Title
Testing Ballast Water Treatment at a Municipal Wastewater Treatment Plant

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Publication Date
2001-09-08

COMPLETION REPORT
CALIFORNIA SEA GRANT COLLEGE PROGRAM
Project Progress Report to NSGCP

Project Number: R/CZ-159

Project Number: Testing Ballast Water Treatment at a Municipal Wastewater Treatment Plant

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Project Initiation Date: 10/1/1999

Project Completion Date: 9/30/2000

Date of This Report: 7/8/2001

PROJECT RESULTS:

The main goal of the project was to investigate the feasibility of treating ships' ballast water in existing municipal wastewater treatment plants (= publicly-owned treatment works or POTWs). The main objectives included identifying and characterizing the limiting factors that could restrict the volume of ballast water that can be treated at POTWs; and test, in a series of laboratory experiments, the effectiveness of standard municipal wastewater treatment in removing or killing ballast water organisms. We worked with staff of the City and County of San Francisco to analyse the effects of treating ballast water at San Francisco's Southeast Treatment Plant (SETP) as a model. We report here on the experimental tests and analyses of the effectiveness of treating ballast water organisms in POTWs.

We identified seven conditions or treatments in WWTPs that have the potential to remove or kill organisms in ballast water. These are: (1) osmotic stress; (2) shear stress in the collection system and pumps; (3) light deprivation; (4) primary settling; (5) low dissolved oxygen (DO) in primary system; (6) secondary settling; and (7) disinfection.

Some of these are briefly discussed below. Our experiments focussed on osmotic stress, disinfection by chlorination, and secondary settling.

Osmotic stress. Organisms arriving in salty ballast (typically in the range of 25-35 ppt) will be subject to osmotic stress when the ballast water is diluted with sewage in the collection system and POTW. At SETP, the average residence time in the collection system plus treatment plant (i.e. the duration of exposure to low salinities) is 11 hours.

Light deprivation. Studies have shown that the concentration of organisms in ballast tanks typically decays exponentially over time (e.g. Gollasch *et al.* 1998). The leading hypothesis is that phytoplankton die off due to lack of light for photosynthesis, and that zooplankton subsequently starve.

Primary Settling. Stokes' Law calculations (which assume spherical particles, quiescent liquid, no flocculation, and dilute solutions such that particles settle independently) suggest settling times for dinoflagellate cysts and diatom spores to be on the order of 10 cm/hr and 100 cm/hr, and removal rates on the order of 10% and 50% respectively. These provide lower bound estimates for removal of these organisms. Removal would be enhanced if cysts or spores adhere to settling sluge particles. Larger an heavier motile organisms stunned or immobilized by osmotic stress would generally be expected to settle faster.

Low dissolved oxygen. In many wastewater treatment plants, the dissolved oxygen in the plant influent is quickly depleted at depth in the primary sedimentation basins, and this may be effective at killing organisms in the influent. Some plants aerate their influent upstream of the plant, primarily to avoid noxious gas (H_2S) generation, and this masy raise the minimum issolved oxygen level. Low issolved oxygen is reported to not be a problem at SETP.

Disinfection. SETP uses sodium hypochlorite (NaOCl) at an average dose of 8.6 mg CL_2/L for disinfection, with an average of 30 min. residence time in the contact basin.

Methods

General Methods

Tests were conducted on a dinoflagellate *Amphidinium carterae*, a diatom *Thalassiosira weisflogii*, a copepod *Tigriopus californicus*, and veliger larvae of the clam *Venerupis philippinarum* (Manila clam). Cultures of *A.carterae* were obtained from Carolina Biological Supply Company and a culture of *T. weisflogii* was obtained from Long Marine Lab in Santa Cruz. These were cultured using Fritz's F/2 media. All cultures were maintained at a constant temperature of approximately 17 degrees Celsius and under 40 watt cool white fluorescent bulbs. The copepod *T. californicus* was obtained from University of California Davis and fed Tahitian Blend algal paste obtained from Reed's Aquaculture. Eleven-day-old larvae of *V. philippinarum* were obtained from Taylor Shellfish of Shelton, Washington and maintained at conditions previously described and gently bubbled with oxygen. The seawater used for cultures and experiments was from Bodega Bay, CA and filtered to 0.2 microns and sterilized by microwave. The seawater was obtained from the USEPA lab in Richmond, California and had a salinity of 34 ppt as measured by refractometer. The diatoms and

dinoflagellates were examined and counted with an Olympus BHTU microscope outfitted for epifluorescence microscopy on an improved Neubauer double ruling bright-line hemocytometer.

Osmotic Stress

T. weisflogii and *A. carterae* cultures with approximately 1 x 104 cells/mL, cells were centrifuged at 2500 rpm for 20 minutes to concentrate the culture to a density of approximately 3 x 105 cells/mL. The cells were then examined under epiflorescence microscopy with green and blue excitation to determine if the cells remained either mobile and/or fluorescing after the centrifuging. Green light is absorbed by the light-harvesting fucoxanthin pigments in diatoms and peridinin pigments in dinoflagellates which fluoresce bright red in cells that are capable of harvesting light. Blue light is absorbed by chlorophyll and fluoresces bright red in cells with functioning chlorophyll pigments.

Following centrifuging, volumes of 0.2 mL, 0.8 mL and 2 mL were pipetted from the concentrated cultures into 6 sterile test tubes for each organism. Deionized water (for treatments) and seawater (for controls) and were added to each tube to bring the volume in each tube to 40 mL, resulting in 0.5%, 2% and 5% dilutions. The resulting salinities for each treatment dilution were thus approximately 0.17, 0.68 and 1.70 ppt respectively, and 34 ppt for all control dilutions. From each treatment dilution, 10 mL were pipetted into 3 separate test tubes, resulting in 3 replicates per treatment dilution. From the each control dilution, 10 mL were pipetted into one test tube, resulting in one control per dilution.

After ten hours, the contents of the test tubes were poured into 100 mL flasks and diluted with 59.65 mL seawater to bring the salinity up to at least 33 ppt and effectively end osmotic stress. Care was taken to rinse the test tubes with seawater to remove all cells. hemocytometer with an Olympus BHTU microscope. Cells were excited with green light 95 to 105 cells were examined and scored for normal/abnormal appearance, motility (dinoflagellates only) and fluorescence under green light from each treatment replicate and control.

A separate culture study was performed to determine if osmotically stressed cells were capable of reproducing. .05 mL, 0.2 mL and .5 mL of *A. carterae* and *T. weisflogii* culture were pipetted from cultures into sterile test tubes (initial culture concentrations approximately 3 x 105 cells/mL and 1 x 105 cell/mL respectively). Deionized water (for treatments) and seawater (for controls) were added to each tube to bring the volume in each tube to 10 mL, resulting in 0.5%, 2% and 5% dilutions. 1 treatment replicate and 1 control of each treatment dilution were performed. After ten hours, 5 mL were pipetted from each test tube into separate 125 mL Erlenmeyer flasks containing 100 mL of F/2 media, bringing salinities up to at least 32 ppt. Flasks were plugged with sterile cheesecloth and placed in the light and temperature conditions previously described. After 32 days, the flasks were examined for color change indicating cell growth and cells were counted in flasks that showed a color change. This procedure was repeated for the remaining flasks, and at 60 days, cells were counted in the remaining flasks.

The copepods (*T. californicus*) were poured onto a 22 micron Nitex mesh filter and washed from the filter into a petri dish by gently spraying the filter with seawater. 8

mL samples were placed in four flasks. 160 mL of deionized water was added to 3 of the flasks for treatments, resulting in 4.8% seawater dilutions. 160 mL of seawater was added to the fourth flask as a control. A similar procedure was followed with 12 day old *V. philippinarum* larvae except that the 8 mL samples were taken directly from the culture flask and not filtered. The salinities for both the copepods and the clam larvae were approximately 1.62 ppt for the treatments and 34 ppt for the controls.

After 5 hours, the copepods and clam larvae were poured from each flask onto 22 micron Nitex mesh filters, washed into petri dishes with seawater and examined under a dissection scope. 100 organisms were examined from each flask and scored as moving or not moving.

Chlorination

Standardization of Solutions

A 1.0 N sodium thiosulfate ($Na_2S_2O_3$) solution was acquired for the purpose of stopping the chlorination reactions and standardizing the sodium hypochlorite solution to be used in the chlorination experiments. The $Na_2S_2O_3$ solution was diluted to approximately 0.025 N and standardized against a 0.025 N standard potassium biniodate ($KH(IO_3)_2$) solution according to Jenkins *et al.* 1980. The concentration of the $Na_2S_2O_3$ solution was determined to be, in fact, 1.0 N. The solution was refrigerated until used the following week.

An *UltraClorox* solution (approximately 70,000 mg/L as Cl_2) of sodium hypochlorite (NaOCl) in sodium hydroxide (NaOH) was standardized against the above sodium thiosulfate stock solition using the iodometric method (Jenkins *et al.* 1980). The stock NaOCl solution was diluted to approximately 5 mg/L as Cl_2 and titrated with $Na_2S_2O_3$ in the presence of glacial acetic acid and a starch indicator. Two titrations were performed and the chlorine concentration of the diluted stock solution was determined to be 5.19 mg/L as Cl_2 using the following equation:

$$\text{mg/L as } Cl_2 = \frac{[\text{Volume of } S_2O_3^{2-} \text{ for sample (mL)} - \text{Volume of } S_2O_3^{2-} \text{ for blank (mL)}] \times S_2O_3^{2-} \text{ Normality} \times \frac{35.5 \text{ mg Cl}}{\text{meq Cl}} \times \frac{1000 \text{ ml}}{\text{liter}}}{\text{Sample Volume (mL)}}$$

The concentration of the *UltraClorox* solution was found to be approximately 71,900 mg/L as Cl_2. The solution was refrigerated until used the following week.

Sodium Thiosulfate Experiment on Diatoms and Dinoflagellates

An experiment was performed to assess the effect of sodium thiosulfate on diatoms and dinoflagellates. For the treatments, 5 mL of 1.0 N $Na_2S_2O_3$ was added to 1 mL of culture for each organism. This corresponds to approximately 2.5 times the concentration used to stop the disinfection reaction in the chlorination experiments. The controls consisted of 1 mL of culture. After 30 minutes, 100 cells were counted from each tube under epifluorescence microscopy as previously described for the osmotic stress experiments.

Chlorination Experiments on Diatoms and Dinoflagellates

Experiments were performed at initial chlorine doses of 5.0 and 8.6 mg/L Cl_2 to assess the effect of chlorination on diatoms (*T. weisflogii*) and dinoflagellates (*A. carterae*). Cell counts were made on diatom and dinoflagellate cultures and the cultures were centrifuged to yield a concentrate of approximately 10^7 cells/mL. In each experiment, 0.05 mL of concentrated culture was placed in a 10 mL test tube, and a mixture of 9.9 mL of secondary effluent from the SF–SEP and 0.05 mL of NaOCl solution at the appropriate concentration (Table A) was added to the test tube. The total BOD_5 of the secondary effluent was 6.0 ppm and the average ammonia concentration was 30.6 mg/L (measurements made by SEP staff on that batch of effluent). For controls, deionized water was substituted for the NaOCl solution. The test tubes were inverted twice for mixing and left for thirty minutes. To stop the disinfection reactions, 5 mL of 1.0 N sodium thiosulfate ($Na_2S_2O_3$) was added to each test tube, including controls. Three treatment and three control tubes were run for each organism at each dose level.

Table 1. Volumes and concentrations in chlorination experiments

Target Dose	Concentrated Culture		NaOCl Solution		Secondary Effluent
(mg/L as Cl_2)	(mL)	(cells/mL)	(mL)	(mg/L as Cl_2)	(mL)
5.0	0.05	1×10^7	0.05	1000	9.9
8.6	0.05	1×10^7	0.05	1720	9.9

Cells were examined under epifluorescence microscopy as previously described for the osmotic stress experiments. In addition, 0.2 mL samples were pipetted from two control and two treatment tubes at each dose level into 125 mL Erlenmeyer flasks containing 100 mL of F/2 media and cultured as previously described for the osmotic stress experiments.

Titrations were performed separately to estimate the residual total and free chlorine concentrations in these experiments. Mixtures of secondary effluent, NaOCl solution, and seawater (substituting for the organism culture) in the same relative proportions and concentrations as in the experiments were placed in 200 mL beakers. After 30 minutes, the total and free Cl_2 residuals were determined using the amperometric titration method (*Standard Methods 4500-Cl D*) with a standard phenylarsine oxide (PAO) as the titrant (instead of $Na_2S_2O_3$). The titrations were performed on a *HACH* digital amperometic titrator using the methods outlined in the instruction manual for free and total Cl_2 forward titrations at 0-10mg/L. Additional titrations were performed on mixtures of the NaOCl solution and de-ionized water at the same dilution as that of the experiments, to check for degradation of the NaOCl solutions over the duration of the experiments.

Settling

To test the ability of activated sludge to remove phytoplankton we compared settling rates in effluent and mixed liquor in 3 experiments: two with the diatom, *Thalassiosira weisflogii* in settling media from the reactor columns and one with the dinoflagellate, *Amphidinium carterae*, in media from a sewage plant.

Thalassiosira weisflogii *in reactor column media*

One experiment was performed in media (effluent and mixed liquor) from the control reactor column to represent non-acclimated conditions. A second experiment used media from the 5% dilution column to represent acclimated conditions. 4 250 mL samples of *T. weisflogii* at a density of approximately 4 x 104 cells/mlwere concentrated by centrifuging at 3000 rpm for 40 minutes to a volume of approximately 10 mL. 0.1 mL of concentrated culture was diluted to 1 mL and cell counts were performed. Culture volumes and initial cell densities are shown in Table 1. In each experiment, one approximately 10 mL concentrated sample was placed in each of two 1 L beakers. 990 mL of effluent and 990 mL of mixed liquor were added to the beakers and stirred using a gang-stirrer at 80 rpm for 20 minutes. 1 mL samples were taken 6 cm from the top of each beaker at 5, 10, 15, 20, 30 and 60 minutes after stirring was stopped. Three cell counts per sample were performed, the results were averaged, and the percent of cells settled was calculated by comparing intial and final cell density at 60 minutes. The initial cell density was estimated by the cell counts performed on the concentrated 10 mL samples. A second approximation of initial cell density was estimated by the cell counts performed on the cultures before centrifuging to determine if the cell count was above 4 x 104 cells/mL. Mixed liquor suspended solids and the zone settling velocity were measured according to methods previously described.

Amphidinium carterae *in sewage plant media*

The third settling experiment was conducted with the dinoflagellate, *Amphidinium carterae* with non-acclimated effluent and mixed liquor from the Marin Sanitation Agency. Two 500 mL samples of *A. carterae* culture at a density of approximately 2 x 105 cells ml-1 were centrifuged for 15 minutes at 2500 rpm and reduced to a volume of approximately 15 mL with a total cell number of approximately 4 x 107 (Table 2). 0.1 mL of concentrated culture was diluted to 1 mL and cell counts were performed using an improved Neubauer double ruling bright-line hemocytometer. The two 500 mL samples were composed of several cultures that were split evenly between the samples to insure similar initial cell densities. The same procedures that were used for measuring the settling of *T. weisflogii* as described above were followed. An additional sample was taken from the effluent beaker immediately after stirring was stopped to obtain an initial cell density.

Table 2. Initial cell densities in 3 replicates of settling experiment.

Experiment	Settling Medium	Concentrated Cell Culture			Cell Density in Media (cells/mL)
		Initial Volume (mL)	Cell Density (cells/mL)	Standard Deviation	
1	Effluent	9.4	8.6×10^5	8.0×10^4	8.1×10^3
1	Mixed Liquor	10	1.0×10^6	1.7×10^5	1×10^4
2	Effluent	9.2	9.5×10^5	2.4×10^5	8.7×10^3
2	Mixed Liquor	10.1	9.5×10^5	2.4×10^5	9.6×10^3
3	Effluent	14.8	2.7×10^6	4.5×10^5	4.0×10^4
3	Mixed Liquor	15.2	2.8×10^6	5.1×10^5	4.3×10^4

Results

Osmotic Stress

Dinoflagellates

Motility and appearance. In all dilutions, the *A. carterae* cells examined from the treatment flasks were non-motile, in contrast to the actively swimming cells examined from the control flasks. Treated cells compared to the control cells, as the cells appeared less pigmented and slightly shriveled.

Fluorescence. Fluorescence levels of treated and untreated *A. carterae* cells are shown in Figure 1. The strongest effects were observed in the most stressful dilution of 0.5% with 23% of the treated cells no longer fluorescing and 42% faintly fluorescing. In the 2% dilution, the percentage of non-fluorescing and faintly fluorescing cells decreased to 6% and 35% respectively. Differences between the percentage of non-fluorescing and faintly fluorescing cells (both bright and faint) in treatments and controls and the percentage of non-fluorescing cells in treatments and controls were analyzed in Chi-square tests (Tables 3 and 4). No significant difference between treatments and controls were observed at 5% dilution. At all dilutions, the cells scored as brightly fluorescing in the controls were generally somewhat brighter than the cells scored as brightly fluorescing in the treatments.

Figure 1. Fluorescence levels of *A. carterae* following 10 hours of osmotic stress at 3 dilutions. Treatment values are means of 3 replicates with standard deviations for each fluorescence level indicated.

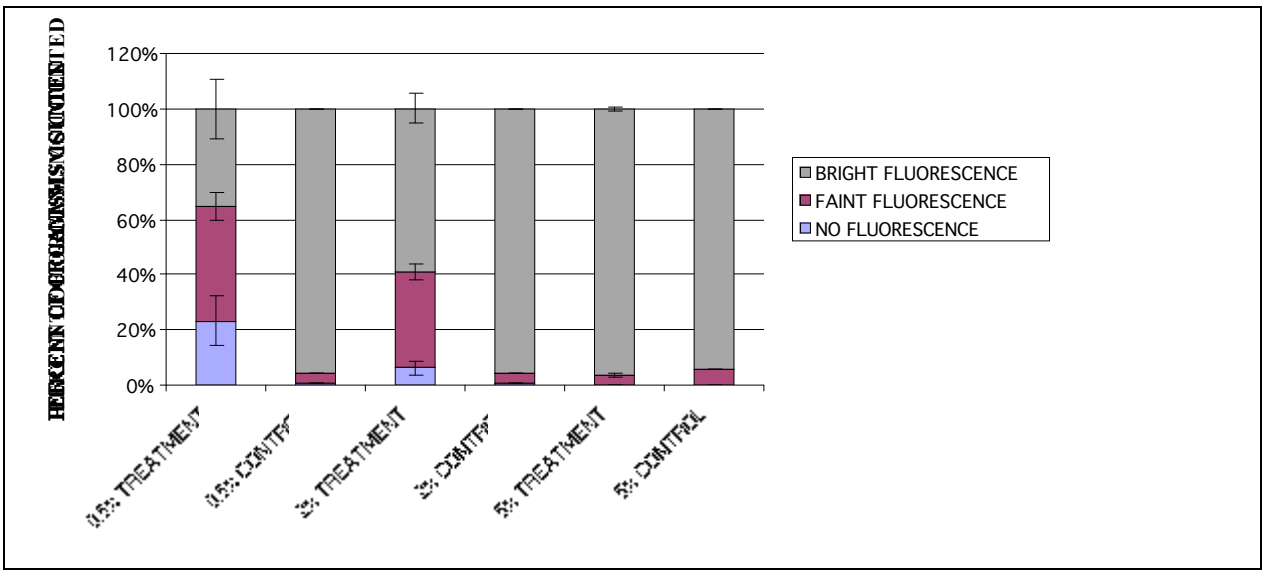


Table 3. Chi-square test results for *A. carterae* osmotic stress tests, comparing the percent non-fluorescing cells in each treatment replicate and the control at 2 dilutions. Values were not computed at the 5% dilution due to identical numbers of non-fluorescing cells between treatments and controls.

Dilution	Replicate	Chi-square	p
0.5%	1	11.06	< 0.0009
0.5%	2	32.11	< 0.0001
0.5%	3	29.41	< 0.0001
2%	1	3.70	< 0.05

2%	2	6.74	< 0.009
2%	3	1.85	< 0.0001

Table 4. Chi-square test results for *A. carterae* osmotic stress tests, comparing percent non-fluorescing and percent faintly fluorescing cells in each treatments and the control at 3 dilutions.

Dilution	Replicate	Chi-square	p
0.5%	1	62.53	< 0.0001
0.5%	2	105.01	< 0.0001
0.5%	3	80.21	< 0.0001
2%	1	37.76	< 0.0001
2%	2	48.66	< 0.0001
2%	3	32.00	< 0.0001
5%	1	.54	< 0.38
5%	2	.99	< 0.33
5%	3	.38	< 0.54

Cell growth. After 32 days, all of the control flasks in each dilution showed a visual color change and cells were counted (Table 5). Treatment flasks were allowed to grow for an additional 30 days, but no cell growth indicated by color change was noted.

Table 5. Cell densities of *A. carterae* cultures grown following 10 hours osmotic stress at 3 dilutions. Means and standard deviations given for 4 counts.

Dilution	Treatment/Control	Time	Mean cell concentration (cells/mL)	Standard Deviation
0.5%	Control	32 days	2.6 x 105	6.2 x 104
2%	Control	32 days	1.5 x 105	1.3 x 104
5%	Control	32 days	2.1 x 105	2.6 x 104
0.5%	Treatment	60 days	0	0
2%	Treatment	60 days	0	0
5%	Treatment	60 days	0	0

<u>*Diatoms*</u>

Appearance. At all dilutions, the treated cells of *T. weisflogii* were slightly shriveled in comparison to control cells and the surface of the frustules had a different appearance than in the control cells.

Fluorescence. Fluorescence results are shown in Figure 2. Significant differences between the fluorescence levels of treatments and controls were observed only at 0.5% dilution. Differences between the percentage of fluorescing cells (both bright and faint) in treatments and controls and the percentage of brightly fluorescing cells in treatments and controls were analyzed in Chi-square tests (Tables 6 and 7). As was found with A. carterae, at all dilutions the cells scored as brightly fluorescing in the controls were

generally somewhat brighter than the cells scored as brightly fluorescing in the treatments.

Figure 2. Fluorescence levels of *T. weisflogii* following 10 hours of osmotic stress at 3 dilutions. Treatment values are means of 3 replicates, with standard deviations for each fluorescence level indicated.

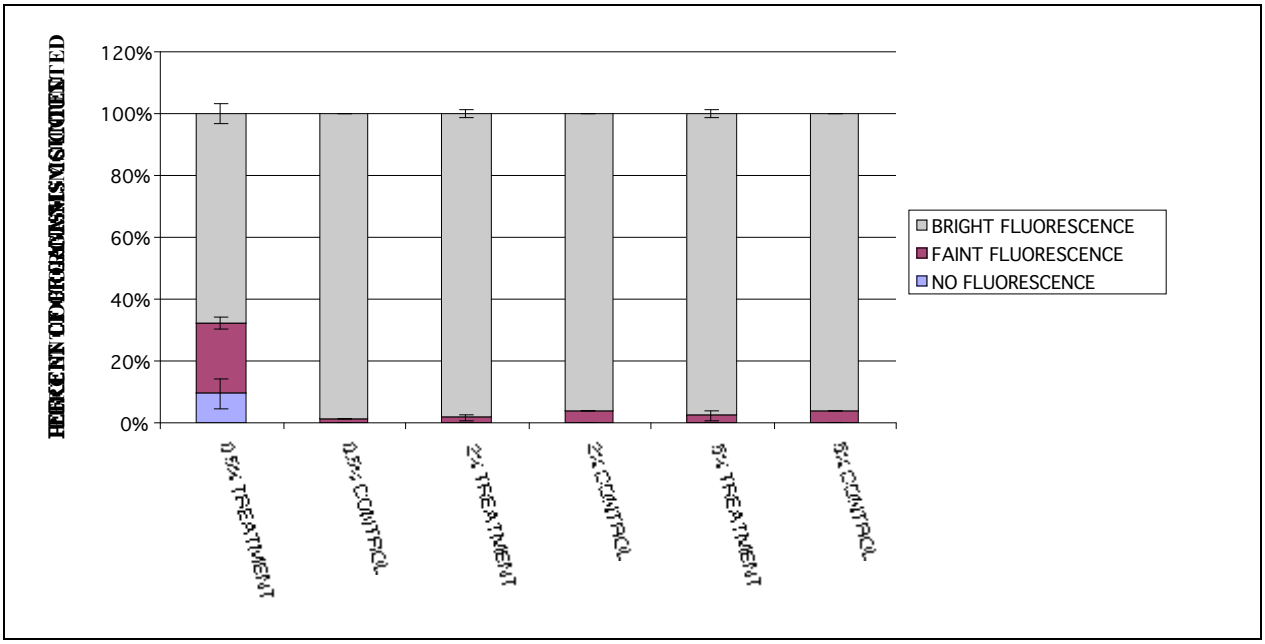


Table 6. Chi-square test results for *T. weisflogii* osmotic stress tests, comparing the percent non-fluorescing cells in each treatment and the control at 0.5%

Dilution	Replicate	Chi-square	p
0.5%	1	6.13	.0129
0.5%	2	7.25	.0071
0.5%	3	16.22	< 0.001

Table 7. Chi-square test results for *T. weisflogii* osmotic stress tests, comparing the percent non-fluorescing and faintly fluorescing cells in each treatment replicate and the control at 3 dilutions.

Dilution	Replicate	Chi-square	p
0.5%	1	7.25	0.0071
0.5%	2	8.33	0.0039
0.5%	3	16.22	< 0.001
2%	1	0.15	0.7004
2%	2	1.85	0.1742
2%	3	1.85	0.1742
5%	1	0.00	n/a
5%	2	0.69	0.4071
5%	3	1.85	0.1742

Cell growth. After 32 days, all of the control flasks showed a color change and cell counts revealed good growth from an initial concentration of no more than approximately 250 cells/mL. (Table 8). Treatment flasks were allowed to grow for an additional 30 days. Although no color change was observed in the 5% dilution (least stressful) flask at 32 days, a change was seen after 60 days and cell counts revealed good growth. No color change was observed in the 0.5% and 2% dilutions at 32 or 60 days, and cell counts at 60 days found no cells present.

Table 8. Cell densities of *T. weisflogii* cultures grown following 10 hours of osmotic stress at 3 dilutions. Means and standard deviations given for 4 counts.

Dilution	Treatment/Control	Time	Cell concentration	Standard Deviation
0.5%	Control	32 days	3.1 x 104	1.6 x 104
2%	Control	32 days	3.0 x 104	1.5 x 104
5%	Control	32 days	5.6 x 104	1.4 x 104
0.5%	Treatment	60 days	0	0
2%	Treatment	60 days	0	0
5%	Treatment	60 days	4.5 x 104	1.2 x 104

<u>*Copepods and Larval Clams*</u>
Motility. All of the *T. californicus* and larval *V. philippinarum* examined from the treatment flasks were non-motile. Organisms observed in the control flasks were actively swimming.

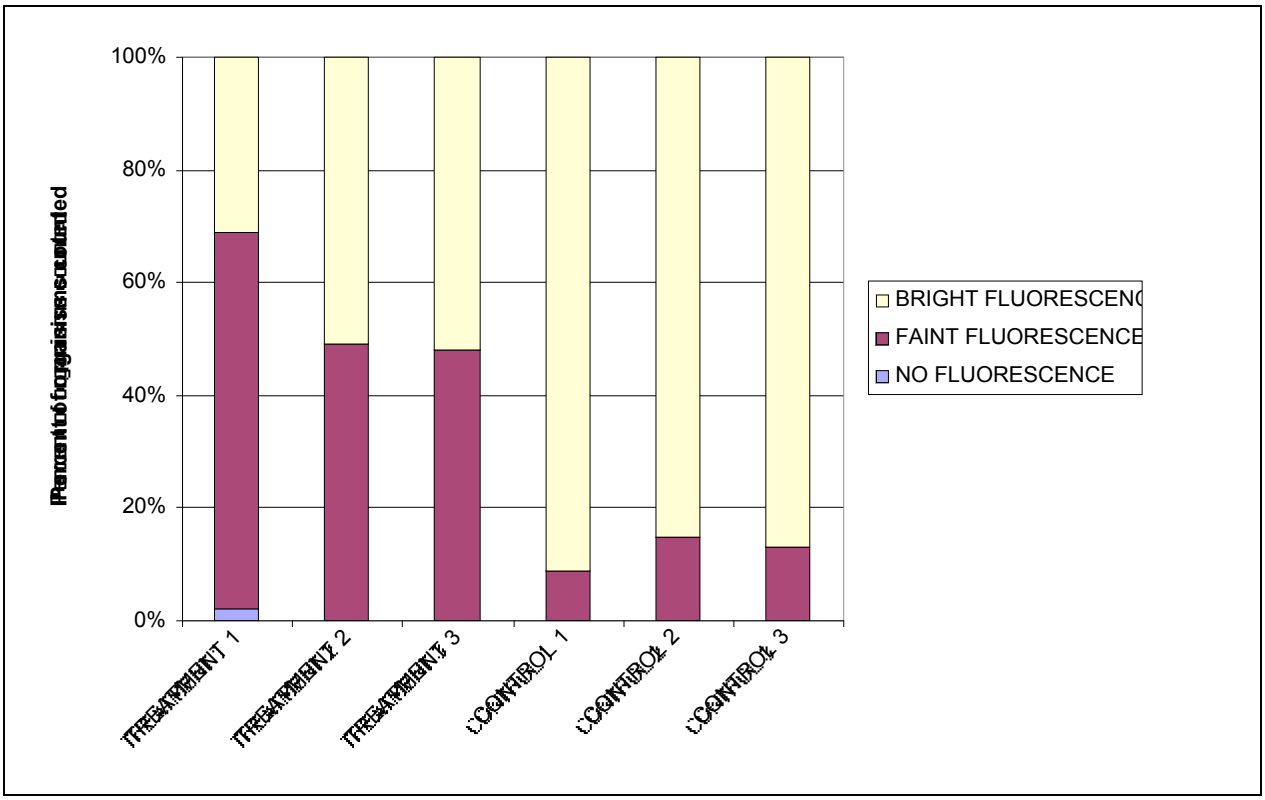
<u>Chlorination</u>
Sodium Thiosulfate experiment on diatoms and dinoflagellates. All dinoflagellates (*A. carterae*) and diatoms (*T. weisflogii*) were normal in appearance and fluorescing brightly following 30 minutes of exposure to sodium thiosulfate. The dinoflagellates were actively moving.

<u>*Dinoflagellates*</u>
Fluorescence. In the 5.0 mg/L dose experiment, all *A. carterae* examined in all treatment and control replicates fluoresced brightly under green light excitation (Table 9). At 8.6 mg/L, the number of faintly and brightly fluorescing cells was significantly different from control cells (Table 9, Fig. 3).

Table 9. Fluorescence by dinoflagellates *Amphidinium carterae* in chlorination experiments

Chlorine Dose	Treatment/ Control	% of cells not fluorescing (± SD)	% of cells faintly fluorescing (± SD)	% of cells brightly fluorescing (± SD)
5.0 mg/L	Treatment	0.0 ± 0.0	3.3 ± 3.5	97.0 ± 4.4
	Control	0.0 ± 0.0	0.3 ± 0.6	99.7 ± 0.6
8.6 mg/L	Treatment	0.7 ± 1.2	54.7 ± 10.7	41.3 ± 10.0
	Control	0.0 ± 0.0	12.3 ± 3.1	87.7 ± 3.1

Figure 3. Fluorescence by dinoflagellates *Amphidinium carterae* in 8.6 mg/L chlorination experiment



Diatoms

Fluorescence. Fluorescence levels in *T. weisflogii* exposed either to 5.0 mg/L or 8.6 mg/L were not significantly different from the control cells (Table 10).

Table 10. Fluorescence by diatoms *Thalassiosira weisflogii* in chlorination experiments

Chlorine Dose	Treatment/ Control	% of cells not fluorescing (± SD)	% of cells faintly fluorescing (± SD)	% of cells brightly fluorescing (± SD)
5.0 mg/L	Treatment	0.0 ± 0.0	5.3 ± 1.2	94.7 ± 1.1
	Control	0.0 ± 0.0	3.3 ± 1.5	96.7 ± 1.5
8.6 mg/L	Treatment	0.3 ± 0.6	4.7 ± 4.0	94.3 ± 4.5
	Control	0.3 ± 0.6	5.0 ± 3.0	95.7 ± 2.1

In all samples examined, the treated dinoflagellates and diatoms did not look healthy as the appearance of the cells differed greatly from that of the control cells, being shriveled and less pigmented. Dinoflagellates were not moving in either the treatment or control samples due to centrifuging.

Cell growth. After 36 days, the diatom control flasks at both doses showed a visual color change and cells were counted (Table 11). Control flasks of *A. carterae* and treatment flasks of *T. weisflogii* and *A. carterae* were allowed to grow for an additional 30 days, but no cell growth indicated by color change was noted.

Table 14. Average cell densities of *T. weisflogii* control and treatment flasks following 30 minutes of chlorine exposure at two doses. Means and standard deviations given for 4 counts. No cell growth indicated by color change was noted in treatment and control flasks of *A. carterae* after 60 days.

Dose (mg/L)	Control/ Treatment	Time	Average Cell Density (cells/mL)	Standard Deviation
5.0	Control	36 days	5.9 x 104	1.5 x 104
5.0	Control	36 days	2.9 x 104	4.3 x 103
8.6	Control	36 days	1.3 x 104	4.7 x 103
8.6	Control	36 days	1.9 x 104	1.4 x 103
5.0	Treatment	60 days	0	0
5.0	Treatment	60 days	0	0
8.6	Treatment	60 days	0	0
8.6	Treatment	60 days	0	0

The titrations showed significant chlorine residuals after 30 minutes of contact with secondary effluent and seawater (Table 15), and indicated that the NaOCl solutions did not degrade significantly over the course of the experiments (Table 16).

Table 15. Chlorine residuals in test titrations after 30 minutes contact.

Target Dose (mg/L)	Free Chlorine Residual (mg/L)	Total Chlorine Residual (mg/L)
5.0	0*	3.4
8.6	1.5†	6.8

* The results of two trials were below the detection limit.

† Average of two trials (1.77 mg/L, 1.31 mg/L).

Table 16. Chlorine concentrations in test titrations to check degradation of NaOCl solutions

	Free Cl_2		Total Cl_2	
Target Dose (mg/L)	Before Tests (mg/L)	After Tests (mg/L)	Before Tests (mg/L)	After Tests (mg/L)
5.0	*	5.2	*	5.5
8.6	8.4	8.3	8.5	8.4

* These titrations were not performed, but the concentrations at the start of the tests were assumed to be approximately 5.0 mg/L based on the Cl_2 concentration determined in the standardization of the *UltraClorox* solution.

Settling

T. weisflogii *in reactor column media*

The two estimates of cell density calculated by counts performed before and after centrifuging fell within the same range. The effects of non-acclimated activated sludge on the settling rates of *T. weisflogii* were significantly different than the settling rates of *T. weisflogii* in effluent (Fig. 4). After 15 minutes, very few cells were observed in the mixed liquor using the described counting methods. The cell density in the effluent samples decreased slightly over the 60 minute sampling period. Approximately 18% of

the cells settled out of the sampling zone of the effluent, while 96% settled out of the mixed liquor. Mixed liquor suspended solids and zone settling velocity measurements are shown in Table 17.

Figure 4: Cell density of *T. weisflogii* in non-acclimated mixed liquor and effluent over time. Error bars represent standard deviation of the average of the 3 counts performed. Closed triangle represents estimated cell density calculated by counts of cultures before centrifuging (approximately 4 x 10 4 cells/mL)

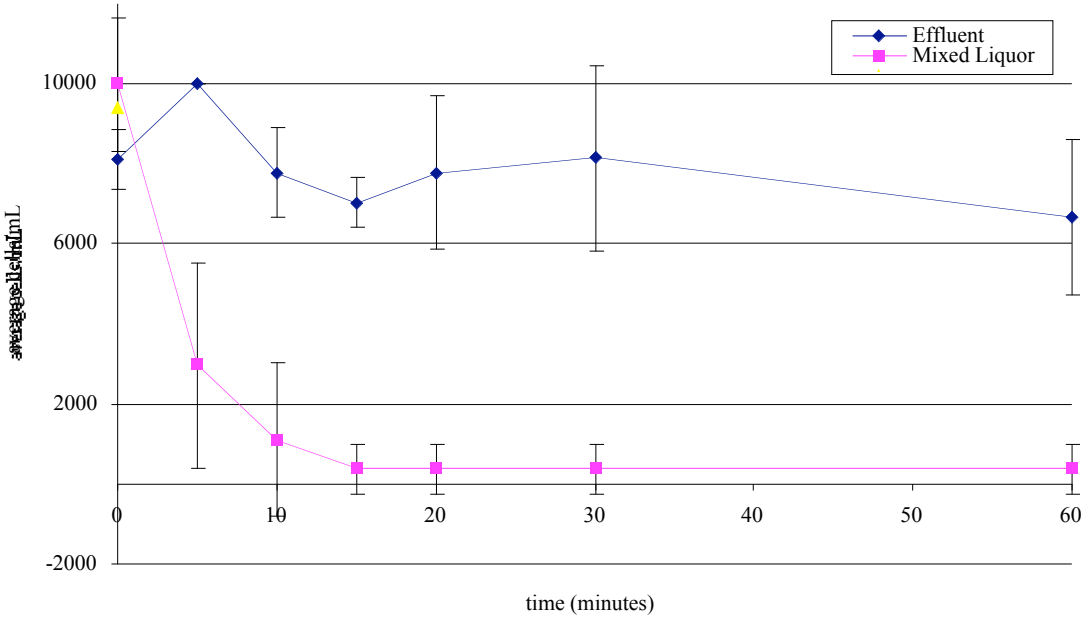


Table 17. Estimates of mixed liquor suspended solids and zone settling velocities in 3 experiments.

Experiment	Mixed liquor suspended solids (mg/L)	Zone settling velocity
1	1654	6.82
2	1600	7.23
3	1200	–

In the second experiment with *T. weisflogii*, using acclimated effluent and mixed liquor, initial cell densities were estimated by cell counts performed on the concentrated 10 mL samples. A second estimate of cell density at time zero estimated by the cell counts performed on the cultures before centrifuging fell within the same range. The cell density in the mixed liquor decreased significantly, but did not reach the low levels observed in the non-acclimated mixed liquor (Fig. 5). In the effluent, the cell density decreased significantly, in contrast to the constant cell densities observed in non-acclimated effluent. Approximately 49% of the cells settled out of the sampling zone in the effluent, while 69% settled out of the mixed liquor (Table 18).

Figure 5: Cell density of *T. weisflogii* in acclimated mixed liquor and effluent over time. Error bars represent standard deviation of the average of the 3 counts performed. Closed triangle represents estimated cell density calculated by counts of cultures before centrifuging (approximately 4 x 10 4 cells / mL)

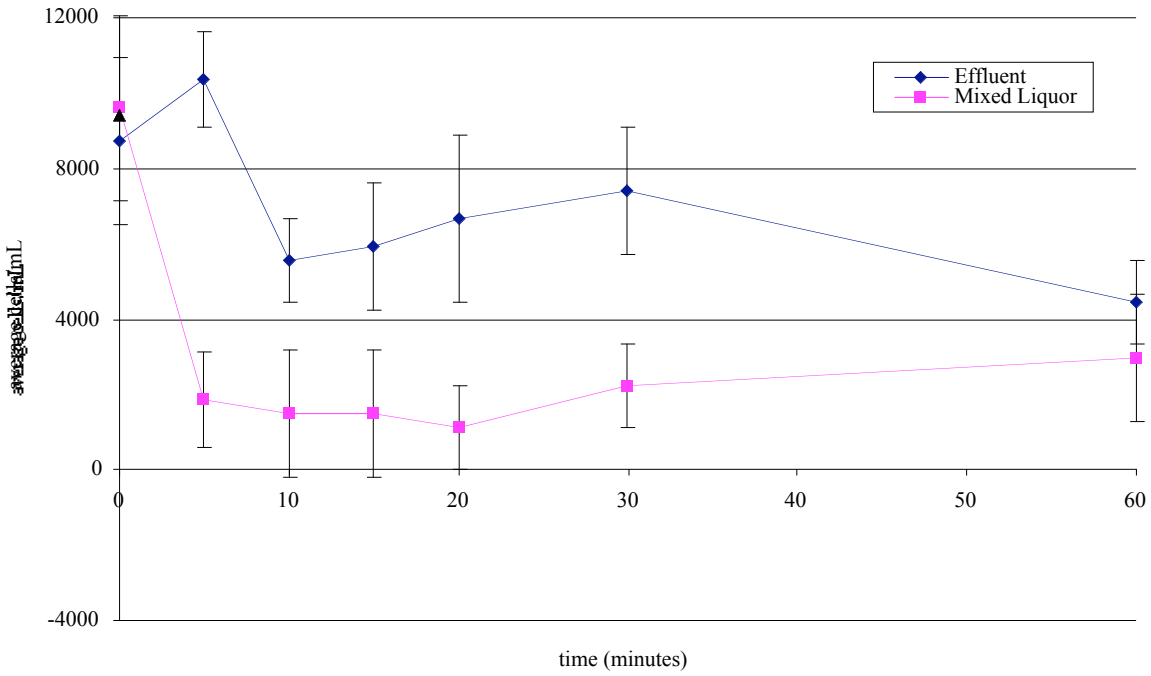


Table 18. Estimate of the percentage of cells that settled calculated from initial cell densities and the final cell densities at 60 minutes. The initial cell density calculated previous to centrifuging was used in Experiment 3.

Experiment	Organism	Media	Percent Settled
1	T. weisflogii	EFFLUENT	17.65
		MIXED LIQUOR	96.30
2	T. weisflogii	EFFLUENT	48.97
		MIXED LIQUOR	69.17
3	A. carterae	EFFLUENT	32.00
		MIXED LIQUOR	65.00

A. carterae *in sewage plant media*

Initial cell densities were estimated by cell counts performed on the concentrated 15 mL samples. These estimates did not fall into the same range as estimates obtained by cell counts performed on cultures before centrifuging. If estimates from the concentrated culture are used, the cell density of the mixed liquor remains constant, while the cell density of the effluent increases. Using the estimate from counts obtained before centrifuging, the cell density of the mixed liquor decreases significantly, while the cell density of the effluent decreases slightly. Approximately 32% of the cells had settled

out of the sampling zone in the effluent, while 65% had settled in the mixed liquor (Fig. 6).

Figure 6: Cell density of *A. carterae* in mixed liquor and effluent over time. Closed triangle represents estimated cell density calculated by counts of cultures before centrifuging (approximately 2 x 10 5 cells/mL). X and diamond represent estimated cell density calculated by counts of 15 mL samples after centrifuging. Error bars represent standard deviation of the average of the 3 counts performed.