample Size: 1.0000E+00
Activity scaling factor: 1.0000E+00/(1.0000E+00* 1.0000E+00) =
1.0000E+00
Detection limit method: Traditional ORTEC method

Random error: 1.000000E+00
 Systematic error: 1.000000E+00
 Fraction Limit: 0.000%
 Background width: best method (based on spectrum).
 Half lives decay limit: 12.000
 Activity range factor: 2.000

ORTEC g v - i(175) Wan32 G53W2.10 02-NOV-2012 13:13:20 Page 2
 Oregon State University Spectrum name: Cs-134sampleNathan.An1

Min. step backg. energy 0.000
 Multiplet shift channel 2.000

Corrections	Status	Comments
Decay correct to date:	NO	
Decay during acquisition:	NO	
Decay during collection:	NO	
True coincidence correction:	NO	
Peaked background correction:	YES	bkgb125-WATERS-7Sep12.Pbc 10-Sep-2012 08:33:32
Absorption (Internal):	NO	
Geometry correction:	NO	
Random summing:	YES	Slope 1.0000E+00
	Net factor	1.0000E+00

Energy Calibration
 Normalized diff: 0.2098

***** UNIDENTIFIED PEAK SUMMARY *****

Peak Centroid	Background	Net Area	Intensity	Uncert	FWHM	Suspected
Channel	Energy	Counts	CountsCts/Sec	1 Sigma	% keV	Nuclide

51.61	10.42	4202.	8675.	14.458	1.51	0.804 HG-203 D
58.58	11.80	11154.	6671.	11.118	2.55	0.805 BI-212 D
67.82	13.64	5773.	2921.	4.869	4.12	0.807 BR-82 D
7041.75	1401.23	7.	727.	1.211	3.86	1.742 BI-214

s - Peak fails shape tests.
 D - Peak area deconvoluted.

***** IDENTIFIED PEAK SUMMARY *****

Nuclide	Peak	Centroid	Background	Net Area	Intensity	Uncert	FWHM
	Channel	Energy	Counts	CountsCts/Sec	1 Sigma	% keV	

CS-134	2388.90	475.35	2559.	1603.	2.672	5.11	1.259D
CS-134	2831.11	563.31	4784.	7246.	12.076	2.76	1.263
CS-134	2862.02	569.46	4890.	12943.	21.572	1.92	1.336
CS-134	3039.94	604.85	3606.	81024.	135.040	0.39	1.327
CS-134	4000.97	796.05	854.	56182.	93.637	0.44	1.482
CS-134	4031.54	802.13	418.	5580.	9.300	1.74	1.465
CS-134	5221.89	1039.00	147.	610.	1.016	6.54	1.398
CS-134	5872.13	1168.42	134.	948.	1.579	4.45	1.613
CS-134	6863.96	1365.84	0.	1471.	2.452	2.61	1.778

s - Peak fails shape tests.
D - Peak area deconvoluted.

ORTEC g v - i(175) Wan32 G53W2.10 02-NOV-2012 13:13:20 Page 3
Oregon State University Spectrum name: Cs-134sampleNathan.An1

***** SUMMARY OF NUCLIDES IN SAMPLE *****

Time of Count Uncertainty 1 Sigma
Nuclide Activity Counting Total
uCi

CS-134	2.9417E-01	3.893E-01%	2.988E+00%
--------	------------	------------	------------

< - MDA value printed.

A - Activity printed, but activity < MDA.

B - Activity < MDA and failed test.

C - Area < Critical level.

F - Failed fraction or key line test.

H - Half-life limit exceeded

----- SUMMARY -----

Total Activity (10.2 to 3186.1keV) 2.942E-01 uCi

***** SUMMARY OF DISCARDED PEAKS *****

1038.50 + CS-134 1167.86 + CS-134 1365.13 + CS-134

! - Peak is part of a multiplet and this area went negative during deconvolution.

? - Peak is too narrow.

@ - Peak is too wide at FW25M, but ok at FWHM.

% - Peak fails sensitivity test.

\$ - Peak identified, but first peak of this nuclide failed one or more qualification tests.

+ - Peak activity higher than counting uncertainty range.

- - Peak activity lower than counting uncertainty range.

= - Peak outside analysis energy range.

& - Calculated peak centroid is not close enough to the library energy centroid for positive identification.

P - Peak background subtraction

Analyzed by: _____
Radiation Center

Reviewed by: _____
Supervisor

Laboratory: Oregon State University

AppendixC: Miscellaneous Calculations

1. The approximate gamma attenuation afforded by a layer of plankton that is exactly the thickness of the Whatman GF/F filter ($420\text{ }\mu\text{m} = 0.042\text{ cm}$) can be described by the following:

End Counting Rate / Initial Counting Rate = $e^{-\mu x}$, where plankton are assumed to have the density of water (1 g/cm^3) due to them being approximately 90% water, and μ/ρ for 0.8 MeV photons in water is $7.857\text{E-}2\text{ cm}^2/\text{g}$ (Shultis and Faw, 2000).

$$\text{End Counting Rate / Initial Counting Rate} = e^{-\mu x}$$

$$\text{Reduction factor} = e^{-(7.857\text{E-}2\text{ cm}^2/\text{g} * 1\text{ g/cm}^3 * 0.042\text{ cm})}$$

Reduction factor = .9967055 or a ~0.33% reduction in counting rates when counted through a $420\text{ }\mu\text{m}$ thick layer of plankton.

2. Assuming 0.1 grams of plankton mass per m^3 of seawater in abundant regions of the world's oceans, the expected mass of plankton in a 10 ml sample is calculated as follows:

$$100\text{ mg} / 1\text{ m}^3 = 100\text{ mg} / 1\text{E}6\text{ ml} = 1\text{E-}4\text{ mg/ml}$$

$$1\text{E-}4\text{ mg/ml} * 10\text{ ml} = 0.001\text{ mg per } 10\text{ ml sample}$$

Assuming that F/2 enrichment increases the growth rates by a factor of 100, the estimated phytoplankton mass in a 10 ml sample is 100 μg .

3. Assuming 0.392 grams/kg of potassium at 35‰ salinity (Turekian 1968) as K^+ , 1 ml of seawater has a density of 1.025 g/ml, adding Cs^+ at 190 bq/ml is equivalent to:

$$190\text{ bq/ml} = 190\text{ disintegrations per second of Cs-134 per } 1\text{ ml of seawater}$$

$$190\text{ dps} / (\ln 2 / 2.0652\text{ years}) / 1.025\text{ grams} = 17428915245\text{ atoms / gram}$$

$$17428915245\text{ atoms/gram} / N_A/\text{gram} = 2.89 \times 10^{14}\text{ mol of Cs/gram of seawater}$$

$$2.89 \times 10^{14}\text{ mol of Cs-134/gram of seawater} * 133\text{ g/mol of Cs-133}$$

$$= 3.88 \times 10^{-12}\text{ grams Cs-134 per gram of seawater or } 3.88 \times 10^{-9}\text{ grams/kg}$$

This addition of Cs^+ is 8 orders of magnitude lower than the existing concentration of K^+ and this is assuming 100% activation of CsCl in the original activation vial. Assuming 1% activation implies that there was 100x more non-radioactive cesium added to the seawater than radioactive cesium- still 6 orders of magnitude lower than existing K^+ concentration.

4. The minimum counts that could be detected above background and control counts to obtain a false negative rate of less than 5% is obtained by the following formula (Knoll 2010):

$N_D = 4.65\sqrt{N_B} + 2.71$ where N_B is the average control counts for the duration of the experiment of 607 counts (see Appendix E).

$N_D = 4.65\sqrt{607} + 2.71 = 117$ counts in five minutes. The corresponding minimum CF that this method can quantify is approximately:

$$N_D / \text{filter efficiency} / \text{plankton mass} / \text{Average water counts} / \text{water efficiency} / \text{water mass}$$

$$CF_{\min} = ((117 \text{ counts} / 11.8 \text{ counts/Bq}) / 0.00052 \text{ grams}) / ((10760 / 6.1 \text{ counts/Bq}) / 10.2677 \text{ grams})$$

$$CF_{\min} = 111.1$$

AppendixD: Calibration Source Results

Table A4: Calibration source calculations

Counts in 5 minutes	Activity (Bq)	Decay-corrected activity (Bq)	Efficiency (counts in 5 mins/Bq)	Uncertainty
Filter				
16422	1600	1374.760563	11.94535284	0.08009272
20034	2000	1718.450704	11.65817556	0.070770757
		Average	11.8017642	0.175859451
Filtrate				
10486	2000	1718.450704	6.102008033	0.05958924

AppendixE: Fluorometry Results

Table A5: Days four and eleven fluorometry results and relative chlorophyll *a* values. Values are in relative fluorescent units.

	Day 4	Day 11
Empty	0.6600	0.6500
Blank 1	0.7450	0.8000
Blank 2	0.8400	0.7650
Plankton 1	224	271
Plankton 2	228	274
Plankton 3		280
Relative Chlorophyll <i>a</i>	225.2075	274.2175

AppendixF: Pipette Calibration Results

Table A6: Pipette calibration trial data and error analysis

Trial	10 ml pipette wt (g)	$\bar{x} - x_{avg}$	$(x - x_{avg})^2$
1	10.0227	0.00542	0.0000293764
2	10.035	0.01772	0.000313998
3	10.0282	0.01092	0.000119246
4	10.0313	0.01402	0.00019656
5	10.015	-0.00228	0.0000051984
6	10.018	0.00072	0.0000005184
7	10.083	0.06572	0.004319118
8	10.0145	-0.00278	0.0000077284
9	10.0046	-0.01268	0.000160782
10	10.0414	0.02412	0.000581774
11	10.0176	0.00032	0.0000001024
12	10.0154	-0.00188	0.0000035344
13	9.9713	-0.04598	0.00211416
14	10.007	-0.01028	0.000105678
15	10.036	0.01872	0.000350438
16	10.027	0.00972	0.0000944784
17	10.0108	-0.00648	0.0000419904
18	10.028	0.01072	0.000114918
19	9.993	-0.02428	0.000589518
20	9.9926	-0.02468	0.000609102
21	10.0075	-0.00978	0.0000956484
22	10.0275	0.01022	0.000104448
23	9.9941	-0.02318	0.000537312
24	9.989	-0.02828	0.000799758
25	10.031	0.01372	0.000188238
26	10.0096	-0.00768	0.0000589824
27	10.0016	-0.01568	0.000245862
28	10.0074	-0.00988	0.0000976144
29	10.0323	0.01502	0.0002256
30	10.026	0.00872	0.0000760384
		Sum	0.012187728
Average	10.01728	$\sigma = \sqrt{(\text{Sum}/30)}$	0.0201558
Seawater equivalent	10.267712	σ^* seawater density	0.0206597

Appendix G: Raw Data

Table A7: Raw Data

	Day 1	Day 2	Day 3	Day 4	Day 8	Day 11
Time of Sampling	1/29/2013 1230 PM	1/30/2013 1135 AM	1/31/2013 1445 PM	2/1/2013 1140 AM	2/5/2013 1300 PM	2/9/2013 1050 AM
Hours since contaminated	23.938	46.583	72.750	94.670	191.000	260.830
Time of Counting	3/21/2013 1200 PM	3/21/2013 1200 PM	3/21/2013 1200 PM	3/21/2013 1200 PM	4/16/2013 1500 PM	4/16/2013 1500 PM
Hours Elapsed between counting/filter	1223.5	1200.5	1173.25	1152.33	1682	1612
Decay fraction	0.954	0.955	0.956	0.957	0.938	0.940
HP1 Filter Net Cts	549	485	555	531	530	587
HP2 Filter Net Cts	607	581	434		492	496
HP Filter Avg Net Cts	578	533	494.5	531	511	541.5
HP Filter Deviation	29	48	60.5	23.043	19	45.5
HW1 Filter Net Cts	575	605	584	587	683	591
HW2 Filter Net Cts	643	625	555	616	629	
HW Filter Avg Net Cts	609	615	569.5	601.5	656	591
HW Filter Deviation	34	10	14.5	14.5	27	24.310
Difference of HP Avg Net and HW Avg Net	-31	-82	-75	-70.5	-145	-49.5
Percent difference	5.363	15.385	15.167	13.277	28.376	9.141
HP Filter Avg Net Cts Uncertainty	44.688	49.031	62.213	27.226	33.015	51.587
HP Filter Efficiency	11.802	11.802	11.802	11.802	11.802	11.802
HP Filter Efficiency Uncertainty	0.176	0.176	0.176	0.176	0.176	0.176
HP Filter Unadjusted Activity	51.327	47.289	43.827	47.024	46.181	48.806
HP Filter Adjusted Activity	-2.753	-7.275	-6.647	-6.243	-13.104	-4.462
HP Filter Mass (grams)	0.0052	0.0052	0.0052	0.0052	0.0052	0.0052
HP Filter Mass Uncertainty	0.000052	0.000052	0.000052	0.000052	0.000052	0.000052
HP1 Filtrate Net Cts	10818	10668	10297	10285	10992	10630
HP2 Filtrate Net Cts	10854	10882	10780	11025	10718	11170
HP FiltrateAvg Net Cts	10836	10775	10538.5	10655	10855	10900
HP FiltrateAvg Net CtsDeviation	18	107	241.5	370	137	270
HW1 Filtrate Counts	11062	11038	11007	11244	10996	11076
HW2 Filtrate Counts	11138	11122	11224	11240	11132	
HW FiltrateAvg Net Counts	11100	11080	11115.5	11242	11064	11076
HW AvgFiltrate Uncertainty	38	42	108.5	2	68	105.242
Difference of HW Filtrate and HP Filtrate Net Counts	-264	-305	-577	-587	-209	-176
Percent difference	2.436	2.831	5.475	5.509	1.925	1.615

Table A7: Raw Data (Continued)

	Day 1	Day 2	Day 3	Day 4	Day 8	Day 11
HP Water Efficiency	6.102	6.102	6.102	6.102	6.102	6.102
HP Water Efficiency Uncertainty	0.060	0.060	0.060	0.060	0.060	0.060
HP Water Activity	1775.809	1765.812	1727.054	1746.146	1778.923	1786.297
HP Water Mass	10.268	10.268	10.268	10.268	10.268	10.268
HP Water Mass Uncertainty	0.021	0.021	0.021	0.021	0.021	0.021
Unadjusted Concentration Ratio	570.710	528.791	501.081	531.757	512.599	539.503
Adjusted Concentration Ratio	-30.609	-81.352	-75.998	-70.601	-145.454	-49.317
Unadjusted CR Uncertainty	30.953	49.124	63.214	31.507	22.713	48.541
Adjusted CR Uncertainty	44.129	48.679	63.085	27.413	33.303	51.422
HW1 Net Activity	1859.4	1850.0	1850.9	1887.7	1846.9	1864.9
HW2 Net Activity	1879.8	1875.6	1886.4	1894.2	1877.6	
HW Avg Net Activity	1869.6	1862.8	1868.6	1890.9	1862.3	1864.9
Uncertainty	46.12	45.95	46.09	46.64	45.94	46.00
HP1 Net Activity	1819.4	1789.4	1734.5	1730.5	1846.3	1791.8
HP2 Net Activity	1830.2	1832.6	1803.4	1806.8	1798.2	1872.6
HP Avg Net Activity	1824.8	1811.0	1769.0	1768.6	1822.2	1832.2
Uncertainty	45.01	44.67	43.63	43.63	44.95	45.19
Diff	44.8	51.8	99.7	122.3	40.0	32.7

AppendixH: Failed methods

This appendix contains just a few informal notes about lessons learned for future work and alternate methods/materials.

Membrane filters- Membrane filters weigh substantially less than glass fiber filters, but are also much more expensive. Glass fiber filters are rigid and can easily be picked up with forceps. Membrane filters reduce the uncertainty in direct mass measurements and are easier to chemically manipulate (NH_4^+ washes), but tear very easily and cling to forceps when they are picked up. Contamination control became extremely difficult when membrane filters were tested.

Weighing GF/F filters- Glass fiber filters weighed around 185 mg when dried, and usually picked up a few milligrams of salt after seawater was ran through them, even after the distilled water rinse. Since the plankton mass was in micrograms it was difficult to directly weigh the plankton mass on each filter without having significant relative uncertainty. Other researchers have rinsed filters with 3% ammonium formate to remove salt precipitates, so this method could be tested.

Not-rinsing filters- See above- salt retention was significant, and may have impacted cesium uptake. Attempts were made to minimize contaminants on the filters other than plankton to minimize the likelihood that the cesium was bound to anything else.

Glass funnel without vacuum suction- Flow rates through the glass fiber filters was much too slow to perform this measurement without vacuum filtration. Filter saturation and then flows bypassing the filters on the edge of the funnel was a concern when this method was attempted.

Un-enriched seawater- While this is closer to an in-situ conditions, growth rates were too low to develop a reasonable population in a reasonable amount of time. Evaporation, nutrient depletion, and water loss to repeat sampling would have been a more significant factor using this method.

