THE USE OF A TILAPIA HYBRID TO REMOVE NITROGEN AND PHOSPHORUS FROM WASTEWATER

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ABSTRACT

This study examined the ability of a fish-periphyton system to remove nitrogen and phosphorus from wastewater. Other studies have shown that systems such as this are capable of removing nutrients; however, they either lacked controls, used fish of mixed age classes, or consisted of only one trial. The goal of this study was to use a one-tank system instead of a series of tanks to examine removal, as well as to compare the removal efficiencies of juvenile and adult fish. Three trials were conducted in the summer of 2003 consisting of three weeks each. Influent flowed in and out of each tank separately (as opposed to a series of tanks as in other studies) and was received by three treatments: algae alone as a control, tanks of 10 adults weighing an average of 100g each, and tanks of 100 juveniles weighing 1g each. Reduction of nitrogen in the influent occurred at an overall rate of 142g day^{-1} $tank^{-1}$ for algae alone, 70.1g day^{-1} $tank^{-1}$ for juveniles, and an effluent increase of 44.4g day^{-1} $tank^{-1}$ for adults. Total phosphorus was not removed on average by any treatment, possibly due to the tanks not being run in an interconnected series. This study suggests that if fish are to be used in a wastewater treatment system, juveniles would be more efficient at removing nitrogen than adults.

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INTRODUCTION

There has been an increasing demand for more efficient wastewater treatment practices both in developed and developing countries as the world's population increases (McGarry 1980, Jewell 1994). Current wastewater treatment practices have reached their maximum efficiency and applicability, and in developing countries, the lack of any type of wastewater treatment only contributes to the myriad health problems experienced there. Wastewater treatment systems that are both efficient and ecological are needed and are currently being examined. Examples of such systems include the use of both natural and created wetlands to treat effluent from wastewater treatment plants (WWTPs) (Dolan *et al.* 1981, Brown 2001), fish periphyton systems (Drenner *et al*. 1987, 1990, 1995, 1997, Rectenwald and Drenner 2000), and algal systems (Vymazal 1988, Adey 1993, Davis 1990).

There have been several studies examining the ability of a fish-periphyton system to remove nutrients from water and wastewater (Drenner *et al.* 1995, Drenner *et al.*1990, Rectenwald and Drenner 1997, Drenner *et al.* 1997) as well as studies that have addressed the ability of algae alone to remove nutrients (Vymazal 1988, Adey 1993). Most studies showed promise in that they were capable of removing impressive amounts of nitrogen and phosphorus from the water; however, the fish-periphyton experiments either lacked adequate controls (Rectenwald and Drenner 1997, Drenner *et al*. 1997), were terminated early, or consisted of only a few weeks of trials or only one trial (Rectenwald and Drenner 1997, Drenner 1997 *et al.*, Drenner 1990, Drenner 1995).

These studies all examined the ability of certain systems to remove nutrients such as nitrogen and phosphorus from wastewater. Inputs of phosphorus and nitrogen into natural waters are a notorious cause of cultural eutrophication. Many studies have

discussed the role of phosphorus in causing the eutrophication of waters. Bricker *et al.* (1999) studied numerous estuaries in the United States and found that nutrient inputs, especially those of nitrogen and phosphorus, lead to decreased light availability and increases in algae, epiphytes, and macroalgae. Edmondson (1970) found that after diversion of sewage from Lake Washington, phosphorus levels within the lake were greatly reduced, whereas nitrogen levels were only minimally reduced. Other improvements in the lake's water quality included reduced algal abundance and increased transparency of the lake. Elser *et al.* (1990) accept that phosphorus inputs are most often responsible for eutrophication of waters, but emphasize that the addition of nitrogen and phosphorus together has a more significant role in eutrophication than either of the nutrients alone. Schindler (1974) enriched small lakes with nitrogen and phosphorus and found that phosphate-enriched lakes became highly eutrophic very quickly whereas lakes enriched with nitrogen and carbon showed no significant differences from prefertilization conditions. Also, when phosphorus was not added, no algal blooms were noted. When the additions stopped, the lakes recovered quickly. Henderson-Sellers and Markland (1987) have discussed the direct positive linear relationship between phosphorus and chlorophyll a levels. Horner and Welch (1981) examined the effect of phosphorus addition on stream periphyton communities, finding that phosphorus enrichment increased the rate of growth and biomass of algae more significantly than nitrogen enrichment. Bothwell (1989) noted that in most lotic periphyton communities, phosphorus is the limiting factor in periphyton growth and that high accumulations of lotic periphyton developed even with very small additions of phosphorus.

Traditional wastewater treatment plants (WWTPs) typically employ three stages of treatment to remove solids, nutrients, and toxins from influent water. A combination

of settlement processes (the settlement of suspended materials and subsequent removal) and biological processes (the use of cells and organisms to convert these materials into cellular materials and later removal) act together to do this. Primary treatment involves the use of bar screens to remove large objects present in the influent, while secondary treatment can have several different steps depending on the size and specific function of the WWTP. A typical design would involve a grit filter to receive influent that has been primarily treated. High flow rates in these filters allow larger particles to settle to the bottom and lighter materials to float and move into settling tanks. A slow flow rate in settling tanks allows a longer water retention time for suspended particles to settle to the bottom and then be removed. Some type of biological treatment then follows, usually rotating contact tanks or trickle filters that use microorganisms (primarily bacteria) to uptake the particles remaining in the water as a food source. These organisms are then removed from the water in secondary settlement and can be used in activated sludge processes. Tertiary treatment polishes the effluent through the use of sand, gravel, or other similar material filters before final discharge into the receiving water body. This step can also involve the use of chemicals to remove nutrients or to eliminate any living cells present (Barnes and Wilson 1976, Cameron and Cross, 1976). Tertiary treatment is usually prohibitively costly to small municipalities or industries; therefore, an ecological system that could eliminate the need for costly chemicals and highly skilled workers would be appropriate for smaller WWTPs.

Tilapias in particular have been used in numerous studies to treat wastewater or remove nutrients in a fish-periphyton or aquatic macrophyte system. Tilapias have been cultured extensively all over the world and many studies discuss both their biology and culture (Fishelson and Yaron 1983, Pullin and Lowe-McConnell 1982, Moriarty *et*

*al.*1973, Jauncey 1981, Santiago *et al.* 1987, Mironova 1974, Dempster 1993, Dempster 1995, Lovell 1979, Meske 1985, Bromage and Roberts 1995). Dempster *et al.* (1993) found that *Oreochromis niloticus* (Nile tilapia) had much higher ingestion rates in treatments in which periphyton and phytoplankton were presented as food, and that these fish preferentially fed on periphyton rather than filter-feeding on phytoplankton. A later study by Dempster *et al.* (1995) found that filter feeding alone failed to support growth in tilapia, and that the fish fed 10 to 40 times more on periphytic mats than on suspended phytoplankton. Hofer and Newrkla (1983) also determined that tilapia feed primarily on algae and detritus through the use of gut studies, as did Huchette *et al.* (2000) using artificial substrate cages. Azim *et al.* (2001) showed that by supplementing available algal substrates with materials such as bamboo and PVC, the use of periphyton alone as food can produce high yields in tilapia pond culture. The fish-periphyton system takes advantage of the diets of tilapias that graze on algae (specifically periphyton) that have taken up nutrients from the wastewater. Consequently, nutrients are effectively removed from the water column and ultimately assimilated by the fish.

Drenner *et al*. (1987) performed a study of the effect of tilapia (*Tilapia galilaea)* on phytoplankton community structure and found that tanks with fish had reduced phosphorus levels and increased levels of nanoplankton as compared to tanks without fish. Drenner *et al*. (1990) again examined the responses of a pond community with nutrient addition and fish. Bluegill (*Lepomis macrochirus*) were used in this study and significantly increased periphyton chlorophyll levels, algae levels, and total phosphorus levels as compared to tanks without fish.

A third study by Drenner *et al*. (1995) specifically examined the feasibility of a fish-periphyton wastewater nutrient removal system. Liquid fertilizer was added to lake

reservoir water to increase nutrient levels. Results showed tanks with stoneroller minnows (*Campostoma anomalum*) had greater total nitrogen and total phosphorus removal than tanks without fish; however, there was no consistent biomass of fish among tanks in one trial. Another ecological wastewater treatment method studied by Drenner *et al.* (1997) used *Tilapia mossambica* and algae to treat wastewater in lanes of tanks. Tanks with fish removed more TN and TP than tanks without fish; however, there were several possible problems with this study. Stocked fish biomass was similar in all tanks but fish of varying size were used, and in a second trial, stoneroller minnows were used in a 24-tank series with no control series, there was continual stocking of additional fish, and tilapia were added later in the trial.

Rectenwald and Drenner (2000) studied the use of a *Tilapia mossambica*-algal system to remove nutrients from actual municipal wastewater. This system used one series of twelve tanks with fish. Removal rates of up to 121 mg TP $m^{-2}d^{-1}$ and 385 mg TN $m^{-2}d^{-1}$ were reported; however, fish of all sizes and ages were stocked in all tanks and the entire system lacked a control treatment of any sort.

Yi *et al.* (2002) used a tilapia-lotus culture system to remove nutrients from old pond mud. After the fish are removed, mud left in the ponds containing nutrients is removed and has been used to fertilize farmland; however, its removal is time- and cost-intensive. Ponds were stocked with fish alone, fish and lotus, and lotus alone. Nutrients were added to all but the lotus only treatment. The lotus-tilapia system was found to remove about 2.4 tons N annually, and 1 ton P annually. The system could be used to culture tilapia for consumption, and the lotus can be used for many commercial purposes.

Other studies have indicated that periphyton-only systems are capable of removing nutrients from wastewater as well. Vymazal (1988) used a trough system with

screens for algal attachment to remove nutrients in a flow-through system. A high rate of flow (312.5 l h^{-1}) was employed to treat water from a local river. Removal rates were up to 70% for phosphate and 24% for nitrate. However, algae present in the effluent would have to be removed prior to release into natural waters.

Adey *et al.* (1993) used algal "floways" with screens for algal attachment and an algal scrubber system with screens of algae in series. Influent consisted of water from a drainage canal and local algae were allowed to grow on the screens to develop a periphyton community. Only phosphorus removal was measured, and removal of TP was approximately 17% for the floway system and approximately 26% for the serial scrubbers. This system also required scraping to remove algae, which could be used for feed or fertilizer. Additionally, Boston (1993) stated that periphyton has been found to uptake and adsorb toxicants using its large surface area on substrata.

Davis *et al.* (1990) studied the ability of periphyton present in a secondary clarifier of a wastewater treatment plant (WWTP) to remove nitrogen and phosphorus. Clay tiles were placed in the clarifier and uptake rates were as high as 160 mg P m^{-2} d^{-1} and 1900 mg N m^{-2} d^{-1}. Lower temperatures and higher current velocity decreased uptake rates, as did grazing; however, numerous studies (Edwards *et al.* 1981, Drenner *et al.* 1987, Drenner *et al.* 1990, Mulholland *et al.* 1991, and Huchette *et al.* 2000) have showed that periphyton communities had increased biomass and were healthier in the presence of grazers.

In addition to wastewater systems involving tilapia, there have been studies in which tilapia are grown for aquacultural purposes in various types of wastewater, including power plant effluent to control algae and aquatic plants (Ferreira and Schoonbee 1983), sewage-fed stabilization ponds in which harvested tilapia were used as

high-protein animal feed (Edwards *et al.* 1981), and the use of chicken manure to culture tilapia (Lovell 1979). Additionally, Kohler and Pagan-Font (1978) examined the use of rum distillation wastes, pharmaceutical wastes, and chicken feed for aquaculture of tilapia and found that these growth media had minimal adverse effects on fish health. Ernst *et al.* (1989) found that using chicken manure as food in red tilapia seawater pools was feasible, although fish productivity was greatly variable in their treatments.

In this study, the ability of juvenile fish (approximately 1.0 gram each) to remove nutrients from wastewater was directly compared to that of older fish (100 to 150 grams each) on a per gram of body weight basis. As juvenile fish have a higher feeding rate demand than older fish (Cho 1990, Jauncey and Ross 1982, Kubaryk 1980, Meske 1985) and more efficiently utilize food for growth than adults at temperatures of 28-30^{o} C (Mironova 1976), I hypothesized that they would be able to remove more nutrients than the older classes on a per unit weight basis. If so, it would be more economically feasible for wastewater treatment plants and industries implementing this system to purchase and stock juveniles than adults.

There are several possible outcomes of this study that would reinforce the aforementioned hypothesis. The juvenile fish could show a higher growth rate than the adults, and thereby assimilate more nutrients in the process of weight gain. Juveniles could show a higher assimilation of nutrients than adults (aside from any increases due simply to growth), therefore removing more nutrients from the influent. Alternatively, nutrients in the effluent of juvenile tanks could be lower than tanks containing adults over the course of the experiment, or the periphyton found in juvenile tanks could contain more nutrients than periphyton found in the adult tanks. It is worth noting, however, that the current system does not attempt to optimize all conditions that would lead to

maximum nitrogen and phosphorus removal from the influent. This study does attempt to provide a direct comparison of the abilities of the different age classes of tilapia (not the abilities of the periphyton or tank design specifically) to remove nutrients from the water column.

METHODS

Wastewater System Design

A fish-periphyton system was implemented at the UNCW Center for Marine Science Aquaculture Facility. The fish-periphyton system (Figs. 1) consisted of nine independent 378.5-liter tanks (approximately 3.366 m^2 internal surface area) with their own inflow and outflow and one 3,028-liter mixing tank.

Approximately 80g of Peters' inorganic fertilizer (2:1 N:P as labeled; 4.6:1 actual) was added to the mixing tank and diluted to concentrations within a range of values of total nitrogen and total phosphorus reported in effluents of wastewater treatment plants within a 100-mile radius of Wilmington, NC (NCDENR public records). The water used in this dilution was tap water available from the system's location at Wrightsville Beach. The target range of values for nutrients was 2 mg/L for total nitrogen and 1 mg/L for total phosphate according to values detected at these wastewater treatment plants. A chlorine test kit was used to measure the amount of chlorine present and the water was dechlorinated as needed using sodium thiosulfate. Refilling and dechlorination of the water in the mixing tank occurred every other day.

The mixing tank supplied water to each 378.5 L tank through a PVC pipe along which nine faucets were placed, allowing each 378.5 L tank to receive its own inflow of water independent of the other tanks. A 946-liter per hour Mag Drive submersible pump in the mixing tank monitored by a timer supplied water to each tank at a rate of approximately 5.68 liters per hour. Each tank also had an airstone placed in a 2" PVC pipe that airlifted water out from each tank at a rate of approximately 5.68 liters per hour and into an adjacent storm drain. The balance between inflow and outflow resulted in a daily tank turnover of approximately 36%.

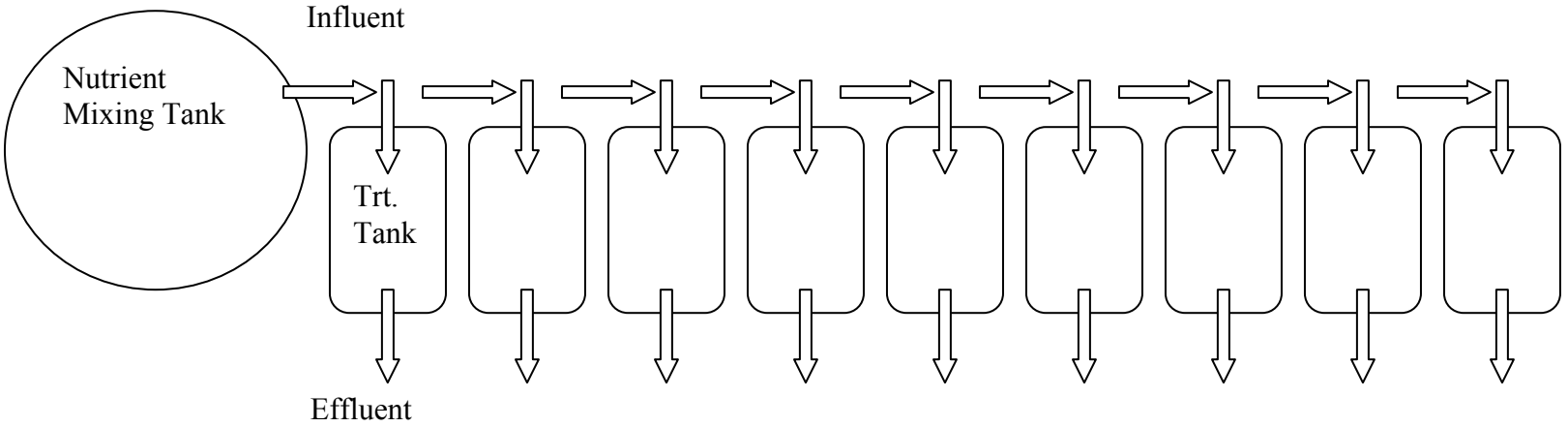

Fig. 1. Schematic of treatment tanks, influent, and effluent.

The tilapia used in this experiment thrive in waters between 28 and 31 degrees Celsius (Prillaman, personal communication), maintained by one 300-watt heater with thermostat (set at 30 degrees Celsius) per tank. A minimum/maximum Sper Scientific thermometer placed in each 378.5-liter tank monitored daily temperature. A corrugated plastic rain screen was placed over the 378.5-liter tanks to reduce rainwater inputs, fish mortality due to jumping, and predation by birds. Tank nets were placed over each tank to confine the fish to their assigned tank. The mixing tank was covered by a plastic tarp elevated at the center by a PVC frame to prevent rainwater inputs.

Rocks covered with algae located in the trickle filter of the James A. Loughlin Wastewater Treatment Plant in Wilmington, NC were collected prior to each trial. Each 378.5-liter tank was filled with tap water and dechlorinated and three rocks of similar size were placed in each 378.5-liter tank (approximately 3.365 m^2 internal surface area) and algae allowed to grow for seven days. Miracle Gro (2:1 N:P ratio as labeled, 4.6:1 actual) was added to each tank in the amount of approximately 20g the same day the rocks were added to promote rapid algal growth. The preliminary studies showed that this was a necessary step to ensure sufficient food for the fish upon their addition. Three 2-foot sections of two-inch diameter PVC piping were placed in each tank to provide additional algal substrate (Azim *et al.* 2001), and three clay tiles (225 cm^2 each) were suspended using cable ties in each tank to obtain measurements of periphyton present (Aloi 1990).

The fish used were hybrids produced by Blue Ridge Aquaculture in Martinsville, Virginia that are most closely related to an *Oreochromis aureus* / *Oreochromis niloticus* hybrid. Juveniles were transported using large plastic bags filled with water and saturated with oxygen and placed inside a transport cooler. A small transport tank with an

aerator was used to move the older class fish. After the 7-day period of algal growth (described in the following paragraph), 100 juvenile tilapia (1.0g mean body weight) were added to each of three tanks, with each tank receiving approximately the same weight of fish. Older tilapia (75 to 150g) were added to each of three tanks at approximately equal amounts of weight per tank, with 10 fish per tank. The fish were counted and weighed prior to stocking and at the conclusion of each trial using an Ohaus portable scale. Fish weights were measured only twice per trial to eliminate unnecessary stress to the fish, once immediately prior to stocking, and at the conclusion of each trial. (Two preliminary trials conducted in August and September 2002 contained 145 juveniles in each juvenile tank and 10 adults per adult tank. Juvenile mortality was a major factor in the second study, probably due to their extremely small size upon transport). The remaining three tanks had no fish in order to assess the ability of algae alone to reduce nutrients. At the time of stocking, dissolved oxygen, temperature, and pH measurements were made in each of the treatment tanks. Tanks with water quality levels most conducive to fish health and safety were stocked with fish. Each trial consisted of seven days of algal growth with no fish or simulated wastewater flow, followed by two weeks of measurements with fish.

Nutrient and Chlorophyll *a* Analysis

The tiles were scraped in straight lines with a microscope slide and algal amounts measured using chlorophyll a analyses expressed in milligrams per square meter according to Welschmeyer (1994) every week beginning at the conclusion of the one week of algal growth. Total algae present were extrapolated from these tiles according to the internal surface area of the tanks and pipes. Half of the tile surface was scraped for

chlorophyll a analysis while the other half was scraped and filtered in a similar fashion for periphyton total nitrogen and total phosphorus analysis by persulfate digestion (Parsons *et al.* 1984, Valderrama 1981). Algae from each half of the tile was filtered through a 47-millimeter type AE glass fiber filter, wrapped in aluminum foil and frozen until later analysis. Suspended algae samples were taken three times weekly in three 50-millileter samples from each tank for chlorophyll *a* analysis. Samples were immediately filtered through a 25mm type AE glass fiber filter and wrapped in aluminum foil and frozen until later analysis using a Turner 10 AU fluorometer after extraction according to Welschmeyer (1994). Three additional 50-milliliter tubes were filled, filtered, and frozen once weekly in a similar manner for suspended algae total nitrogen and phosphorus analysis according to the aforementioned method.

Total phosphorus in the outflow was measured using persulfate digestion according to Parsons *et al.*, (1984) and Valderrama (1981). Three 50-milliliter samples were taken in acid-washed polyethylene bottles from each tank's wastewater tube daily and from the nutrient mixing tank (influent) daily and also when mixing the fertilizer (the tank was usually refilled every other day) to ensure proper concentration and kept frozen until auto analyzer analysis. Total nitrogen in the effluent was measured according to Valderrama's (1981) methods using persulfate digestion and auto analyzer analysis using the same samples as for total phosphorus. All water quality samples were taken at the same time daily for the duration of the trials and subsequently analyzed using an Alpkem autoanalyzer. Also, nutrient levels were measured in the effluent of each tank prior to the addition of fish on the days when fish were stocked in order to ascertain baseline nutrient levels in the tanks. No attempts were made to "normalize" these initial nutrient levels in terms of nutrient removal after the initial addition of 20g Miracle Gro as aforementioned.

Removal of nutrients from the influent was calculated by graphing the influent and effluent data and determining the day of the trial at which at least one treatment had effluent nutrient levels lower than nutrient levels in the influent. After this point, removal was calculated on a daily basis by subtracting the nutrient levels in the effluent from the nutrient levels in the influent.

Three adult fish and several juveniles were sacrificed and euthanized with MS-222 and frozen for tissue total nitrogen and total phosphorus analysis immediately after transport at the beginning of each trial. These samples were compared to fish tissue nutrients at the end of each trial (also euthanized and frozen) for TN and TP content. Whole adult fish were homogenized in a Hamilton Beach blender individually. For each tank, a composite sample of three fish from each tank containing adults was made by taking a small amount of homogenized material from each fish and mixing well. Juveniles were processed by tank by taking approximately half the juveniles present at the conclusion of each trial and homogenizing simultaneously into a composite sample. Fish euthanized immediately after transport (both adult and juvenile) were also prepared in the manner described above. Fish tissue nitrogen was analyzed according to EPA method 351.3 for total Kjeldahl nitrogen, tissue phosphorus analyzed according to EPA method 365.2 (880 nm) for total phosphorus, and total solids (to determine wet weight to dry weight ratios) according to EPA method 160.3.

There were five replications of the entire system: two in July and August 2002 that served as a preliminary study, and three trials used in reporting data in summer 2003: June 16-July 7, July 11-August 2, and August 4-25. Replication of entire trials was essential in understanding the inherent variability found in biologically-based systems such as this. The preliminary study's main goal was to determine the functionality of the

treatment system and was not used in reporting data. A two-way ANOVA by repeated measures was used to assess any significant effects using treatment and day as factors and effluent nutrient levels as the dependent variable over the experimental period. Additional one-way ANOVAs were used to determine significant differences between adult and juvenile tissue nutrient levels, with age class as factors and tissue nutrient levels as the dependent variable.

Frost's (1972) equations for algal growth and grazing were used to determine the amount of periphyton chl*a* consumed per fish per day:

(1) $C_2 = C_1 e^{k (t2-t1)}$

where C_2 is the periphyton chl*a* concentration at the end of time interval t1-t2 (in days) in each tank containing algae only; C_1 is the periphyton chl*a* concentration at the beginning of the interval, e is the base of the natural log, and k is the algal growth constant expressed as mg chl*a* day^{-1}. This equation was rearranged to solve for k:

$(\ln(C_2/C_1))/t_2-t_1$

(2) $C_{2*} = C_{1*} e^{(k-g)(t2-t1)}$

C_{2*} is the periphyton chl*a* concentration in each tank with fish present at the end of the time interval, and C_{1*} is the periphyton chl*a* concentration at the beginning of the experimental period. The grazing constant is g, expressed as mg chl*a* grazed $fish^{-1}$ day^{-1}. The equation was rearranged to solve for g:

$g = k - (\ln(C_2/C_1))/t_2-t_1$

Average tank periphyton chl*a* concentrations were also determined across all trials for the adult and juvenile treatments:

$\langle C \rangle = (C_1 [e^{(k-g)(t_2-t_1)}-1])/(t_2-t_1)(k-g)$

where <C> is the average periphyton chl*a* concentration over the trial period.

Additionally, ingestion was calculated by first using the filtration equation:

$F = Vg/N$

where F is the volume swept clear, V is the volume of the tank and N is the number of fish present in that particular tank. F was then used to calculate ingestion of chl*a* per fish:

$I = \langle C \rangle * F$

where I is the amount of chl*a* eaten per fish per day.

RESULTS

Overview

A two-way ANOVA (Table 1) by repeated measures indicated that there were significant differences in the amount of effluent nitrogen ($p= 0.001$) among treatments, but there was no significant difference in effluent phosphorus levels ($p=0.8071$) among treatments. Also, daily effluent nutrient levels were significantly different for effluent nitrogen ($p=0.0074$), but not for effluent phosphorus ($p=0.093$). One-way ANOVAs examining the change in fish tissue nutrients between adults and juveniles from day 1 to day 14 showed that the change in nitrogen levels found in fish tissue was significantly different between juveniles and adults, as were changes in phosphorus from the beginning to the end of the trials (Table 2).

Fish survival was 100% for the adults in trials 1 and 2; however, one fish in trial 1 jumped outside its assigned tank into another tank of adults. There was greater mortality in trial 3, with an entire tank of 10 fish dead on day two of the trial and later, one fish dead due to jumping outside its tank, resulting in a 90% survival for this tank. Juvenile survival was fairly high given their small size upon transport (approximately 1g each), with 79.0% survival in trial 1, 76.3% survival in trial 2, and 89.3% survival in trial 3 (all percentages are out of 300 total fish per trial). On average, all fish in all trials lost weight, notably the adults. Juvenile fish in some individual tanks gained weight; however, on average, juveniles also lost some minimal weight from the beginning to the end of each trial (Table 3).

Table 1.) Results of two-way ANOVA: treatment vs. effluent nutrient levels, day vs. effluent nutrient levels.

	df (treatment, error)	F	p
Treatment vs. effluent nitrogen	2, 4	62.43	0.001
Day vs. effluent nitrogen	3, 5	13.82	0.0074
Treatment vs. effluent phosphorus	2, 4	0.23	0.8071
Day vs. effluent phosphorus	3, 5	3.78	0.0930

Table 2.) One-way ANOVA results for Δ fish tissue nutrients: day 1- day 14.

	n (treatment, error)	df	F	p
Δ Tissue N, Adults vs. Juveniles	1, 4	1	183794.4	<0.0001
Δ Tissue P, Adults vs. Juveniles	1, 4	1	45.6251	0.0025

Table 3.) Average fish weights in grams averaged over all trials ± standard deviation. Weights were averaged across all three trials for the day one mean and for the day 14 mean.

	Day 1	Day 14
Adults	113 ± 17.6, n=80	105 ± 12.6, n=79
Juveniles	1.16 ± 0.167, n=900	1.14 ± 0.147, n=734

Nitrogen Removal and Assimilation

Removal of nitrogen from the influent varied from trial to trial (Table 4). Removal rates for all trials were calculated by graphing the influent and effluent for each treatment by trial and determining the point in the trial at which at least two treatments removed nutrients consistently. For trial one, this period occurred in the last four days, trial two in the last ten days, and trial three in the last eight days (Figs. 2-7). Juveniles removed an average of 70.1mg nitrogen day^{-1} (0.623mg $gram^{-1}$ of body weight at an average weight of 1.14g) and adults on average did not remove nitrogen, rather, they added an average of 44.4mg of nitrogen day^{-1} to the effluent (0.045mg $gram^{-1}$ of body weight at an average weight of 105g). All removal rates were calculated using weights of fish on day 14 for each trial. The control tanks of periphyton alone showed the highest nitrogen removal in general over all trials, with an average daily removal of 142mg (0.149mg removed mg^{-1} periphyton nitrogen). Although phytoplankton was sampled, its contribution to total chl*a* biomass was less than 2%, and was not used to calculate removal.

Nitrogen concentrations in the influent, effluent, periphyton, phytoplankton, and fish tissue are found in Table 5 for the beginning and end of each trial. In general, periphyton nutrient concentrations decreased across the three treatment types from the beginning to the end of each trial; however, the amount of periphyton nitrogen present increased in juvenile tanks in trials 2 and 3, as well as in adult tanks in trial 3. There was no obvious accumulation of nitrogen in the periphyton that would explain removal of nitrogen from the water column. Periphyton nitrogen averaged across all trials decreased in the algal control tanks from 2090 mg $tank^{-1}$ on day 1 to 888 mg $tank^{-1}$ on day 14, from 2250 mg $tank^{-1}$ to 1110 mg $tank^{-1}$ in the adult tanks, and 1240 mg $tank^{-1}$ to 1090 mg $tank^{-}$

^{1} in the juvenile tanks. Phytoplankton nitrogen averaged across all trials increased in algae-only tanks from 4.43 mg tank^{-1} on day 1 to 12.6 mg tank^{-1} on day 14, decreased in adult tanks from a day 1 mean of 5.88 mg tank^{-1} to a day 14 mean of 5.06 mg tank^{-1}, and increased in juvenile tanks from a day 1 mean of 5.67 mg tank^{-1} to a day 14 mean of 7.69. Fish tissue nitrogen averaged across all trials decreased in adults from 18.4mg g^{-1} on day 1 to 17.7 mg g^{-1} on day 14, while juvenile tissue nitrogen increased from 9.5 mg g^{-1} on day 1 to 20.9 mg g^{-1} on day 14 (Figure 8).

Phosphorus Removal and Assimilation

Phosphorus removal (Figs. 9-14) was calculated using the same time frame as aforementioned with nitrogen removal. Phosphorus removal from the influent occurred most consistently across the treatments in trial 3 (Table 6); however, when the removals for all three trials were averaged, there was addition of phosphorus to the effluent by all three treatments. The control treatment of periphyton added an average of 40.1 mg day^{-1} to the effluent, juveniles added 20.2 mg, and adults 6.62 mg.

Phosphorus amounts in influent, effluent, periphyton, phytoplankton, and fish tissue from the beginning to the end of each trial are found in Table 7. Periphyton phosphorus decreased across all trials when averaged by treatment from 128 mg tank^{-1}on day 1 to 111 mg tank^{-1} on day 14 in the algal control tanks, 284 mg tank^{-1} to 126 mg tank^{-1} in tanks containing adults, and 177 mg tank^{-1} to 69.9 mg tank^{-1} in juvenile tanks. Phytoplankton phosphorus increased in algae-only tanks from a day 1 mean of 3.16 mg tank^{-1} to a day 14 mean of 5.23 mg tank^{-1}, decreased in adult tanks from a day 1 mean of 5.12 mg tank^{-1} to a day 14 mean of 3.55 mg tank^{-1}, and decreased in juvenile tanks from a day 1 mean of 4.00 mg tank^{-1} to a day 14 mean of 3.85 mg tank^{-1}. Fish tissue

phosphorus averaged across all trials in adults increased from 2.39 mg g^{-1} on day 1 to 4.66 mg g^{-1} on day 14. Juvenile tissue phosphorus increased from 2.60 mg g^{-1} on day 1 to 6.92 mg g^{-1} on day 14 (Figure 15).

Periphyton chl*a*

Initial periphyton chl*a* levels in the algae-only tanks was 182 mg chl*a* m^{-2}, 463 mg m^{-2} in juvenile tanks, and 693 mg m^{-2} in adult tanks. Tanks with algae alone showed an increase in chl*a* levels until about day 7 (475 mg m^{-2}), whereafter chl*a* levels decreased to approximately those of day 1 by day 14. Tanks with juveniles and adults showed a similar pattern in decrease in chl*a* from day 1 to day 7, with a sharp increase to day 14 for adults, and a smaller increase for juveniles. Levels from day 1 to day 7 were used to calculate ingestion for adults and juveniles according to the aforementioned Frost (1972) equations.

Phytoplankton chl*a*

Phytoplankton levels in algae-only tanks increased until approximately day 12 and then leveled off until the end of the trial, with an average of 23.7 μg chl*a* $liter^{-1}$ on day 1 increasing to a day 14 average of 71.3 μg chl*a* $liter^{-1}$. Phytoplankton in both the adult and juvenile tanks decreased from day 1 of the trials to day 14, with adult tanks containing a day 1 mean of 36.6 μg chl*a* $liter^{-1}$ that decreased to a day 14 mean of 7.44 μg chl*a* $liter^{-1}$, and juvenile phytoplankton stocks decreased from a day 1 mean of 36.9 μg chl*a* $liter^{-1}$ to a day 14 mean of 23.9 μg chl*a* $liter^{-1}$.

Water Quality

The average dissolved oxygen across all trials was 4.82 mg O_2 $liter^{-1}$, while the average pH was 8.17. The average minimum temperature averaged across days 1-14 (days with fish) of all trials was 27.3 $\pm$ 1.67 s.d. and the average maximum temperature was 31.6 $\pm$ 1.78 s.d.

Table 4.) Average nitrogen influent, mg $tank^{-1}$ day^{-1}; Removal rates, mg TN $tank^{-1}$ day^{-1}. Negative values indicate addition of nitrogen to the effluent (no removal).

	Avg. Influent	Algae	Adults	Juveniles
Trial 1	1020	60.0	-133	213
Trial 2	984	118	-12.1	-123
Trial 3	969	250	12.0	122

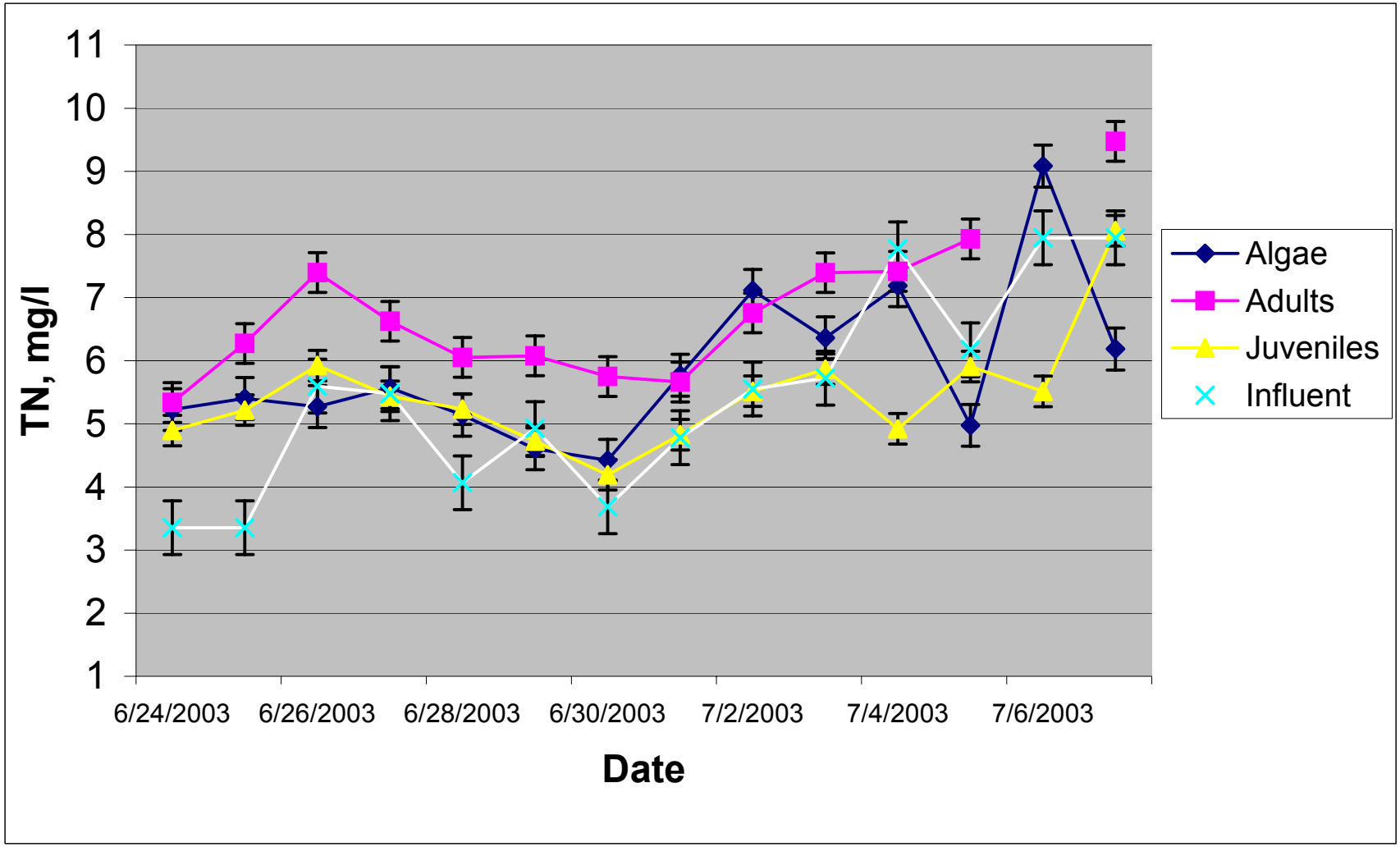

Figure. 2.) Trial 1 daily influent and average daily effluent TN levels ± standard error.

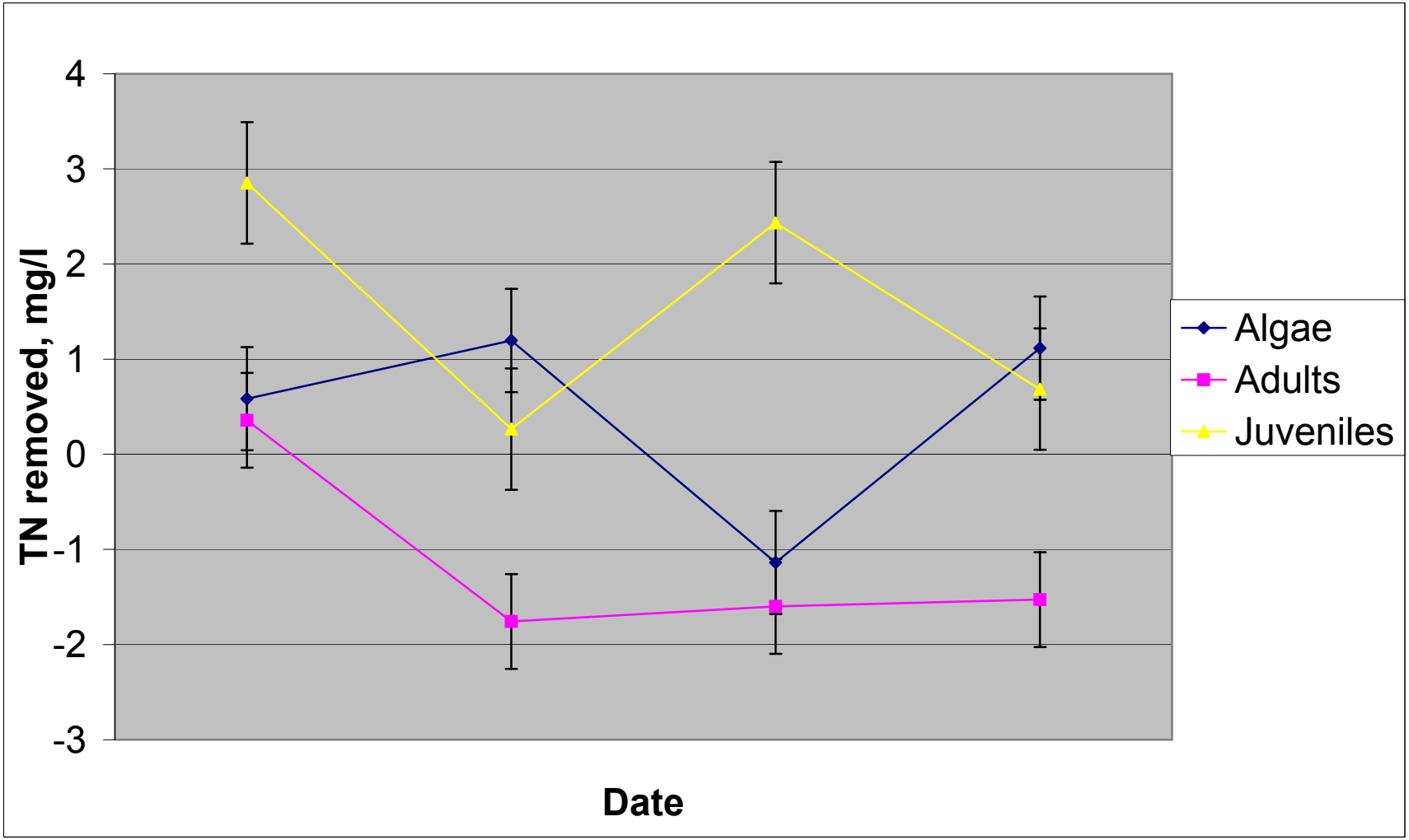

Figure. 3.) Trial 1 daily TN removal during the calculated removal period of 7/4/03-7/7/03 ± standard error.

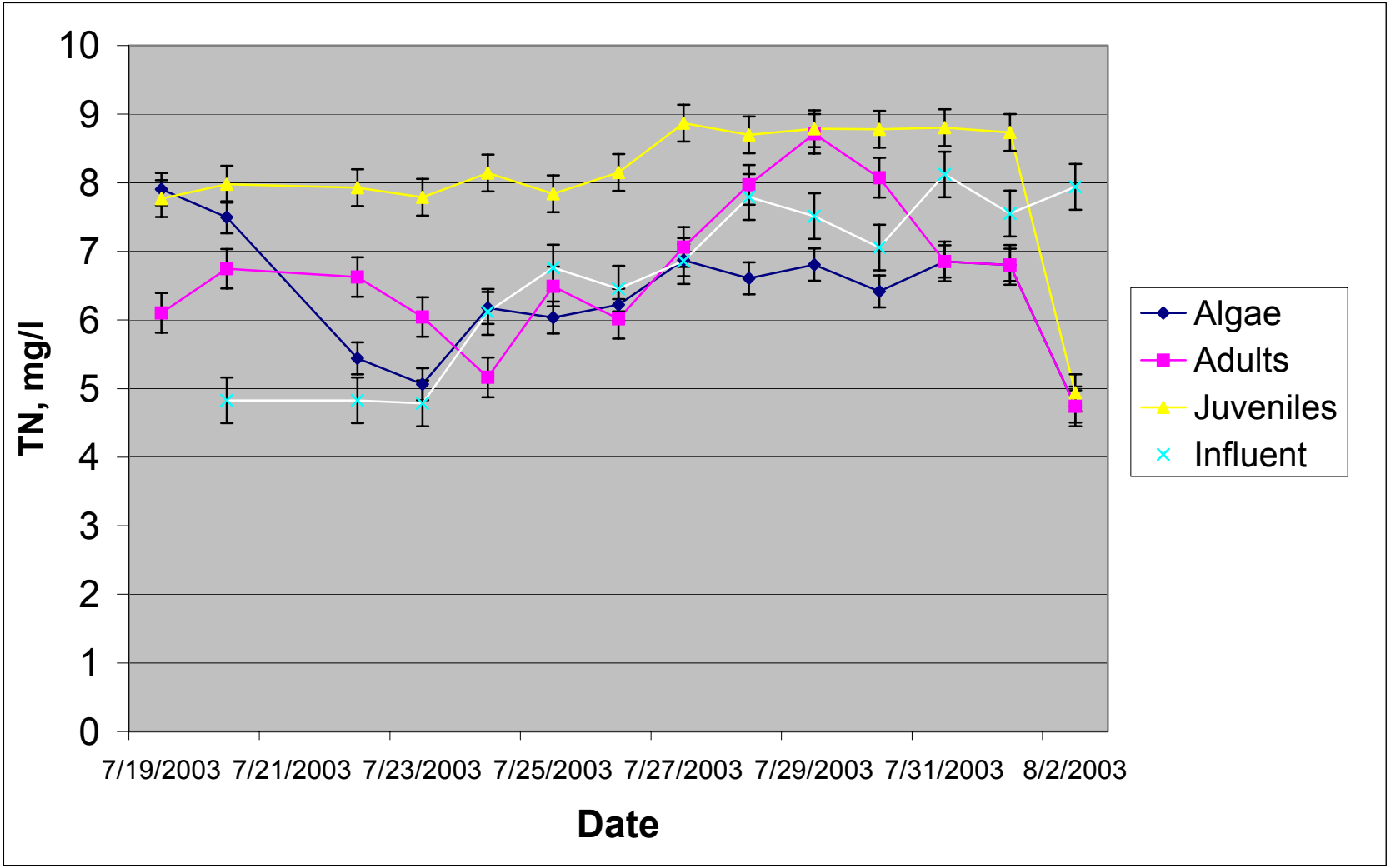

Figure 4.) Trial 2 daily influent and average daily effluent TN levels $\pm$ standard error.

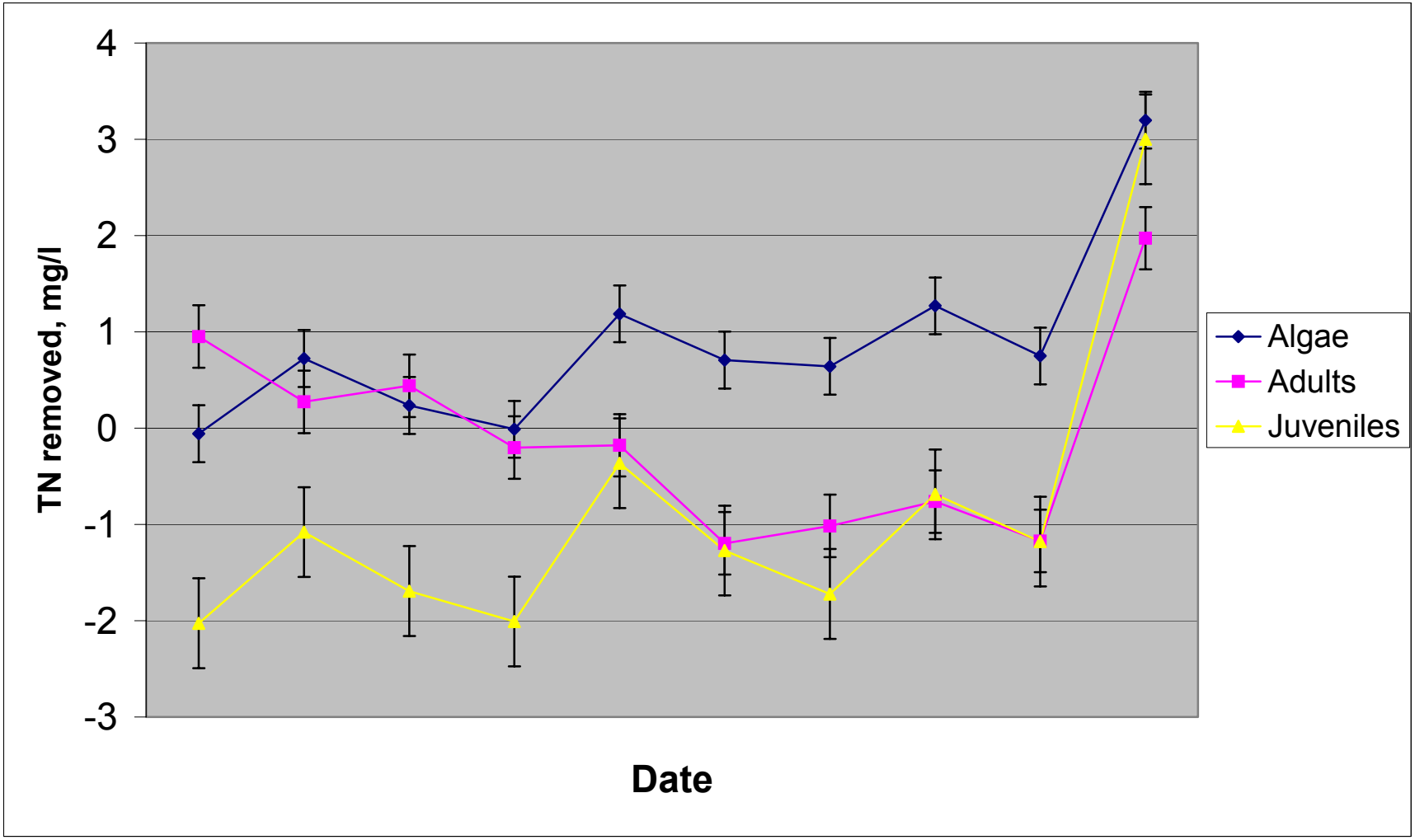

Figure 5.) Trial 2 daily TN removal during the calculated removal period of 7/24/03-8/2/03 ± standard error.

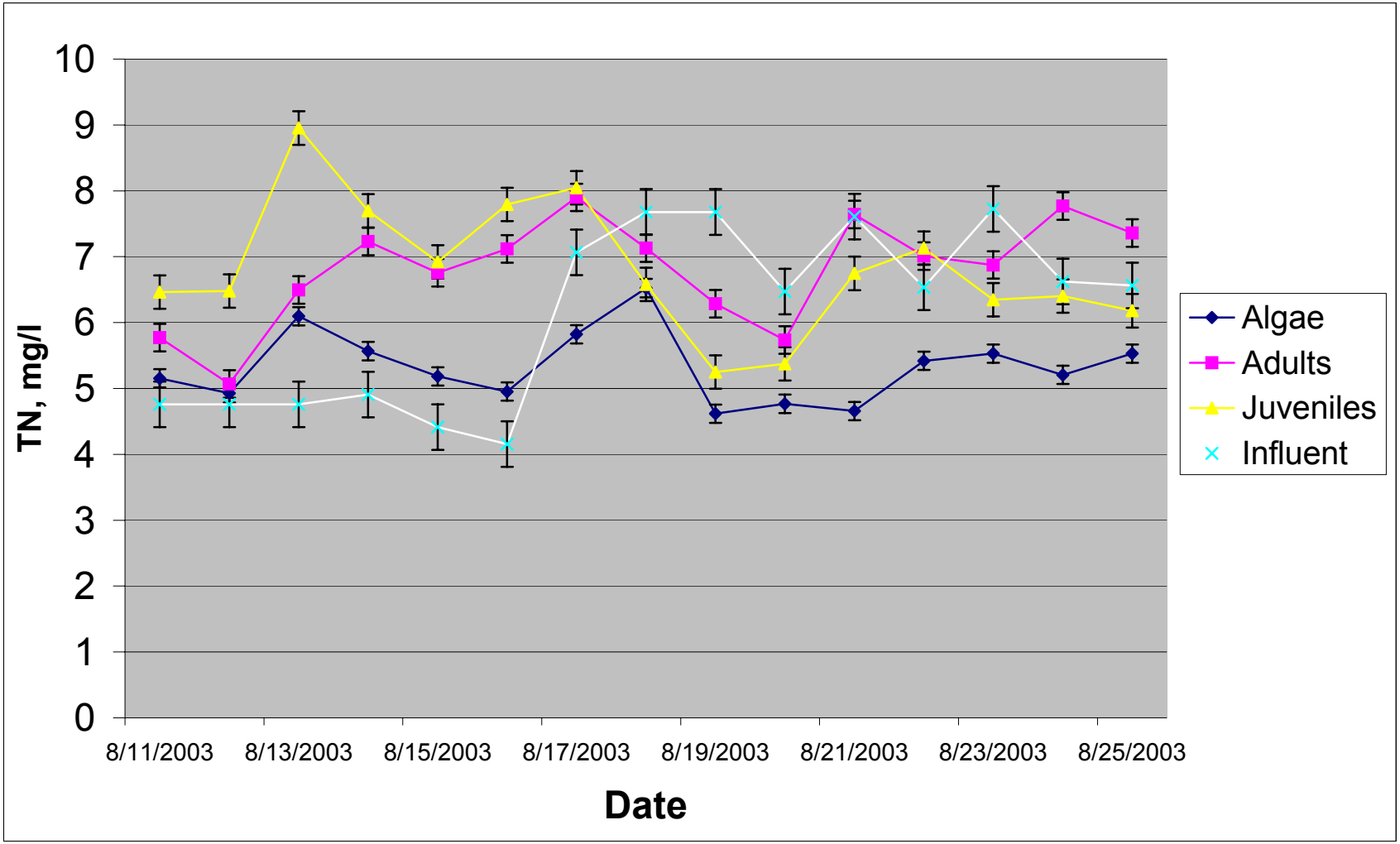

Figure 6.) Trial 3 daily influent and average daily effluent TN levels ± standard error.

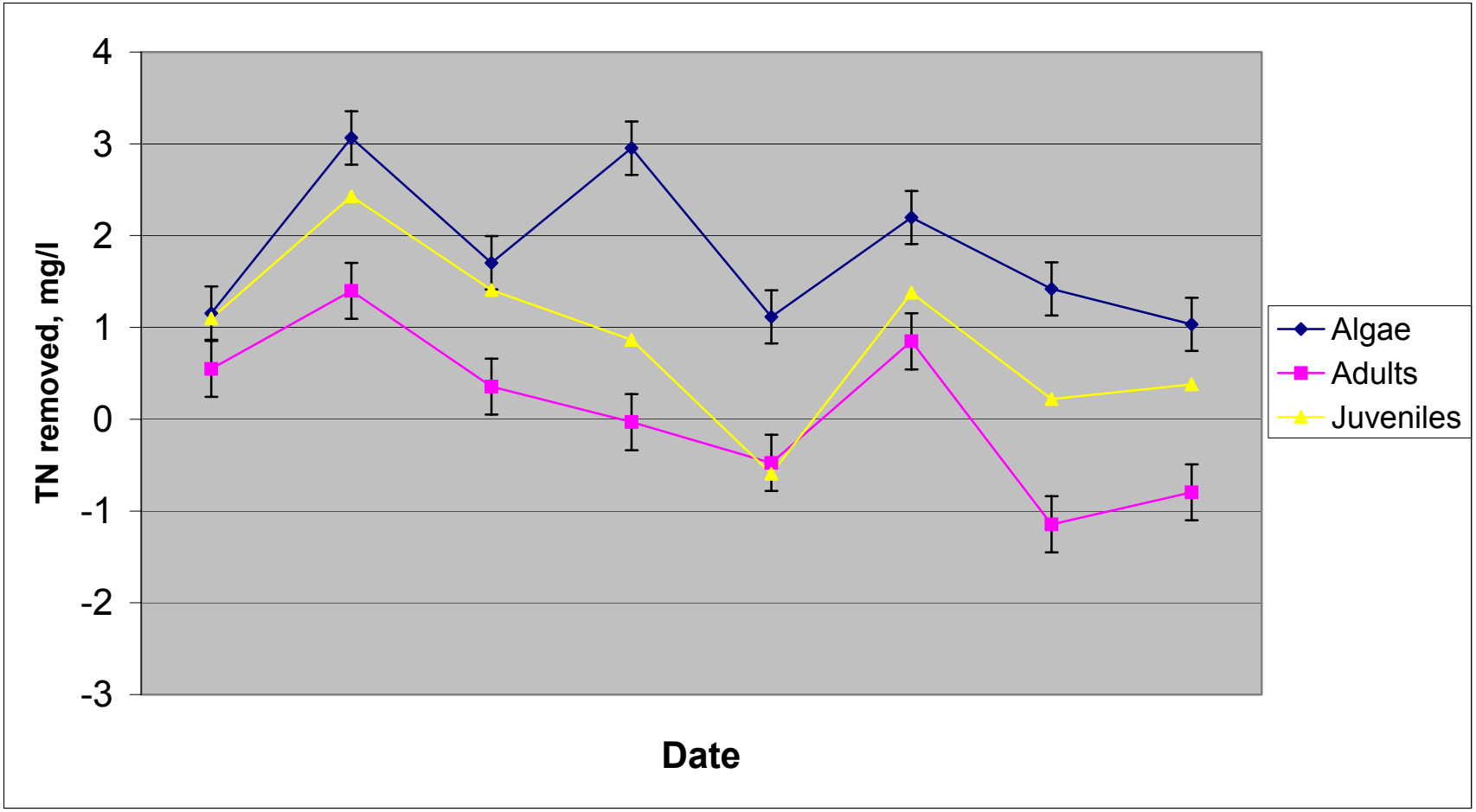

Figure 7.) Trial 3 daily TN removal during the calculated removal period of 8/18/03-8/25/03 ± standard error.

Table 5.) TN, mg tank^{-1} day^{-1}; influent and effluent ± standard deviation

		Influent	Effluent	Periphyton	Phytoplankton	Fish
Trial 1 Beg.	Algae	686± 217	712±169	4180	2.11	N/A
End		1080±217	843±169	837	8.29	N/A
Beg.	Adults	686±217	728±155	5910	3.62	24500
End		1080±217	1290±155	1410	3.05	21200
Beg.	Juveniles	686±217	586±123	1720	3.28	1290
End		1080±217	990±123	728	3.66	2190
Trial 2 Beg.	Algae	658±164	1060±119	1150	4.34	N/A
End		1080±164	646±119	964	12.8	N/A
Beg.	Adults	658±164	920±147	1390	4.59	17600
End		1080±164	813±147	1050	4.70	16500
Beg.	Juveniles	658±164	1090±137	1250	4.56	981
End		1080±164	673±137	1740	11.5	1720
Trial 3 Beg.	Algae	649±183	702±73.3	950	6.82	N/A
End		895±183	754±73.3	863	16.6	N/A
Beg.	Adults	649±183	779±110	692	9.43	18400
End		895±183	1000±110	884	7.44	14900
Beg.	Juveniles	649±183	881±134	740	9.18	1020
End		895±183	843±134	819	7.94	1940

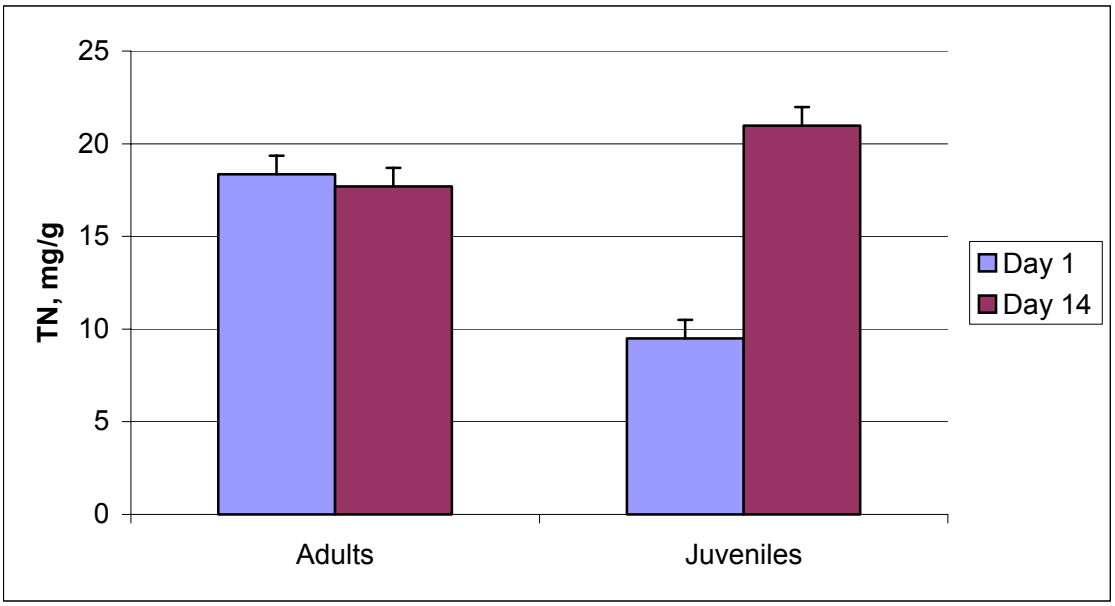

Figure 8.) Fish tissue nitrogen averaged over all trials. Adults day 1 mean=18.4±0.027 s.d.; Adults day 14 mean=17.7±0.023 s.d.; Juveniles day 1 mean=9.49±0.030 s.d.; Juveniles day 14 mean=21.0±0.018 s.d.

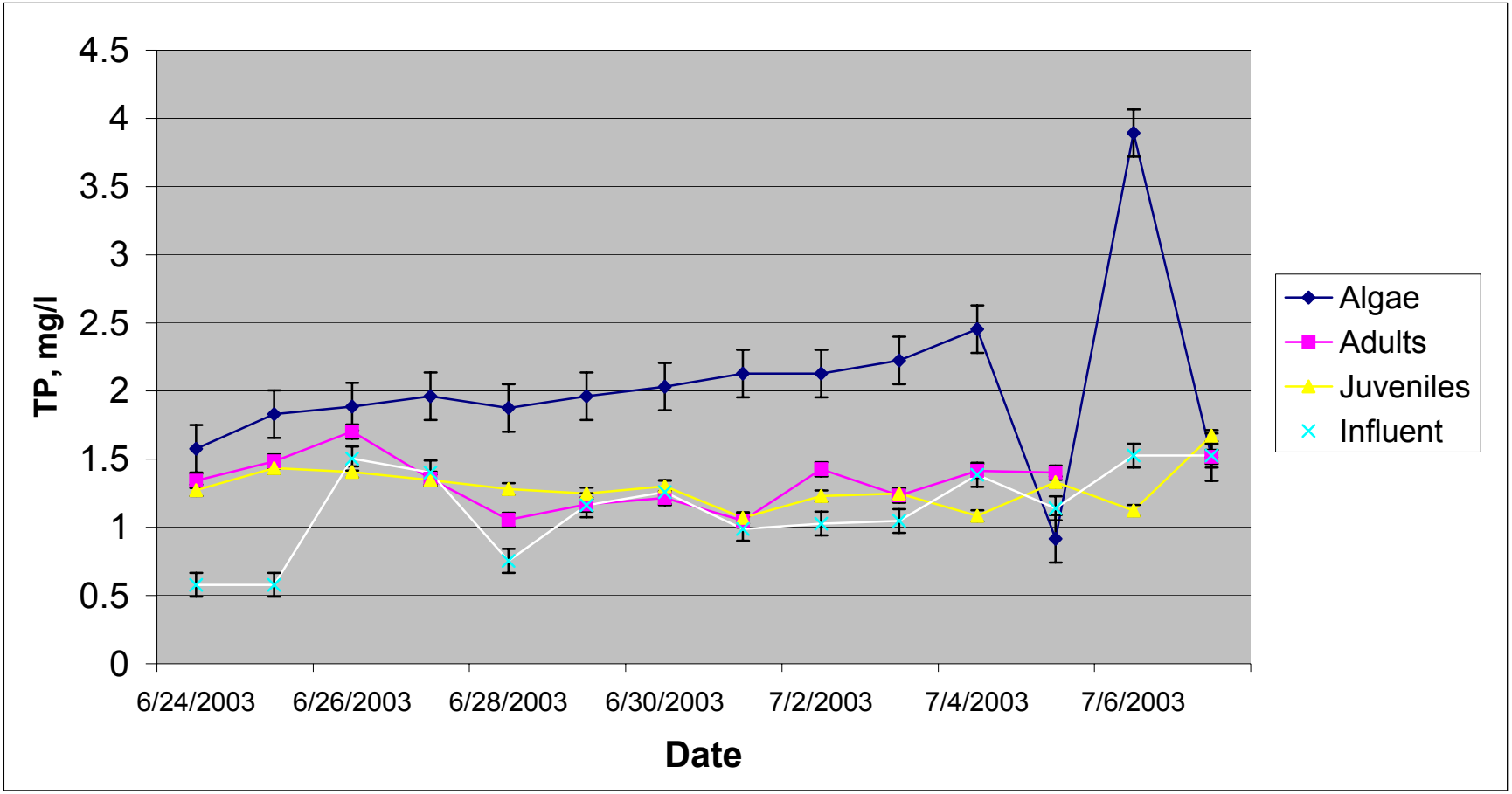

Figure 9.) Trial 1 daily influent and average daily effluent TP levels $\pm$ standard error.

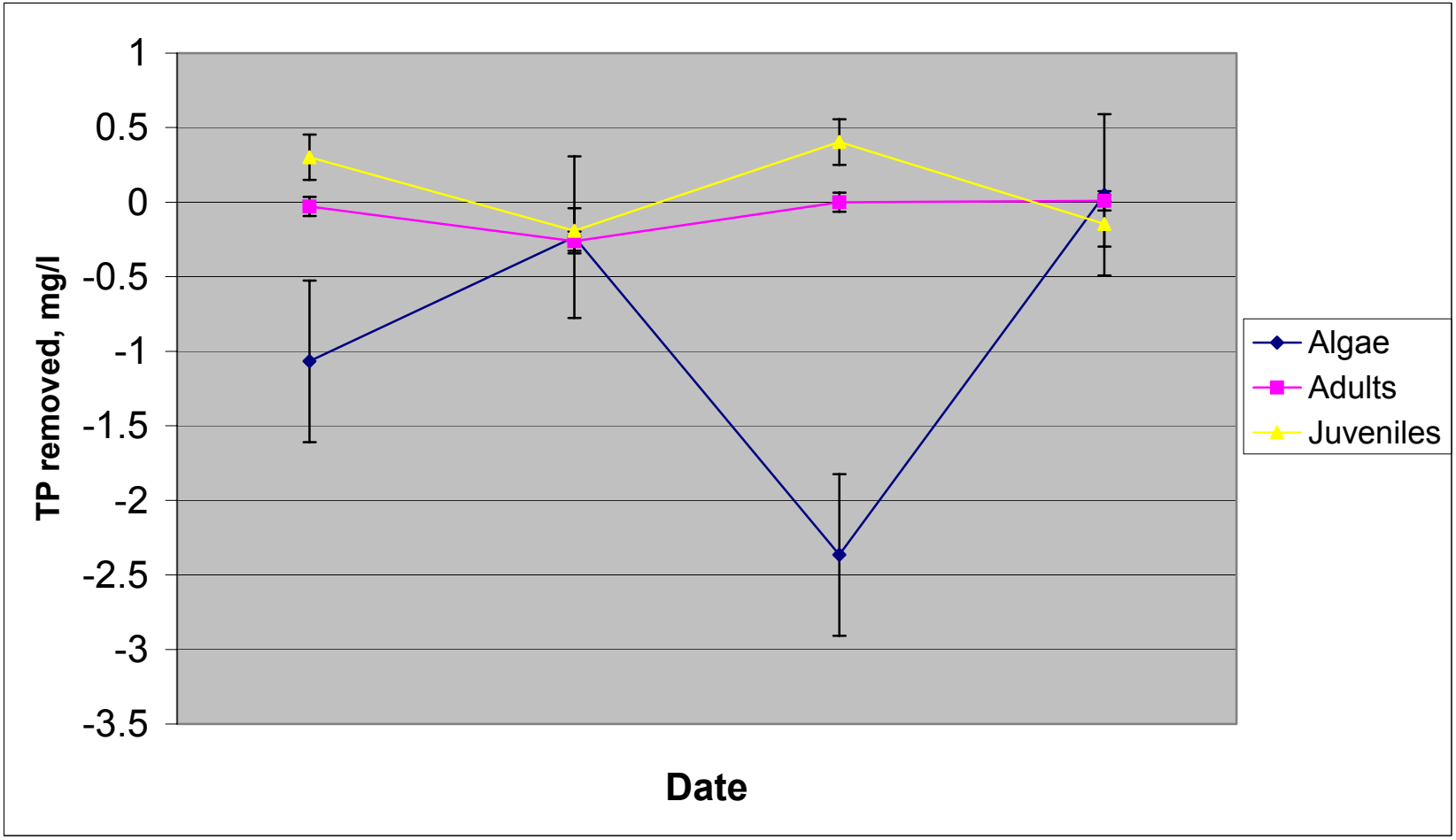

Figure 10.) Trial 1 daily TP removal during the calculated removal period of 7/4/03-7/7/03 ± standard error.

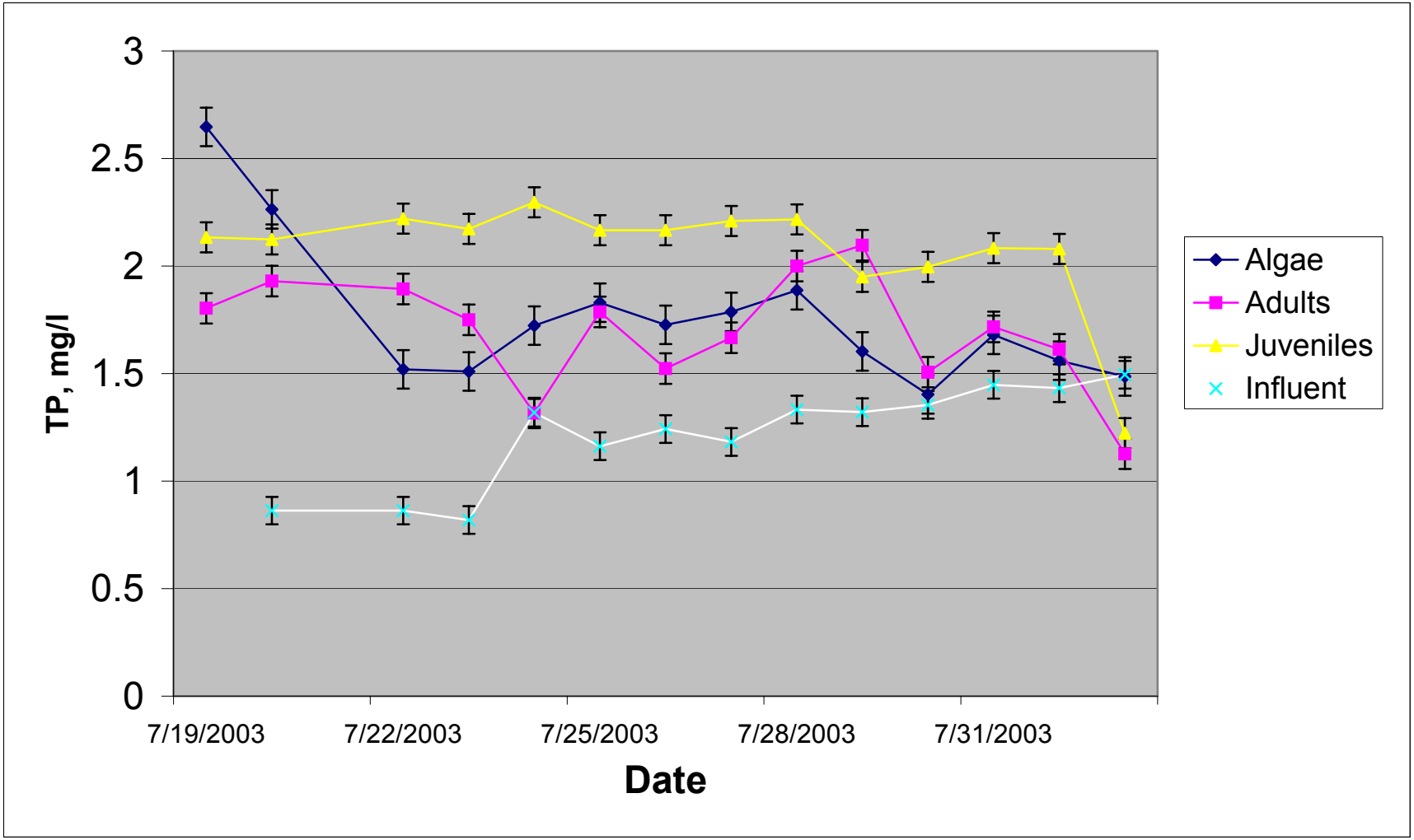

Figure 11.) Trial 2 daily influent and average daily effluent TP levels ± standard error.

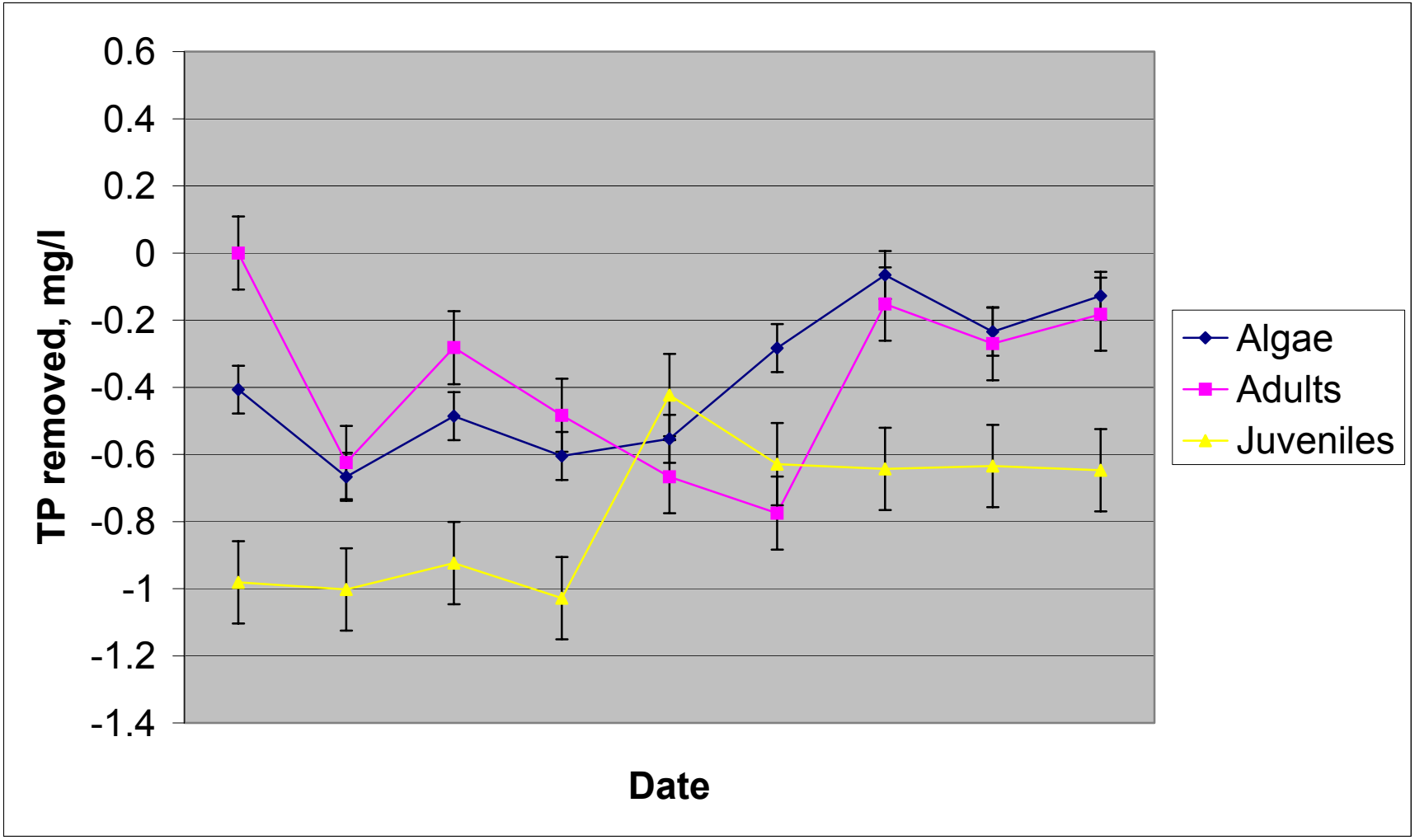

Figure 12.) Trial 2 daily TP removal during the calculated removal period of 7/24/03-8/2/03 ± standard error.

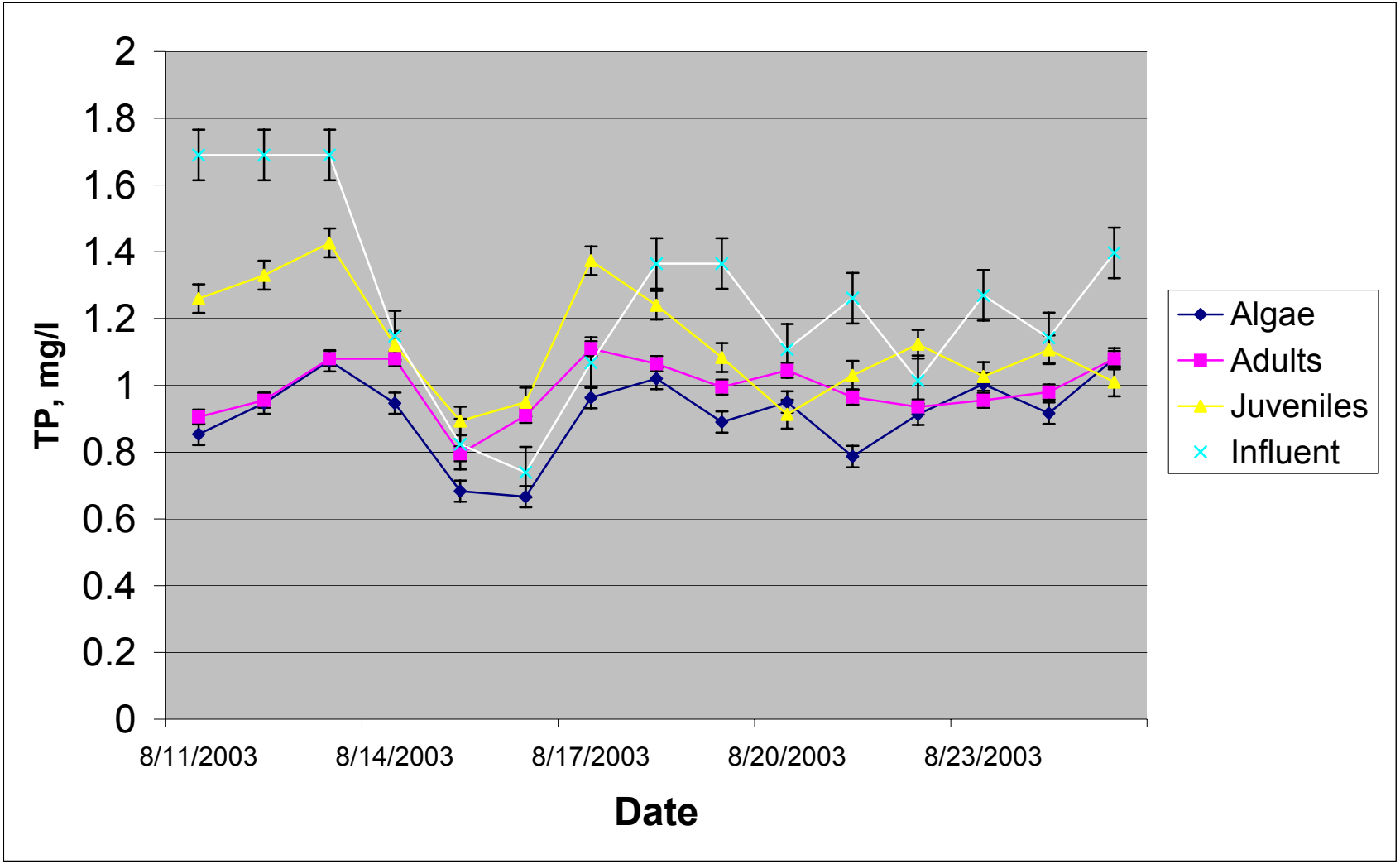

Figure 13.) Trial 3 daily influent and average daily effluent TP levels ± standard error.

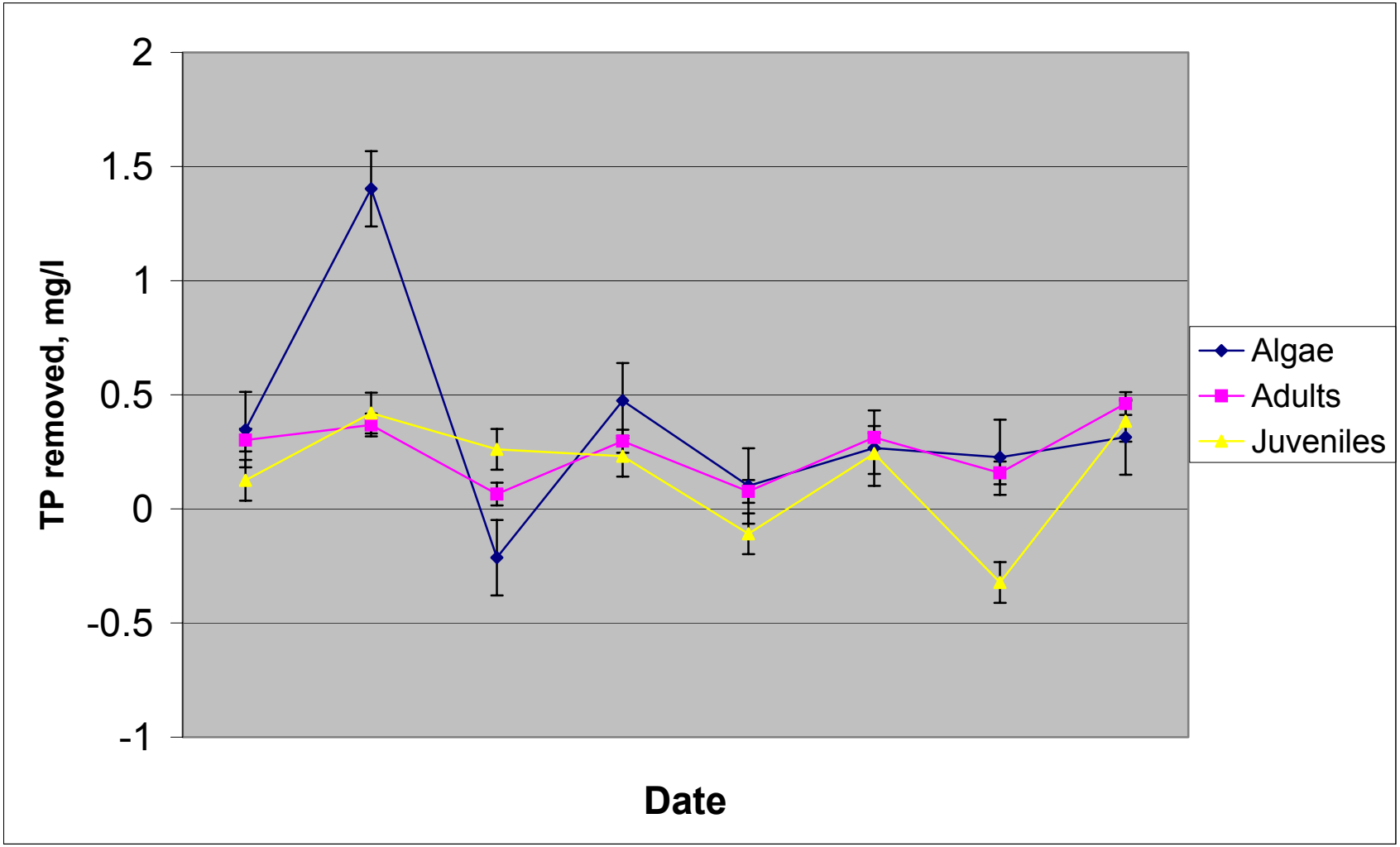

Figure 14.) Trial 3 daily TP removal during the calculated removal period of 8/18/03-8/25/03 ± standard error.

Table 6.) Average phosphorus influent, mg $tank^{-1}$ day^{-1}; Removal rates, mg TP $tank^{-1}$ day^{-1}; negative values indicate addition of phosphorus to the effluent (no removal).

	Avg. Influent	Algae	Adults	Juveniles
Trial 1	190	-123	-12.8	12.5
Trial 2	181	-46.7	-41.8	-94.2
Trial 3	169	49.7	34.7	21.0

Table 7.) TP, mg tank^{-1}; influent and effluent ± standard deviation

		Influent	Effluent	Periphyton	Phytoplankton	Fish
Trial 1 Beg.	Algae	118±44.7	215±88.8	237	0.630	N/A
End		208±44.7	327±88.8	67.4	5.51	N/A
Beg.	Adults	118±44.7	183±25.6	290	2.96	3380
End		208±44.7	207±25.6	147	3.76	5820
Beg.	Juveniles	118±44.7	152±21.1	285	2.31	466
End		208±44.7	228±21.1	58.8	3.47	740
Trial 2 Beg.	Algae	118±31.6	361±45.6	130	3.50	N/A
End		204±31.6	204±45.6	191	5.03	N/A
Beg.	Adults	118±31.6	246±36.1	430	6.38	2230
End		204±31.6	154±36.1	130	3.63	4330
Beg.	Juveniles	118±31.6	291±35.6	136	2.78	243
End		204±31.6	167±35.6	69.2	4.30	562
Trial 3 Beg.	Algae	230±40.0	116±16.8	167	5.35	N/A
End		190±40.0	147±16.8	75.0	5.16	N/A
Beg.	Adults	230±40.0	123±11.8	130	6.03	2330
End		190±40.0	127±11.8	101	3.27	3680
Beg.	Juveniles	230±40.0	171±22.7	110	6.91	219
End		190±40.0	138±22.7	81.8	3.76	627

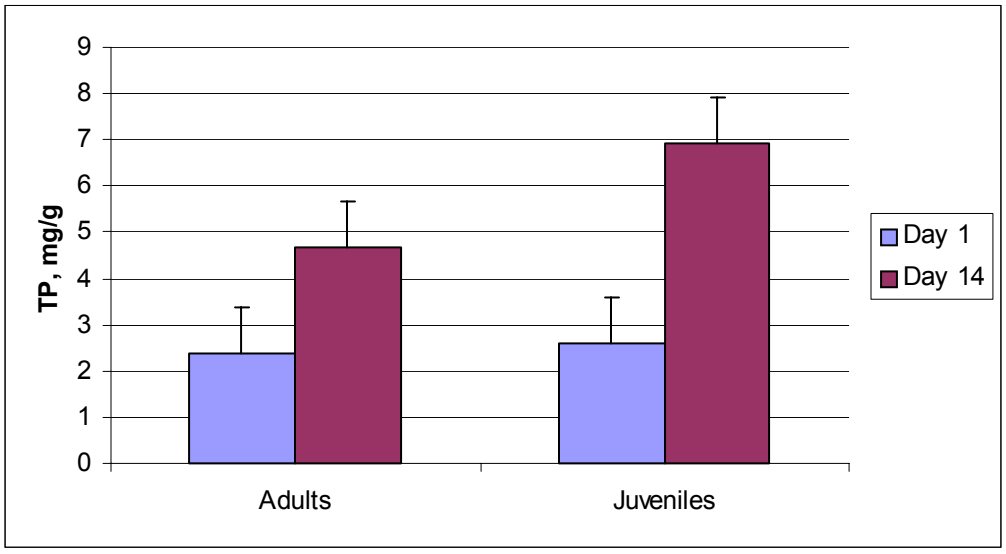

Figure 15.) Fish tissue phosphorus averaged over all trials. Adults day 1 mean=2.39±0.126 s.d.; Adults day 14 mean=4.66±0.296 s.d.; Juveniles day 1 mean=2.60±0.736 s.d.; Juveniles day 14 mean=6.92±0.256 s.d.

DISCUSSION

It was expected that the juvenile tilapia would have a greater nitrogen and phosphorus assimilation rate, thereby a greater nutrient removal rate than the adults per unit weight. Since both nitrogen and phosphorus are necessary nutrients for the growth and development of fish (Committee on Animal Nutrition 1993), some minimal extraction and assimilation is to be expected. Also, the tanks with fish should have a greater nutrient removal rate than the tanks without fish. Adey (1993) used algae only to treat wastewater and noted phosphorus removal; however, this method requires vacuuming or manual removal of the algae before release into natural waters. The fish/periphyton method uses the fish primarily to remove the algae from the water and uptake nutrients in the process.

The primary goal of this study was to compare the abilities of juveniles and adults to remove nitrogen and phosphorus from wastewater. Juveniles removed an average over all trials of 70.1 mg nitrogen day^{-1} (or 8.47% nitrogen removed from influent) from the influent at a rate of 0.623mg g^{-1} body weight. Adults failed to remove nitrogen from the influent, adding 44.4mg of nitrogen day^{-1} to the effluent (0.045mg $gram^{-1}$ body weight, addition of 5.36% to nutrients in influent). This could be due to the decrease in periphyton abundances after day 7. With periphyton levels at their lowest, nutrient removal ability by the algae was reduced; however, the levels of periphyton remaining were still eaten to some extent by the adults. These two factors resulted in very little uptake of nutrients by algae coupled with excrement by the fish causing increased nutrient levels above that of the influent. Although periphyton nutrients did not show any great increase from day 1 to day 14 of the trials (perhaps due to sloughing of periphyton

from the walls of the tank due to vigorous aeration out through the effluent), fish tissue nutrients, especially in juveniles, changed dramatically. Juveniles experienced an approximate two-fold increase in nitrogen per gram of body tissue despite losing weight from day 1 to day 14, suggesting that fish assimilation of nitrogen was responsible for the final removal of nitrogen from the water column. Juveniles gained an average of 851mg of nitrogen $gram^{-1}$ of body tissue $tank^{-1}$ over each trial, or 60.8mg day^{-1} (54.8% increase in tissue N per fish). Phytoplankton nitrogen levels also increased in juvenile tanks from a day 1 mean of 5.67 mg N $tank^{-1}$ to a day 14 mean of 7.69 mg N $tank^{-1}$ compared to a decrease in mean phytoplankton nitrogen in adult tanks of 5.88mg N $tank^{-1}$ to a day 14 mean of 5.06 mg N $tank^{-1}$. Phytoplankton chl*a* levels were also higher on average throughout the course of all trials in juvenile tanks, suggesting that adult fish successfully filter-fed on the suspended phytoplankton in comparison with juveniles. This removal of available algae for nutrient uptake could have also caused less nutrient removal by adult tanks. Although the adults visibly fed on suspended phytoplankton in the tanks, studies by Dempster *et al.*(1993, 1995) have shown that adults cannot maintain weight on a diet of phytoplankton alone, and tend to lose weight when presented a diet of phytoplankton alone and that juveniles also feed primarily on periphyton rather than suspended phytoplankton.

Although the highest removal rates for TN were for periphyton alone in this study, similar removal for juveniles could possibly be achieved by stocking at increased densities. Only 100 1g fish were stocked into each tank, whereas maximum stocking densities for this size fish range up to 600 m^{-3} in tanks or ponds with a moderate growth of algae (Coche 1982). Tanks used in this experiment could therefore support up to 265 fish per tank, and at a removal rate of 0.623mg g^{-1} fish tissue, could remove up to 245 mg

tank^{-1} TN from the water column. Stocking densities of this magnitude may not be practical: increased fish densities also lead to increased feces and therefore a necessary increase in flow rate or the use of manual processes to clear the feces from the tank.

Since juvenile fish sequestered so much nitrogen in their tissues over the course of these trials in comparison to adult fish (which neither gained tissue nitrogen nor removed it from the water column), it is suggested that they be used instead of adults in future studies.

Adult tissue nitrogen did not show any real change from day 1 to day 14 (Fig. 8), possibly due to their weight loss and lack of available food after about day 7, and a decrease in overall weight (Table 3). This loss of both weight and tissue nutrients could account for the increase in nitrogen in adult tank effluent levels. The adults in this experiment failed to remove nitrogen or phosphorus possibly due to the effects of their intense grazing. By day 5 and 6 of the trials, periphyton were virtually absent from the walls of the tank, and phytoplankton chlorophyll levels were also low. Mid-way through the trial, periphyton chl*a* levels began to increase; however, the growth of periphyton was not enough to support weight gain of the fish. From visual observations, it was noted that at this point in the adult tanks, the formerly green algae-dominated community of periphyton changed into a brown algae-dominated community, possibly leading to decreased palatability to the fish. Huchette *et al* (2000) found that grazed periphyton communities were younger, healthier, and more productive. These findings may have held for tanks containing juveniles in the current study (which could often be seen actively eating periphyton from the tank walls). Using the aforementioned Frost (1972) equations, it was determined that juveniles in this study (using data averaged for periphyton chl*a* across all trials and juvenile tanks) were capable of ingesting a daily

average of 271 mg chl*a* m^{-2} day^{-1} with an average daily standing crop of 140 mg chla m^{-2}. Although periphyton chl*a* levels in juvenile tanks decreased from day 1 and showed only a minimal increase after day 7, periphyton nitrogen levels increased over the course of the trial in trials 2 and 3. This increase in nutrients (and therefore removal from the water column) could be due to grazer enhancement as suggested by Huchette *et al.* (2000) and Mulholland *et al.* (1991).

TN removal rates from influent reported here using a one-tank system are similar to those in Rectenwald and Drenner, 2000, who reported up to 108mg TN removed per day (23% removed) and Drenner *et al.*, 1995, which used multiple tanks interconnected in series. Other studies using algae or algae and fish did not report TN levels (Adey 2000, Drenner *et al.*1990, Drenner *et al.* 1997). Unlike the current study, nutrient removal was greatest in tanks with fish; however, other studies used fish of varying sizes and age classes, notably Rectenwald and Drenner. Mouth-fry were not removed from adults prior to stocking, resulting in a fish population including breeding adults and very small fry.

Influent TN and TP in this study were comparable to amounts used in other studies (Rectenwald and Drenner 2000, Drenner *et al.* 1995); however, these studies, as well as Adey (1993) focused primarily on phosphorus removal, with removal rates for TP up to 120 mg TP m^{-2} d^{-1} for tanks with fish (Rectenwald and Drenner 2000) and 139 mg TP $m^{-2}d^{-1}$ with algae alone (Adey 1993). Although fish tissue phosphorus increased in both juveniles (62.4% increase) and adults (48.7% increase) from day 1 to day 14 of the trials (Figure 9), this system failed to remove TP from the water column, possibly a result of the one-tank system used here. Tanks showed an increase in phosphorus over that of the influent levels, due possibly to intense grazing of algae. With very little algae left to

uptake nutrients and continual excrement production by the fish, increased phosphorus levels here are not surprising. A study by Drenner *et al.* (1990) using mesocosms with fish found that the presence of bluegill can increase TP levels; however, this study was slightly different from the current study.

The aforementioned algal treatment studies used long raceways of algae (Adey) or multiple tanks of fish connected in series, with the first tank's effluent used as influent for the second tank, the second tank's effluent used as influent for the third tank, and so on (Rectenwald and Drenner 2000). Rectenwald and Drenner reported increased removal of TP in tanks located later in the series, and an increase over initial influent levels in the first few tanks in the series; however, the current study did not attempt to optimize system efficiency, rather, it examined the efficiencies of different size fish.

The average one-week lag in nutrient removal abilities for fish in this study could be due to the drastic change in diet from pelleted food at Blue Ridge Aquaculture to a natural diet of algae only presented during the experiment. Since removal of nutrients from the influent is dependent on uptake by the algae and subsequent consumption by the fish that then assimilate the nutrients, an inability to properly process the new diet may be temporarily problematic. In this study, availability of periphyton also appeared to be a problem, especially for adults around days 7 and 8, after which adults generally were not observed grazing periphyton. The adults grazed the periphyton so low so fast that it took the remainder of the experimental period (approximately 7 days) for periphyton levels to return to initial conditions. Using Frost's (1972) grazing equations based on algal growth rates, it was determined that adults on average (using data across all trials and tanks of adults) were capable of eating 4,320 mg m^{-2} chl*a* $fish^{-1}$ day^{-1}, while the average cell concentration in the tanks was much lower: the daily average over all trials was 321 mg

chl*a* m^{-2}. However, if a similar periphyton-fish system was implemented at a wastewater treatment plant, algae present in biofilter tanks could be pumped to fish tanks as supplement food.

The current study did not attempt to optimize certain aspects of this system with respect to nutrient removal. With careful preparation and consideration of a number of possible optimization steps, further studies could show an increase in the amounts of nutrients removed. Connecting tanks in series as shown in previous studies could enhance removal instead of using a single-tank system as presented here. Other ways to optimize the system include determining the appropriate amount of algae needed per fish per tank (by using a preliminary grazing study and Frost's (1972) equations) and therefore increasing tank surface area or adding substrata such as bamboo or PVC for increased algal colonization. By increasing total periphyton present, primary nutrient removal could be increased, as well as the number of fish stocked into each tank. The dearth of available food in adult tanks in this study is an example of inadequate food (periphyton) possibly leading to the failure of the system to remove nutrients. This study used a fairly low flow rate of approximately 5.68 liters $hour^{-1}$, resulting in a daily tank turnover of approximately 36%. With adequate aeration and monitoring of water quality (pH, temperature, ammonia), flow rates in future studies could be reduced further to increase tank retention time of influent water; however, this results in less volume being treated on a daily basis and would require an increase in tank size or number to treat the desired volume of influent. Drenner *et al.*'s (1995) results suggest that increasing water temperature leads to increased nutrient removal. Since tilapia are tolerant of high temperatures (up to 32^{o} C), their use would be conducive to increasing the temperature in treatment tanks by use of heated greenhouses or submersible heaters (as used in this

study). Caulton (1982) reports that tilapia assimilate a higher portion of their food at increased temperatures. If fish are to be used specifically in wastewater treatment systems, results from this study suggest that juveniles be used to increase nutrient removal. This study (even with its lack of certain optimization steps) suggests that there is very little or no optimization required in order to achieve increased nutrient accumulation in juveniles. By utilizing juveniles, both nutrient removal from wastewater and fish growout can be accomplished. If the wastewater used contains no heavy metals or other harmful toxins, fish could be used for human or animal consumption.

The importance of this system and others like it is increasing. The great need for economical and ecologically based systems has been expressed by numerous scientists and authors (Henderson-Sellers and Markland 1987, McGarry 1980, Dolan *et al.* 1981, Skillicorn *et al.* 2002). This system would provide an affordable and efficient means of nutrient removal and may also be able to provide food with use in developing countries.

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