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Aquatic Botany 74 (2002) 343–367

Aquatic botany

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Effects of ammonia in pulp mill effluents on estuarine phytoplankton assemblages: field descriptive and experimental results

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Received 27 February 2001; received in revised form 8 May 2002; accepted 27 August 2002

Abstract

A field descriptive and field/laboratory experimental program was carried out to evaluate the effects of ammonia (NH_3) discharged by a pulp mill on estuarine plankton. Field data indicated that ammonia concentrations in the receiving system (Amelia Estuary; range, 0.19–0.43 mg l^{-1}) were significantly higher than those taken in the reference system (Nassau Estuary; range, 0.09–0.11 mg l^{-1}). Significantly reduced chlorophyll *a* concentrations were noted in the Amelia system, and these varied inversely with ammonia concentrations. There were periodic reductions of light transmission in areas affected by mill effluents and in upper areas of both estuaries due to postulated urban storm water runoff. Field surveys indicated that whole water and net phytoplankton numbers and species richness were significantly lower in the Amelia system. Zooplankton numbers were significantly lower at various Amelia stations, whereas there were no significant differences in zooplankton species richness between the two study areas. The field results indicated that the most likely system-wide differences of water quality that could account for the noted biological responses were the relatively high ammonia concentrations in the Amelia system. Field ammonia levels in the Amelia system were significantly associated with observed impairment of key indices of phytoplankton assemblages in areas affected by mill discharges, especially during summer periods of maximal impact. Laboratory microcosm experiments with *Skeletonema costatum* indicated adverse effects of ammonia on chlorophyll indicators. Microcosm results indicated that ammonia had a stimulatory effect on *S. costatum* at mean concentrations of 0.06 mg l^{-1} with initial adverse effects of ammonia within a range of 0.1–0.24 mg l^{-1} and major effects at concentrations >0.46 mg l^{-1}. Mesocosm experiments with ammonia indicated stimulatory effects from 0.11 to 0.14 mg l^{-1} with inhibition of phytoplankton growth at 0.20 mg l^{-1}. The difference between stimulatory effects and

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PII: S0304-3770(02)00150-X

inhibition of ammonia on *S. costatum* and phytoplankton assemblages was relatively small. Microcosm and mesocosm experiments with pulp mill effluents resulted in a broad range of responses to ambient ammonia concentrations indicating undetermined interactions of the effects of ammonia with other components of the effluents. Within the context of the noted ranges of impacts in both the field and the laboratory, it was suggested that long-term average ammonia concentrations in the Amelia River-estuary at the outfall station should not exceed 0.11 mg l^{-1} with short-term concentrations not exceeding 0.20 mg l^{-1}. The pulp mill in the Amelia River-estuarine system has undertaken a restoration program based on these ammonia limits.

Keywords: Phytoplankton: Zooplankton: Pulp mill: High salinity estuaries: Ammonia: Light transmission

1. Introduction

Studies of coastal phytoplankton assemblages indicate that combinations of water circulation, temperature, salinity, light and nutrients, along with predation and interspecific competition, represent important controlling factors in any given area (Bricker et al., 1999; Howarth et al., 2000; Livingston, 2000). Differences in essential nutrients and the physiological state of indigenous microalgal assemblages contribute to the complexity of the relationships of phytoplankton and water quality in coastal systems.

The Amelia and Nassau River-estuaries (Fig. 1) are located in coastal northeast Florida, and are characterized by extensive marsh development and relatively high salinities. Tidal ranges approximate 2–3 m. The study area is a maze of channels and bayous with direct connections to the Atlantic Ocean. Major parts of the Nassau system are within a state park, and preliminary water quality analyses indicated relatively high water quality (Livingston, 1996). The climate along this part of the coast is mild. Annual rainfall averages around 120 cm with peaks during summer months. A sulfite pulp mill discharges effluents (≈114.000 m^3 per day) into the Amelia River-estuary (Fig. 1). Mill effluents currently are discharged into a 125,000 m^2 mixing zone on outgoing tides. Dennison et al. (1993) found that effluent-receiving areas of the Amelia system were characterized by low dissolved oxygen and pH, high water color, and low primary production relative to reference sites. Secchi depths were relatively low and total organic carbon (TOC) levels were relatively high in receiving areas of the Amelia estuary. Generally, phytoplankton and zooplankton numbers and diversity in the Amelia system were comparable to those in reference areas elsewhere (Dennison et al., 1993). The Florida Department of Environmental Regulation (FDER, 1991) reported high (~1.7 mg l^{-1}) concentrations of ammonia in areas affected by the pulp mill; recent analyses (Livingston, 1996) corroborated these findings. There were also indications of low phytoplankton species richness in the discharge areas (FDER, 1991).

Ammonia toxicity to marine phytoplankton has not been well established. The US Environmental Protection Agency (1976) proposed a limit of 0.02 mg l^{-1} as unionized ammonia for protection of freshwater aquatic life. Recent water quality criteria are based on relatively few data (US Environmental Protection Agency, 1989). Admiraal (1977) showed that toxicity to phytoplankton is due to ammonia (NH_3) rather than ammonium (NH_4^+), and that concentrations of 0.247 mg l^{-1} ammonia retarded growth of seven species of benthic diatoms. Concentrations of 0.039 mg l^{-1} ammonia reduced reproduction of a red macroalga,

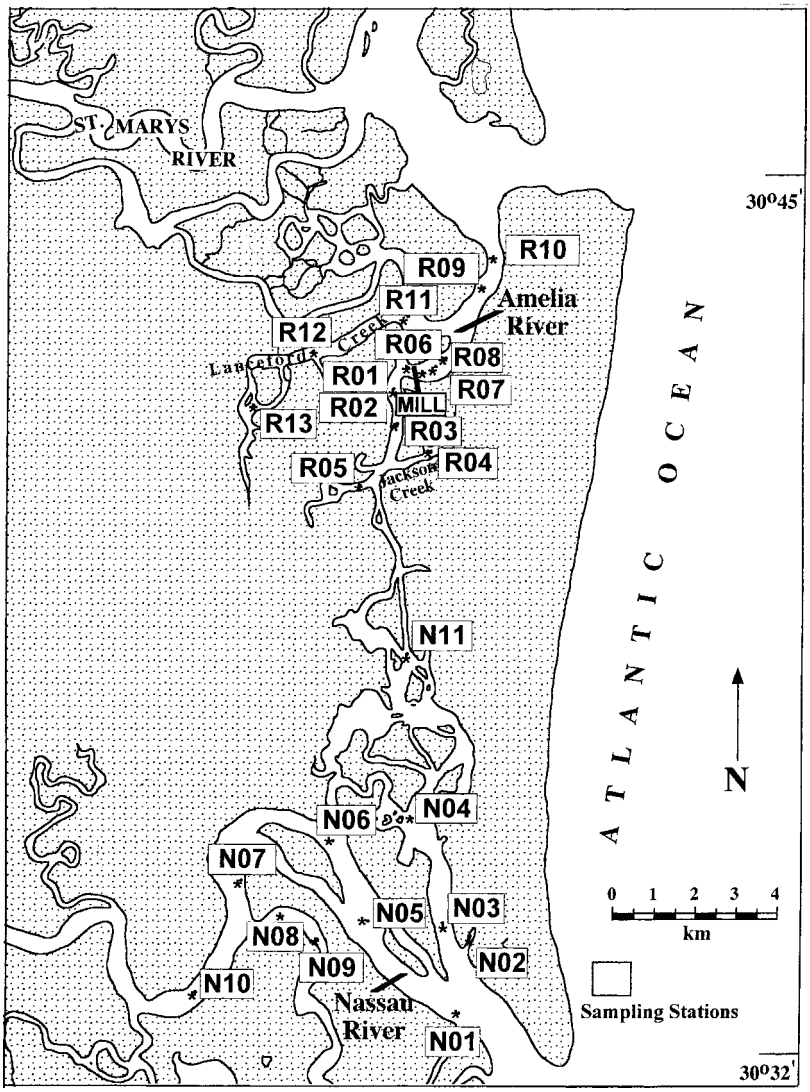


Fig. 1. Locations of sampling sites for the Amelia and Nassau River-estuary study (1994–1995; 1997–1998).

Champia parvula (Admiraal, 1977). These concentrations were within the range of ammonia found in polluted parts of the Amelia system (FDER, 1991). Ammonia is also an important nutrient for coastal phytoplankton, with studies that indicate preferential uptake by individual plankton species that sometimes leads to blooms (Admiraal and Peltier, 1980; Flemer and Livingston, 1998; Livingston, 2000; US Environmental Protection Agency, 1989). Ammonia has been shown to be a selective factor in the species composition of benthic diatoms due to species-specific variation of the ammonia toxicity (Van Raalte et al., 1976; Sullivan,

1978; Admiraal and Peltier, 1980). Thus, the potential effects of ammonia discharges on coastal phytoplankton can be both stimulatory and inhibitory with species-specific responses to ranges of ammonia concentrations.

Ammonium toxicity in water can be due to the effects of both the ionized (NH_4^+) and unionized (NH_3) forms with the relative concentration of each dependent on ambient pH and temperature (Körner et al., 2001). Unionized ammonia toxicity increases with increased pH and temperature (US Environmental Protection Agency, 1989). Whitfield (1974) defined the relationships of the ionized and unionized forms under different conditions of temperature, atmospheric pressure, and pH. Downing and Merkens (1955) found that the unionized form is the most toxic as it is uncharged and therefore traverses the cell membrane more readily. Some authors (Clement and Merlin, 1995) attributed toxicity to NH_3 only. Other studies (Monselise and Kost, 1993) attributed toxicity to both forms. With respect to the effects of ammonium on duckweed (*Lemna gibba*), Körner et al. (2001) did not have a firm conclusion regarding the relative toxicity of ionized (NH_4^+) and unionized (NH_3) ammonium. In this study, we determined the ammonium concentrations as unionized ammonia with the pH of the study areas being relatively constant (mean, 7.64; S.D., 0.32; Livingston, 1996).

Based on a 12-month field analysis (1994–1995), Livingston (1996) found that the Nassau system was an adequate (i.e. unpolluted, with comparable habitat distribution) reference area for studies of the Amelia system. Determinations of water quality and phytoplankton/zooplankton distributions in the Amelia and Nassau River-estuaries indicated that ammonia was present in significantly high concentrations in the Amelia system. A combined field descriptive and field/laboratory experimental program (1997–1998) was then established to determine the effects of ammonia on phytoplankton assemblages. Specific research questions for this study were (1) whether pulp mill effluents were associated with observed reductions of phytoplankton assemblages in the Amelia system and (2) whether ammonia and/or light transmission were responsible for such effects.

2. Methods and materials

Preliminary analyses for water quality factors together with light transmission data (spectroradiometric determinations) were used to delineate the distribution of mill effluents so that isopleths of important variables could be determined and associated gradients verified through factor-specific spatial differentiation. Stations were determined that defined the distribution of mill effluents in the receiving area. Matching stations, chosen for comparability of habitat characteristics (temperature, salinity), were established in the Nassau River-estuary as reference sites for comparative analyses (Fig. 1). Data were taken monthly over two 12-month sampling periods (1994–1995; 1997–1998). Microcosm and mesocosm experiments with pulp mill effluents and ammonia were carried out during the 1997–1998 sampling period under conditions approximating those observed in the field.

2.1. Water quality and light transmission

Detailed descriptions of methods for the collection of physical/chemical field data are given by Flemer and Livingston, 1998; Livingston (1979, 1982, 2000), and Livingston

et al. (1997, 1998, 2000). Field data (temperature, salinity, conductivity, dissolved oxygen, pH) were taken with Datasonde four multiprobes (AMJ, Inc.). Dissolved oxygen anomaly was calculated from the field measurements as the difference between the measured dissolved oxygen and the oxygen solubility at the observed temperature and salinity (Weiss, 1970). Chemical analyses were based on protocols of the American Public Health Association (APHA, 1989); these included chlorophyll *a* (APHA Method 1002-G), Biochemical Oxygen Demand (APHA Method 405.1), true (NCASI) color (colorimeter, Pt–Co units), and nutrients (ammonia, nitrite, nitrate, particulate and dissolved organic nitrogen, total nitrogen, orthophosphate, particulate and dissolved organic phosphorus, total phosphorus). Ammonium was measured as ammonia with an ion electrode (US EPA, 1983, method 350.3). This method has limited sensitivity to interference from humic materials and paper mill effluents (Flemer and Livingston, 1998). Particulate Organic Carbon was analyzed according to methods by Parsons et al. (1984). Turbidity was determined with a ratio turbidimeter. Light penetration depths were taken using standard Secchi disks. Field light transmission data were taken with a Li-Cor LI-1800UW Underwater Spectroradiometer. The underwater light field was characterized by incident radiant flux per unit surface area as quanta $m^{-2}\,s^{-1}$. Samples included 3–5 scans (replicates) taken at 1–2 nm intervals that were averaged for each reading. Flux measurements were taken for photosynthetically active radiation (400–700 nm; PAR) and individual wavelengths. For any series of collections, field samples were taken during relatively calm conditions between the hours of 10:00 and 14:00 h. Multiple air light readings were taken to correct for short-term radiation variability during light measurements.

2.2. Biological sampling

Net phytoplankton samples were taken with two 25 μm nets (bongo configuration) in duplicate runs for periods of 1–2 min. Repetitive (3) 1 l whole water phytoplankton samples were taken at the surface. Phytoplankton samples were immediately fixed in Lugol's solution in its acid version (Lovegrove, 1960). Samples were analyzed by methods described by Prasad et al. (1990) and Prasad and Fryxell (1991). Zooplankton were taken with two 202 μm nets (bongo configuration) in duplicate runs. Samples were preserved with 10% formalin. Plankton identifications were made to species (Prasad and Livingston, 1987).

Methods used for the comparison of monthly data (water quality, biological factors) were developed to determine significant differences between matching Amelia and Nassau sites (polluted and unpolluted) over the 12-month study periods (Livingston et al., 1998; Livingston, 2000). For independent, random samples from normally distributed populations, the parametric *t*-test was used to compare the sample means. For cases where one or both of the data sets violated the assumption of normality, a data transformation was made to bring the data into normality. Tests were also developed to compare two serially correlated populations of numbers taken at subject stations by calculating differences of the observations and plotting the autocorrelations (months) of the differences. If differences were not serially correlated, we applied the Wilcoxon sign-rank test to compare (0.05 confidence level) the two sets of numbers. Table 1 was constructed of the means where the statistical test could be run without serial autocorrelations.

Table 1
Annual mean whole water and 25 μm phytoplankton and zooplankton counts (number of cells per liter), species richness (SR), and Shannon–Wiener diversity (SH) in matched stations in the effluent-receiving Amelia River (stations R1–R13) and reference Nassau River (N1–N8) during 1994–1995 and 1997–1998

Station pair	Whole water phytoplankton			25 μm Phytoplankton			Zooplankton		
	Number of cells per liter ($\times 10^5$)	SR	SH	Number of cells per liter ($\times 10^4$)	SR	SH	Number of cells per liter ($\times 10^3$)	SR	SH
(A) 1994–1995									
R1–N4	1.4–3.1*	14.2–17.8*	1.6–1.51	3.4–9.3	32.5–38.0	1.8–1.8	7.3–1.8*	11.6–10.8	1.1–1.0
R4–N2	1.2–2.4*	13.6–16.8*	1.5–1.5	3.6–4.1	35.1–33.3	2.1–1.9	4.1–9.2*	10.3–11.0	1.2–1.0
R8–N3	1.7–2.9*	14.3–19.7*	1.5–1.5	4.3–6.2*	36.2–42.1*	1.9–1.8*	1.2–1.7	11.9–11.8	1.3–1.0
R10–N1	1.3–3.3*	15.4–18.9	1.6–1.3*	5.1–9.6	37.7–42.7	2.0–1.7	6.0–1.9*	12.0–11.2	1.5–1.2
R11–N5	1.6–3.4	15.2–17.7	1.5–1.3	4.5–9.2	39.1–40.8	2.0–1.8	1.3–9.9	11.9–11.4	1.1–1.2
R12–N6	1.1–2.2*	14.0–17.1	1.6–1.6	3.5–7.7	36.0–37.3	2.1–2.0	1.3–1.6	9.6–10.0	1.0–1.1
R13–N8	2.0–2.8*	11.9–15.4*	1.6–1.4	3.3–7.7*	33.1–35.8	2.1–1.8	5.5–1.5*	8.9–9.8	1.0–0.9
(B) 1997–1998									
R01–N04	2.4–2.9	27.3–34.8*	1.8–2.1						
R08–N03	2.5–3.4	31.3–35.6	2.1–2.1						
R11–N05	1.9–2.7*	28.9–37.1*	2.0–2.2						
R12–N06	1.4–3.2*	25.2–32.2*	1.9–1.9						
R13–N08	1.4–2.2*	23.7–32.0*	1.7–2.0						

Differences among paired stations were tested with parametric *t*-tests and Wilcoxon tests.
* Significant $P < 0.05$.

Field data were analyzed using a Principle Components Analysis (PCA) as a preliminary review of the water quality variables (Livingston et al., 1998). The PCA was used to reduce the physical–chemical variables into a smaller set of linear combinations that could account for most of the total variation of the original set. Significant principal components were then applied to regression models with phytoplankton and zooplankton abundance and species richness as dependent variables. Residuals were tested for independence using serial correlation (time series) analyses and the Wald–Wolfowitz (Wald and Wolfowitz, 1940) runs test. A chi-square test was run to evaluate normality. Statistics were run using SAS™, Systat™ and SuperAnova™.

2.3. Experimental methods

A combination of background field monitoring, controlled laboratory experiments using microcosms of *Skeletonema costatum* (Grev.) Cleve and field mesocosm experiments (multispecies) was used to evaluate the effects of pulp mill effluents and ammonia on plankton assemblages in the Amelia River-estuary. Measured solutions of ammonia were used to evaluate the effects of ammonia by itself relative to the effects of ammonia as part of the whole mill effluent. Target concentrations for the ammonia experiments were based on known field concentrations in polluted areas of the Amelia system. Concentrations of pulp mill effluents were determined by field color analyses at ambient conditions at station R1 in the Amelia system. We carried out one microcosm test (29 June to 4 July 1998) using lab-cultured *Skeletonema* with measured injections of ammonia, and two tests (17–22 July 1998; 28 August to 1 September 1998) with pulp mill effluents with ammonia concentrations approximating those in the field. We performed six larger-volume mesocosm tests in the field with natural phytoplankton assemblages taken from the reference Nassau area. Two tests (19–21 August 1997; 27–29 October 1997) were run with measured injections of ammonia and 4 tests (20–22 May 1998; 24–26 June 1998; 4–6 August 1998; 23–25 September 1998) were carried out with pulp mill effluents that were added to basal mixtures to approximate ammonia concentrations determined in the field. For all tests, ammonia dosages were tested daily and ammonia was added where necessary to maintain target concentrations.

Laboratory microcosm tests were established using 18 1000 ml Erlenmeyer flasks in a randomized block array. The basal mixture (700 ml) was offshore water enriched with nitrate, orthophosphate, and silicon dioxide. Each flask was inoculated with the lab-cultured test species (*S. costatum*). After addition of ammonia solution or mill effluent, ammonia concentrations (five treatments and a control) were measured with an Orion ammonia-sensitive electrode (Flemer and Livingston, 1998). Experiments were run at 22 ± 1 °C. Growlight@ lights were used; light levels were checked with a spectroradiometer for treatment comparability. Experimental light levels were comparable to those taken in the field (PAR, 0.5 m: 275–300 $\mu E\ m^{-2}\ s^{-1}$). Day lengths of 10:14 h (light:dark) were used for the experiments. Water quality collections were taken daily for each test. Chlorophyll *a* concentrations (indicative of *Skeletonema* abundance) were analyzed using a Wetlabs fluorometer. Test results were determined for days 1, 3, and 5 of the test period.

Field mesocosms were established in the Amelia River-estuary with water and natural phytoplankton assemblages taken from the Nassau system. Zooplankton were removed by passing water through a 64 μm plankton net. A standard mesocosm, run as a closed

system, was a 20-l clear, polypropylene cubitainer fitted with closure adapters that allowed acceptance of the Hydrolab datasonde for monitoring purposes. Mesocosms were suspended 0.1 m below the water surface in the Amelia system in areas distant from mill effects. Five treatments (using ammonia or mill effluent) were established with a control. Three replicates per treatment were randomly distributed in a meshed frame for containment. Through experimentation, we established an effluent/ammonia spiking routine at 1-day intervals. Mesocosms were monitored individually for ammonia, color, chlorophyll *a*, dissolved oxygen and pH on a daily basis. Ammonia concentrations were determined to ascertain the concentration of inoculants. Color was assayed to determine the concentration of the mill effluent. Chlorophyll *a* was taken as an index of phytoplankton growth. The maximum duration of the tests was 2–3 days as determined by a series of preliminary tests.

A one-way ANOVA model was used to analyze the microcosm results. Six treatments, with the first as the control (no added ammonia), were arranged in a randomized (six treatments by three replicates) experiment. The variable of interest was chlorophyll *a* (representative of numbers or biomass of *S. costatum*). The same experiment was repeated during three time periods: 29 June to 4 July as experiment 1, 17–22 July (experiment 2), and 28 August to 1 September (experiment 3). Multiple comparison tests were performed on the experimental results. Based on the recommendation by Kirk (1995), *post hoc* contrasts were tested by Tukey's HSD (honestly significant difference) test, [Tukey is sufficient]. The SAS statistical software was used for the analysis. Statistical assumptions were tested using residual box plots. We used scattergrams of the residuals versus the fitted values of the response variable Y and scattergrams of cell means versus the standard deviations. In addition, interactive bar charts were constructed showing cell means with standard deviation error bars. In no case were the residuals considered other than random and we therefore used no transformations for the data analysis.

3. Results

3.1. Water quality data

There were no consistently significant differences in surface temperature, salinity, Secchi depths, BOD, DOC, TSS, silica, TP, POC, or sulfide between cognate station pairs during the survey periods (1994–1995; 1997–1998). Surface water color was significantly ($P < 0.05$) higher at stations R03, R04, R10, N06, N09, and N11 than their paired matches during 1994–1995 and at stations R01, R08, and R11 during 1997–1998. Color gradients arranged as distance from the effluent discharge indicated the mill as the source (Fig. 2). However, mean color was highest in the upper parts of both estuaries during winter months of increased rainfall. Surface turbidity was significantly ($P < 0.05$) higher at stations R03, R04, R14, N08 and N10 during 1994–1995 and was significantly higher at stations N04 and N08 during 1997–1998. There were no significant differences in mean orthophosphate concentrations among stations during both sampling periods although the upper Nassau system (stations N07, N08, N09, N10) had uniformly higher concentrations of orthophosphate than the paired stations in the Amelia system. Total phosphorus (TP) was significantly higher at

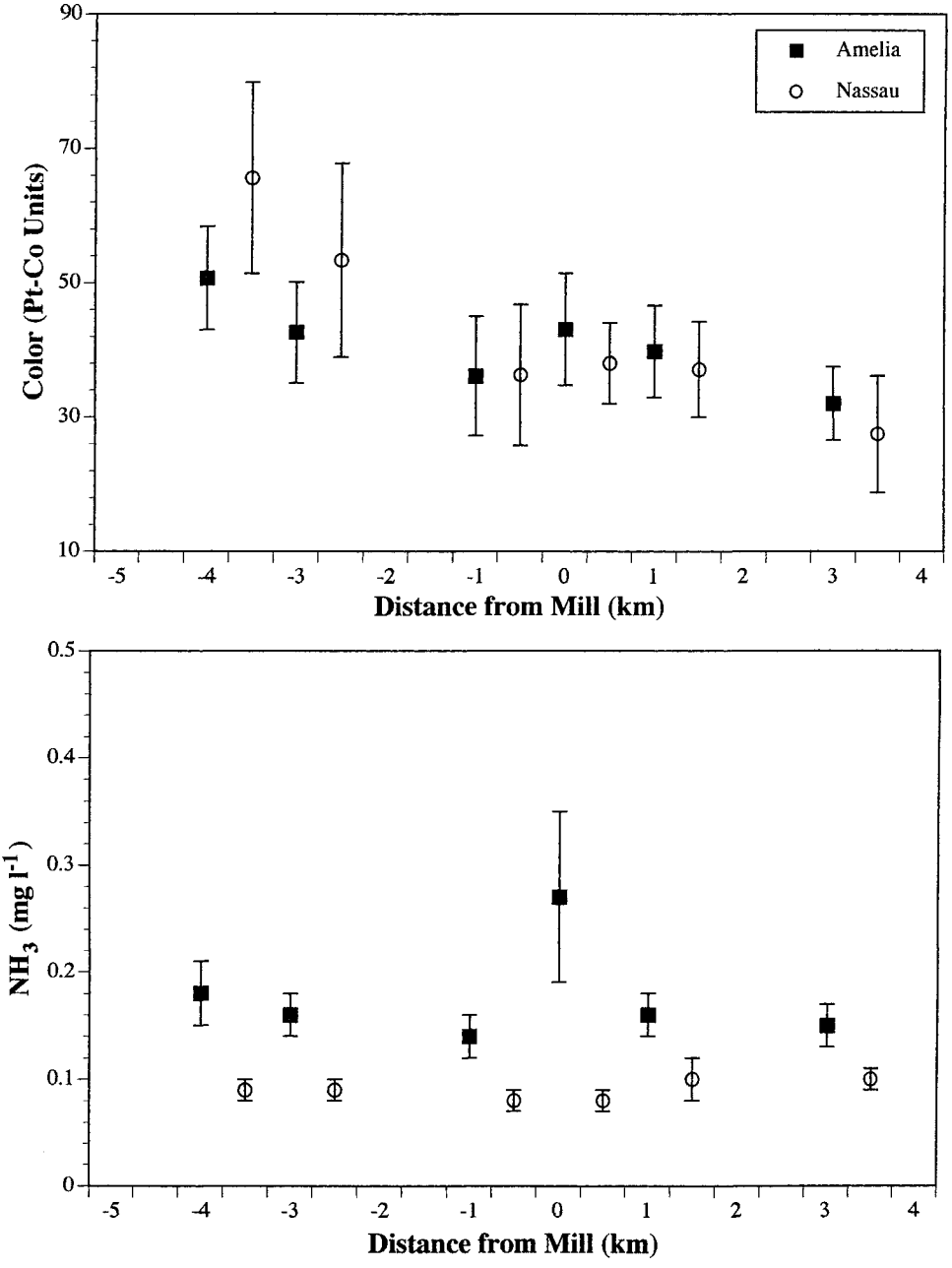


Fig. 2. Gradients (negative = up-river and positive = down-river in km from mill outfall) of surface color (Pt–Co units) and surface ammonia (mg l^{-1}) shown as 12-month means (±one standard error) taken from November 1994 through October 1995.

stations R01, R04, R05, and R11 during 1994–1995 and was higher at stations R02, R03, R09 N08 and N10 during 1997–1998.

During 1994–1995, surface ammonia concentrations were significantly ($P < 0.05$) higher at all stations in the Amelia system with the exception of R04 and R12 (Fig. 3). The relatively high ammonia concentrations near the mill outfall and gradients of surrounding stations indicated the pulp mill as the source (Fig. 2). Mean annual surface ammonia concentrations ranged from 0.19 to 0.43 mg l^{-1} in the Amelia estuary during 1997–1998. No such gradient was noted in the Nassau estuary with annual means ranging from 0.09 to 0.11 mg l^{-1}. The highest ammonia concentrations in the Amelia system appeared during spring/summer months during both sampling periods (Fig. 3). Mean nitrite/nitrate concentrations near the outfall (stations R01, R03, R06) followed this trend, although differences were not statistically significant in the upper parts of the respective study areas. Surface total nitrogen was generally higher throughout the Amelia system during both sampling periods with significant ($P < 0.05$) differences at stations R01, R02, R03, R06, R09, and R11. Mean surface chlorophyll *a* concentrations were generally lower in the lower Amelia River-estuary than matched stations in the Nassau system during both sampling periods, and were significantly reduced ($P < 0.05$) at stations R01, R08, R11 and R12 during 1997–1998. Spatial and temporal chlorophyll *a* trends followed (inversely) those of ammonia.

3.2. Light transmission

Light data (Fig. 4) indicated that during 1994–1995, there were no major differences in light penetration between the Amelia and Nassau systems. Although, euphotic depths at station R01 were lower than at station N04 during 1997–1998, this was not consistent throughout the entire sampling period. In three of the eight noted readings, the differences were negligible. When viewed as differences in euphotic depths at different wave lengths, there were no significant differences between paired stations N04 and R01. The lowest euphotic depths (and highest extinction coefficients) in both systems were noted during February 1998, a period of low chlorophyll *a* concentrations. With the exception of station R11 at the 430 nm level, light extinction coefficients in the Amelia system were not significantly higher than those in the Nassau system. There were no significant reductions of euphotic depths in the Amelia system.

In both systems, there was evidence of a "gelbstoff shift" (Livingston et al., 1998) whereby humic substances absorb light at lower wave lengths. Extinction coefficients were significantly higher and euphotic depths were significantly lower in the upper Nassau system where the highest levels of color were noted (Fig. 2). The highest light extinction coefficients were noted at station N08. Although, there was thus no evidence of a significant mill effect on light transmission in the Amelia River-estuary relative to the reference system, the upper parts of the Nassau River-estuary were subject to the effects of runoff that affected both color and light transmission.

3.3. Phyto- and zooplankton

Nearly 250 species of whole water phytoplankton were identified in the two study areas during the 1994–1995 survey. Numerical abundance of phytoplankton was reduced in the

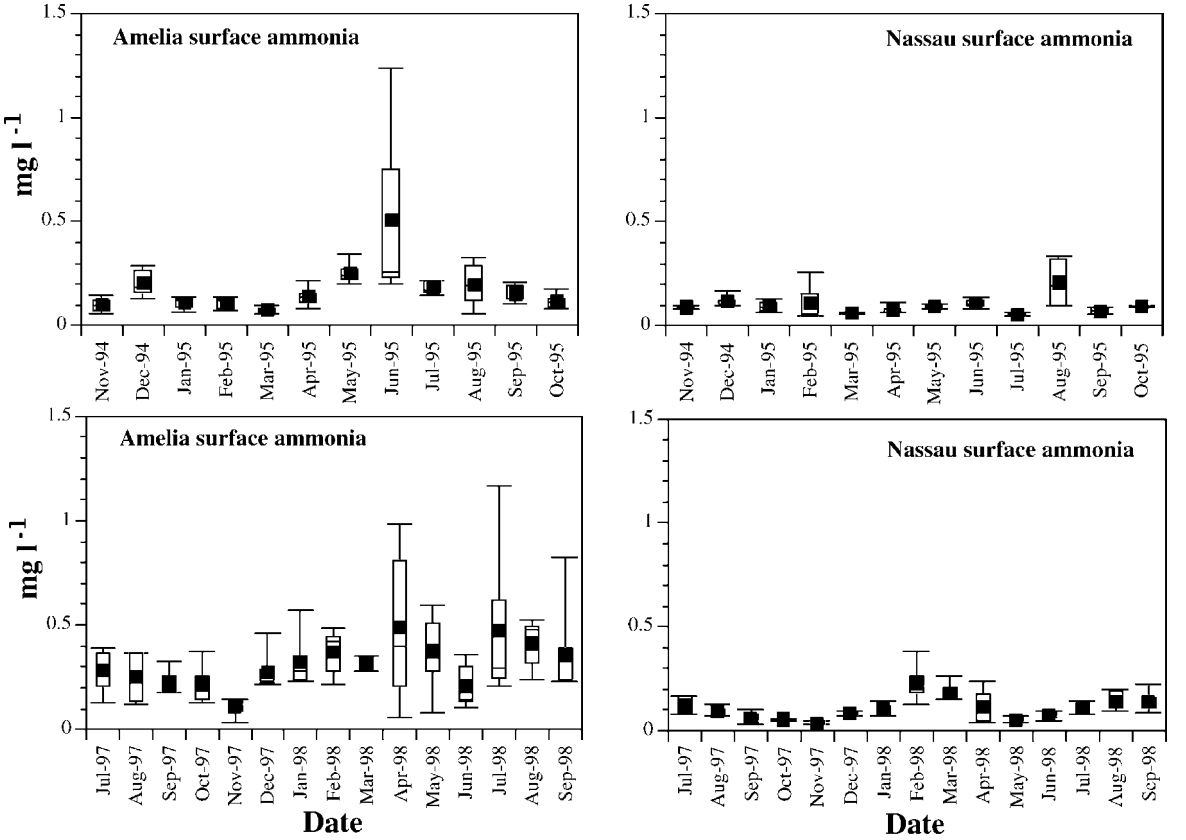


Fig. 3. Surface ammonia data ($mg l^{-1}$) taken monthly in the Amelia and Nassau systems from November 1994 through October 1995 and from July 1997 through September 1998. Data are presented as means with the 10th, 25th, 75th and 90th percentiles.

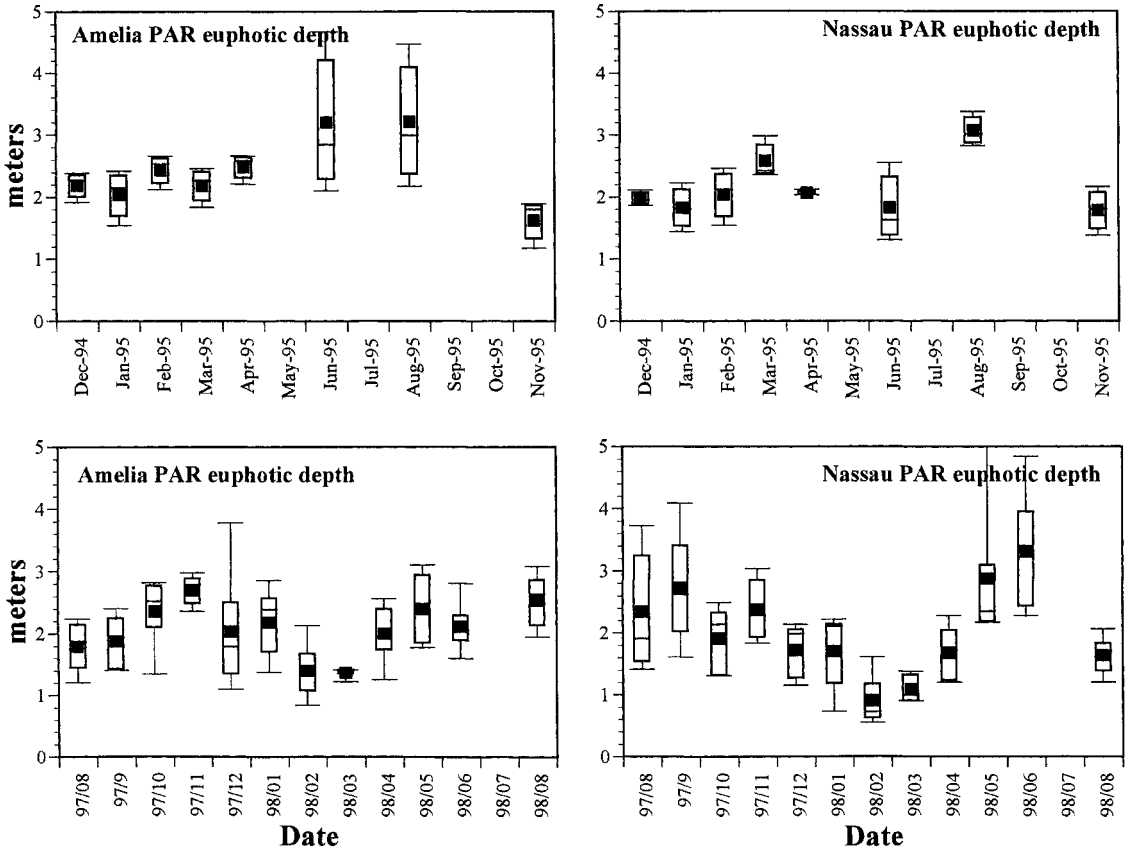


Fig. 4. Euphotic depths (PAR) in the Amelia and Nassau systems during 1994–1995 and 1997–1998. Data are presented as means with the 10th, 25th, 75th and 90th percentiles.

Amelia system relative to the reference area, and totaled only about 57% of the phytoplankton numbers found in the Nassau system. *S. costatum* was dominant in both study areas. Other dominants included *Cylindrotheca closterium*, *Thalassionema nitzschoides* and *Asterionellopsis glacialis*. Major reductions were noted for *S. costatum*, *A. glacialis*, *T. nitzschiodes*, and *Pseudonitzschia* sp. in the Amelia system relative to the reference area.

The 1997–1998 results were similar to those of 1994–1995. Of the 10 top dominant species, representing over 75% of the numbers of phytoplankton taken during the 1997–1998 survey, seven such species had considerably higher numbers in the Nassau system than in the Amelia system. The top dominant in the Nassau system was *S. costatum* whereas phytoplankton assemblages in the Amelia system were dominated by *Chaetoceras socialis*. Cryptophytes and nannoflagellates were somewhat higher in the Amelia system than the reference system. Nannococcoids were noted primarily at the outfall station. Blooms of *Navicula* sp. were found at station R13 during July 1998. In addition to *S. costatum*, several species were notably higher in the Nassau system; these included *Thalassiosira proschikinae* and *T. decipiens*, *Asterionellopsis japonica*, *C. closterium*, *T. nitzschoides*, *Chaetoceros curvisetus* and *Chaetoceros laciniosus*.

With the exception of station R13, densities of diatoms (Class Bacillariophyceae) were lower in the Amelia system than the Nassau system during both sampling periods. The silicoflagellates were often more abundant in the Nassau system. The cryptophytes (Division Cryptophyta), green algae (Division Chlorophyta), dinoflagellates (Division Dinophyta), and blue–green algae (Division Cyanophyta) were generally found in higher concentrations in the Amelia system.

Phytoplankton numbers and species richness of the net (25 μm) and whole water phytoplankton were higher in the Nassau system during 1994–1995 (Table 1); such differences were statistically significant ($P < 0.05$) in the whole water phytoplankton, but not the net phytoplankton. The most pronounced differences of phytoplankton numbers and species richness were noted during warm months (March–July 1995). Shannon–Wiener diversity tended to be similar among the various station combinations with no significant differences except at station R10. Zooplankton numbers were significantly lower (stations R01, R03, R04 and R10) in the Amelia system during 1994–1995 (Table 1). The most pronounced zooplankton differences between the two study areas occurred during March and April 1995. Zooplankton species richness was not significantly ($P > 0.05$) different between the two systems (Table 1).

During 1997–1998, phytoplankton numbers and species richness were generally lower at Amelia stations (Table 1); such differences were usually statistically significant ($P < 0.05$). Numerical abundance data showed that the primary reductions at station R01 occurred during November and December 1997 and April, July and August 1998. Higher numbers were noted at station R01 during June 1998; this increase occurred during the period of relatively lower ammonia concentrations. Differences of phytoplankton numbers and species richness between stations R11 and R12 and their matching stations were significant. Significantly higher phytoplankton numbers were noted at station R13 than at its Nassau equivalent, a result of the July 1998 *Navicula* bloom. Species richness, however, was significantly lower at station R13 than station N08.

3.4. Statistical analyses

Detailed descriptive (Fig. 5) and statistical analyses were carried out with field data taken during spring-summer 1994–1995 using factors that were significantly different between the two study areas. Particular attention was given to warm water periods when phytoplankton differences between the study areas were greatest. Water color and Secchi depths were comparable between the two systems. Chlorophyll *a* concentrations were somewhat lower in the Amelia system during April, May, and June 1995 although the reduction of this factor were not as pronounced as in phytoplankton (Fig. 5). Ammonia concentrations were higher in the Amelia system during April, May, June, and July with somewhat higher concentrations in the Nassau system during August although overall averages were generally higher in the Amelia system. The occurrence of high ammonia tended to be the primary factor associated with reduced phytoplankton numbers in the Amelia system. This was generally true of phytoplankton species richness indices (Fig. 5). Zooplankton numerical abundance followed the phytoplankton trends (Table 1). With the exception of August 1995, concentrations of 0.1 mg l^{-1} ammonia appeared to the dividing line between the two study areas.

A PCA/regression analysis (Table 2) was run with the 1994–1995 data. This analysis was run two ways: (1) for all stations and all dates over the 12-month sampling period, and (2) for data taken during warm months of the year. The analysis run over the entire sampling period indicated that whole water phytoplankton numbers were negatively associated with color and positively associated with salinity and chlorophyll *a*. During summer months, whole water phytoplankton numbers were negatively associated with ammonia, and positively associated with temperature and chlorophyll *a*. During the 12-month period, net phytoplankton numbers varied negatively with color and BOD, and positively with salinity, chlorophyll *a*, and DOC. Net phytoplankton numbers were negatively associated with ammonia and positively associated with temperature and chlorophyll *a* during summer months. Whole water phytoplankton species richness was negatively associated with color and BOD during the 12-month period and negatively associated with TN during the summer months. Net phytoplankton species richness was negatively associated with color and BOD during the 12-month period, and was negatively associated with ammonia during summer months. Thus, there appeared to be a negative response of phytoplankton numbers and species richness to ammonia during warmer periods.

Zooplankton numbers varied negatively with ammonia and total nitrogen and positively with Secchi depths during the 12-month period, and were negatively associated with ammonia and sulphides and positively associated with temperature and chlorophyll *a* during summer months. Zooplankton species richness during the 12-month period was positively associated with high Secchi depths and negatively associated with turbidity and TSS. During summer months, zooplankton species richness was positively associated with temperature and negatively associated with color and sulphide.

3.5. Laboratory microcosms

The microcosm experiments were designed to evaluate the effects of ammonia on growth of *S. costatum*, and to determine the potential influence of mill effluents (with ambient

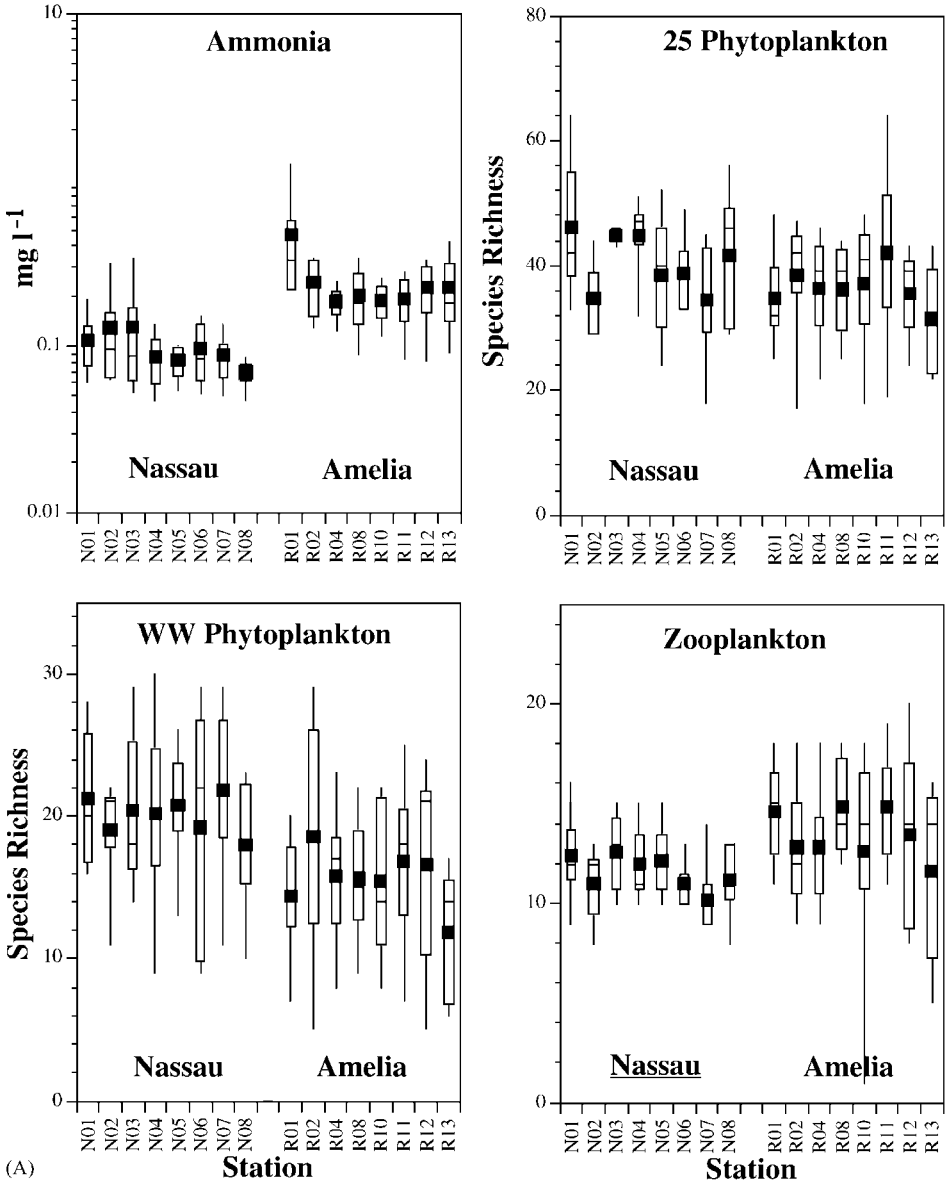
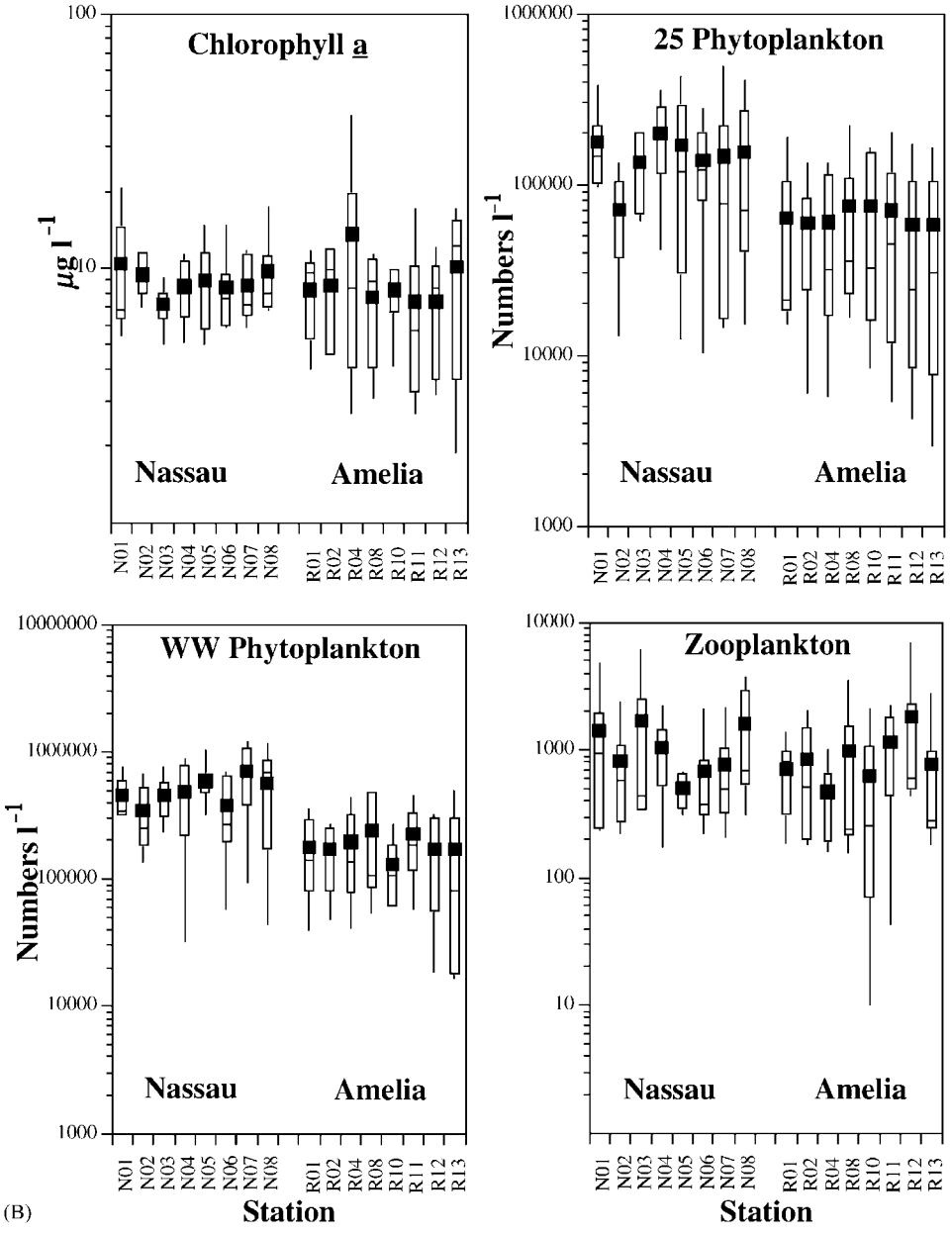


Fig. 5. (A) Surface ammonia, net (25 μm) and whole water (WW) phytoplankton species richness and zooplankton species richness taken during spring-summer of 1994–1995. (B) Surface chlorophyll *a*, net (25 μm) and whole water (WW) phytoplankton cells per liter and zooplankton numbers per liter taken during spring-summer of 1994–1995. Data are presented as means with the 10th, 25th, 75th and 90th percentiles.



(B)

Fig. 5 (*Continued*).

Table 2
Results of principal components/regression analyses of field data for whole water phytoplankton, net phytoplankton, and zooplankton (numbers of cells per liter, number of taxa) taken in the Amelia and Nassau River-estuaries over a 12-month-period (1994–1995) and during summer months (1994–1995)

Dependent variable	Independent variable	r^2	Significance	Sign
(A) 12-month-period (1994–1995)				
Whole water phytoplankton				
Number of cells per liter	Salinity	0.46	0.0001	+
	Chlorophyll *a*	0.46	0.0001	+
	Color	0.46	0.0001	–
Number of taxa	Color	0.54	0.0001	–
	Salinity	0.54	0.0001	+
	Chlorophyll *a*	0.54	0.0001	+
	BOD	0.54	0.0035	–
	DOC	0.54	0.0035	+
Net phytoplankton				
Number of cells liter	Color	0.59	0.0001	–
	Salinity	0.59	0.0001	+
	Chlorophyll *a*	0.59	0.0001	+
	BOD	0.59	0.0030	–
	DOC	0.59	0.0030	+
Number of taxa	Color	0.59	0.0001	–
	Salinity	0.59	0.0001	+
	Chlorophyll *a*	0.59	0.0001	+
	BOD	0.59	0.0035	–
	DOC	0.59	0.0035	+
Zooplankton				
Number of cells per liter	Ammonia	0.28	0.0059	–
	Secchi	0.28	0.0012	+
	TN	0.28	0.0059	–
Number taxa	Secchi	0.37	0.0012	+
	Turbidity	0.37	0.0012	–
	TSS	0.37	0.0009	–
(B) Summer period (1994–1995)				
Whole water phytoplankton				
Number of cells per liter	Ammonia	0.50	0.0001	–
	Temperature	0.50	0.0001	+
	Chlorophyll *a*	0.50	0.0001	+
Number of taxa	Temperature	0.19	0.0393	+
	Secchi	0.19	0.0184	+
	Chlorophyll *a*	0.19	0.0393	+
	TN	0.19	0.0184	–
Net phytoplankton				
Number of cells per liter	Ammonia	0.58	0.0001	–
	Temperature	0.58	0.0001	+
	Chlorophyll *a*	0.58	0.0001	+
Number of taxa	Ammonia	0.19	0.0434	–

Table 2 (*Continued*)

Dependent variable	Independent variable	r^2	Significance	Sign
Zooplankton				
Number of cells per liter	Temperature	0.58	0.0148	+
	DOC	0.34	0.0035	–
	Chlorophyll *a*	0.34	0.0035	+
	Ammonia	0.34	0.0035	–
	Sulphide	0.58	0.0148	–
Number of taxa	Temperature	0.55	0.0001	+
	Color	0.55	0.0087	–
	Sulphide	0.55	0.0087	–

Independent variables listed in order of predominance.

field ammonia concentrations) on such effects. The results are shown in Fig. 6. For the first two experiments, Tukey's HSD test indicated that there were no significant differences among the six treatment means during day 1. For day 1 in experiment 3, Tukey's HSD showed that means of treatments 5 and 6 were higher than the control. In experiment 1, there were no significant differences of chlorophyll *a* among treatment means by day 3. By day 5, Tukey's test showed that there were significant differences of chlorophyll *a* between controls (treatment 1) and each of the ammonia treatments. All three tests indicated that there was also a significant difference between chlorophyll *a* concentrations in treatments 1, 2, and 3 and treatments 4, 5, and 6. Chlorophyll *a* concentrations decreased gradually with increasing ammonia concentrations (Fig. 6, top). The addition of ammonia increased microalgal production; the final chlorophyll *a* concentrations were highest at mean ammonia concentrations around 0.06 mg l^{-1}. Chlorophyll concentrations were significantly lower at ammonia concentrations of from 0.11 to 0.24 mg l^{-1} and much lower at concentrations over 0.46 mg l^{-1}.

Results of experiment 2 (pulp mill effluents) indicated that, by day 3, controls and treatment 6 had significantly lower chlorophyll than treatments 2, 3, 4, and 5. This result showed ammonia stimulation of *S. costatum* at relatively high concentrations of ammonia (0.06–0.62 mg l^{-1}). By day 5, there were significant reductions of chlorophyll at mean ammonia concentrations of 0.71 mg l^{-1}. Compared to experiment 1 (ammonia only), these results indicated a difference in the action of ammonia on the growth of *S. costatum* in the presence of mill effluents.

The third experiment (with pulp mill effluents) showed that, by day 3, there was significant stimulation of chlorophyll production at mean ammonia concentrations of 0.07 mg l^{-1}. All three statistical tests indicated that mean chlorophyll *a* of treatment 2 was significantly higher than means of the control and other treatments by day 3. By day 5, controls were higher than treatments 3, 4, 5, and 6. Tukey's test showed that controls were significantly higher than treatments 4, 5 and 6. These results showed adverse effects on *S. costatum* at mean ammonia concentrations of 0.27 mg l^{-1}. These differences were significant at mean ammonia concentrations of 0.46 mg l^{-1}. The results of experiment 3 resemble those of experiment 1 although the shape of the chlorophyll curves during day 5 was somewhat different. Based on the differences of the results of the ammonia and pulp mill effluent

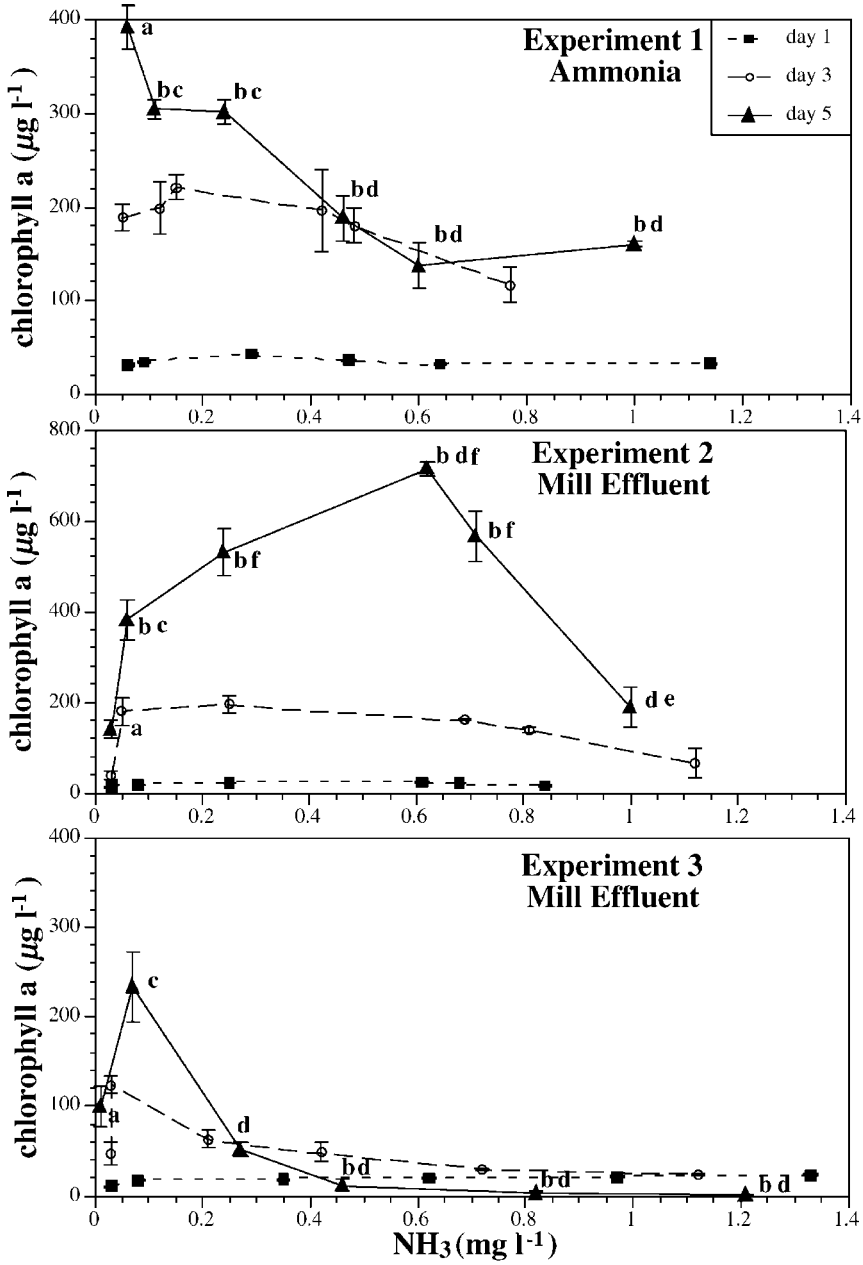


Fig. 6. Results of the microcosm experiments with *S. costatum* spiked with ammonia (experiment 1) and pulp mill effluents (experiments 2 and 3). Ammonia concentrations and chlorophyll *a* as a function of ammonia are shown as means ± one standard error (three replicates) per treatment over the 5-day duration of each experiment. Data were taken on day 1, day 3, and day 5 of each experiment. Treatment 1 was the control for the experiments. Multiple comparisons for day 5 are presented lettering intaking sign. Experiment 1: Treatment 1(a) significantly ($P < 0.05$) different from treatments 2(b), 3(b), 4(b), 5(b), 6(b). Treatments 1(a), 2(c), 3(c), significantly ($P < 0.05$) different from treatments 4(d), 5(d), 6(d). Experiment 2: Treatment 1(a) significantly ($P < 0.05$) different from treatments 2(b), 3(b), 4(b), 5(b). Treatment 2(c) significantly ($P < 0.05$) different from treatment 4(d). Treatment 6(e) significantly ($P < 0.05$) different from treatments 3(f), 4(f), 5(f). Experiment 3: Treatment 1(a) significantly ($P < 0.05$) different from treatments 4(b), 5(b), 6(b). Treatment 2(c) significantly ($P < 0.05$) different from treatments 1(a), 3(d), 4(d), 5(d), 6(d).

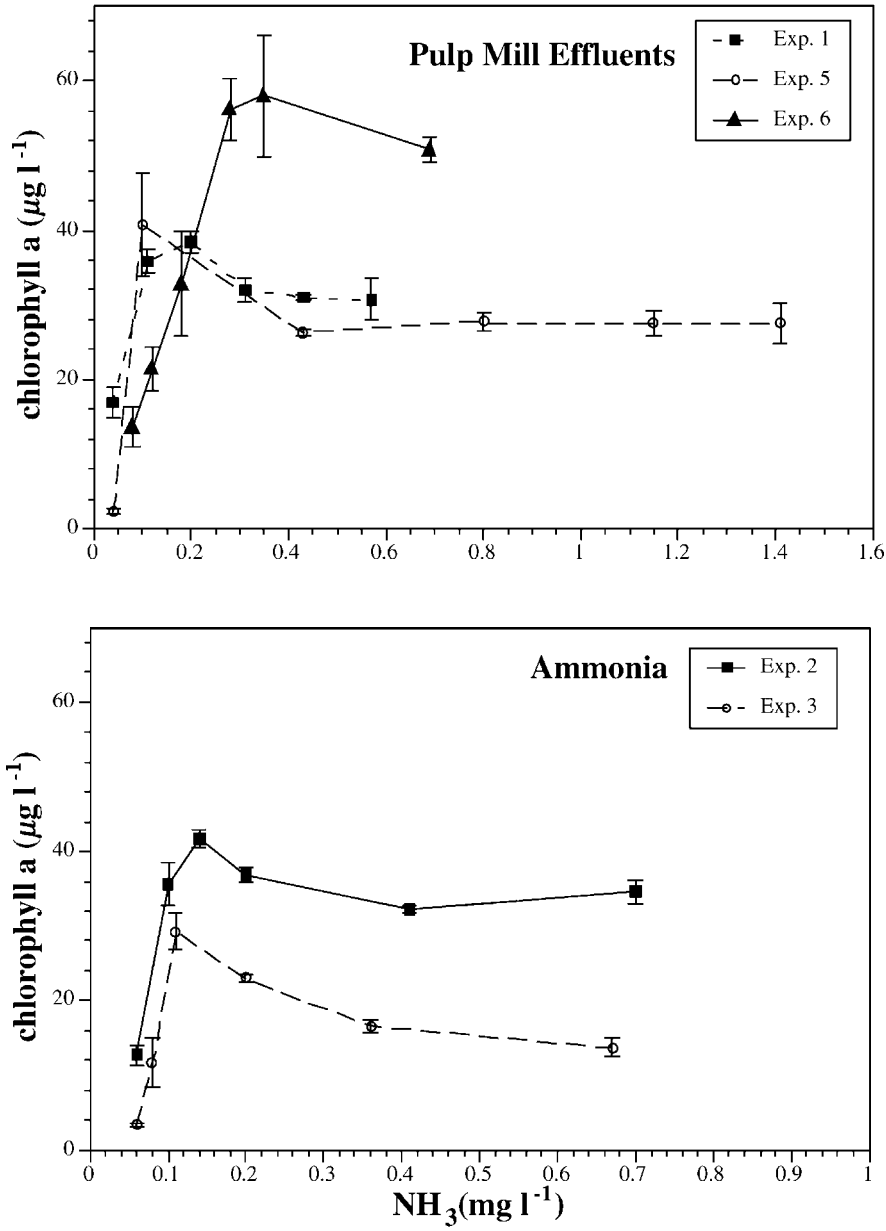


Fig. 7. Results of the phytoplankton mesocosm experiments spiked with ammonia (experiments 2 and 3) and pulp mill effluents (experiments 1, 5 and 6). Ammonia concentrations and chlorophyll *a* data (as an indicator of phytoplankton numbers) are shown as means ± one standard error. There were three replicates per treatment over the 3-day duration of each experiment. Chlorophyll *a* data taken on day 3 of each experiment are shown. Treatment 1 was the control for the experiments.

tests, it is likely that factors other than ammonia in the mill effluents affected *S. costatum* growth.

3.6. Field mesocosms

Compared to untreated controls, there was usually an increase of chlorophyll *a* at the lowest ammonia concentrations indicating stimulatory effects of ammonia (Fig. 7). Chlorophyll *a* reductions were seen as indicators of ammonia inhibition.

Results of experiment 1 (mill effluent, mean temperature of 30 °C; Fig. 7) showed chlorophyll *a* peaks at mean ammonia concentrations of 0.20 mg l^{-1} with inhibition commencing at mean ammonia concentrations of 0.31 mg l^{-1}. Results of experiment 5 (mill effluent, mean temperature of 30.3 °C) indicated increased chlorophyll *a* (nutrient enhancement) at mean ammonia concentrations of 0.10 mg l^{-1} with inhibition noted at 0.43 mg l^{-1} ammonia. Experiment 6 (mill effluent, mean temperature of 28.7 °C) indicated ammonia stimulation at 0.36 mg l^{-1} and ammonia inhibition at 0.69 mg l^{-1}. The results of the last two experiments showed somewhat higher toxicity thresholds for ammonia than the first pulp mill experiment. The results of mesocosm experiment 4 (not shown in Fig. 7) reflected extremely high ammonia concentrations in the mill effluent during the experimental period (24 June 97: average concentrations at 4.8 mg l^{-1}). There was no asymptotic point of the chlorophyll *a* response in the various treatments, and the results were therefore not included in the final analyses.

The second mesocosm experiment (ammonia; mean temperature of 21.7 °C) gave results that resembled those of experiment 1 (mill effluents). Chlorophyll *a* peaked at 0.14 mg l^{-1} ammonia with inhibition at 0.20 mg l^{-1} ammonia. Experiment 3, run with ammonia concentrations at a mean temperature of 28.4 °C, gave similar results to those of the first two experiments with stimulation at 0.11 mg l^{-1} ammonia and inhibition at 0.20 mg l^{-1} mean ammonia concentrations. Water temperature did not appear to have a major effect on the results of the mesocosm experiments.

4. Discussion

The basic questions in this paper concern the effects of pulp mill effluents and associated ammonia concentrations on plankton assemblages in a high salinity estuary. These questions should be answered within the context of major loading of ammonia by a paper mill into a physically variable coastal environment. Phytoplankton abundance and species richness in the Amelia and Nassau systems were seasonally variable, with peaks usually occurring during summer months although there was evidence of winter increases of phytoplankton abundance. Zooplankton abundance peaked during spring months, which coincided with declines of phytoplankton abundance. Summer increases of phytoplankton numbers were correlated with relatively low zooplankton numbers. Nutrient limitation experiments (Livingston, unpublished data; Figs. 6 and 7) indicated that nitrogen was the chief limiting nutrient to phytoplankton in the Nassau and Amelia River-estuaries during all seasons.

The diatom *S. costatum* is ubiquitous in coastal waters world-wide, and is frequently dominant in inshore phytoplankton blooms (Bonin et al., 1986; Young and Barber, 1973;

Stockner and Costella, 1976; Hulburt and Rodman, 1963; Hulburt and Corwin, 1970). In these studies, *S. costatum* was often dominant due to its relatively high growth rate under a wide range of temperature–light conditions. There is also evidence that *S. costatum* grows well under high nutrient conditions, and it can use organic phosphorus as a source of growth. It can also assimilate organic molecules such as urea (Round, 1981). It grows in New York Bight waters where massive amounts of chemicals were dumped (Young and Barber, 1973). This species was a major constituent of the phytoplankton community in the Nassau and Amelia systems during different times of the year. However, *S. costatum* abundance was severely reduced in the Amelia system compared to the reference Nassau system.

Field analyses showed that water color, temperature, and salinity play important roles in seasonal changes of phytoplankton associations in the Amelia and Nassau systems. However, during warm periods, ammonia was a leading factor associated with reductions of phytoplankton numbers and species richness (Table 2). Field results indicated that phytoplankton abundance and species richness were significantly lower in the Amelia system relative to the Nassau system.

Extremely high concentrations of ammonia in the Amelia system and the general trends of reduced phytoplankton and zooplankton abundance and phytoplankton species richness during periods of high ammonia concentrations provided the basis for the experimental program. We used laboratory microcosms and field mesocosms to determine whether or not ammonia had a toxic effect on plankton assemblages, and to analyze possible effects of mill effluents on ammonia impacts. Microcosm results indicated that ammonia had a stimulatory effect on *S. costatum* at mean concentrations of 0.06 mg l^{-1} with negative effects of ammonia occurring within a range of 0.1–0.24 mg l^{-1} and major impacts at concentrations >0.46 mg l^{-1}. Mesocosm experiments with ammonia indicated stimulatory effects from 0.11 to 0.14 mg l^{-1} and inhibition of phytoplankton growth beyond 0.20 mg l^{-1}. The difference between stimulatory effects and inhibition of ammonia on *S. costatum* and phytoplankton assemblages was relatively small. A comparison of Figs. 6 and 7 indicates that microcosm and mesocosm results regarding ammonia effects showed a slight shift to higher inhibition concentrations of ammonia in the field tests. A similar comparison for mill effluent effects showed variable but similar results. Also, mill effluents were somewhat less toxic than pure ammonia, which indicates that effluent components other than ammonia were in some way related to the observed response of the phytoplankton. It is possible that increased nutrients such as orthophosphate and nitrate in the effluent altered the inhibitory effects of ammonia on phytoplankton.

Results of microcosm and mesocosm experiments with ammonia were generally consistent with field estimates of ammonia effects on phytoplankton. Light penetration and temperature could have obfuscated ammonia effects on phytoplankton in the field. Increased ammonia concentrations during winter (high color, low temperature) did not have as pronounced adverse effects as those during summer months. Multivariate statistical analyses of the field data (Table 2) confirmed that multiple factors determined phytoplankton distribution and there were seasonal differences of plankton response to ammonia. Reduced zooplankton numbers in the Amelia system could have been related to changes of phytoplankton assemblages rather than direct effects of ammonia since zooplankton species richness did not appear to be affected by high ammonia concentrations.

The presence of blooms in upper parts of the Amelia estuary complicated direct evaluations of the influence of ammonia since urban storm water effects were indicated both in terms of water quality and phytoplankton response. Since the lower experimental inhibitory concentrations of the ammonia tests were comparable to the field results, concentrations of 0.20 mg l^{-1} can be taken in our view as conservative estimates of ammonia toxicity. The field results also presented a more representative analysis of the effects of long-term exposure to ammonia than the short-term experimental tests. Based on projections of ammonia inhibition of phytoplankton at 0.20 mg l^{-1}, a restoration effort was initiated by the pulp mill that was consistent with comparable levels of ammonia concentrations in the Amelia system (range of average ammonia concentrations: 0.19–0.43 mg l^{-1}) relative to the reference Nassau River-estuary (range of average ammonia concentrations: 0.09–0.11 mg l^{-1}). Recommended long-term average ammonia concentrations at station R01 (Amelia River-estuary) were set at 0.11 mg l^{-1} ammonia with short-term average increases not exceeding 0.20 mg l^{-1}. The pulp mill undertook a restoration program based on these estimates.

5. Conclusions

High ammonia concentrations represented a major factor associated with pulp mill discharges into the Amelia River-estuary relative to conditions in the reference Nassau River-estuary. There were no significant increases in light extinction coefficients of the Amelia system although there were periodic increases of color and associated reductions of light penetration in areas affected by mill effluents. Significantly reduced chlorophyll *a* concentrations were noted at various Amelia stations. Phytoplankton numbers and species richness were significantly reduced in the Amelia system relative to the Nassau system. The most significant associations of reduced phytoplankton numbers and species richness with ammonia concentrations occurred during summer months, indicating temperature as a modifying factor for ammonia impact. Phytoplankton distribution in the upper parts of both estuaries was somewhat anomalous due to postulated impacts of urban storm water runoff in the form of increased nutrients and phytoplankton blooms.

Results of microcosm experiments with *S. costatum* and ammonia indicated that the difference between ammonia stimulation and inhibition was relatively small. Concentration ranges of 0.06–0.24 mg l^{-1} had stimulatory effects with adverse effects of ammonia occurring between 0.11 and 0.24 mg l^{-1}. Phytoplankton mesocosm tests indicated ammonia stimulation of phytoplankton at concentrations ranging from 0.11 to 0.14 mg l^{-1} with inhibition at concentrations beyond 0.20 mg l^{-1}. Field data were generally consistent with experimental results. Mesocosm experiments with mill effluents gave more diverse results, a possible result of effects of other components on ammonia toxicity. Ammonia concentrations that could be considered inhibitory were difficult to determine with any accuracy using such data. Seasonal differences in the effects of ammonia on phytoplankton in the field added to the complexity of setting exact limits to ammonia loading by the mill. Within the context of the noted ranges of impacts in both the field and laboratory results, it was recommended that long-term average concentrations in the Amelia River-estuary at

station R01 should not exceed 0.11 mg l^{-1} ammonia with short-term average increases not exceeding 0.20 mg l^{-1}.

Acknowledgements

The authors are grateful for technical assistance with transmission and scanning electron microscopes provided by Ms. K. A. Riddle (Department of Biological Science, Florida State University). Mrs. A. Black and Mr. D. Watson (Histology Division, Department of Biological Science, Florida State University) aided in specimen preparation and serial thin sectioning. Database management was organized by Mr. P. Homann. E. Tokar and D. Tudor provided administrative support. The authors would also thank the anonymous reviewers for their contribution to the manuscript. The authors were also grateful for the careful review of the ms by the journal editor.

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