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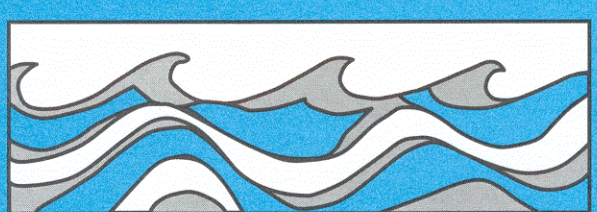
Walter T. Trial
Eugene B. Welch



Water Resources Series
Technical Report No. 78
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by

Walter T. Trial and Eugene B. Welch

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Office of Water Research and Technology
through the Washington Water Research Center

NOTICE

Effective July 1982, and No. 77, the Water Resources Report series supersedes the Charles W. Harris Hydraulics Laboratory Technical Report series. The last publication in the Harris Laboratory series was Technical Report Number 76, June 1982. Requests for reports in either series should be addressed to:

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ADDENDUM

The discussion in paragraphs 1 and 2, p. 44 and 2, p. 49 needs clarification. The rates of nitrification reported from changes in $NO_3^- + NO_2^-$ concentration *in situ* were calculated using the first order growth equation, $N_t = N_o e^{kt}$. For N_t = 183 $\mu g\ l^{-1}$, N_o = 80 $\mu g\ l^{-1}$ and t = 0.106 days, the rate contant (k) is 7.8 day^{-1}. These concentrations were determined by subtracting dilution corrected concentrations from those observed. The assumption for using that equation is that nitrifying bacteria increase in proportion to the $NO_3^- + NO_2^-$ formed.

The more conventional procedure for determining the first order rate using $NO_3^- + NO_2^-$ accumulation is $N_t = N_o(1 - e^{-kt})$, where N_o is the ultimate concentration possible or the maximum NH_4^+ concentration available and N_t is the $NO_3^- + NO_2^-$ content at time t. This equation assumes that nitrification is limited (controlled) by the NH_4^+ concentration. For a theoretical maximum NH_4^+-N concentration, after complete dilution, of 1960 $\mu g\ l^{-1}$ (Table 6, 1953+7), and an observed accumulation of 103 $\mu g\ l^{-1}$ $NO_3^- + NO_2^-$-N (183 - 80 $\mu g\ l^{-1}$) over a 0.106 day travel time, K = 0.52 day^{-1}. That is similar to the rate calculated by Yake (1981) where he expressed N_o and N_t as effluent concentrations corrected for dilution, i.e., 13 and 1.9 mg l^{-1}, respectively.

Our principal contention is that the nitrification rate in the Duwamish is more realistically expressed by a zero order reaction. Whether nitrifying bacteria are presently limited by NH_4^+ has not been demonstrated, however.

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ACKNOWLEDGEMENTS

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INTRODUCTION

The Duwamish River estuary is located in King County, Washington and discharges to the salt waters of Elliot Bay and Puget Sound at the City of Seattle. Prior to 1906 the freshwaters of the White, Black and Green Rivers entered the Duwamish estuary. Diversion of the White River in 1906 and the Black River in 1916 reduced the size of the Duwamish drainage area by one-fourth to its present 777 km^2. The Howard Hanson Dam, constructed upstream on the Green River in 1962 at Eagle Gorge, provides flood control and augments summer low flow by release of water from the reservoir to maintain a minimum instream flow of 3.11 m^3 sec^{-1} (110 cfs) downstream of the City of Tacoma's municipal diversion. This flow has been declared inadequate for fish requirements by the State Game Department (DOE, 1980) and a minimum flow of 8.49 m^3 sec^{-1} (300 cfs) has been established by the State Instream Resources Protection Program (WAC 173-509).

The Green-Duwamish River basin, as it is sometimes referred to, has an annual runoff of approximately 117 cm (46 inches). Annual average streamflow is approximately 45.3 m^3 sec^{-1} (1600 cfs). Seasonal runoff has been characterized by Richardson et al. (1968) as high winter, moderate spring, and sustained low flows during summer months. The upper basin is important as a municipal water supply for the City of Tacoma while the tidally influenced lower Duwamish is an important industrial area. It has been estimated that runs of Duwamish steelhead and salmon are worth more than $10 million annually (METRO, 1981).

The lower portion of the estuary (below approximately river km 10) has been dredged to an average depth of 10-15 m and averages 160-230 m in width (Harper-Owes, 1981a). The upper portion of the estuary (km 21-10) has been characterized as a tidally influenced river that experiences flow reversals during low freshwater flow and high tides (Fischer, 1968). A saltwater wedge is present throughout the dredged portion during low tide except during periods of high river flow. At high tide and during periods of low river flow the saline wedge reportedly may extend upstream as far as river km 16 (Santos and Stoner, 1972). Tidal range of the Duwamish averages about 3 m at Elliot Bay.

The river km index system used in this study places river km 0 at the northern tip of the west bank of the West Waterway (Dawson and Tilley, 1972). Other workers (Bernhardt, 1981; Yake, 1981) have utilized an index that assumes river km 0 to be located 1.4 km upstream of this point.

The Duwamish estuary reportedly is the single most important site of pollution in Puget Sound (Malins, personal communication) with the two most important pollutants recovered from sediments being arsenic and lead. Histopathological effects of these and other pollutants on bottom fish in the estuary have been reported. Pelagic cod and salmon examined from Elliot Bay showed no such pathology (Malins et al., 1982). Others have catalogued pollutant loads to the estuary (Harper-Owes, 1981b) and the source of such pollutants appears to be chiefly related to industrial activity along the lower estuary.

This study focused on the lower 21 km of the Green-Duwamish River system and examined the fate and potential effect of ammonia discharged to the estuary from the Renton Wastewater Treatment Plant (RTP) located at river km 20.5. The plant is a secondary treatment facility utilizing an activated

sludge process with a design capacity of 94.6 m^3 min^{-1}. Waste sludge is transported to the West Point, Seattle treatment plant for digestion and disposal. Dechlorination of discharged effluent is by means of SO_2 injection. The ammonium concentration of the effluent characteristically ranges from 10-15 mg N l^{-1} and during extremely low river flow periods (approximately 6 m^3 sec^{-1}) the effluent may presently comprise one-fourth of the total flow.

Ammonia has been shown by many authors to be toxic to aquatic life (Warren, 1962; Ball, 1967; Emery and Welch, 1969; Thurston and Russo, 1981; and Buckley, et al., 1979). A criterion of 20 µg l^{-1} as unionized ammonia (NH_3) has been established (USEPA, 1976) and includes a ten-fold safety factor for sublethal chronic effects on fish and other life forms. Much higher concentrations are required for short-term lethal effects. The concentration of unionized ammonia in solution is a function of the concentration of total ammonium, pH, temperature and the ionic strength of the solution. Thus, each factor should be considered when determining the toxicity or potential toxicity of a given discharge to the receiving stream.

This study was designed to examine nitrification in the estuary and to evaluate the potential for toxicity of unionized ammonia to migrating and resident fish. This was initiated because of a proposal to double the quantity of effluent discharged to the estuary by the year 2000 (METRO, 1978). Recently METRO (Municipality of Metropolitan Seattle) has decided to remove the entire effluent from the estuary and discharge it to Puget Sound. Nevertheless, a better understanding of nitrification in the Duwamish and its effect on the toxicity potential of ammonia as well as oxygen depletion should contribute to the prediction of such effects in other estuaries and similar flowing water systems.

Specifically, the objectives in this study were to: 1) identify zones and sites of nitrification within the Duwamish River estuary downstream from the RTP; 2) determine if sediments in the estuary act as a source of ammonium to overlying waters; 3) determine to what extent, if any, nitrification may be inhibited by salinity; and 4) evaluate the potential toxic effect of unionized ammonia discharged to the estuary due to expansion of the RTP.

MATERIALS AND METHODS

Nitrifying Bacteria

Viable autotrophic nitrifying organisms of the genus Nitrosomonas and Nitrobacter were enumerated by the MPN (Most Probable Number) method (Alexander, 1965) using the media described by Matulewich et al. (1975). Water and sediment samples were collected intermittently during 1980 and 1981 (8/28/80, 7/30/81, and 11/19/81) from six estuary stations located at the following river kilometers; 5.6, 7.7, 10.0, 13.2, 17.9, and 21.0 (Figure 1). Stations were selected based on their equal distribution throughout the estuary, ease of access and on historical usage of the sites in previous studies (Santos and Stoner, 1972).

Surface water samples were collected using sterile plastic "whirlpacs" while a Van Dorn sampler was used for subsurface samples. Sediment samples were collected with an Ekman dredge or piston corer and material from the sediment-water interface (0 - 1 cm) was placed in sterile "whirlpacs" for later analysis. All samples were placed on ice for transport to the laboratory where they were stored at 4° C for 10-12 hours prior to analysis.

Serial ten-fold dilutions of each innoculum (10 ml) were prepared using sterile phosphate buffer (APHA, 1976). One ml portions of each dilution were then transferred to three replicate tubes of each dilution containing 6 ml of sterile nitrite or ammonium medium. Tubes were then incubated in the dark at 28 $\pm$ 1° C. The tubes were examined every 15-20 days for a total duration, in nearly all cases, of at least 60 days for the presence or absence of viable nitrifying organisms by utilizing a spot plate and NO_2^- test reagent (Strickland and Parsons, 1972). A small amount of inoculated medium was placed in a

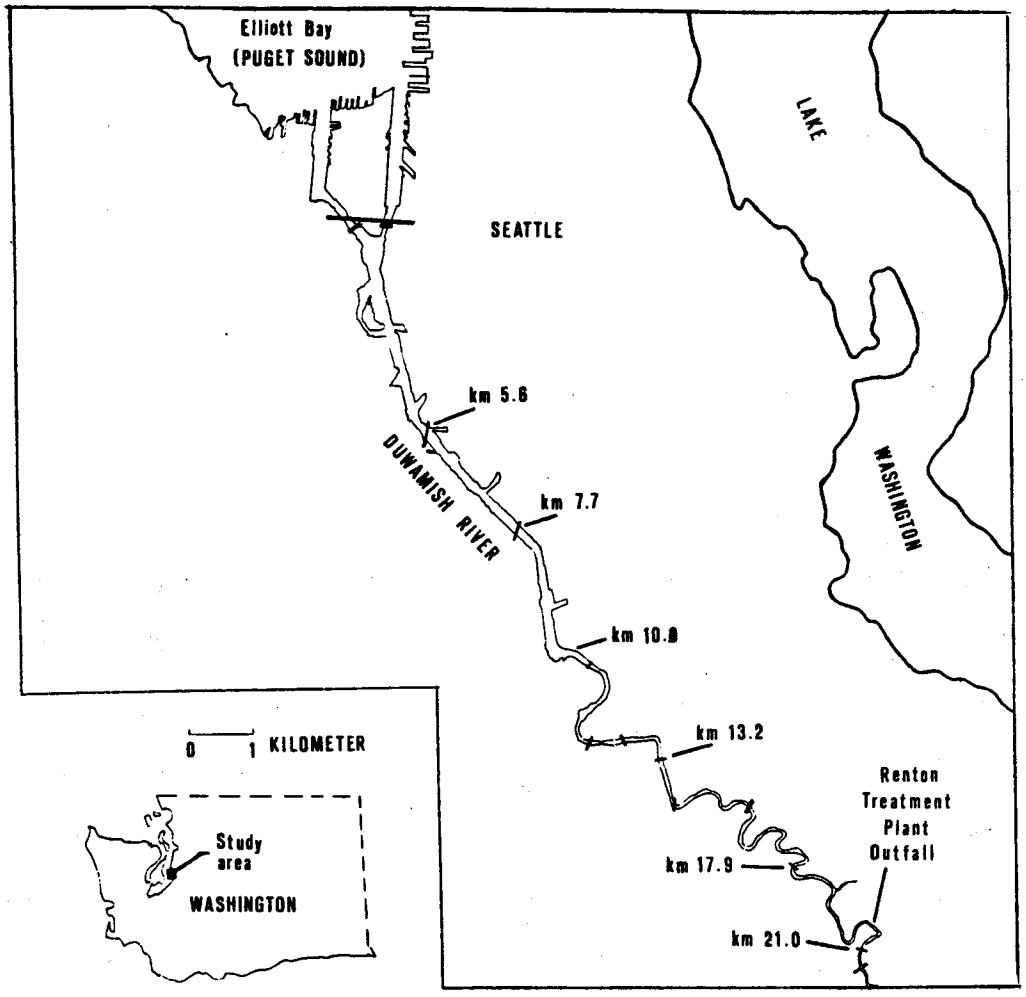

Figure 1. Duwamish River estuary study area and sampling stations.

spot plate depression and one or two drops of NO_2^- test reagent were added. Depending on the media, the presence or absence of red color is indicative of nitrifying activity. Nitrite medium showing no color upon the addition of NO_2^- reagent would be considered positive for active Nitrobacter spp. (NO_2^- oxidized to NO_3^-), while the presence of a red color in NH_4^+ medium would indicate active Nitrosomonas spp. (NH_4^+ oxidized to NO_2^-). MPN values were computed according to isolation and enumeration methods of USEPA (1978).

Nitrite and ammonium media had an initial salinity of 6 $^o/oo$. In order to test the inhibitory or stimulatory effect of salinity on the organisms recovered from the estuary the media were modified by increasing the salinity to 12 $^o/oo$ and 19 $^o/oo$ with filtered seawater (previously shown to be low in NO_2^- at less than 10 $\mu g\ l^{-1}$). Samples of sediment and water were collected from four estuarine stations and were examined using the previously described MPN methods.

Interstitial Ammonium

Replicate cores (3.5 cm ID) were collected from six estuary stations intermittently during low flow periods of 1980 - 1981. A piston corer was utilized on the lower estuary deep water stations (5.6, 7.7, 10.0 km) while hand-held polycarbonate coring tubes (3.5 cm ID) were used on the shallower three upriver stations (13.2, 17.9, 21.0 km). Collected samples were returned to the laboratory where incremental core sections (0-3, 3-6, 6-9 cm) were removed from the core tubes. Pore water was squeezed and filtered (0.45 μm membrane filters) from each section under a nitrogen atmosphere at ambient temperature. The interstitial waters were then analyzed for NH_4^+-N and $NO_2^-+NO_3^-$-N.

Analytical

Water column samples were filtered through 0.45 μm membrane filters (Millipore Corp.) and stored frozen in 60 and 120 ml polyethylene bottles prior to analysis. Ammonium nitrogen (as NH_3-N) was analyzed using the colorimetric, automated phenate procedure (USEPA, 1979) while $NO_2^-+NO_3^-$-N was determined by the colorimetric, cadmium reduction method (USEPA, 1979). Total Kjeldahl nitrogen was analyzed by the colorimetric, semi-automated block digester, AA II method (USEPA, 1979). The nitrogen samples were analyzed using a Technicon AA II Auto Analyzer and standard manifolds for nitrate, nitrite, ammonia and total nitrogen.

In situ measurements of specific conductance, salinity and temperature were recorded in the water column at one meter intervals using a YSI (Yellow Springs Instruments) Model 33 S-C-T meter. The pH was measured on samples collected using a portable meter (Extech Model 609). Dissolved oxygen was measured using the azide modification of the iodometric method (APHA, 1976).

Spatial and Temporal Surveys

In order to examine *in situ* changes of ammonium over time and travel down the estuary, diurnal surveys were conducted during low flow periods of 1981. The first was a 23-hour time of travel drogue survey conducted on September 2-3, 1981. At high tide (8:23 p.m.) on this date a drogue was placed in the river approximately 200 meters above the effluent outfall of the Renton sewage treatment plant. The drogue was started in midstream, but sometimes had to be returned to midchannel due to eddies and bends in the river that tended to slow its downstream progress. While following the drogue downstream by boat, water samples were collected and filtered for chemical analysis every hour

throughout the 23-hour period. In addition the following parameters were measured; temperature, pH, salinity, specific conductance, and dissolved oxygen. The following chemical analyses were performed on the samples collected; total Kjeldahl nitrogen, NH_4^+-N, NO_2^--N, and NO_3^--N.

On October 15-16, 1981, a 24-hour survey was conducted in which samples were collected every hour at an estuary station located 9.6 km downstream of the Renton Sewage Treatment Plant discharge and 10.4 km above the estuary mouth. Physical and chemical determinations were similar to those in the September 2-3, 1981 survey.

In Vitro Sediment Experiments

Bottom sediments were collected (25 August 1981) with an Ekman dredge at midstream at the head of commercial navigation in the estuary (km 10.0). These surface sediments were combined and homogenized. Three liter glass beakers were utilized as experimental chambers in which to observe the evolution/removal of ammonium from sediments to/from overlying waters and for the effect(s) of salinity on nitrification. Approximately 400 g (dry weight) of sediment were placed in each of 12 chambers and water from two different estuary sites, i.e. different salinity, was added to replicate chambers. Salinity of water in one series of six chambers was approximately 4 $^o/oo$ and in the second series about 20 $^o/oo$. Each series of six chambers was then treated according to the following: two received addition of N-Serve, (1 mg l^{-1}, Dow Chemical) to inhibit nitrification, two received streptomycin (70 mg l^{-1}) to inhibit all microbial activity (protein synthesis) and the final two chambers remained untreated.

The chambers, with sediment and water added, were allowed to equilibrate for 24 hours, after which filtered, ammonia-free air was slowly bubbled

(without disturbing sediments) through the water of the chambers to maintain aerobic conditions throughout the experiments. Chambers were darkened, covered with aluminum foil to limit evaporation and incubated at 22 $\pm$ 3° C. At days 1, 3, 6, 9, 15, and 23, 100 ml of water were removed from each chamber, filtered and analyzed for NO_2^--N, NO_3^--N, and ammonium. Removed water was replaced with filtered water corresponding to the salinity of waters added to the tanks on day zero. The concentrations of nitrogen species were plotted for replicate chambers over the 23-day period and rates of ammonium evolution/removal and nitrification were calculated from observed data.

RESULTS AND DISCUSSION

Nitrification

The Process

There are seven generally recognized genera of nitrifying bacteria noted in Bergey's Manual (Buchanan and Gibbons, 1974). The two most important genera are considered to be Nitrosomonas and Nitrobacter. The oxidation reactions carried out by these organisms are as follows:

$$\text{Nitrosomonas: } NH_4^+ + 1\text{-}1/2O_2 \rightarrow NO_2^- + H_2O + 2H^+$$

$$\text{Nitrobacter: } NO_2^- + 1/2O_2 \rightarrow NO_3^-$$

There are intermediate products produced but reportedly their conversion is rapid and they do not tend to accumulate under fully aerobic conditions (Alexander, 1965). Direct measurements of O_2 utilization by Nitrosomonas and Nitrobacter (Wezernak and Gannon, 1967) are shown below and differ only slightly from the actual stochiometric values:

1.) 3.22 mg O_2 mg^{-1} NH_4^+-N oxidized to NO_2^--N

2.) 1.11 mg O_2 mg^{-1} NO_2^--N oxidized to NO_3^--N

Total 4.33 mg O_2 mg^{-1} NH_4^+-N oxidized to NO_3^--N

Rheinheimer (1974) has noted that the efficiency of these reactions is very low, e.g. the efficiency of Nitrobacter winogradsky is reportedly about 10-11 percent. The nitrifying bacteria are obligate chemoautotrophs, i.e. they do not use organic nutrients as energy or carbon sources. Relative to other bacteria, growth of these organisms is quite slow. Nitrosomonas europaea has been shown to have a generation time of seven hours (Alexander, 1965). Lees (1954) has shown the optimum pH for Nitrobacter to be 7.7 while O_2 uptake

dropped to near zero at a pH of approximately 9.5. For Nitrosomonas, the pH optimum was 8.6 with the rate of O_2 uptake falling to near zero at pH 9.6. It was also noted that ammonia oxidation proceeded rapidly at pH levels down to 6.5. Srna and Baggaley (1975) reported optimum pH for nitrification to be 7.45 with a range of 7.0 to 8.2.

Zones and Sites of Nitrification

Autotrophic nitrifying bacteria were isolated from sediments and the water column in the estuary during 1980 and 1981 in order to identify the site(s) of nitrifying activity and to determine if nitrifying bacteria may be inhibited (or enhanced) by salinity. During low freshwater flows and high tidal conditions the salt-water wedge extends upstream to approximately km 16. Such an occurrence might be expected to affect nitrifer activity (and, as noted later, ammonium release from sediments) if the nitrifiers normally acclimated to freshwater, are intolerant to saline conditions. It was noted in studies conducted in the estuary in 1979 that lower in vitro rates of nitrification were obtained for saline estuarine waters than for freshwater (Welch and Trial, 1979).

Sediment and overlying water column samples were collected from six estuary stations on 8/28/80 for enumeration of nitrifying bacteria. Comparison of MPN estimates for water and sediment nitrifiers at successive sites between km 21.0 and 5.6 indicated that nitrifiers were anywhere from about one to five orders of magnitude more abundant in sediments than in the water phase (Table 1). Sediment nitrifiers generally showed a decrease in numbers as one moved downstream with water column numbers showing a general decline below km 10.0 (Figure 2).

Table 1. Abundance of sediment and water column nitrifying bacteria from samples collected 8/28/80 and reported as MPN ml^{-1} after 127 days incubation. Sediments from Stations 13.2, 17.9, and 21.0 were collected at near-shore sites at low tide while sediments at Stations 10.0, 7.7, and 5.6 were collected from deep water using a piston cover.

NITROSOMONAS

Station	5.6	7.7	10.0	13.2	17.9	21.0
Surface	---	---	76	46	29	< 4
1 m	29	46	---	---	---	---
B + 1 m	4	< 4	29	---	---	---
Sediment	760	2,860	28,600	45,700	286,000	198,000

NITROBACTER

Surface	---	---	10	10	10	< 4
1 m	4	4	---	---	---	---
B + 1 m	< 4	< 4	10	---	---	---
Sediment	4,570	2,860	11,000	28,600	286,000	76,000

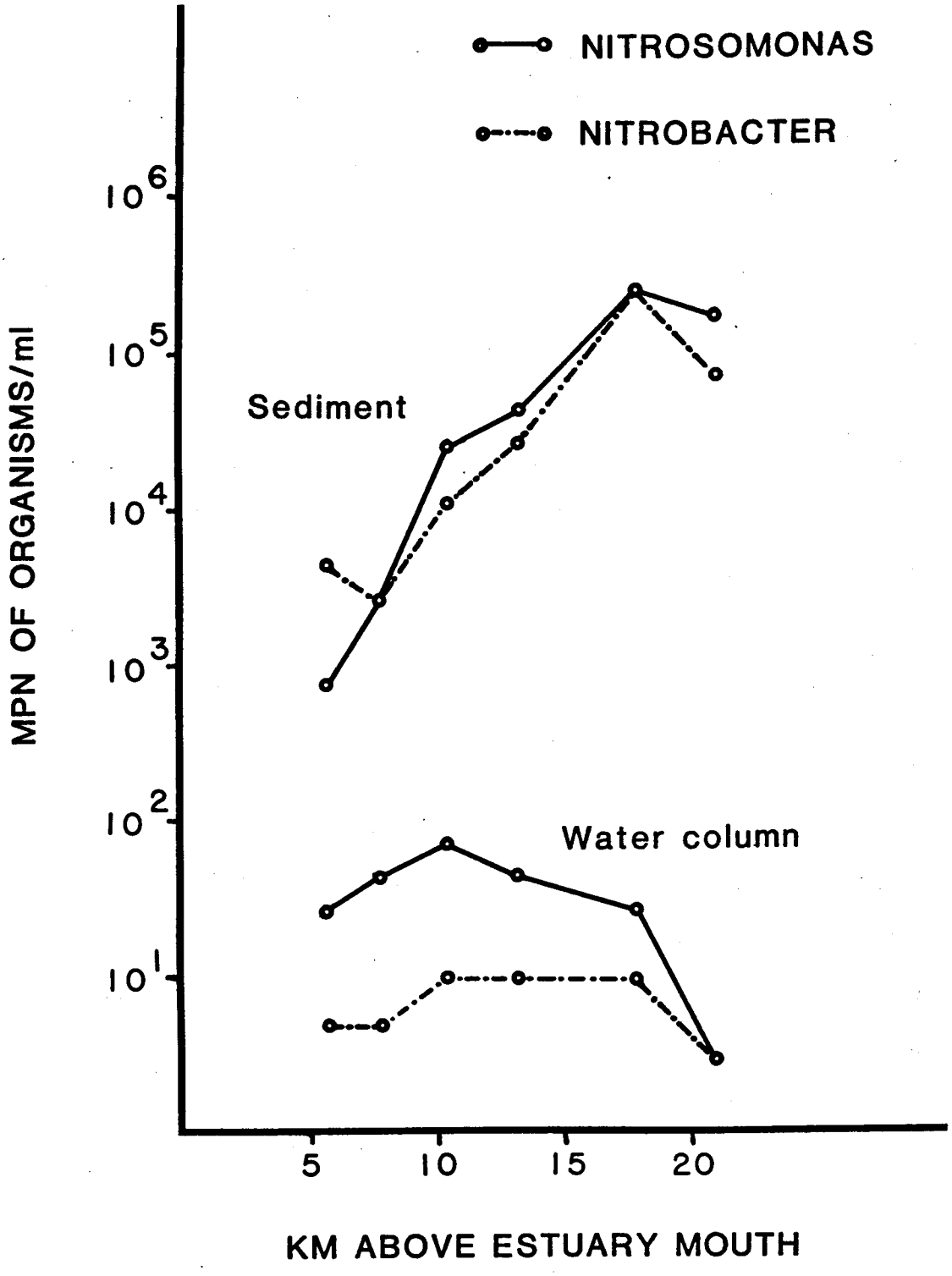

Figure 2. Abundance of Nitrosomonas and Nitrobacter spp. isolated from sediments and water column of the Duwamish River estuary, 28 August 1980.

The high numbers of sediment nitrifiers found at km 21.0, a station upstream from the effluent outfall, was surprising since water column ammonium concentrations were previously shown to be fairly low (< 50 μg l^{-1}) (Welch and Trial, 1979) as were concentrations of NO_2^--N (< 10 μg l^{-1}). Upriver NO_3^--N concentrations (approximately 400 μg l^{-1}) may be indicative of upper basin nitrification and are typical of Puget Sound area freshwater. Groundwater inputs may also be expected to contribute significantly to NO_3^--N present.

In this regard Billen (1975) notes that simple counts of nitrifying bacteria cannot be used to indicate the activity of the organisms since dormant bacteria may be present in sediments or water. Simple counts, such as reported here, can be used to demonstrate the potential for activity and combined with measured rates of nitrification can demonstrate the important sites of nitrification within the system.

If one accepts the argument of Tuffey, et al. (1974) that nitrification does not occur in the water phase to any significant degree until numbers of nitrifiers reach 10^3 to 10^4 organisms ml^{-1} then it could be argued that significant nitrification does not take place in the water column of the Duwamish since numbers did not exceed 10^2 in samples collected during the 8/28/80 survey. This was a period of low flow (8.6 m^3 sec^{-1}) and relatively moderate water temperature (17.5° C). However, the occurrence and increase of NO_2^- and NO_3^--N in the river below the effluent outfall indicated that nitrification was proceeding at what appeared to be a fairly rapid rate. The bulk of ammonium oxidation appears to have taken place at the sediment-water interface in the estuary as evidenced by the large numbers of nitrifiers recovered from estuarine sediments. This observation appears consistent with that of Curtis

et al. (1975) who studied nitrifying bacteria in the Trent River Basin, England and estimated that 80 percent of the NH_4^+ oxidation occurred in the river sediments.

The relatively short downstream travel time in the upper estuary (e.g., at river discharge of 10 m^3 sec^{-1} and low tide a parcel of water travels from km 20.9 to 11.1 in six hours) tends to preclude the growth of nitrifiers in the water column because generation times are at least that long. Thus, nitrifiers observed in the water column probably originate from populations in sediment.

The lower estuary has a longer water residence time and may favor water column nitrifier activity but as discussed later such activity may be affected by salinity, toxicity and dilutional effects of saline water.

Effects of Salinity

Salinity effect(s) on sediment nitrifers was examined by sampling sediments at various estuary sites which were expected to experience incursions of the saltwedge during high tides and low freshwater flows. Sediments collected on 8/1/81 were analyzed for nitrifier abundance. Salinity of the media was altered by the addition of filtered (0.45 μm) saline water that was low in ammonium (50 $\mu g \cdot l^{-1}$), nitrite and nitrate nitrogen (control tubes showed no reaction for nitrite). Salinity in normal ammonium and nitrite media was 6 $^{o}/oo$. Adjusted media (plus saline water) had salinities of 12 $^{o}/oo$ and 19 $^{o}/oo$.

Results of this enumeration experiment revealed that lower abundance of ammonium oxidizers was obtained from the higher saline media and river sediments above 10.0 km, while for sediment at km 10.0 the higher saline media

yielded a greater abundance of ammonium oxidizers (Figure 3). Additionally, nitrite oxidizers were shown to yield lower numbers in the higher salinity media at all four stations sampled (Figure 4).

If these results, which are based on long-term effects (incubation time of 30 days), are extrapolated to short-term (i.e., six hours) in situ effects in the estuary it is probable that nitrification activity in sediments would decrease during upstream incursion of the salt-wedge. River flow reversal caused by high tide coupled with a decrease in nitrifier activity could act to maintain high river ammonium levels during periods of low river discharge.

Finstein and Bitzky (1972) have observed a series of relationships between nitrifier activity and marine salts; effects ranged from inhibition at low salt concentrations as well as in the absence of salts and a gradient of effects occurred at intermediate concentrations. They also noted that the environment of origin of the nitrifiers appeared to determine their ability, or lack of it, to nitrify when subjected to various salt concentrations. The organisms studied by Finstein and Bitzky were shown to acclimate towards an increase of salts but not towards a lack of salts.

Longitudinal salinity distribution measured in the Duwamish estuary by Dawson and Tilley (1972) has indicated the wide range of salinity between tidal cycles that can occur over sediments during low freshwater discharge ($11.1\ m^3\ sec^{-1}$) and high tidal range (4.1 m). For sediment at river km 10.0 the range is approximately 2 $^{o}/oo$ to > 25 $^{o}/oo$ and for sediment at km 13 the salinity range is < 1 $^{o}/oo$ to 14 $^{o}/oo$ (Figure 5).

Nitrifying bacteria isolated from km 10.0 appeared to be halophilic which is consistent with the fact that saline wedge waters are present in the uppermost dredged portion of the estuary during low river discharge. It is at this

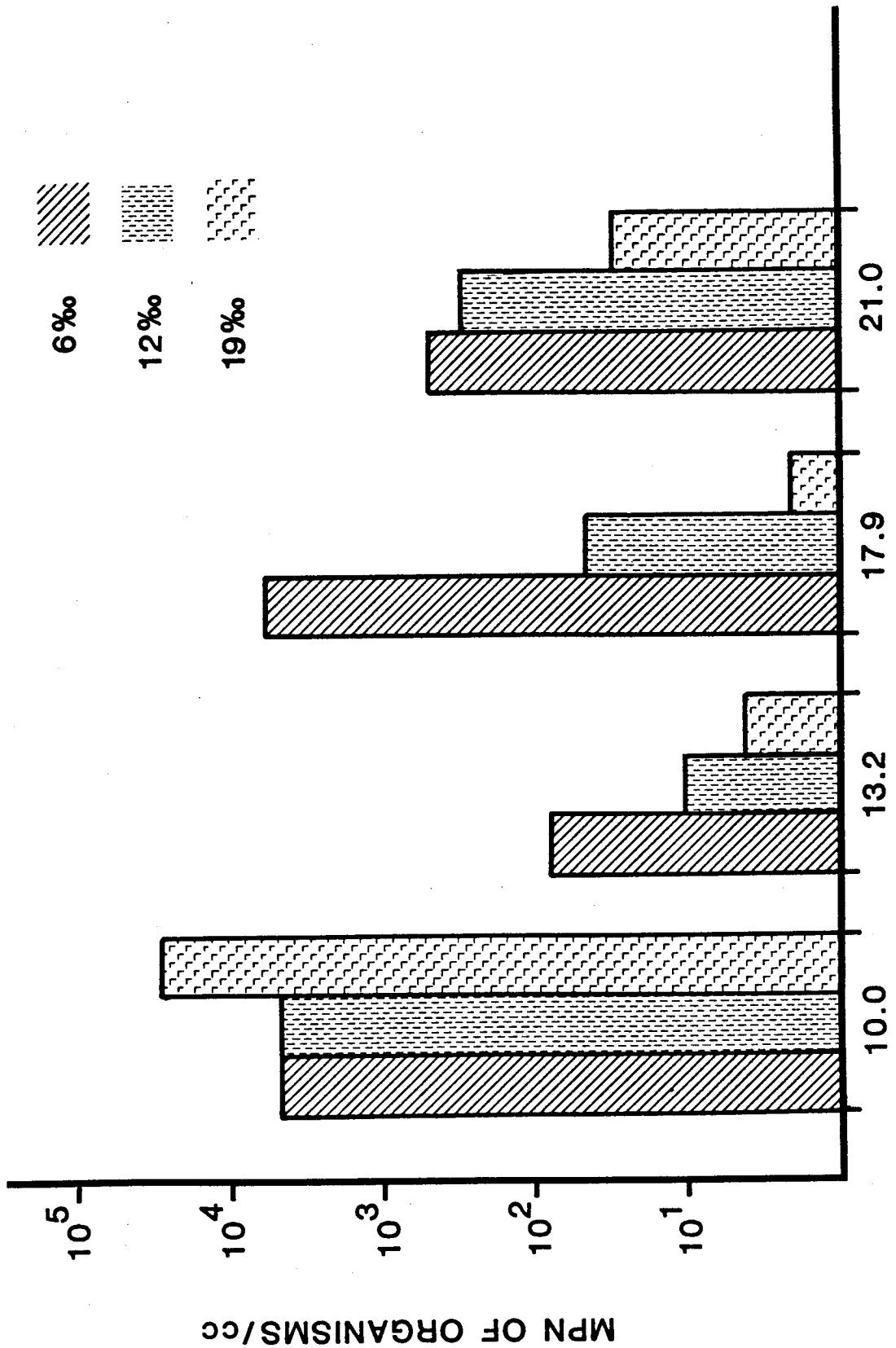

Figure 3. Nitrosomonas spp. isolated from sediments of four stations of the Duwamish River estuary (30 July 1981) and cultured in media of 6, 12, and 19 o/oo salinity.

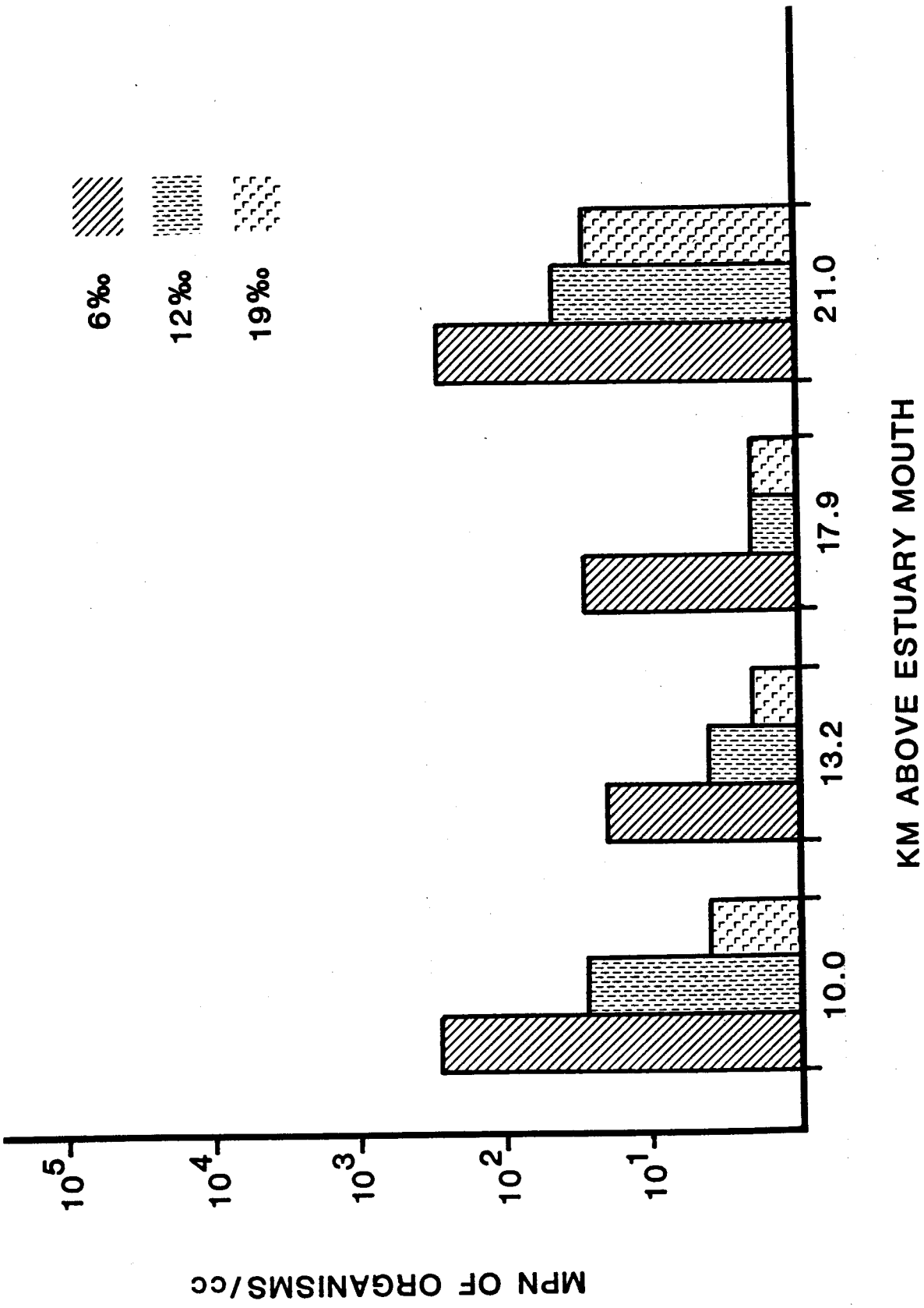

Figure 4. Nitrobacter spp. isolated from sediments of four stations of the Duwamish River estuary (30 July 1981) and cultured in media of 6, 12, and 19 °/oo salinity.

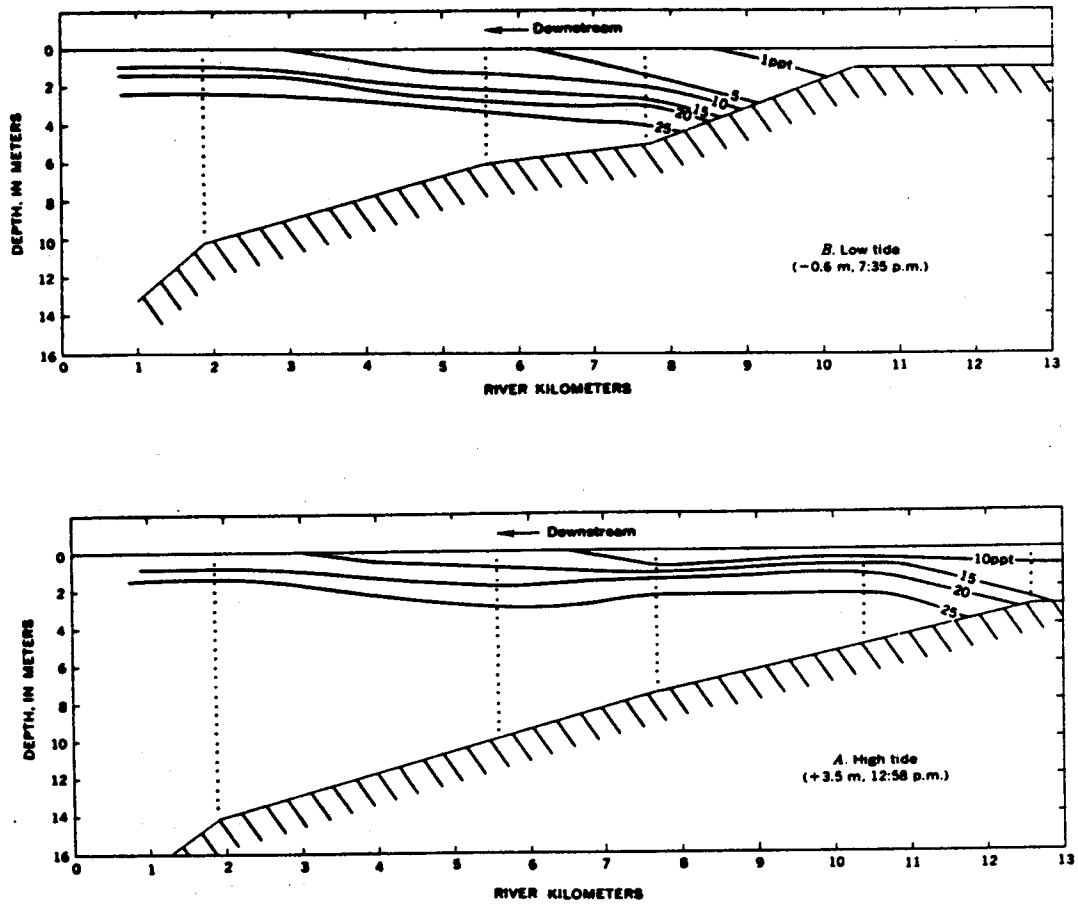

Figure 5. Longitudinal salinity profiles measured in the Duwamish River estuary at low (- 0.6 m) and high (+ 3.5 m) tide, July 1968. (from; Dawson and Tilley, 1972.).

point in the estuary that the generally narrow and relatively shallow freshwater portion of the river enters the widened, deeper dredged channel of the lower estuary.

A final examination of salinity effects on nitrifiers collected from the estuary was made on water column samples collected on 11/19/81 in order to verify the observations from the 8/28/80 survey in the lower estuary which had indicated low activity. River flow upstream of the effluent discharge was recorded at 21.5 m^3 sec^{-1} on this date (USGS records) while instream temperature varied from 8.5 to 10.5° C. Surface water samples were collected from five estuarine stations. In addition, water at one meter above the bottom was collected at two of these stations in the lower estuary.

Again, enumeration methods were the same as previously noted with the exception that regular media (6 °/oo salinity) and adjusted media (19 °/oo) were used for testing saline effects on nitrifiers.

Results indicated that low numbers of nitrifying bacteria were present in the water column at the time of the survey. Nitrite oxidizers were recovered only in media of 6 °/oo salinity and then only at very low abundance at two surface stations (Table 2). Ammonium oxidizers from surface waters of the four uppermost stations were shown to be adversely affected by the 19 °/oo salinity media (a positive response in one case) while samples 1 m above the bottom at the lower two stations and in both media showed identical MPNs as did the surface waters sampled at the lowest estuary station (km 7.7). Salinity addition did not enhance recovery of the low numbers of water column nitrifiers collected from the lower estuary. Low abundance of nitrifiers observed

Table 2. Abundance of water column nitrifying bacteria from samples collected 11/19/81 and reported as MPN ml^{-1} after 63 days incubation. High (19 °/oo) and low (6 °/oo) salinity media utilized to observe inhibition or enhancement by salinity.

RIVER km	7.7	10.0	13.2	17.9	21.0	
Surface	29	46	46	46	4	} NITROSOMONAS 6 °/oo Salinity
Bottom + 1 m	< 4	4	---	---	---	
Surface	29	29	76	< 4	< 4	} NITROSOMONAS 19 °/oo Salinity
Bottom + 1 m	< 4	4	---	---	---	
Surface	< 4	4	< 4	7	< 4	} NITROBACTER 6 °/oo Salinity
Bottom + 1 m	< 4	< 4	---	---	---	
Surface	< 4	< 4	< 4	< 4	< 4	} NITROBACTER 19 °/oo Salinity
Bottom + 1 m	< 4	< 4	---	---	---	

in November could have been expected as witnessed by the previously low numbers found in the survey on 8/28/80. The fact that counts were even lower than in August may have been a result of dilution; 8.61 vs. 21.5 m^3 sec^{-1} river discharge at the time of the 8/28/80 and 11/19/81 surveys, respectively. Similarly, Somville (1978) attributed a decrease in nitrification rates near the mouth of the Scheldt estuary to dilution and to an increase in salinity.

Effects of Temperature

Another factor involved in the lower recovery of nitrifying bacteria for the 11/19/81 survey is believed to be temperature; 17.4 vs. 8.5° C mean surface temperature for the 8/28/81 and 11/19/81 surveys respectively. Rheinheimer (1974) has noted in studies on the Lower Elbe that numbers of nitrifying bacteria were strongly related to water temperature. A significant increase in numbers occurred only above 10 to 15° C and during summer months small temperature flucuations (2 - 3° C) were shown to elicit positive and negative responses in bacterial numbers. Rheinheimer also noted that Nitrobacter is more strongly inhibited by light and poisons than is Nitrosomonas. This may also be important in the Duwamish as noted later in this report.

Temperature has been shown to have a significant effect on nitrifier activity and thus on rates of nitrification. Srna and Baggaley (1975) have reported in situ effects of rapid temperature changes on nitrification rates. A rise of 4° C resulted in a 50 percent increase in NH_4^+ oxidation while a 1° C drop caused a 30 percent decrease. For NO_2^- oxidation, a 4° C temperature rise caused a 12 percent increase and a 1.5° C drop decreased the rate by eight percent. White et al. (1977) have noted that no measurable nitrification occurred in a New York stream receiving nitrogenous wastes when the temperature fell below 17° C.

The relationship between temperature and nitrifier growth rate can be described by the following equation (Stratton and McCarty, 1967):

$$K_T = K_{20}\,\theta^{(T-20)}$$

where: K_T = rate at temperature T

K_{20} = rate at 20° C

θ = 1.09 and 1.06 for Nitrosomonas and Nitrobacter, respectively

A somewhat different temperature effect has been reported for those sediment bacteria responsible for converting organic N to NH_4^+. Rheinheimer (1974) has noted that the numbers of putrefying bacteria in water bodies subjected to sewage have often been shown to be greater in winter than in summer. The large increase in numbers of putrefactive bacteria during winter months, which could be due in part to lower death/grazing rates, reportedly helps to offset the decrease in activity due to temperature and results in winter ammonium production that is comparable to or in some cases higher than that observed in summer. Rheinheimer (1974) also showed that numbers of nitrifiers followed a seasonal cycle that was the exact reverse of putrefactive bacteria.

Sediment-water studies discussed later have indicated the high degree to which ammonification (putrefactive activity) presently exists in sediments collected from a portion of the Duwamish estuary. If numbers of putrefactive bacteria were shown to increase with the onset of lower stream temperatures, although no such studies have been conducted, higher instream NH_4^+-N concentrations than expected could result due to reduced nitrification at that time. Fall streamflow in the Duwamish might normally be expected to substantially dilute the Renton Sewage Treatment Plant effluent and to reduce the effect(s) of ammonia on fish. However, daily low river flows in October and November

1981 were 9.34 and 13.6 m^3 sec^{-1}, respectively, although mean monthly discharges were 22.4 and 22.2 m^3 sec^{-1}, respectively. Thus, it may be that higher estuarine ammonium concentrations could occur during fall, in some years, rather than summer low flow periods.

In Vitro Sediment/Water Experiments

Ammonium Release from Sediment

Previous studies in the Duwamish estuary have shown net increases in water column ammonium as one moves downstream (Welch and Trial, 1979; Bernhardt, 1981). It was hypothesized that such an observed increase in waters of the lower estuary may be due to sediment release of ammonium (Welch and Trial, 1979).

Studies by Brezonik (1973) have shown sediments from an unpolluted estuary (Waccasassa Estuary, Florida) to remove ammonium from overlying water. He concluded that non-biological factors such as sorption and diffusion were the predominant mechanisms responsible. Studies in a eutrophic lake (Bivins Arm, Florida) have shown the sediments to act at various times, depending on the overlying water concentration, as a source and as a sink for ammonium (Brezonik, 1973). In vitro studies by Rowe et al. (1975) showed sediments from shallow near-shore ocean waters to evolve large amounts of ammonium (36 - 1790 µg N m^{-2} h^{-1}) and the production to be highly temperature dependent.

Release of nitrogen from coastal benthic sediments has also been observed by Hartwig (1976) in the LaJolla Bight. Calculations by Rowe et al. (1975) showed that nutrient regeneration in the New York Bight accounted for more than 200 percent of the nitrogen required by phytoplankton photosynthesis.

Toetz (1970), in studies of NH_4^+ adsorption from solution onto clay particles, found that only when the NH_4^+-N concentrations were high (1.8 mg l^{-1})

was adsorption detected. He has noted that in mixtures of ions with differing valences, preferential adsorbance occurs for those ions with the higher valence. So, the quantity of any cation adsorbed to clay particles will depend upon the concentration and valence of that cation, the number of counter ions already adsorbed, and the cation exchange capacity of the clay particles.

For the purposes of this study it was decided to examine sediments of the lower estuary collected at km 10.0, the head of the dredged channel, because it is in the lower estuary that ammonium evolution was believed to occur. The sediment-water chamber studies were not an attempt to recreate *in situ* conditions *per se* but rather to observe generally the effect(s) of salinity on nitrification and on possible evolution of ammonium from Duwamish estuary sediments.

Three liter beakers received estuarine sediment from km 10.0 and river water collected above the Renton Treatment Plant (salinity < 1 $^{o}/oo$), or water collected from the lower estuary (salinity 20 $^{o}/oo$), to yield final chamber salinities of about 4 $^{o}/oo$ and 20 $^{o}/oo$. Sediments in each of the 12 chambers remained undisturbed after the initial addition of water. After two days, sediments exhibited a light colored oxidized surface while underlying sediment appeared blackened and reduced. Initial NH_4^+-N concentration in the water column of the chambers ranged from 830 to 2540 $\mu g\ l^{-1}$. Ammonium, NO_2^-, and NO_3^- nitrogen release/removal were monitored over a period of 23 days.

In untreated control chambers it was observed that more NH_4^+ was released from sediments underlying the higher saline water than from sediments under the less saline water (Figure 6). This may have been due, in part, to the fact that putrefactive organisms responsible for ammonium release were normally acclimated to a higher salinity than that present in the lower salinity

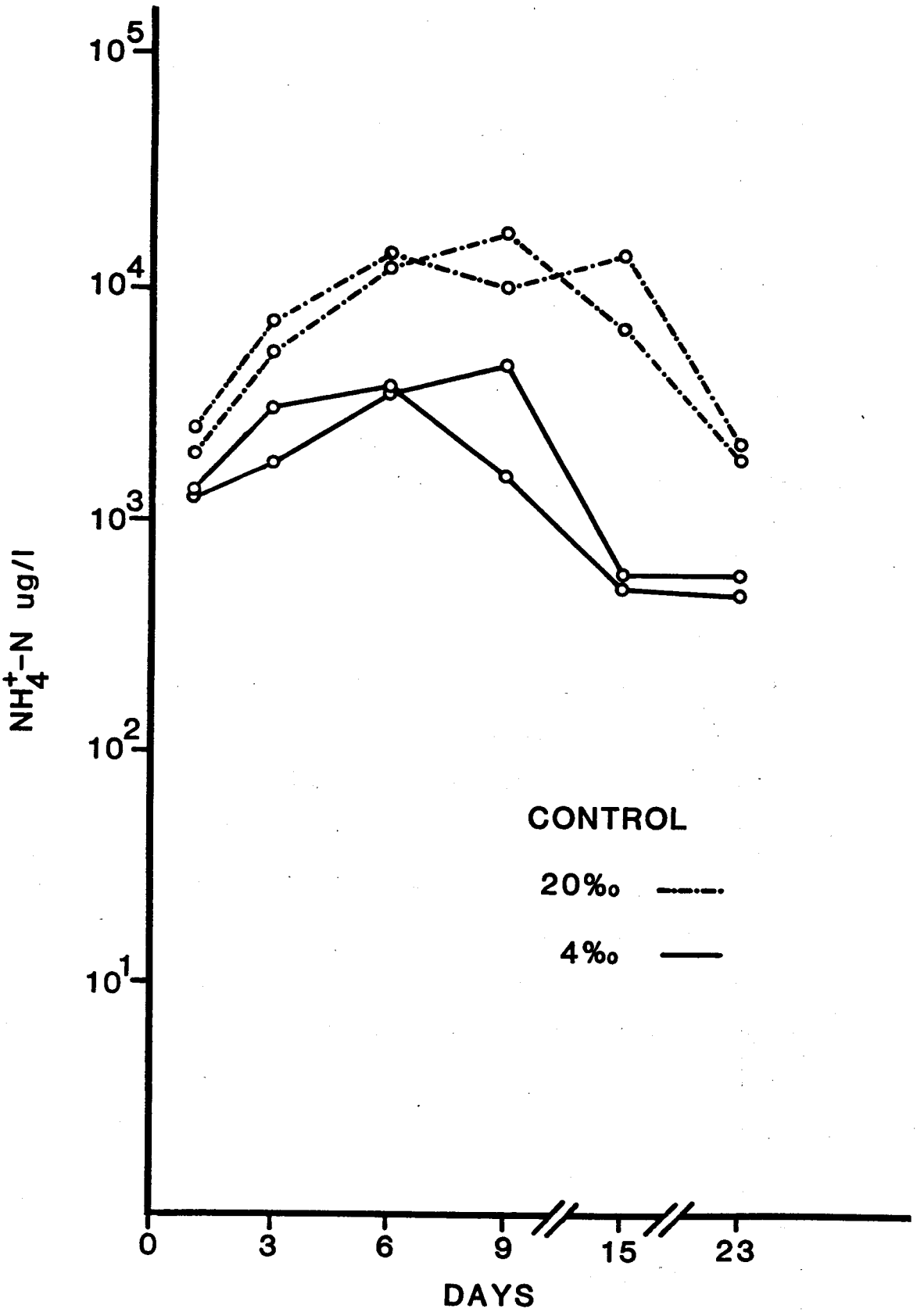

Figure 6. In vitro observations of NH_4^+-N in water over sediments of the Duwamish River estuary (km 10.0). Data are from replicate sediment-water chambers (3 ℓ) incubated at 22 ± 3° C with overlying water salinity of 20 and 4 °/oo.

chambers. However, when all microbial activity was inhibited, as shown later, ammonium release to higher saline waters was increased, thus suggesting a diffusional response. Nonetheless, at both salinities NH_4^+ was generated by sediments from the lower Duwamish estuary. The general decrease in NH_4^+ observed after day nine may be accounted for by loss to the atmosphere and/or by ammonium oxidation as NO_2^-+NO_3^--N levels were shown to increase (Figure 7) as NH_4^+-N decreased. Ammonia loss to the atmosphere did not appear to be appreciable since, as noted later, NH_4^+ levels continued to increase in the absence of nitrification.

A nitrification inhibitor, N-Serve, was added at 1 mg l^{-1} to the second set of sediment-water chambers in order to observe conditions when ammonium was not oxidized. However, based on observed NO_2^-+NO_3^--N increases and NH_4^+-N decreases (Figures 8 and 9), inhibition of nitrification appeared to be unsuccessful. N-Serve apparently was not added in sufficient concentration to inhibit the nitrifying bacteria. Comparison of N-Serve chambers with untreated control chambers showed similar changes in NO_2^-+NO_3^--N with time. In fact, NO_2^-+NO_3^--N levels reached higher final water column concentrations in the N-Serve treated chambers than in controls.

Streptomycin was added to the third set of chambers for the purpose of observing sediments and water where all biological activity (protein synthesis) had been inhibited. This treatment appeared to inhibit NH_4^+ and NO_2^- oxidation for the first 15 days as shown by a general decrease in NO_2^-+NO_3^--N concentrations through that period (Figure 10). Denitrification may have been taking place in deeper sediments below the oxidized sediment-water interface during this period and acting, in three of four chambers, to remove nitrate and nitrite from the water column. However, NO_2^--N was not shown to accumulate in the water column during the 15 days as might be expected during

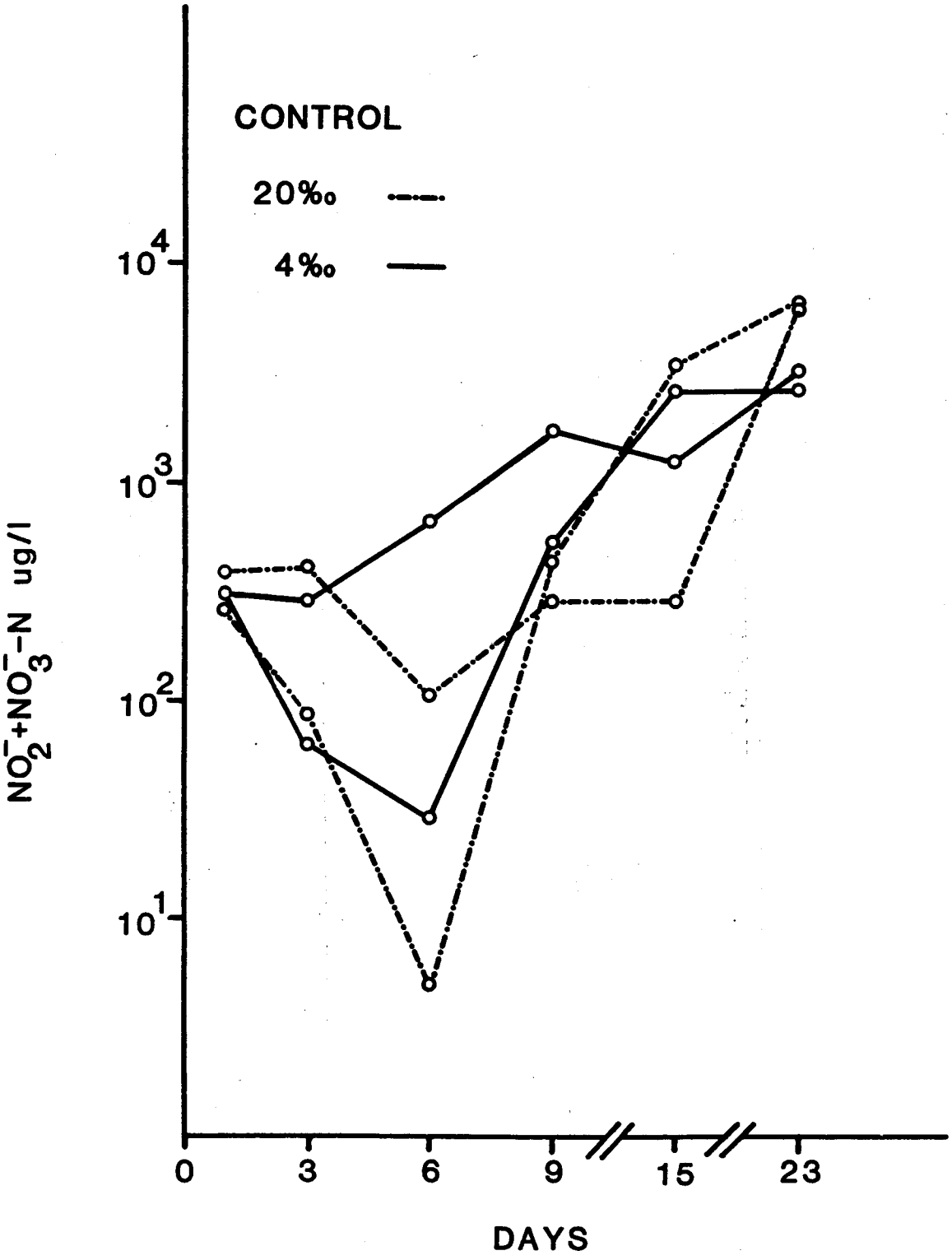

Figure 7. *In vitro* observations of $NO_2^-+NO_3^-$-N in water over sediments of the Duwamish River estuary (km 10.0). Data are from replicate sediment-water chambers (3 ℓ) incubated at 22 ± 3° C with overlying water salinity of 20 and 4 °/oo.

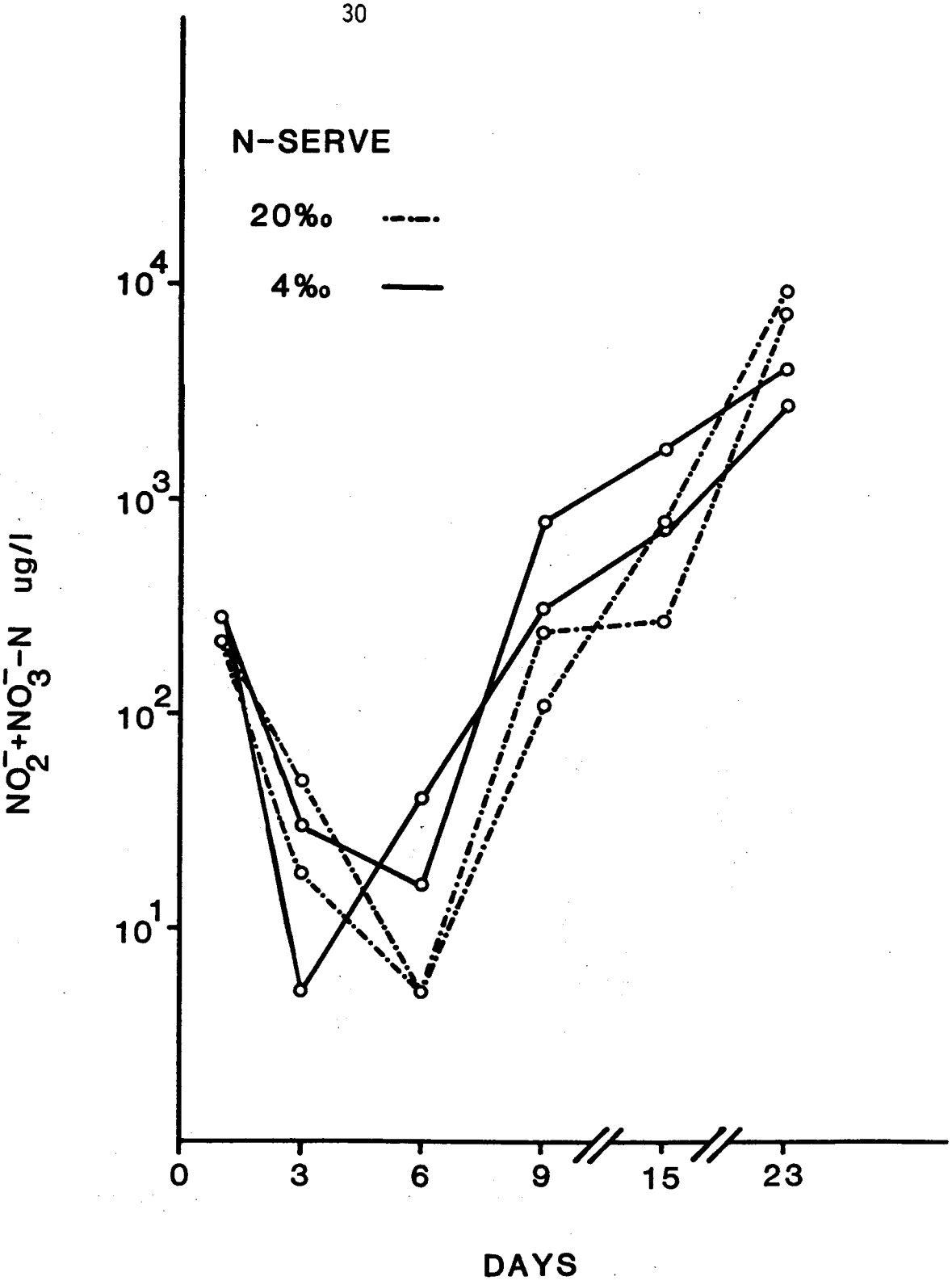

Figure 8: In vitro observations of $NO_2^-+NO_3^-$-N in water over sediments of the Duwamish estuary (km 10.0). Data are from replicate sediment-water chambers (3 ℓ) treated with 1 mg $ℓ^{-1}$ N-Serve and incubated at 22 ± 3° C with overlying water salinity of 20 and 4 °/oo.

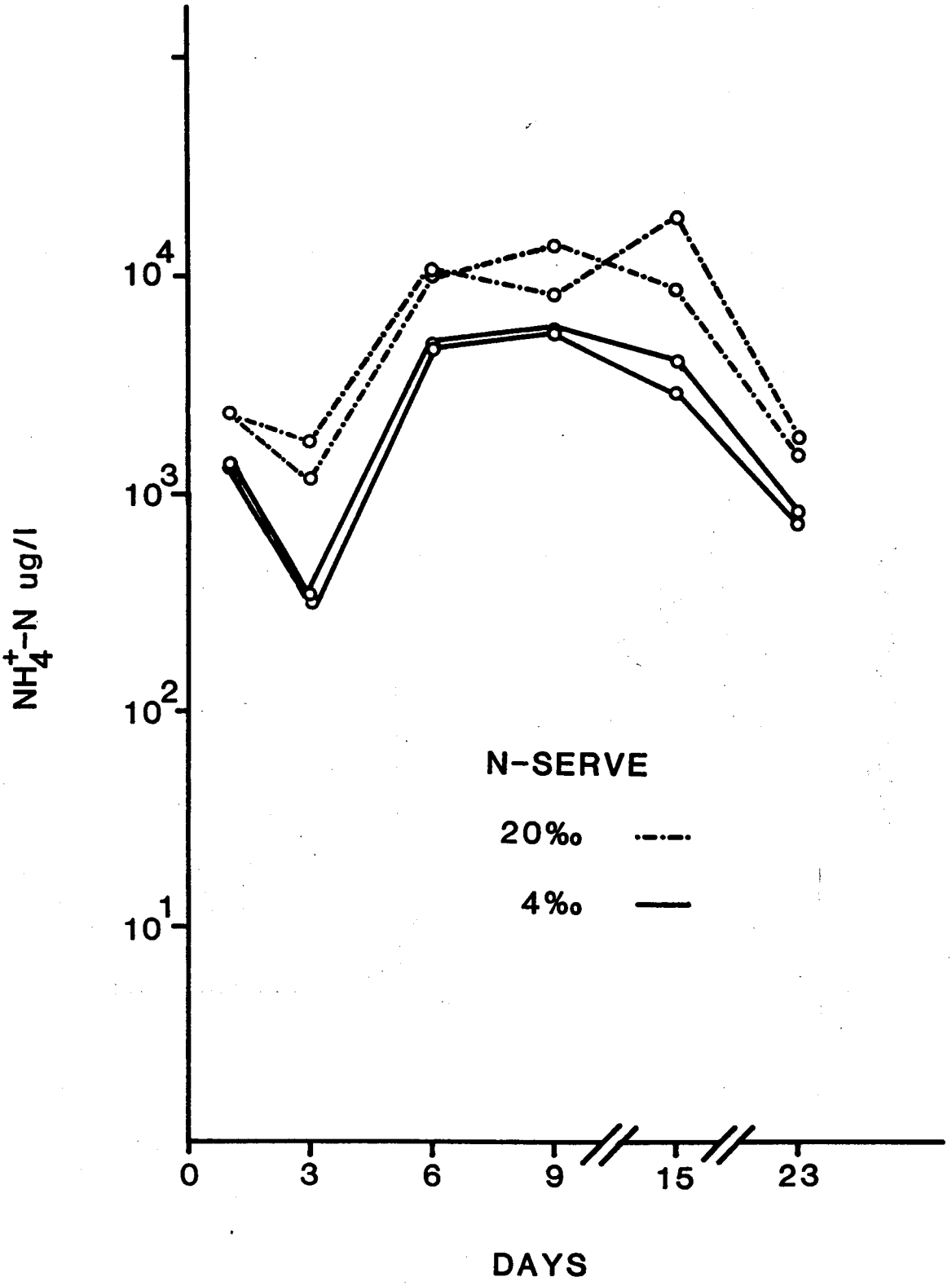

Figure 9. In vitro observations of NH_4^+-N in water over sediments of the Duwamish estuary (km 10.0). Data are from replicate sediment-water chambers (3 ℓ) treated with 1 mg ℓ^{-1} N-Serve and incubated at 22 ± 3° C with overlying water salinity of 20 and 4 ‰.

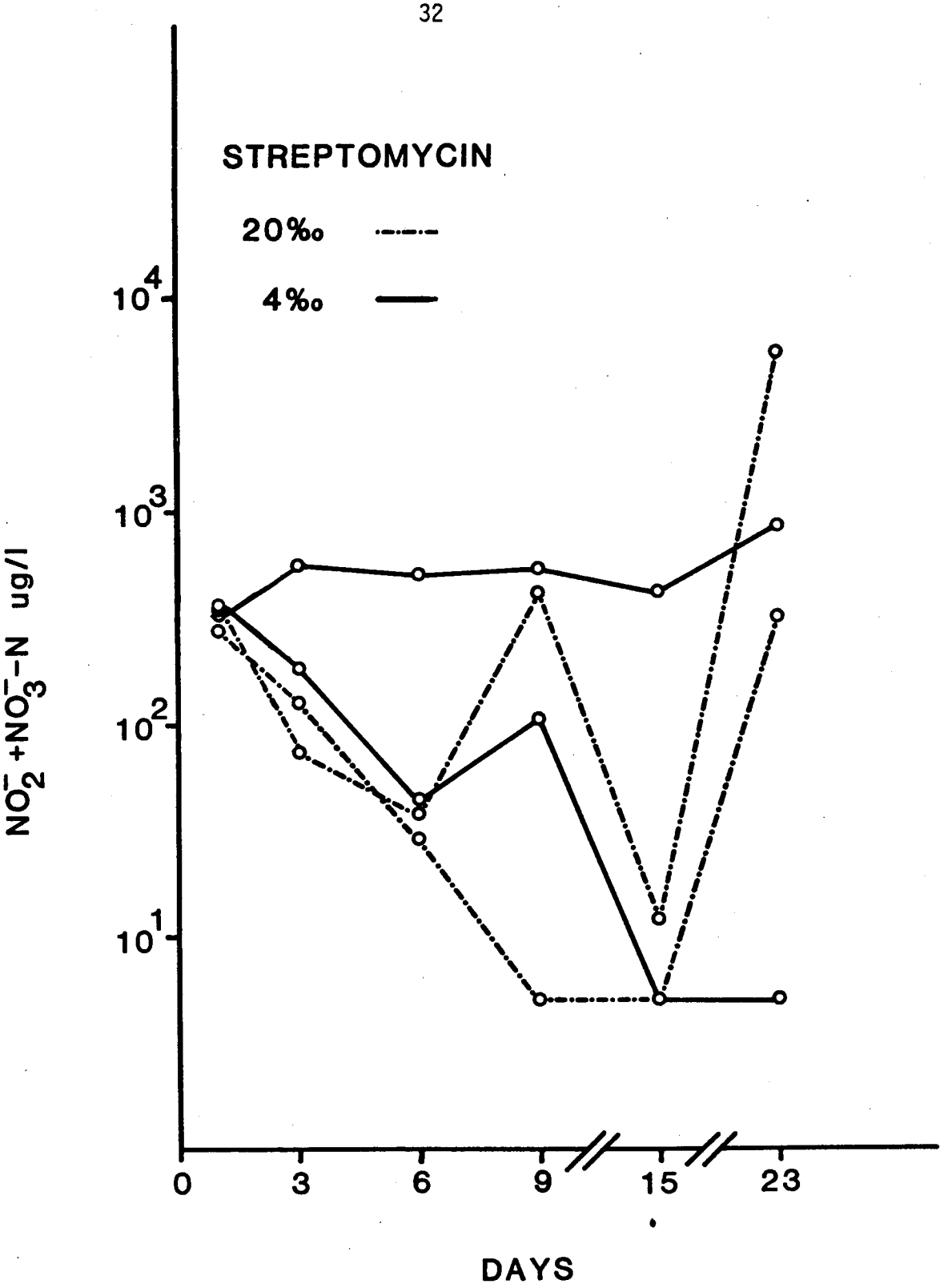

Figure 10. In vitro observations of $NO_2^- + NO_3^-$-N in water over sediments of the Duwamish River estuary (km 10.0). Data are from replicate sediment-water chambers (3 ℓ) treated with 70 mg ℓ^{-1} streptomycin and incubated at 22 ± 3° C with overlying water salinity of 20 and 4 °/oo.

denitrification. Ammonium nitrogen concentrations generally increased through day 23 at both salinity levels with the higher salinity chambers showing a higher mean net flux of NH_4^+ from sediment (Figure 11). (In half of the chambers an initial drop in water column NH_4^+ was noted between day one and three. Such a decrease may be the result of short-term chemical processes, i.e. sorption, initially predominating over long-term gradual increases from biological decomposition.)

Release of NH_4^+ from sediments was extended and apparently enhanced in the streptomycin-treated chambers. This observation was similar to preliminary sediment-water experiments conducted during the summer of 1980 in which NH_4^+ release was shown to increase substantially in chambers treated with streptomycin over that in control chambers (unpublished data).

The flux of ammonium nitrogen was calculated from the change in the water column mass of NH_4^+-N per unit area per time for each replicate chamber at each treatment (Table 3). The range of flux rates for untreated control chambers, prior to the onset of nitrification, was 3.5 - 31.1 mg m^{-2} day^{-1}, (0.13 mg m^{-2} h^{-1}) a range that falls within that observed by Rowe et al. (1975) for in situ ammonium release from near-shore ocean sediments. These rates are somewhat lower than those observed in sediments of a eutrophic lake and highly eutrophic stream by Fillos and Swanson (1975) where release rates of 5 and 15 mg NH_4^+-N m^{-2} hr^{-1}, respectively, were observed. N-Serve- and streptomycin- treated chambers showed a similar range of ammonium flux. It should be noted that ammonium nitrogen flux in the control chambers was 2-10 times higher in the high (20 $^o/oo$) than low (4 $^o/oo$) saline water chambers. The higher flux rate to high salinity overlying water is probably related to the greater ionic gradient enhancing diffusion and possibly to the presence of adsorbed counter ions in the higher saline sediment.

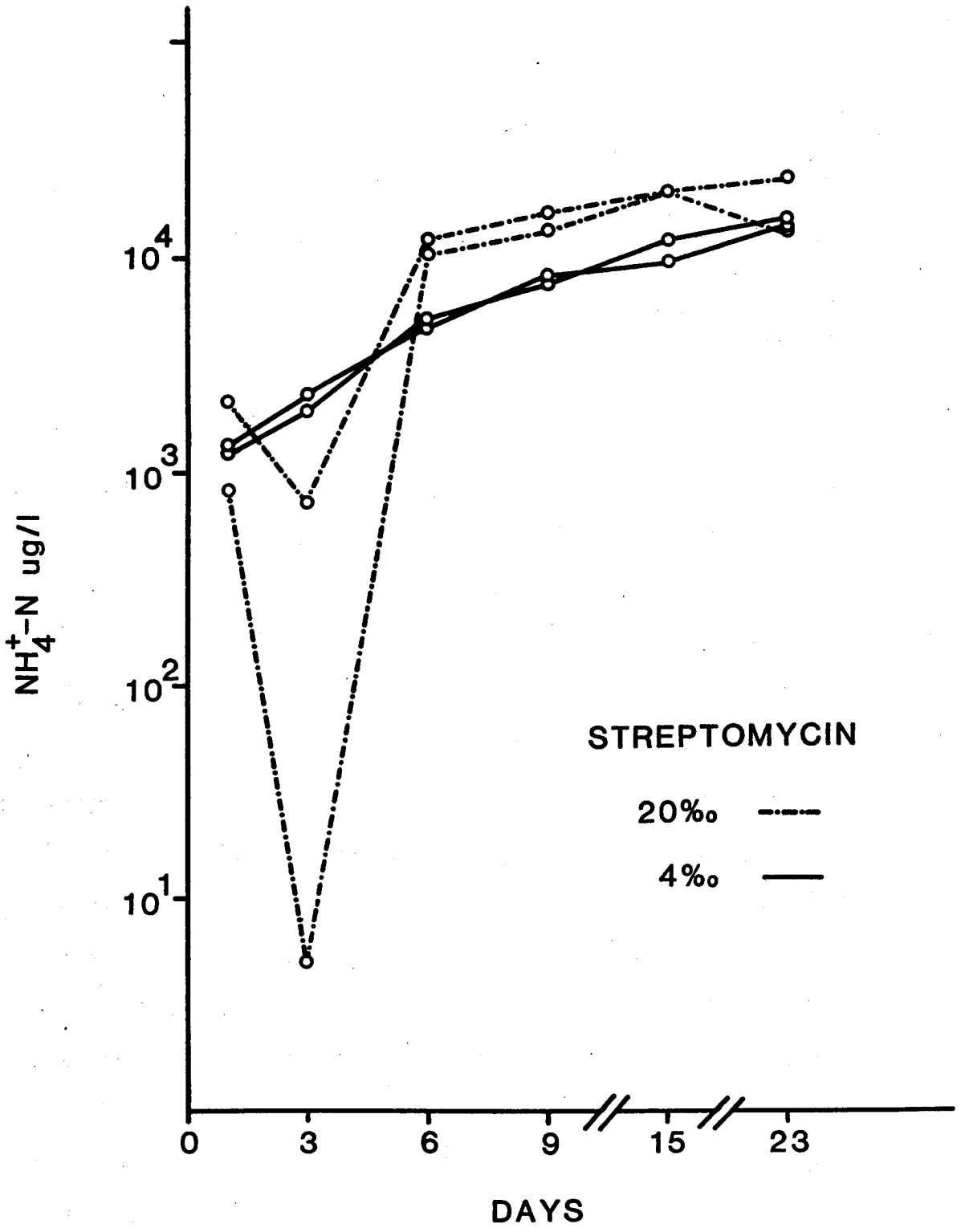

Figure 11. In vitro observations of NH_4^+-N in water over sediments of the Duwamish River estuary (km 10.0). Data are from replicate sediment-water chambers (3 ℓ) treated with 70 mg $ℓ^{-1}$ Streptomycin and incubated at 22 ± 3° C with overylying water salinity of 20 and 4 °/oo.

Table 3. *In vitro* ammonium flux from sediments at km 10.0 to overlying water of 4 °/oo and 20 °/oo salinity. Treatments include control, N-Serve (1 mg l^{-1}), and Streptomycin (70 mg l^{-1}). Flux is reported as µg m^{-2} hr^{-1} at 22 ± 3° C.

	Treatment and Salinity		Day 3	Day 6	Day 9	Day 15	Day 23
1.	A Control	4 °/oo	457	246	- 308	- 116	- 2
2.	B "	4 °/oo	130	314	160	- 440	- .4
3.	A "	20 °/oo	1296	1248	- 532	404	- 802
4.	B "	20 °/oo	922	1208	762	- 1166	- 340
5.	A N-Serve	4 °/oo	- 278	798	120	- 288	- 150
6.	B "	4 °/oo	- 274	830	122	- 174	- 230
7.	A "	20 °/oo	- 154	1622	- 312	1100	- 1136
8.	B "	20 °/oo	- 322	1534	568	- 58	- 498
9.	A Streptomycin	4 °/oo	184	600	324	496	204
10.	B "	4 °/oo	362	370	504	144	308
11.	A "	20 °/oo	- 244	1902	442	728	- 506
12.	B "	20 °/oo	- 400	2086	584	460	240

Summary of the above rates, mean ± 1 SEM. n = 2.

			Day 3	Day 6	Day 9	Day 15	Day 23
1.	Control	4 °/oo	294 ± 116	230 ± 59	-74 ± 165	-278 ± 115	- 1.2 ± 0.6
2.	"	20 °/oo	1109 ± 132	1228 ± 14	115 ± 457	-381 ± 555	- 571 ± 163
3.	N-Serve	4 °/oo	-276 ± 1	814 ± 11	121 ± 1	-231 ± 40	- 190 ± 28
4.	"	20 °/oo	-238 ± 59	1578 ± 31	128 ± 311	521 ± 409	- 817 ± 226
5.	Streptomycin	4 °/oo	273 ± 63	485 ± 81	414 ± 61	320 ± 124	256 ± 37
6.	"	20 °/oo	-312 ± 62	1994 ± 65	513 ± 50	594 ± 95	- 133 ± 264

The substantial release of ammonium nitrogen in chambers treated with streptomycin could have been due to the fact that denitrification and nitrification were simultaneously inhibited. Denitrification in control chambers may have been acting to obscure observed nitrification and thus actual ammonium nitrogen released by sediments because nitrate and nitrite formed would tend to be reduced by denitrifiers in deeper anoxic sediments.

In situ ammonium evolution to overlying waters from sediments of the Duwamish is probably due largely to diffusion through a concentration gradient enhanced by salinity. The activity of benthic macro- and meiofauna in sediments (bioturbation) and freshwater and tidal currents are also no doubt important. In the case of benthic bioturbation, it was observed in control chambers that biological disturbances did, in fact, occur. In the case of tides and currents it can be observed that tidal currents in the estuary are driven by two high and two low tides diurnally. In addition, turbulent mixing processes have been identified in the estuary that include an internal hydraulic jump (Partch and Smith, 1978). Finally, as data later presented herein will show, strong diffusional gradients exist between sediments and overlying water in the lower estuary.

In Vitro Nitrification Rates

Nitrification rates for sediment-water chambers were calculated using a simple zero-order reaction rate based on the surface area of sediments exposed to overlying waters. This rate equation was also used to calculate in situ nitrification as noted and presented later. After the initial lag period (six days), rates of nitrification were calculated for replicate sediment-water chambers and are presented in Table 4. Rates were also calculated based on the surface area of sediments exposed to overlying waters (Table 4). These

Table 4. *In vitro* rates of nitrification calculated from $NO_2^- + NO_3^-$-N in water over sediments collected from river km 10.0. Treatments include controls, N-Serve 1 mg l^{-1}, and Streptomycin 70 mg l^{-1}.

Treatment	Salinity	mg m^{-2} day^{-1}
Control	4 °/oo	2019
Control	4 °/oo	1995
Control	20 °/oo	4938
Control	20 °/oo	4668
N-Serve	4 °/oo	2106
N-Serve	4 °/oo	3213
N-Serve	20 °/oo	5782
N-Serve	20 °/oo	7323
Streptomycin	4 °/oo	278
Streptomycin	4 °/oo	- 1
Streptomycin	20 °/oo	4205
Streptomycin	20 °/oo	225

rates were variable depending on experimental conditions. The initial lag period prior to the onset of nitrification was believed to be a function of homogenizing the sediments prior to incubation. A sufficient nitrifier population was apparently re-established and began to nitrify after about six days.

In untreated control chambers the mean nitrification rate was shown to be higher at 20 ^{o}/oo salinity than at 4 ^{o}/oo. This was also observed in N-Serve treated chambers where inhibition of nitrification was unsuccessful. Streptomycin appeared to effectively curtail bacterial activity and appreciable, sustained nitrification could only be shown to occur, if at all, after day 15 in chambers with 20 ^{o}/oo salinity.

The high surface/volume ratio of sediment and chamber walls/water that existed in the experimental chambers does not allow direct extrapolation of these results to in situ estuarine conditions. Additionally, denitrification reactions in sediments may have acted to obscure nitrification rates obtained. That higher rates of nitrification were obtained in chambers with higher salinity indicates that the activity of sediment nitrifiers collected from the estuary at km 10.0 is decreased by freshwater. Comparison of mean rates obtained from two replicates, each of high and low saline water, revealed that the rate of nitrification decreased by approximately one-half when the salinity of the overlying water was decreased from 20 ^{o}/oo to 4 ^{o}/oo.

Sediment Ammonium Content

Interstitial waters from six estuary stations were examined for NH_4^+-N and NO_2^-+NO_3^--N during low river discharge periods of 1980 and 1981. The results of those analyses indicated that ammonium in surficial sediments

(0-3 cm) of the lower estuary (km 10.0 and below) was present in concentrations from 3 to 73 times higher than that of overlying waters (Table 5). Such a gradient implies that diffusion of ammonium should be from sediments to water in this portion of the estuary. Core data from 1980 indicates that at river stations located above km 10.0 the reverse gradient exists, i.e., water column ammonium concentrations were shown to be from 1.2 to 8.7 times that of interstitial sediment (Table 5). Thus, in the upstream portions of the estuary (above about km 10.0) to below the effluent outfall (km 20.5) the net movement of ammonium would appear to be from the water to sediment. Above the outfall the gradient again reverses because water column NH_4^+-N has been shown to be generally at or below detection limits during low flow (Welch and Trial, 1979), while sediments showed higher interstitial ammonium concentrations (61 - 450 $\mu g\ l^{-1}$ for sediments collected in 1980 and 1981).

Interstitial concentrations of NH_4^+-N for the three core strata (0-3, 3-6, 6-9 cm) from 1980 and 1981 are presented in Figures 12 and 13 respectively. Samples collected during 1980 showed pronounced differences in individual core concentration gradients of NH_4^+-N while cores examined in 1981 all showed an increase in mean interstitial NH_4^+-N with depth.

The high ammonium concentrations observed in sediment pore waters of the lower estuary are thought to be a result of ammonification taking place in organic-rich sediments. In this regard, a sediment organic carbon content of up to 7.6 percent (mean value) has reportedly been measured in the lower estuary (Harper-Owes, 1981b). It appears that, as a result of sedimentation in the lower estuary, organic matter settles out, is buried, and subsequently

Table 5. Comparison of pore water ammonium from surficial sediments (0 - 3 cm) to overlying water column ammonium for various dates and locations on the Duwamish estuary.

Date	River km	Sediment NH_4^+-N ($\mu g\ l^{-1}$)	Overlying Water Column NH_4^+-N ($\mu g\ l^{-1}$)
8/28/80	5.6	890	290
"	5.6	1,370	290
10/1/81	5.6	820	24
"	5.6	680	24
"	7.7	1,760	24
"	7.7	820	24
"	10.0	12,600	580
"	10.0	2,400	580
8/28/80	13.2	1,000	2,100
"	13.2	1,750	2,100
"	17.9	240	2,100
"	17.9	1,200	2,100
"	21.0	240	---
"	21.0	450	---
11/2/81	21.0	61	< 5

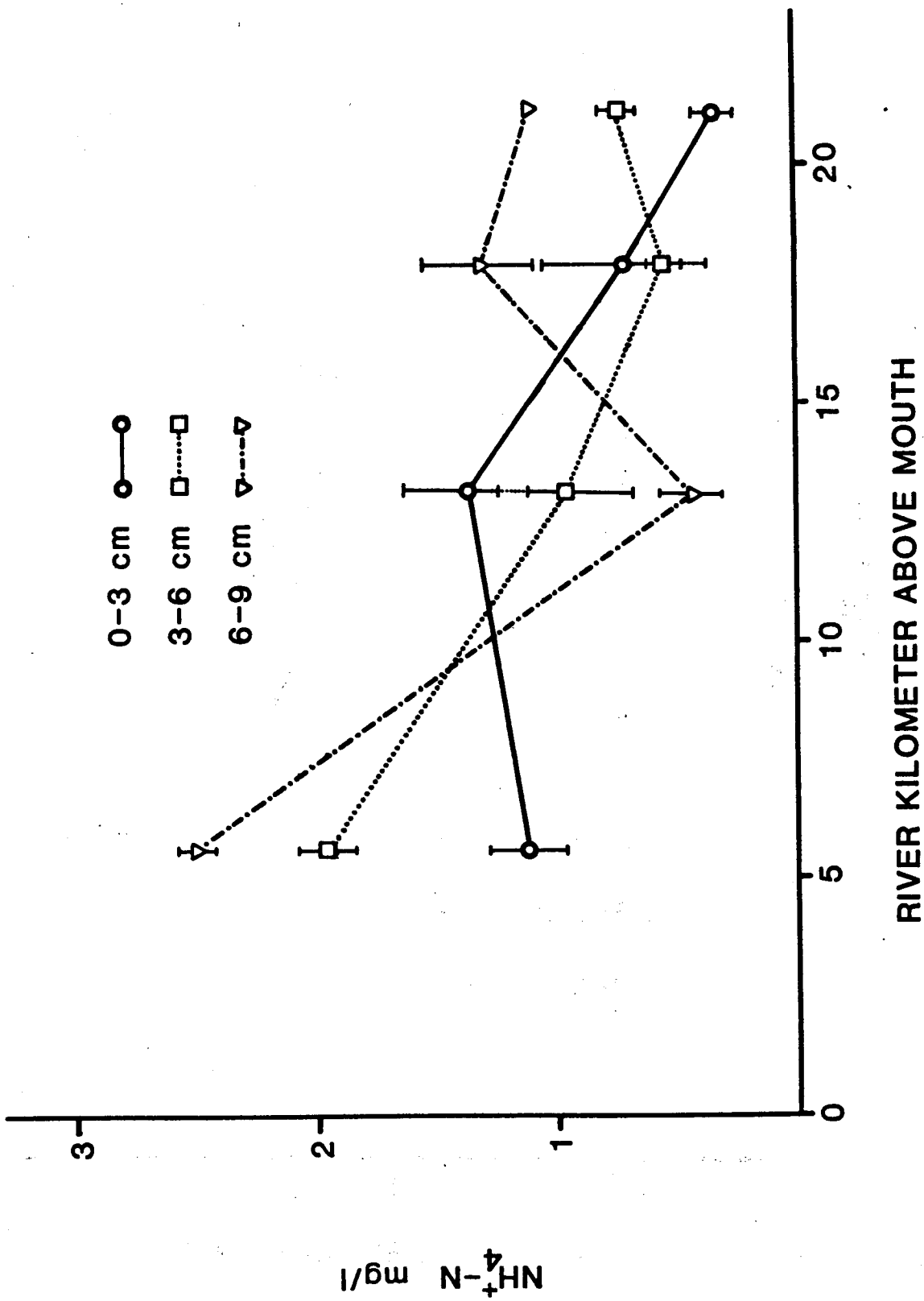

Figure 12. Interstitial NH_4^+-N observed at various depths in Duwamish River estuary sediments from four stations (km 5.6, 13.2, 17.9, and 21.0) during low flow (28 August 1980) ± 1 SEM (n = 2).

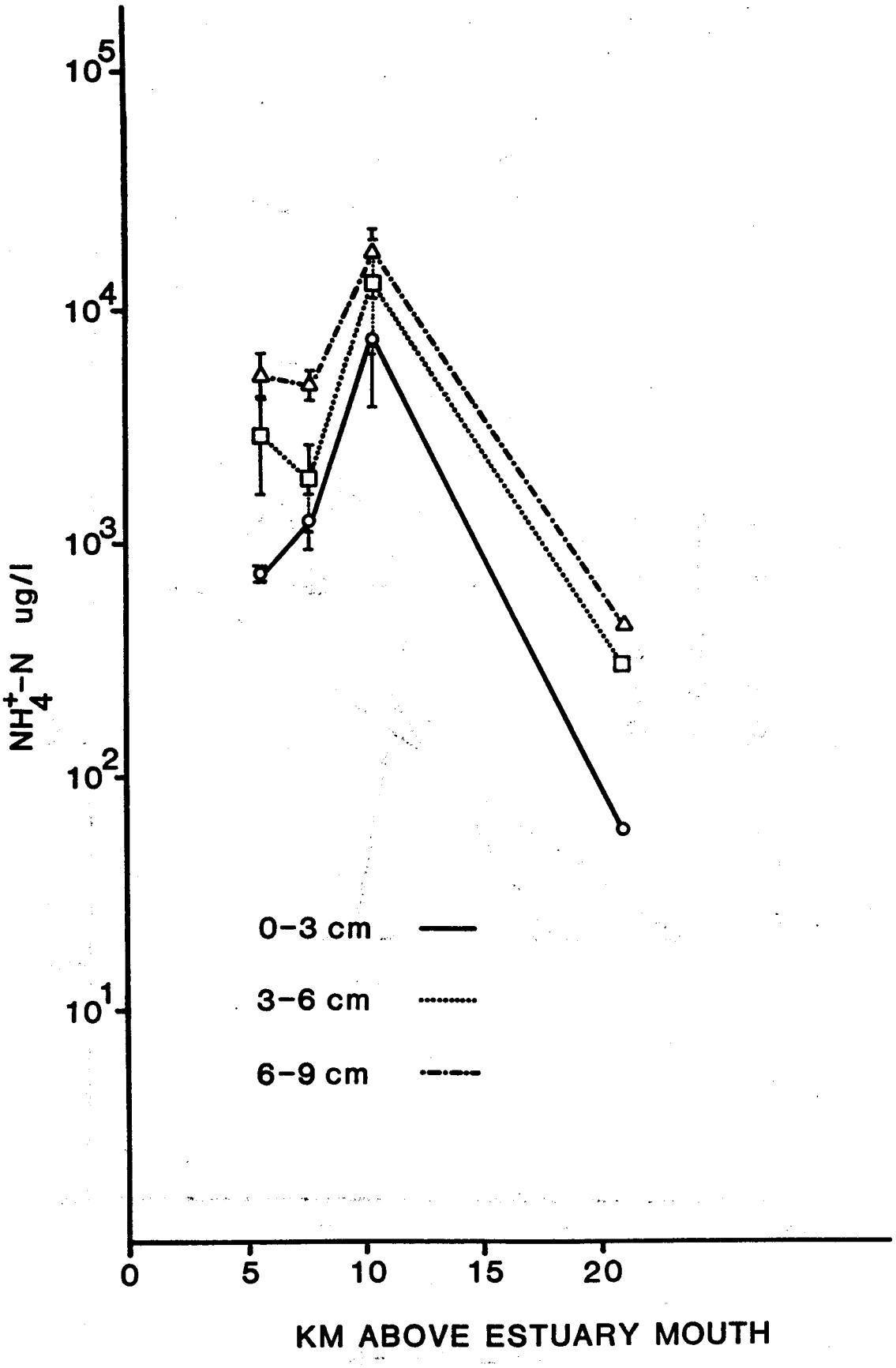

Figure 13. Interstitial NH_4^+-N observed at various depths in Duwamish River estuary sediments from four stations (km 5.6, 7.7, 10.0, and 21.0) during low flow (1 October 1981) $\pm$ 1 SEM (n = 2).

decomposed by putrefactive microorganisms thus producing high concentrations of interstitial ammonium.

In Situ Spatial and Temporal Observations

Drogue Survey and *In Situ* Rates of Nitrification

In situ rates of nitrification in the estuary were calculated based on the observed increase in oxidation products of ammonium (NO_2^- and NO_3^--N) in a single parcel of water as it moved downstream of the treatment plant outfall on September 2-3, 1981. Freshwater flow on these dates were 10.3 and 9.8 $m^3\ sec^{-1}$, respectively. This survey was similar to drogue surveys conducted on the estuary by other workers in 1979 (Bernhardt, 1981; Yake, 1981).

Specific conductance was utilized as a conservative tracer for determination of the fraction of effluent in a single parcel of water as it moved down the estuary. Such a tracer allows one to calculate the expected amount of nitrate and nitrite nitrogen at a given point in the river if the conductivity of the effluent and upstream river are known. By subtracting the expected from the actual concentration of NO_2^- and NO_3^-, the amount of NH_4^+ oxidized at time t can be estimated.

Initial rates of nitrification were calculated over hours two through five of the float survey. Beyond this time and point in the estuary (km 13.3) salinity effects obscured specific conductance readings and made accurate calculation of the effluent fraction impractical. A short distance downstream of this point, as shown by previous observations herein, estuary sediments are capable of releasing ammonium into the water column. This factor would further obscure rate calculations since more available substrate, especially at the site of activity (sediment-water interface), might be expected to result in more oxidation products.

The rate of substrate (ammonium) utilization, k_n, was calculated independently of bacterial numbers and generation times by plotting the rate of production of oxidized nitrogen in mg l^{-1} day^{-1} between hours two and five. The slope of a plot of n NO_3^- and NO_2^--N vs. time yielded rates of 7.8 day^{-1} for ammonium oxidation and 6.1 day^{-1} for nitrite -N oxidation at 19° C.

Some of the higher literature values reported for k_n are those noted by O'Connor and DiToro (1970) for the Flint River, Michigan (2.5 day^{-1} at 28° C at a river flow of 5.8 m^3 sec^{-1}) and by Wezernak and Gannon (1968) for the Clinton River, Michigan (0.258 hr^{-1} at 24° C and river flow of 2.8 m^3 sec^{-1}). Thus, the value of the first order rate constant k_n reported herein is abnormally high for describing nitrification in terms of nitrifier bacterial kinetics in a given volume. It does, in terms of a first-order reaction, describe the observed substrate utilization taking place in the Duwamish. The unusually high rate per volume results because the active nitrifiers are in the sediment and not a unit volume of river water. Activity by the sediment nitrifiers artificially inflates the rate calculation per volume.

According to Tuffey et al. (1974) such a reaction might be better described in terms of a zero-order rate. This is because large numbers of nitrifiers present as surface growth on sediments react with the substrate present (NH_4^+) at a rate that is relatively independent of the substrate concentration, i.e., the substrate concentration downstream of the outfall remains relatively high and does not appear to limit nitrifier growth. (Ammonium nitrogen measured in the water column during the drogue survey of 9/2/82 did not drop below 1.5 mg l^{-1} between km 20.5 and 9.7.) Moreover, the first order rate per volume depends on logistic growth of populations in that volume.

Stratton and McCarty (1969) have noted that even though nitrification is commonly described using first-order kinetics it may be a less than desirable

tool for predictive purposes. They described a zero-order rate equation of the form $^{dy}/dt = b$ that takes the integrated form of the equation for a straight line ($y = bt - c$). A linear plot of substrate utilization, y, vs. time, t, for the float survey conducted herein yielded a slope, b, of 0.040 mg l^{-1} hr^{-1} or 0.96 mg l^{-1} day^{-1}.

The rate of substrate utilization was also calculated based on surface area of the river sediments to account for populations in sediment. An estimate of stream bottom surface area was made using mean sea level (MSL) stream cross-sectional measurements made by Fischer (1968) (Figure 14). Mean depth for these calculations was assumed to be 1 m. A rate of ammonium substrate oxidized was then calculated using the following expression:

$$r = \frac{1}{s}\frac{dN}{dt}$$

where: s = surface area, m^2

N = mass of product produced, mg

t = time, days

During the float survey of 9/2/82 a rate of ammonium oxidation of 970 mg m^{-2} day^{-1} was calculated for hours two through five (km 19.2 - 13.3). This rate includes water column nitrification which, based on the low numbers of nitrifiers observed previously in these waters, appears to account for only a small fraction of the ammonium oxidized. Assuming a mean depth of 1 m, sediment nitrifiers in this river section outnumber overlying water column nitrifiers by as much as ten to one.

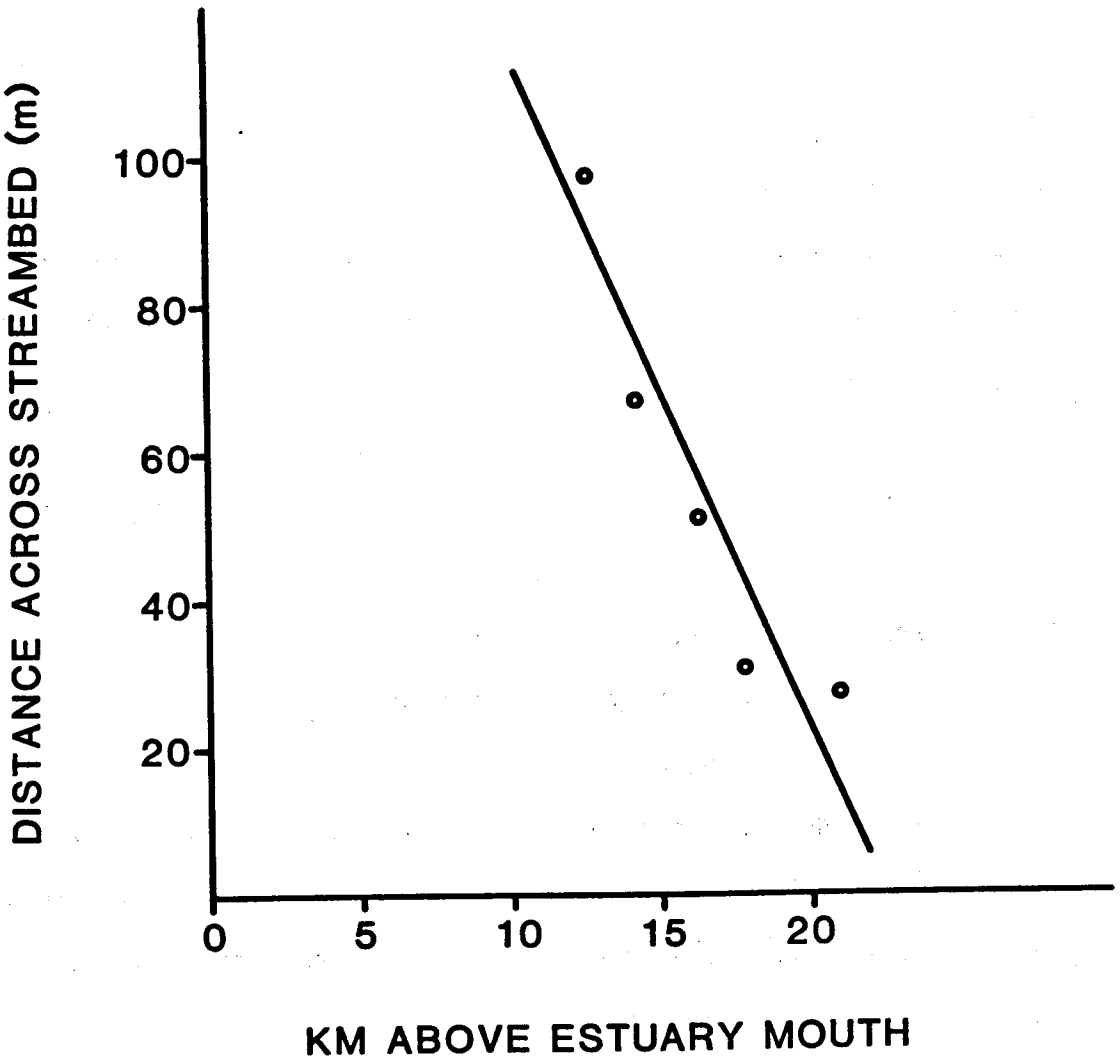

Figure 14. Mean sea level (MSL) measurements of distance across streambed channel of the Duwamish River estuary made by Fischer, 1968. These data were used to estimate the stream bottom surface area available for nitrification over a distance of 5.9 km (km 19.2 - 13.3) in the estuary.

Based on a river to effluent diluted concentration of ammonium (1960 mg m^{-3}) on this day and expected decreases in instream ammonium due to nitrification and saline dilution, the predicted fraction of NH_4^+-N removed over an 11 km reach (km 20.5 - 9.5) was shown to be 13 percent (Table 6). The observed fraction of NH_4^+-N removed over the same reach was 19 percent.

From the bacteriological data presented earlier, and from the time of travel drogue study discussed later, it can be shown that appreciable nitrification in the Duwamish may be considered to occur only where large surface active populations of nitrifiers exist and where dilutional and salinity effects do not overshadow or inhibit nitrification. Such a condition appears to exist in the Duwamish only above about km 10.0. Below this point the mean residence time for a parcel of water at low freshwater flow is on the order of eight days (Harper-Owes, 1981a) and although nitrifiers are present in Duwamish estuary sediments below km 10.0 the large volume of mostly saline water (2×10^7 m^3 at MLLW) tends to dilute out and/or inhibit observable NH_4^+ oxidation. Also, the small number of nitrifiers present in the water column in this region precludes significant nitrification there.

Since nitrification in the Duwamish is most closely associated with sediments rather than the water column, an increase in the instream concentration of ammonium may not necessarily result in a further depletion of dissolved oxygen. In this regard, an incompletely realized potential for nitrification has been noted by Cirello et al. (1979) in the Passaic River, New Jersey. In their study, an increase in the concentration of instream ammonium elicited no further increase in the concentration of nitrate. This observation was reportedly related to a decrease in water column nitrifying bacteria due to their settling out. However, previous studies on the Passaic (Matulewich and Finstein, 1978) have shown sediment nitrifying bactera to far outnumber those in

Table 6. The observed (9/2/82) and predicted change in instream ammonium over a 11.0 km reach of the upper Duwamish with a travel time of 1.3 km hr^{-1}, river flow of 10 m^3 sec^{-1}, initial diluted river plus effluent concentration of 1960 mg m^{-3} and assuming a zero-order rate of NH_4^+-N oxidized of 970 mg m^{-2} day^{-1}.

River km	Time of Travel (hrs)$^{\Delta}$	NH_4^+-N Loss Due to Nitrification*	NH_4^+-N Loss Due to Saline Dilution*	NH_4^+-N Diluted Effluent Concentration*	NH_4^+-N Observed Concentration*
20.5	.17	7	---	1953	4986
19.2	.85	34	---	1919	2106
17.1	.85	34	---	1885	3700
15.5	.85	34	---	1851	3096
13.4	.85	34	---	1817	2549
11.0	.85	34	---	1783	1555
9.8	.85	34	---	1749	1596
9.5	.85	34	29	1686	1596

Predicted fraction of effluent NH_4^+-N removed over 10.9 km = $100 \frac{281}{1960}$ = 13 percent

Observed fraction of effluent NH_4^+-N removed = $100 \frac{364}{1960}$ = 19 percent

$^{\Delta}$ Corrected for surface flow (Kittrell, 1969).

* mg m^{-3}

the water column. Therefore, it appears that in the Duwamish River as in the Passaic, an increase in ammonium may not cause an expected increased oxygen demand even if the numbers of sediment nitrifiers are high.

According to Alexander (1965) the autotrophic nitrifying bacteria are markedly sensitive to toxic compounds. Thus, another factor that may act to reduce observable ammonium oxidation in the estuary below km 10.0 is the inhibition of nitrifying bacteria by high concentrations of heavy metals and other toxicants in sediments and the water column. Recent studies by Malins et al. (1982) have shown mean sediment concentrations of lead and arsenic to be 330,000 and 130,000 ng g^{-1}, respectively, and aromatic hydrocarbon concentrations to be 14,000 ng g^{-1}.

Instream nitrification rates calculated on a volume basis by Yake (1981) for the Duwamish River were relatively low compared to those of 6.1 and 7.8 day^{-1} reported here. He calculated rates of 0.42 and 0.30 day^{-1} at 18° C and 15.4° C respectively, for two different surveys conducted in the summer and fall of 1979. Rates were calculated using a first-order rate reaction based on nitrogenous oxygen demand (NOD), which was in turn calculated from observations of NO_2^- and NO_3^- nitrogen at time t, and utilizing the effluent concentration of ammonium-nitrogen as an ultimate river NOD. Use of the full effluent NH_4^+ concentration may present an unrealistic picture of the actual oxidation rate(s) since the nitrifiers in sediments downstream of the discharge would never be in contact with such a high concentration. Maximum ammonium concentrations observed in the river were usually not more than one-third the effluent concentration. An exception to this occurs during river flow reversal but, except for those periods, dilution of the effluent with river water quickly reduces the initial ammonium concentration available to the downstream sediment nitrifiers. By utilizing a lower ultimate NOD the

resulting substrate utilization curve exhibits a greater slope and thus higher rate of nitrification based on the observed increases of nitrite and nitrate.

Flow reversal at the RTP effluent outfall lasts for only a few hours (probably no more than three) and the cell division rate of nitrifying bacteria is normally rather slow (2-3 times per day). For these reasons the established populations of nitrifiers in the Duwamish sediments downstream of the outfall would not be able to respond quickly enough to the higher instream ammonium concentration observed at flow reversal to warrant using that concentration to calculate a first-order reaction rate for nitrification. A more reasonable approach would be to utilize a diluted effluent concentration of ammonium based on river flow and effluent discharge. (A diffuser at the outfall is designed to effect mixing of effluent with river water). An even better approach might be to use an ammonium concentration measured at a point downstream of the outfall that assures a more reasonable mixed concentration of ammonium that is most representative of that available to the sediment nitrifying bacteria and, as noted previously, a zero-order reaction rate expressed on an areal basis.

In vitro rates of nitrification were previously calculated for waters collected from several sites in the Duwamish estuary and incubated in Erlenmeyer flasks at 20° C (Welch and Trial, 1979). Those rates varied from 0.28 to 0.45 day^{-1} but now appear to only have accounted for nitrification observable in the water column (and probably some growth on bottle surfaces as well) and did not take into account the large numbers of sediment nitrifiers present.

Nitrification and Tidal Dynamics: Float Survey

The float survey of the Duwamish was also used to record Kjeldahl-N NH_4^+-N, NO_2^-+NO_3^--N, over the 23-hour time of travel (Figures 15-17).

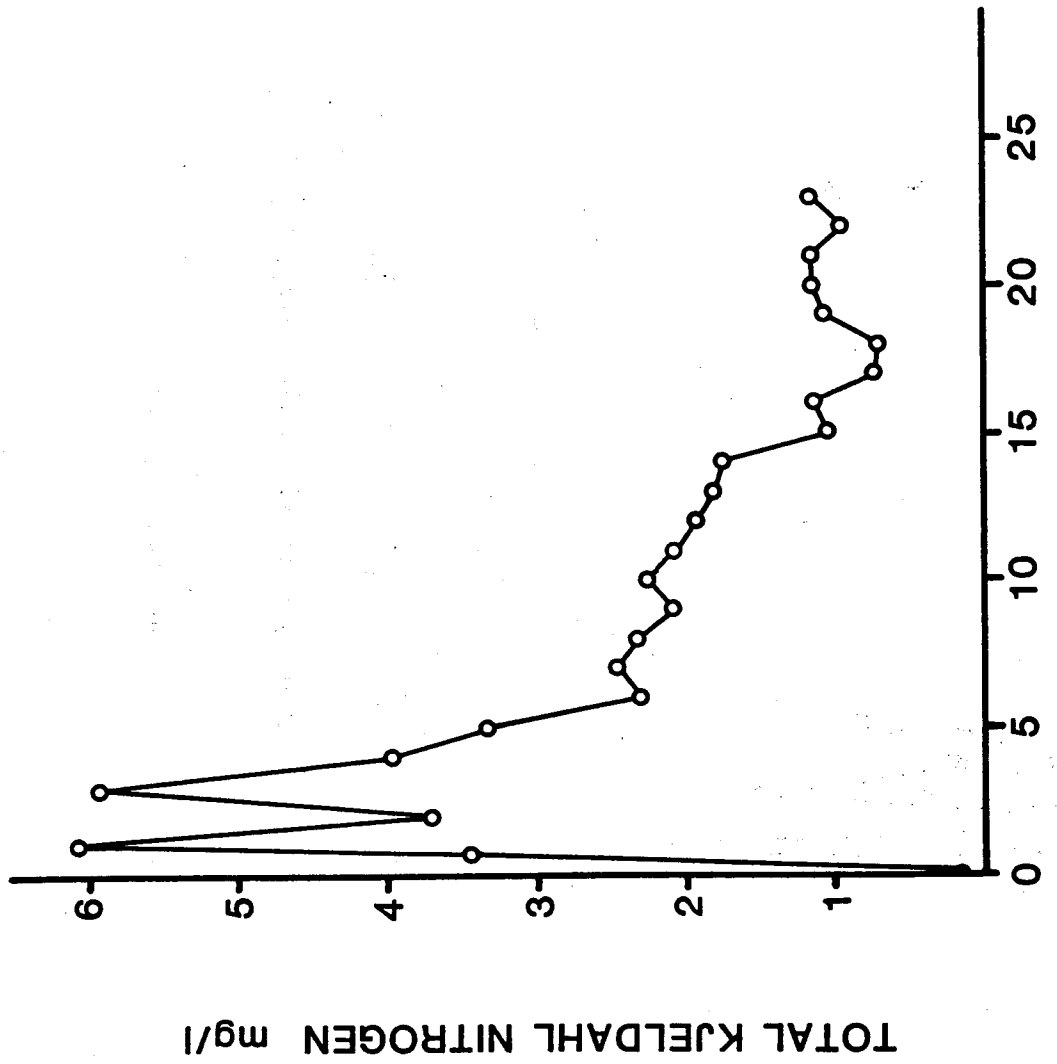

Figure 15. Total Kjeldahl nitrogen (mg ℓ^{-1}) observed in surface waters of the Duwamish River estuary during the time of travel survey of September 2-3, 1981.

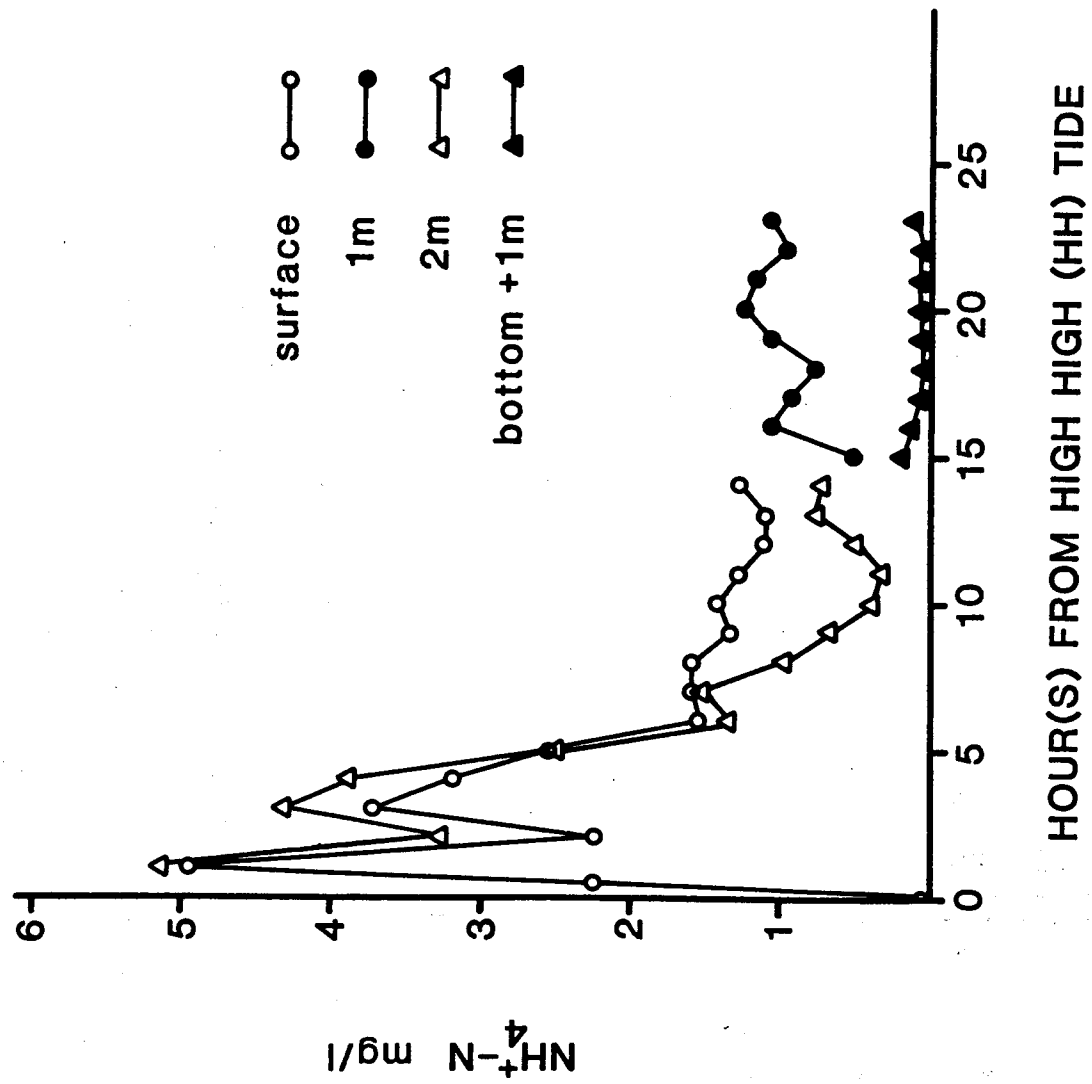

Figure 16. NH_4^+-N observed at surface, 1 m, 2 m, and bottom + 1 m during the time of travel survey of September 2-3, 1981.

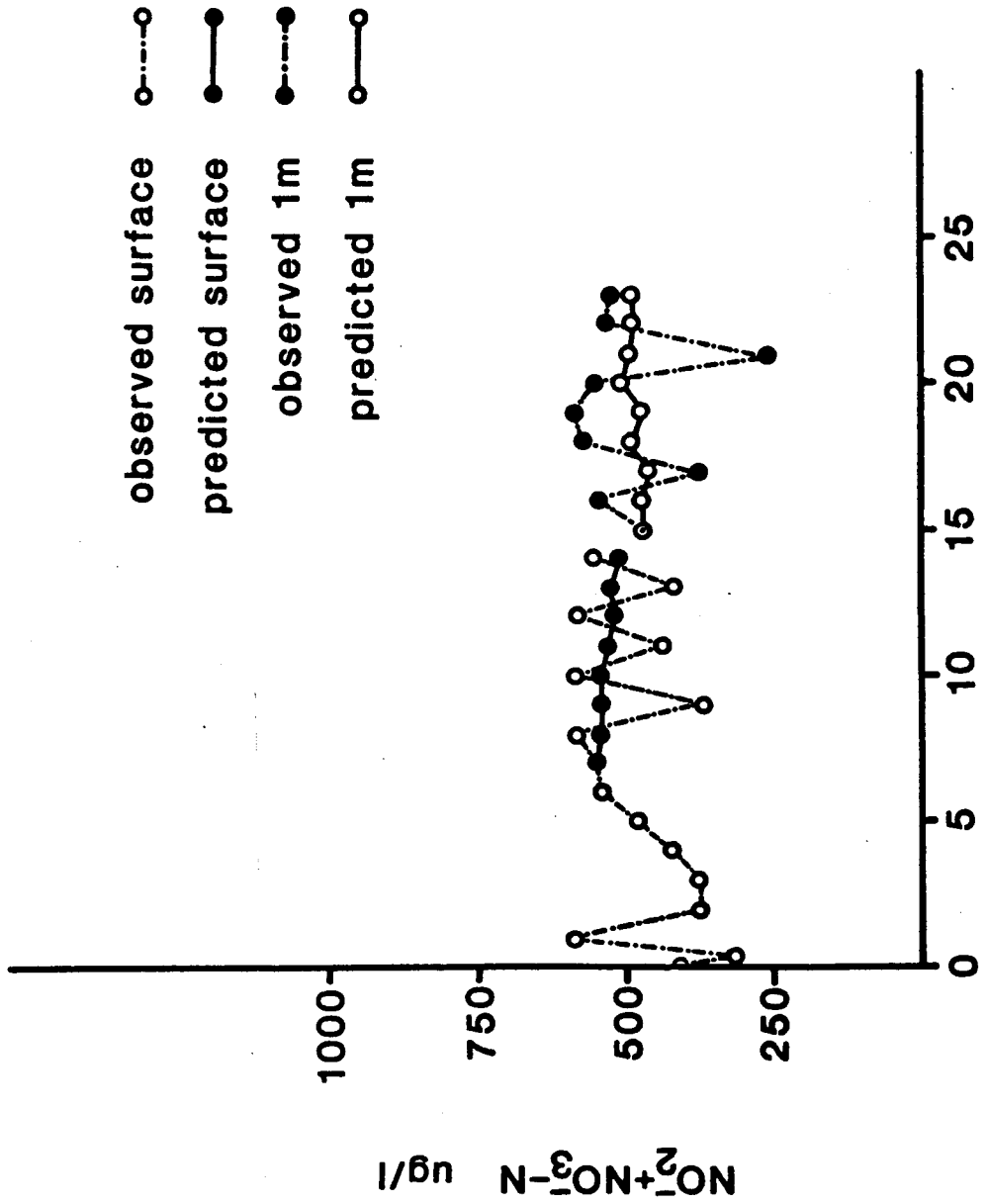

Figure 17. Observed and predicted concentration of $NO_2^-+NO_3^-$-N ($\mu g\ \ell^{-1}$) at surface and 1 m of the Duwamish estuary during time of travel survey of September 2-3, 1981. Predicted values were calculated based upon loss of $NO_2^-+NO_2^-$-N due to saline dilution.

Observed concentrations of $NO_2^-+NO_3^-$-N and NH_4^+-N were plotted against the expected concentrations assuming only saline dilution (Figures 17-19). After salinity increased above one part per thousand (hour eight) surface concentrations of $NO_2^-+NO_3^-$-N fluctuated considerably above and below the expected concentrations (Figure 17).

This may have been due in part to complex mixing and entrainment of saline wedge waters with surface waters as the parcel of water moved downstream. The concentration of $NO_2^-+NO_3^-$-N at 2 m showed a somewhat more stable behavior probably a result of more complete mixing at that depth. It was then observed that for a three-hour period (hours 8 - 11) $NO_2^-+NO_3^-$-N concentrations appeared to remain below the expected diluted concentration. During this period the downstream movement of the drogue slowed and stopped due to the effect of high tide (Figure 20). Beginning at hour 11 the drogue, still under the influence of high tide, began to move upstream until about hour 13 when low tide turned flow back downstream. Thus, during the period of time that the drogue velocity was slowed and stopped (between km 9.5 and 9.9) $NO_2^-+NO_3^-$-N in the water parcel at 2 m was decreasing possibly due to denitrification occurring in sediments or possibly due to dilution with saline waters low in $NO_2^-+NO_3^-$-N.

As the block of water began to move again, this time in an upstream direction due to the influence of high tide, it was observed that the concentration of $NO_2^-+NO_3^-$-N began to increase. From hour 11 through hour 14 the concentration of these combined species increased above expected concentrations. Such an increase indicates active nitrification. However, a corresponding decrease in ammonium was not observed and, in fact, between hours 11 and 14 an increase in NH_4^+-N above the expected level took place at 2 m. Otherwise, NH_4^+-N generally remained below expected levels at surface and 2 m. The observed increase

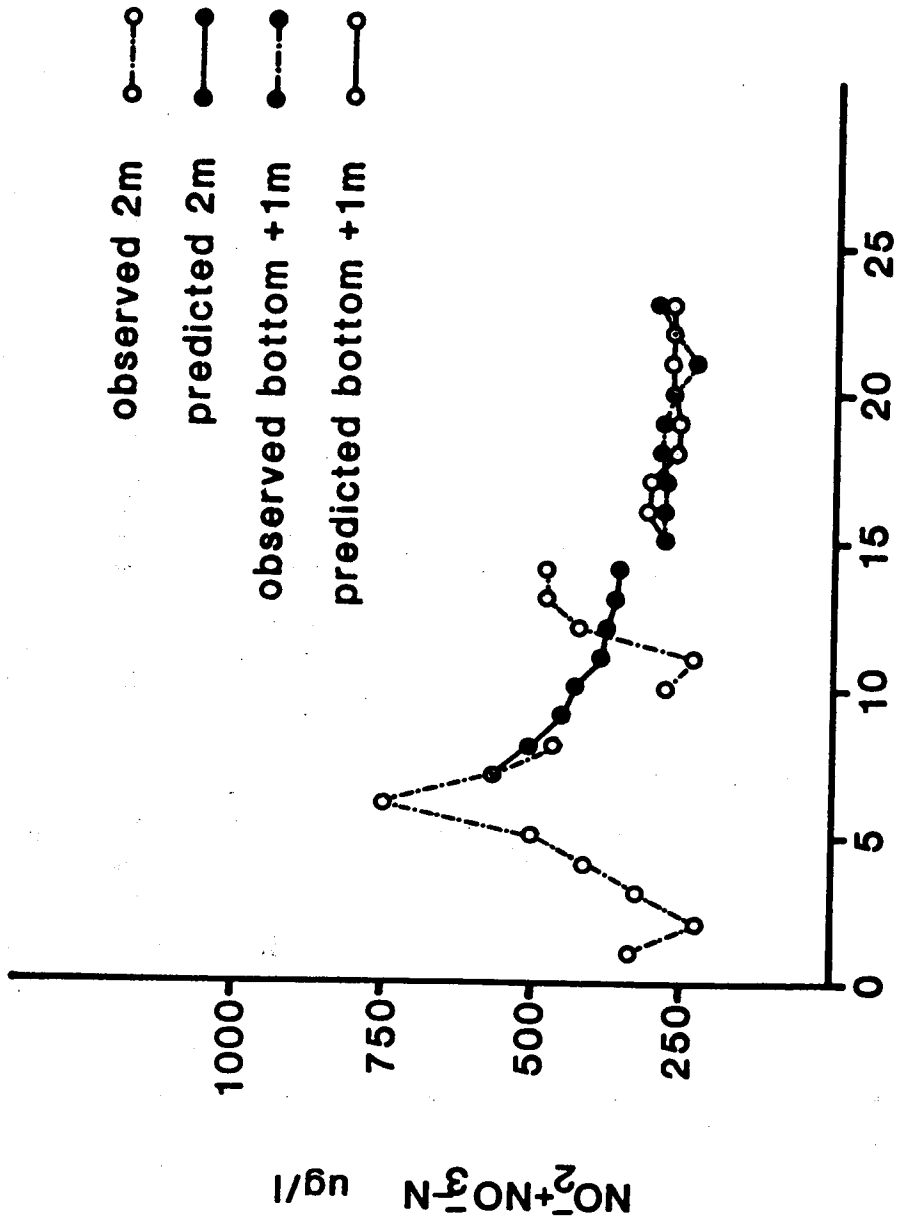

Figure 18. Observed and predicted concentration of $NO_2^-+NO_3^-N$ ($\mu g\ \ell^{-1}$) at 2 m + bottom + 1 m of the Duwamish estuary during time of travel survey of September 2-3, 1981. Predicted values were calculated based upon loss of $NO_2^-+NO_2^-N$ due to saline dilution.

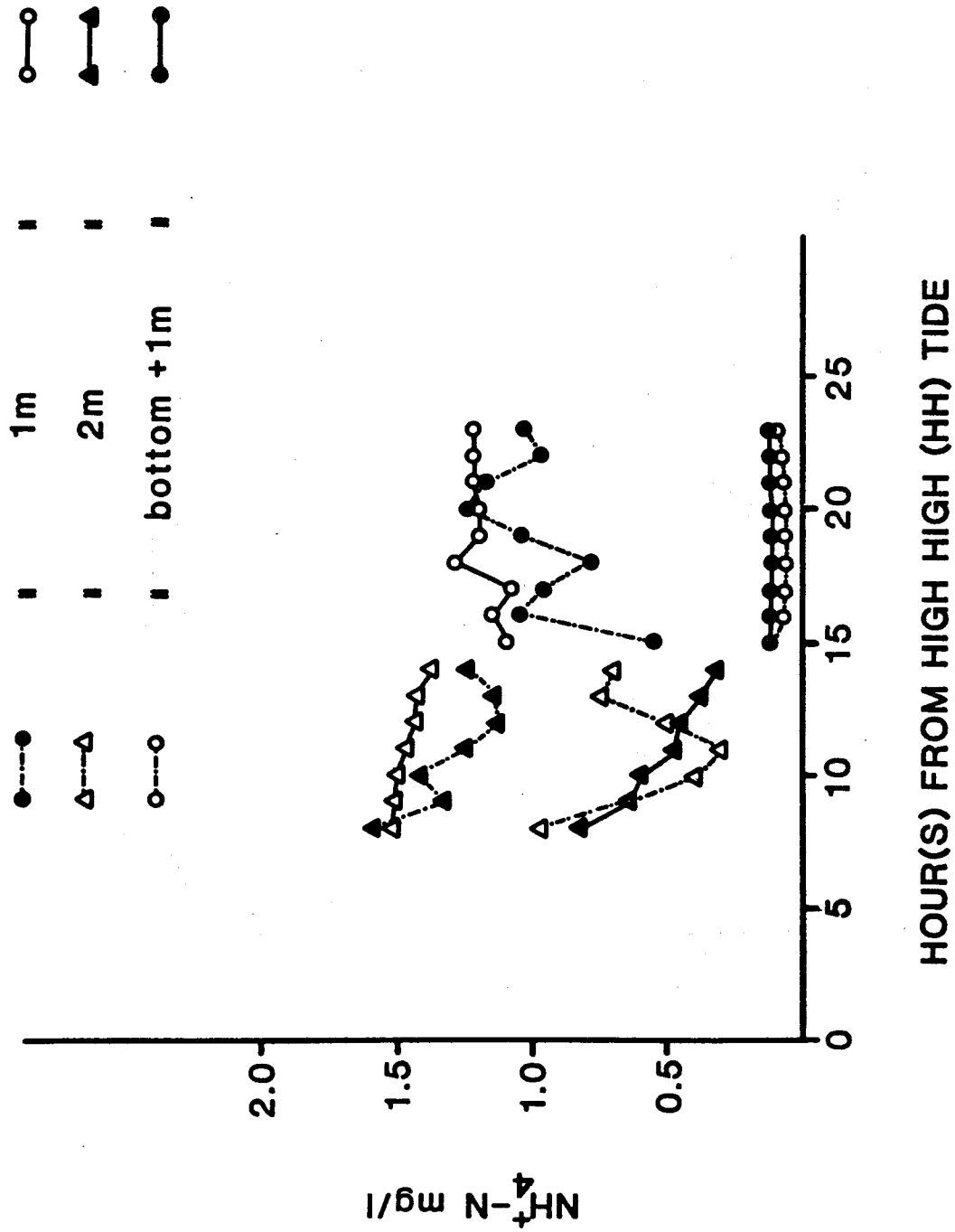

Figure 19. Observed and predicted concentration of NH_4^+-N ($\mu g\ \ell^{-1}$) at surface, 1 m, 2 m, and bottom + 1 m of the Duwamish estuary during time of travel survey of September 2-3, 1981. Predicted values were calculated based upon loss of NH_4^+-N due to saline dilution.

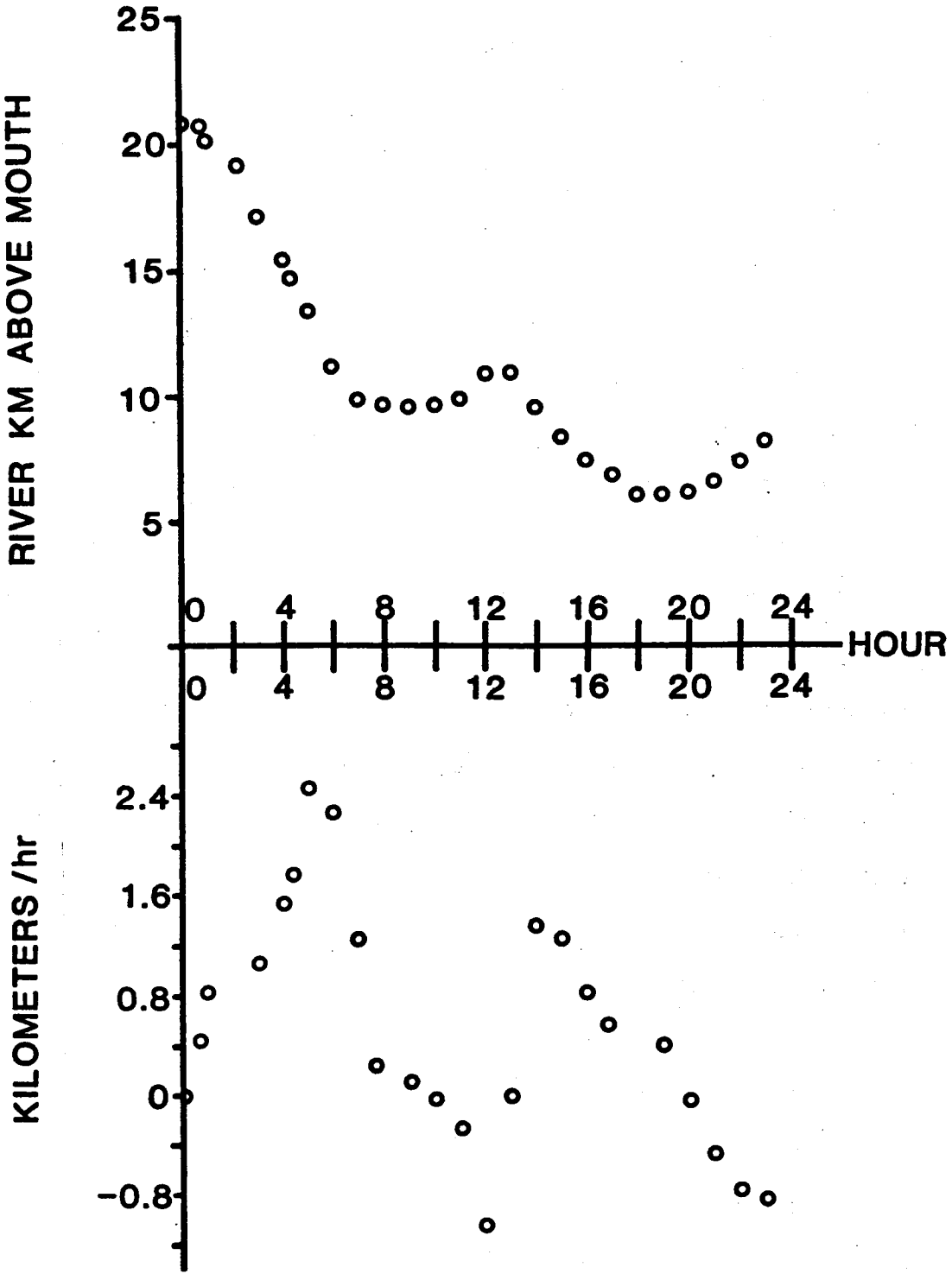

Figure 20. Time of travel survey of September 2-3, 1981. Plotted are; drogue movement over time for various river km and; drogue velocity (km hr^{-1}).

between hours 11 and 14 was believed to have been a result of ammonium release from sediments rather than a simple mixing of low ammonium salt water with high ammonium freshwater because salinity ranged from about 21-25 $^{o}/oo$ salinity in these samples. Also, during this period, the drogue moved between river km 10.9 and 9.5, an area where sediments were previously shown in this study to release ammonium to overlying waters. Additionally, turbulent mixing processess in the Duwamish estuary have been shown to be related to critical flow occurring during ebb tide (Partch and Smith, 1978). The effect(s) of these and other mixing processes are also believed to have influenced the observed data although to what extent is not known.

The above observations point to the complex interactions of nitrogen cycling in the estuary. While nitrification is taking place as witnessed by an increase in $NO_2^-+NO_3^-$-N, ammonification in the sediments may also be acting to increase the concentration of substrate (NH_4^+). Denitrification may also be occurring in the organic-rich anoxic sediments and reducing the concentration of $NO_2^-+NO_3^-$-N that might otherwise be observed. Finally, algal and bacterial assimilation are reducing both ammonium and nitrate-nitrite levels in the water column, although this is generally assumed to be small compared to the ammonium oxidized.

Stationary Survey

A 24-hour stationary survey was designed to observe changes in river ammonium concentrations at a fixed river station. The site chosen for observations was located at river km 10.4, a distance of 10.1 km downstream of the effluent outfall and a short distance (0.4 km) upstream of the dredged, widened channel of the lower estuary.

As noted previously, river flow reversal occurs in the Duwamish during high tide ($\geq$ 3.1 m) and low river flow ($< 8.5\ m^3\ sec^{-1}$) (Bernhardt, 1981). Such a condition can result in a "triple dose" of effluent being discharged to a block or plug of river water in the vicinity of the Renton Treatment Plant outfall, i.e., a plug of water receives effluent on the way downstream, on the way back upstream after flow reversal occurs and again on the way back downstream. Even if flow reversal does not occur directly at the outfall, river flow is often slowed and stopped at and below this point depending on tide height and river flow.

Seasonal periods of low flow ($< 8.5\ m^3\ sec^{-1}$) and thus triple dosing events had passed by the time this survey was conducted. However, the data obtained were nevertheless useful for predicting river concentrations under various tidal and river flow conditions. The survey was conducted during the period 11:30 AM 15 October to 11:30 AM 16 October 1981. River discharge was 22.1 and 20.3 $m^3\ sec^{-1}$ on the respective dates. Tidal heights are presented in Figure 21. Ammonium concentrations measured at surface and 2 m increased every 12 hours coinciding with low tide (Figures 22 and 23). The increase in $NO_2^-+NO_3^-$-N towards the end of high tide (hours six through seven) probably was caused by <u>in situ</u> nitrification (Figure 22). Water that had passed and in which nitrification had taken place was now being transported back upstream. Water with lowered NH_4^+ and raised NO_3^- levels was then sampled during the tide reversal period. The NH_4^+-N drop and $NO_2^-+NO_3^-$-N rise continued for two to three hours until midway between high high (HH) and high low (HL) tide at hour ten. (Instream temperature during this period averaged 11.9° and 12.6° C at surface and two meters, respectively.) This same occurrence did not, however, take place at the end of the next high tide. For a period of 3.5 hours past the second high tide, NH_4^+-N and $NO_2^-+NO_3^-$-N fluctuated in a manner

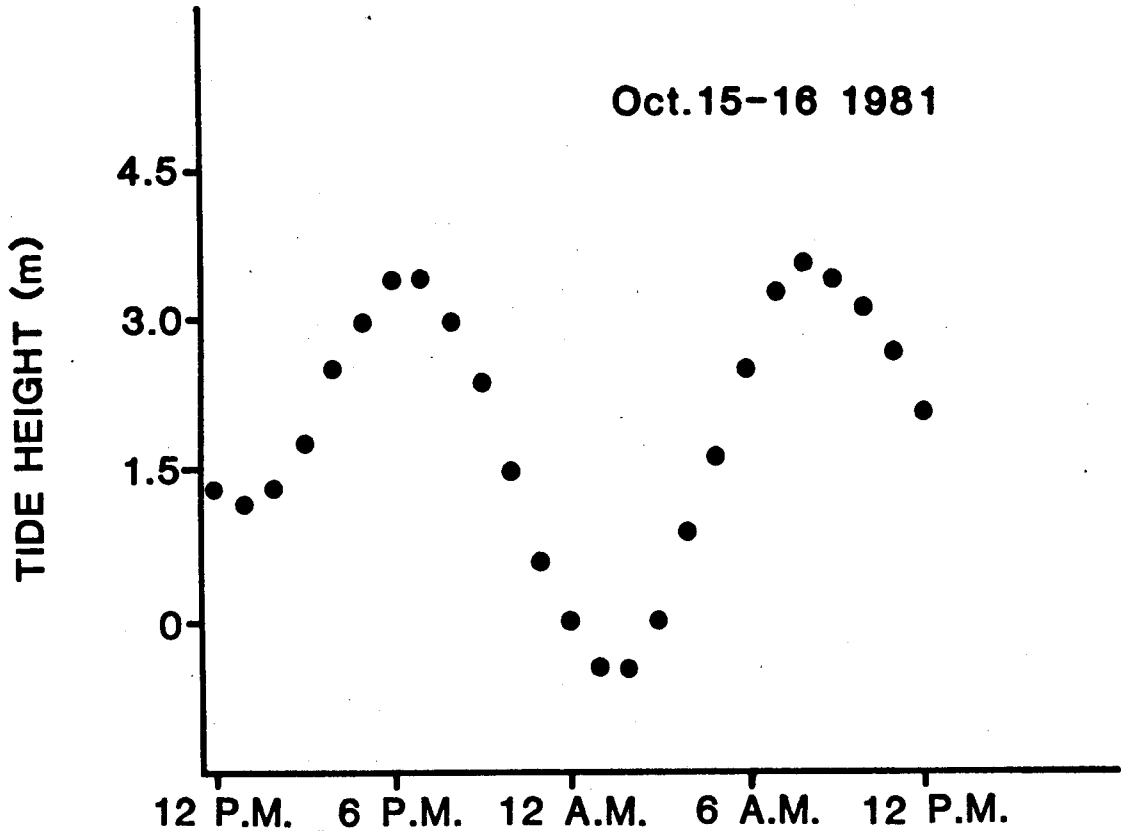

Figure 21. Stationary survey of October 15-16, 1981. Variation in tide height over the duration of the survey (11:30 AM, October 15 to 11:30 AM, October 16).

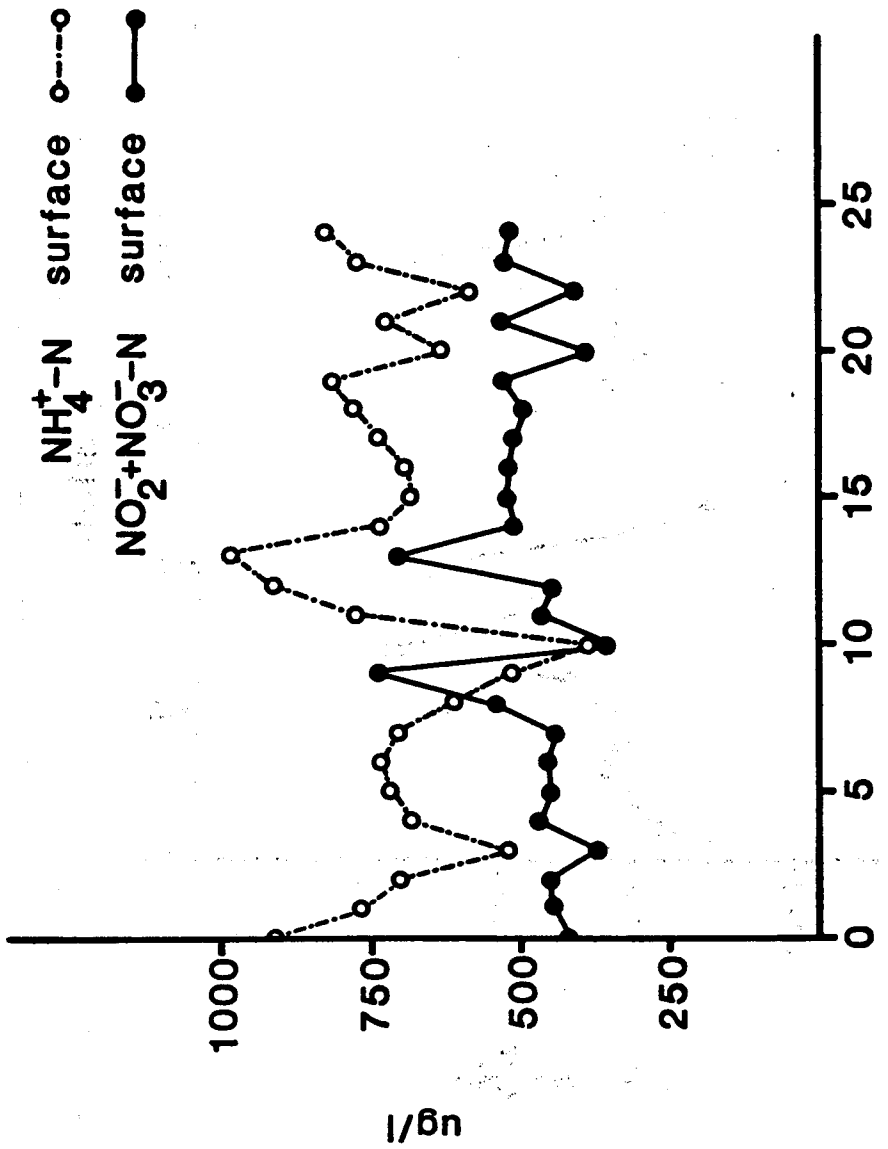

Figure 22. Observed surface concentrations of NH_4^+-N and $NO_2^-+NO_3^-$-N ($\mu g\ \ell^{-1}$) during the stationary 24-hour survey of October 15-16, 1981 at km 10.4.

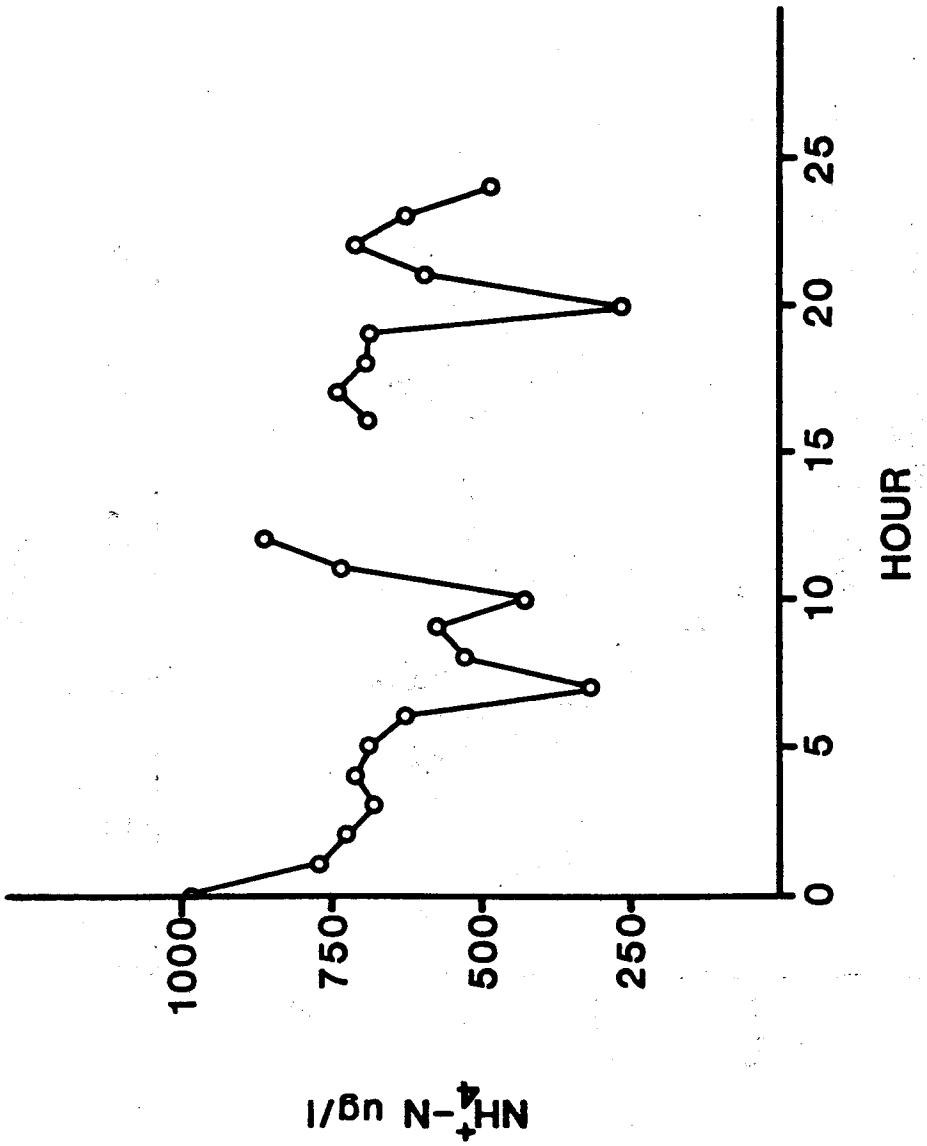

Figure 23. Observed concentration of NH_4^+-N ($\mu g\ \ell^{-1}$) at 2 m during the stationary 24-hour survey of October 15-16, 1981 at km 10.4.

consistent with dilutional effects and gave no clear indication of nitrification activity (Figure 22). A difference in the two high tides was noted; the first was preceeded by a low low (LL) tide of 0.43 m. Thus, the smaller tidal exchange (2.38 m) resulted in nitrification while the higher tidal exchange (4.02 m) did not.

The explanation of both observations can be related to the resident excursion time of effluent in the river. In the case of nitrification, river flow reversal prolongs the contact time of effluent ammonium with sediments and, as previously shown, surficial sediment is the major site of nitrification in the estuary. Thus, the decrease in ammonium concentration and increase in oxidation products (NO_2^-, NO_3^-) would be greatest in the water residing longest over any portion of sediment. The same argument could be made for water column nitrification, but laboratory experiments described earlier have shown nitrifier numbers in the water column to be low.

Based on river and effluent mean discharge on the day of the survey, a dilution factor of 12.5:1 existed which, assuming complete mixing, would result in a river ammonium concentration of about 1 mg l^{-1}, a level approached during both low tide observations. Thus, the dilutional effect of saline water appeared to be largely responsible for the lower NH_4^+-N concentration observed during the second high tide period especially for samples collected at 2 m (Figure 24).

Ammonia Toxicity

Factors Affecting Toxicity

The toxicity of aqueous ammonia solutions to aquatic life has been documented in various reviews (Tsai, 1975; USEPA 1973, 1976). Unionized ammonia, generally represented as NH_3, is known to be the toxic fraction and its

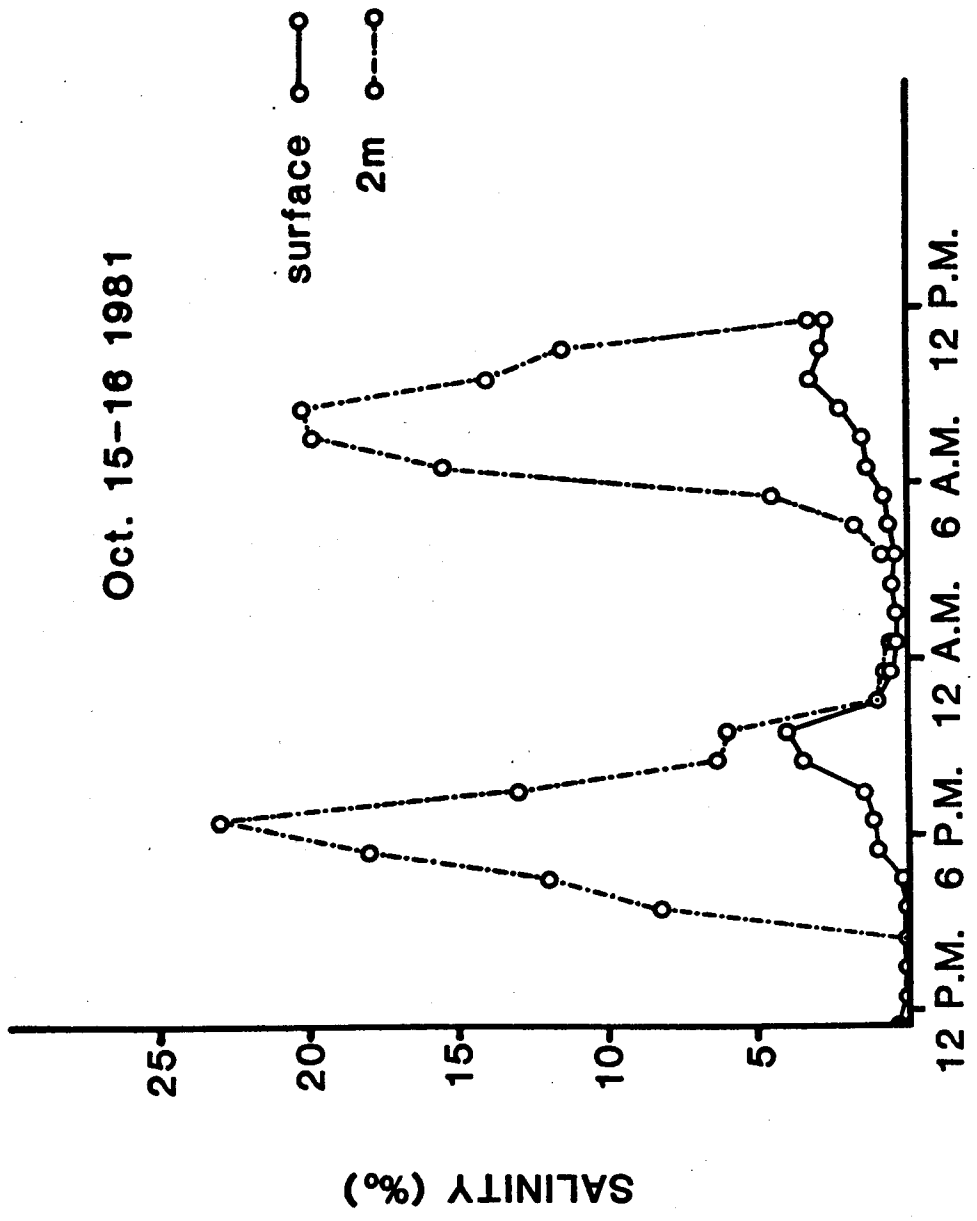

Figure 24. Salinity observed at surface and 2 m in the Duwamish estuary at km 10.4 over the 24-hour period 11:30 AM, October 15 to 11:30 AM, October 16, 1981.

concentration in solution is most notably a function of pH and the concentration of ammonium (Trussel, 1972). This is a result of the equilibrium reaction of NH_3 gas in water:

$$K = \frac{[NH_4^+][OH^-]}{[NH_3]}$$

The higher the pH the greater the percentage of unionized ammonia present in solution, e.g., at 20° C and a pH of 8.0 the percent unionized ammonia in aqueous solution is three while at pH 9.0 the percent NH_3 is 28. Lowering the temperature from 20° to 10° C at pH 8.0 reduces the percent NH_3 from three to two. Trussel (1972) has developed a table from which one may ascertain the percentage of unionized ammonia in aqueous (freshwater) ammonia solutions at differing pH and temperature. As Trussel (1972) further notes, ammonia toxicity is also to a lesser degree a function of temperature, dissolved oxygen, bicarbonate alkalinity, and the free CO_2 concentration of the water.

The unionized ammonia molecule by virtue of its lack of charge is able to penetrate biological cell membranes quite easily. Ammonia in water reportedly kills fish by preventing the normal excretion of ammonia through the gill surface while sublethal levels are reported to cause hyperplasia of gill tissue, O_2 blood level reduction, increased blood pressure, increase in lactate and increased occurrence of acidosis (Tsai, 1975). Herbert and Shurben (1965), in studies with salmonids, showed resistance to lethal concentrations of ammonium that increased with salinity up to 10.2 $^{o}/oo$ but decreased when salinity was increased above this level. One hundred percent seawater in all cases provided greater protection than freshwater. These studies were conducted at pH 7.45. One might surmise that the resistance effects noted were due to changes in the activity of the ammonium as a result of a "salting-out" effect of ions present in seawater. Or, if the ambient ammonium competes with sodium ions

for inward transport at the cellular level then higher saline waters may simply provide more sodium ions to outcompete ammonium. Whitfield (1974) has presented information with which to calculate unionized ammonia concentrations in seawater at a given pH and ammonium concentration.

A water quality criterion of 20 $\mu g\ l^{-1}$ has been established as unionized ammonia (16 $\mu g\ l^{-1}$ as unionized ammonia nitrogen, NH_3-N) for the protection of freshwater aquatic life (USEPA, 1976).

The toxicity of ammonia to aquatic life in the Duwamish estuary can be said to be a function of pH, temperature, ammonium loading, and saline dilution. The latter three are primarily a function of freshwater river flow, RTP effluent concentration and discharge, and tidal effects. The major factor affecting ammonia toxicity in the estuary, elevated pH, appears to be a function of algal bloom activity which is in turn a function of various physical and chemical factors.

Algal Blooms and Elevated pH: Earlier Observations

During the mid and late 1960s and early 1970s summer algal blooms on the lower Duwamish estuary (km 7.7) resulted in surface pH values that approached 9.0 (Figure 25). The cause of these late-summer blooms was thought to be due to low river flow and minimum tidal conditions resulting in a stable water column allowing maximum light availability; a condition considered necessary to produce such a large biomass of phytoplankton (70 $\mu g\ l^{-1}$ chl *a*) in a normally turbid, rapidly flushed system (Welch, 1969; Welch *et al.*, 1972). Since about 1976, however, the incidence of algal blooms in the lower estuary has markedly declined. This decline is noticeably coincident with the diversion of a sewage lagoon effluent from the upper river, which may have provided "seed" that contributed to the success of earlier blooms (Harper and Owes, 1981a).

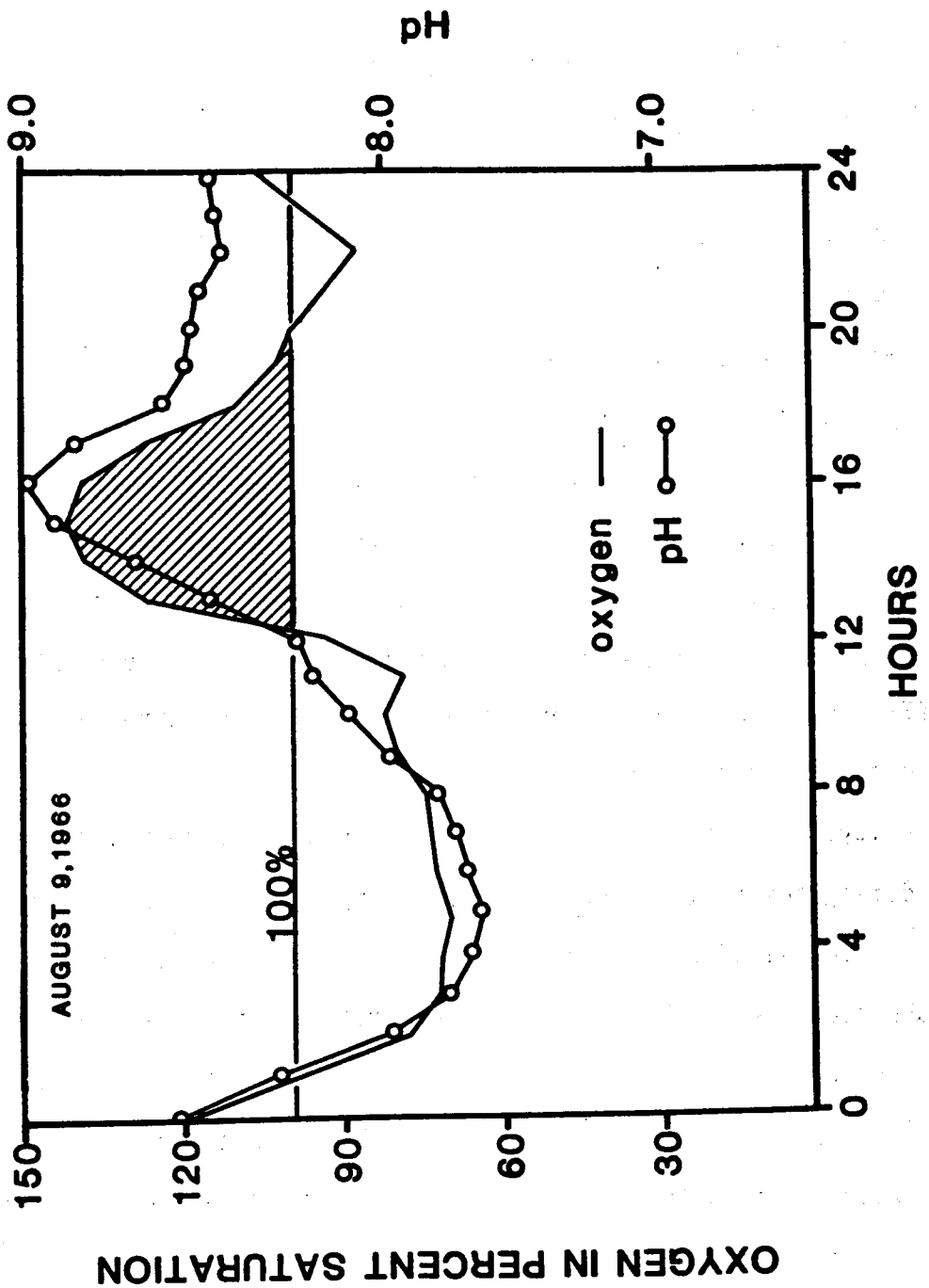

Figure 25. Oxygen and pH diurnal change during an algal bloom in August 1966 at km 7.7 in the Duwamish River estuary.

Nonetheless, the potential for future blooms and coincident high pH conditions probably should not be disregarded. This is especially true because dominant algae in the blooms of 1965-66 were plankton diatoms (Welch, 1969) and diatoms are usually not abundant in sewage lagoons (Palmer, 1977). Metro water quality data collected from an automated monitor located at km 7.7 has indicated nine algal blooms occurring between 1970 and 1977 (based on O_2 saturation) during which maximum pH ranged from 8.25 to 9.2 with freshwater river flow ranging from 9.91 m^3 sec^{-1} to 18.4 m^3 sec^{-1} (James Buckley, written communication).

For a worst case scenario, mean ammonium concentration of surface waters (nine low tide observations) from km 5.6 to 16.2 during the summer of 1979 were used to predict the unionized ammonia content of those waters given an increased pH as a result of algal blooms (Welch and Trial, 1979). If a bloom had occurred raising the pH to 8.5, unionized ammonia could have approached the acute lethal limit for several fish species of approximately 0.4 mg l^{-1} (USEPA, 1973) and at pH 9.0 the concentration would have approached 0.5 mg l^{-1} thus exceeding the lethal limit.

In reality, unionized ammonia content did not approach the 20 µg l^{-1} EPA criterion during the 1979 study, even though NH_4^+-N concentrations reached 2200 µg l^{-1}, because pH did not exceed 7.2. Based on the concentrations of ammonium observed during the 23-hour float survey and the 24-hour stationary survey conducted in this study, unionized ammonia did not exceed the criterion of 20 µg l^{-1}. However, studies by Bernhardt (1981) have shown the criteria to have been exceeded above km 15 and km 19 to the RTP outfall during time of travel studies conducted in September and October of 1979, respectively.

Harper-Owes (1982) have recently estimated a frequency of occurrence of surface water unionized ammonia for two Duwamish sites based on METRO monitor

data, annual average river and 1980 RTP discharge, and "triple-dosing" effects of river flow reversal. For the months of August and September concentrations of 42 $\mu g\ l^{-1}$ and 56 $\mu g\ l^{-1}$ unionized ammonia at km 13 and km 8, respectively, could be expected to occur $\leq$ 5 percent of the time.

Toxicity Predictions

A worst case scenario for the Duwamish River estuary should include a consideration of: 1) the exposure period for migrating adult salmon; 2) the effect of nitrification/sediment ammonium release; 3) projected increases in RTP discharge; and 4) the potential for phytoplankton blooms and resulting high pH.

Adult chinook salmon move through the estuary during the late summer, low flow period when toxic levels of unionized ammonia are most apt to occur. Although little is known specifically about their movements, for this analysis they would be assumed to move through the "critical area" (km 6 - 12) over a period of a few hours. Phytoplankton blooms have persisted in the critical area for a period of one to two weeks, moving upstream on flood tide and down on ebb, with the highest plankton concentration and pH occurring between km 7 - 10 at low tide (Welch, 1969). Migrating salmon may actually avoid such conditions, but lacking specific knowledge no avoidance is assumed. Because high pH is driven by photosynthesis, the maximum is reached during the day and the minimum during predawn hours. During the 1966 bloom pH remained between 8.3 and 9.0 for nearly 12 hours in the day, dropping to 7.6 by early morning (Figure 25). Thus, if avoidance occurs, and assuming only a few hours is necessary to traverse the critical area, fish moving at night would probably avoid toxic conditions. On the other hand, resident fish and salmon that may hold

for a day or more in the critical area would be trapped as pH rose in excess of a full unit during midday.

Nitrification has been shown to occur primarily in the surficial sediments and the rate per unit area of substrate would probably not increase as ammonium concentration increased. The in situ rate of 970 mg m^{-2} day^{-1} at 19° C determined to exist now in the reach from about km 10 to 20 (actually calculated for km 13.3 to 19.2) may therefore be expected to represent the maximum loss of ammonium for further increases in RTP discharge. With continued exposure in a given volume, bacterial growth would normally be expected to increase at some rate until the substrate is consumed. But because sediment bacterial (nitrifier) populations are exposed to a given volume of water containing substrate for only a brief time, the amount of substrate removed is expected to remain a function of contact time. The sediment bacterial population then is probably space and/or diffusion limited rather than substrate limited.

Sediment release of ammonium is considered insignificant in the upper river part of the estuary between about km 10 and 20 because the concentration in the water is greater than that in sediment interstitial water. Thus, water column ammonium should decrease in this region due to nitrification and dilution.

Estimates of maximum NH_4^+-N content occurring in the critical area (km 6 - 12) under increasing waste discharge scenarios were made based on the maximum concentration observed there in 1979 -- 2.2 mg l^{-1}. Effluent flow at that time was about 1.6 m^3 sec^{-1} and at a low river flow of 6.3 m^3 sec^{-1} comprised 20 percent of the river or a 4:1 dilution ratio (four parts river, one part effluent). That concentration would increase by a factor of 1.7 if effluent flow is doubled by the year 2000 and by 2.5 if ultimate plant capacity is

attained. These factors were estimated using 15 mg l^{-1} as an effluent NH_4^+-N content and 0.04 mg l^{-1} as a background river value in order to calculate mixed river-effluent concentrations. In doing so, the fractional decrease in river ammonium concentration resulting in the critical area value of 2.2 mg l^{-1} in 1979 due to sea water dilution and nitrification would be assumed to persist with high waste input. This assumption is inconsistent with the previous rationale regarding constant quantity removal by nitrification, but the procedure was used for convenience and in lieu of actual experimental observations demonstrating a constant rate per unit area accross a substrate concentration gradient.

Figure 26 shows worst case scenarios for phytoplankton bloom conditions in 1966 and 1975, during which maximum pH was 9 and 8.2 - 8.5, respectively. During 1966 the pH ranged between 9.0 and 8.3 for roughly half of the 24-hour period. The occurrence of a bloom is clearly the most important element determining toxicity now or under higher loading. Although blooms have not occurred in the past five or six years, failure to be certain of the cause for that decline necessitates anticipating their return for a worst case scenario. An intense plankton bloom with a doubling of effluent input would clearly result in lethal conditions especially for holding and resident fish in the critical area and possibly even for those taking several hours to traverse the area during the midday period if they lacked an avoidance tendency. While there could theoretically be some sublethal damage result without a bloom, the period of exposure for either holding fish or traversing fish is considered too short for the 0.016 mg l^{-1} chronic limit to be a pertinent criterion in this situation.

Although the 1981 observations represent only one 24-hour stationary survey at km 10.4, instream concentrations of unionized ammonia were predicted

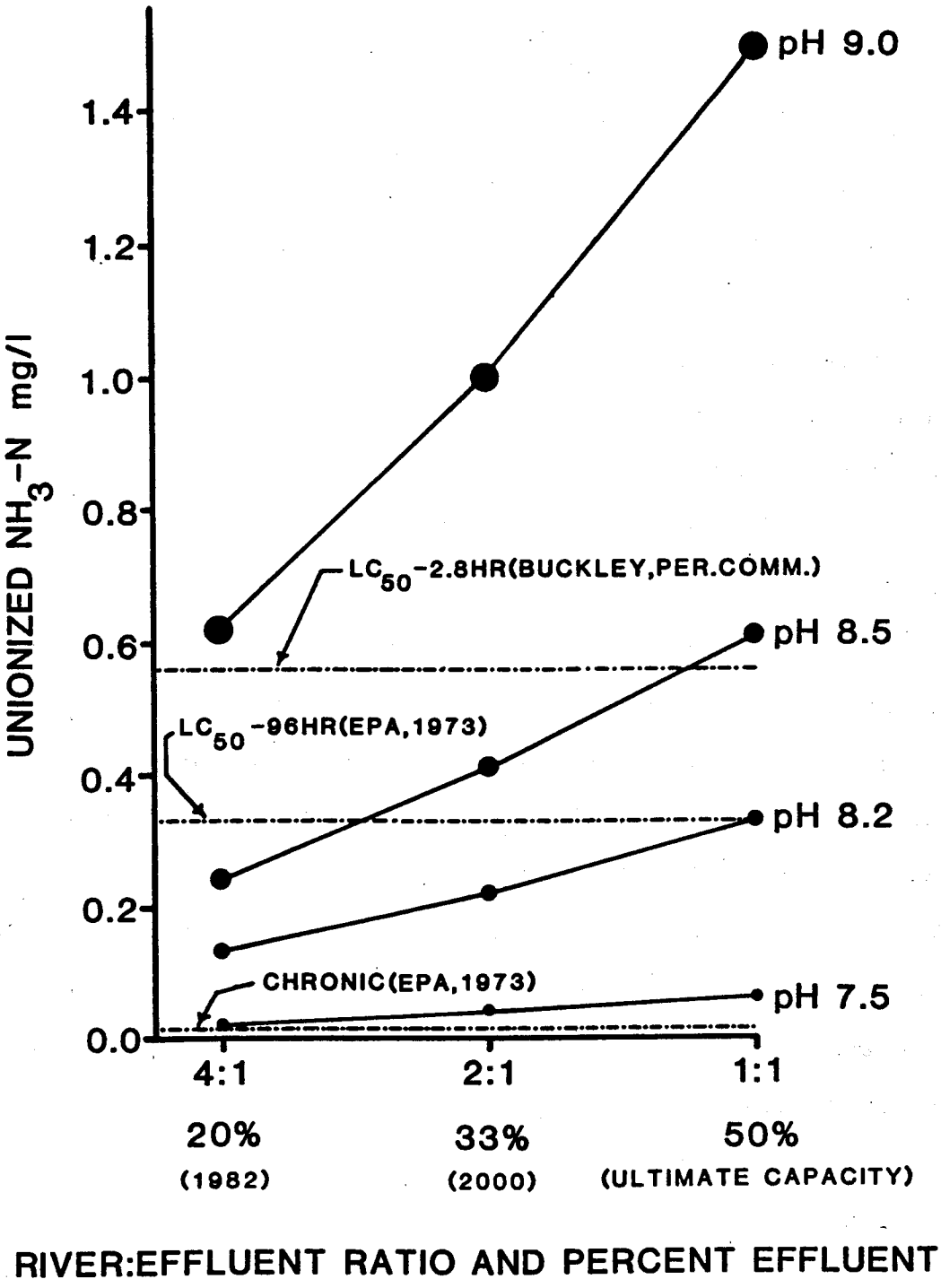

Figure 26. Projected low flow unionized ammonia concentrations at 20° C that could occur in the Duwamish River estuary and nominally exist from km 6 - 12 during phytoplankton blooms (which have persisted from one to two weeks with pH reaching a maximum of 9.0 in 1966 and 8.2 - 8.5 in 1975).

from that data set for various river discharges, temperatures, and pH levels (Table 7) to compare with 1979 results. From these calculations it can be seen that pH is by far the most critical parameter governing the concentration of unionized ammonia present. If the data from this survey are applied to low river discharge (6.3 m^3 sec^{-1}) and one assumes a corresponding increase in the concentration of ammonium then, at 20° C and above, the instream unionized ammonia concentration would exceed the criterion of 16 ug l^{-1} NH_3-N to protect against a chronic effect. That projected low-flow NH_4^+-N concentration (2420 µg l^{-1}) is slightly greater than the maximum observed in 1979 and used in Figure 26. At the same low flow and assuming the effluent discharge is increased to 6.3 m^3 sec^{-1} (RTP maximum design capacity) and temperature and pH remain as observed in the 1981 survey, the mean 24-hour concentration of unionized ammonia nitrogen at km 10.4 would be 30 µg l^{-1} (Table 7). (Correction for salinity was not made for these calculations since mean surface salinity during the survey was 1.3 $^{o}/_{oo}$.) Extrapolations to higher pH give values of unionized ammonia similar to those based on the 1979 data.

Table 7. Predicted unionized ammonia nitrogen (NH_3-N) concentration given various river discharge, pH, temperature, and RTP effluent discharge and based on instream measurements at km 10.0 on 10/15-16/81.

River Flow m^3 sec^{-1}	NH_4^+-N Instream $\mu g\ l^{-1}$	Instream pH	Instream Temp. °C	Percentage of NH_3 Present	Conc. of NH_3-N $\mu g\ l^{-1}$
21.2(1)	720(2)	7.2(3)	11(2)	.38	3
"	"	"	15	.48	3
"	"	"	20	.71	5
"	"	"	25	.99	7
"	"	8.0	11	2.12	15
"	"	"	15	2.65	19
"	"	"	20	3.83	28
"	"	"	25	5.20	37
"	"	8.5	11	6.40	46
"	"	"	15	7.98	57
"	"	"	20	11.18	80
"	"	"	25	14.90	107
"	"	9.0	11	17.78	128
"	"	"	15	21.42	154
"	"	"	20	28.47	205
"	"	"	25	35.76	257
6.3	2,420(5)	7.2(3)	11	.38	9
"	"	"	15	.48	12
"	"	"	20	.71	17
"	"	"	25	.99	24
"	"	8.0	11	2.12	51
"	"	"	15	2.65	64
"	"	"	20	3.83	93
"	"	"	25	5.20	126
"	"	8.5	11	6.40	155
"	"	"	15	7.98	193
"	"	"	20	11.18	271
"	"	"	25	14.90	361
"	"	9.0	11	17.78	431
"	"	"	15	21.42	519
"	"	"	20	28.47	690
"	"	"	25	35.76	866
21.2	2,670(6)	7.2	11	.34	9
6.3	8,970(6)	"	"	.34	30

(1) Initial instream mean flow (10/15-16/81)

(2) Mean 24-hour surface value

(3) Median 24-hour surface value

(4) Based on Trussel (1972)

(5) Concentration if river flow reduced to 6.3 m^3 sec^{-1}

(6) Concentration if effluent flow increased to 6.3 m^{-3} sec^{-1}

SUMMARY AND CONCLUSIONS

The purpose of this research was to determine the rate and location of the nitrification process in the Duwamish River estuary and the degree to which that process contributes to a decrease in ammonium discharged from the Renton Treatment Plant and thereby reduces the potential for ammonia toxicity to migrating salmonids. The prinicpal findings were as follows:

1.) Nitrifying bacteria were primarily associated with sediments rather than the overlying water. Nitrifier MPNs in sediment from river km 21.0-13.2 were higher than levels in samples from the overlying water column by from one to two orders of magnitude. From nitrifier MPN levels and time of travel estimates, significant nitrification in the Duwamish apparently occurs only between km 20.5 and about km 10.0.

2.) The nitrification rate between 19.2 and 13.3 km was best represented by a zero order, sediment based process and was thus expressed on an areal basis. The rate was determined from changes in surface $NO_2^-+NO_3^-$-N content over this reach at 19 C and expressed per unit of sediment surface as 970 mg m^{-2} day^{-1}. Projecting that rate over the reach from km 20.5 to 9.5 indicates that 13 percent of the non-dilution decrease in ammonium concentration could be accounted for by nitrification while a decrease of 19 percent was observed. Because nitrification in the Duwamish is best described using a zero order rate and is primarily associated with the sediment an increase in ammonium concentration would not be expected to increase the nitrogenous oxygen demand in the upper estuary. Nitrification, and thereby oxygen removal, depends upon the contact of ammonium and

oxygen in the overlying water with organisms in the surficial sediments and not on the ammonium content of the water and nitrifier growth therein.

3.) Ammonium content in the surficial layer (0-3 cm) of sediments cores was from 3 to 73 times greater than the overlying water in the lower estuary (km 10.0, 7.7, and 5.6). The sediment water gradient was reversed in the upper estuary (km 13.2 to 17.9) where ammonium content in the water was 1.2 to 8.7 times greater than that in the surficial sediment pore water. Therefore, release of ammonium from sediments to overlying water would be expected only in the lower estuary. Sediments collected from km 10.0 released ammonium to overlying water in vitro at a rate of 3.5 to 31.1 mg m^{-2} day, prior to the onset of nitrification.

4.) Nitrification rates in vitro decreased when sediments collected from the lower estuary (km 10.0) were exposed to overlying water low in salinity (4 $^{o}/oo$) compared to water high in salinity (20 $^{o}/oo$). This observation agreed with results of culture experiments in which nitrifying bacteria isolated from sediments upstream from km 10.0 were inhibited (lower MPN) by high salinity media while growth of those isolated from sediment at km 10.0, a normally high saline area, was enhanced by high salinity media. Nitrifier populations obviously adapt, within limits, to the salinity ranges encountered in the estuary. Nevertheless, the extreme range in salinity encountered in the lower estuary, together with the intermittent contact of high ammonium water in the surface, low salinity layer with sediments, no doubt greatly minimize nitrification and thus ammonium removal from the water column in the lower estuary.

5.) A threat of ammonia toxicity was not present at ammonium concentrations and pH levels observed during this study. However, with increased effluent discharge approaching the RTP ultimate capacity of 6.3 m^3 sec^{-1}, and even with the expected discharge by 2000 (3.1 m^3 sec^{-1}), a definite threat will exist, but only if phytoplankton bloom activity results in pH levels approaching 8.5. In fact, the toxicity threat is so dependent on the phytoplankton caused pH rise that a bloom raising pH to 8.5 at the current effluent discharge (1.6 m^3 sec^{-1}) rate would result in low flow, unionized ammonia-N concentrations of near 300 µg l^{-1} in the critical reach (km 6 to 12) during some fraction of the day. This approaches the 96-hour LC_{50} for salmonids. The 2.8-hour LC_{50} (560 µg l^{-1}) would be reached in a bloom such as occurred in 1966 when pH rose to 9.0. However, a pH averaging about 8.2 would have existed for about onethird of the diurnal cycle during that bloom, thus resulting in only marginally lethal conditions for migrating fish. Doubling the discharge by the year 2000 together with a bloom raising the pH to 8.5 would definitely produce lethal conditions for short-term exposure (hours) in the 6 - 12 km reach. Extrapolating from existing ammonium concentrations in the critical reach and effluent increases also assumes proportional increases in ammonium loss through nitrification (now 13 percent) which probably would not occur because the rate is not considered ammonium concentration dependent but is limited by sediment surface contact. Therefore, this scenario probably underestimates toxicity at higher effluent discharges.

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APPENDIX A

Physical and Chemical Parameters Recorded for Diurnal Time of Travel Survey of September 2-3, 1981 on the Duwamish River Estuary.

Hour	Depth (m)	Sp. Conductance at 25° C	Temp. (°C)	pH	D.O. ($mg\ \ell^{-1}$)	Kjehldahl-N ($\mu g\ \ell^{-1}$)	NO_2+NO_3-N ($\mu g\ \ell^{-1}$)	NH_4^+-N ($\mu g\ \ell^{-1}$)
0	Surf.	197	18.3	7.45	9.3	178	424	21
.75	Surf.	367	18.3	6.50	---	3,450	322	2,220
1	Surf.	347	19.1	6.35	8.1	6,060	586	4,990
1	2	317	19.1	6.35	8.5	4,330	298	5,160
2	Surf.	312	18.3	----	8.3	3,700	370	2,110
2	2	329	18.3	6.80	8.7	3,900	174	3,260
3	Surf.	346	19.2	6.70	8.1	5,940	370	3,700
3	2	346	19.2	6.75	7.9	6,140	328	4,300
4	Surf.	329	18.7	6.75	7.7	3,980	418	3,100
4	2	328	18.7	6.70	7.6	5,050	418	3,860
5	Surf.	307	18.3	6.75	7.2	3,360	480	2,550
5	2	282	18.3	6.75	7.1	3,390	508	2,490
6	Surf.	417	17.5	6.75	7.1	2,300	544	1,560
6	2	484	17.5	7.15	---	---	754	1,340
7	Surf.	778	17.5	6.75	6.6	2,430	550	1,600
7	2	856	18.7	6.75	---	1,890	568	1,560
8	Surf.	985	18.0	6.80	6.6	2,330	580	1,600
8	2	10,900	17.8	6.65	---	1,900	466	993
9	Surf.	1,070	17.5	7.15	6.6	2,090	---	1,320
9	2	21,800	17.1	6.70	---	1,260	---	627
10	Surf.	1,250	17.5	6.90	6.5	2,240	580	1,400
10	2	24,900	16.8	----	---	1,010	280	398
11	Surf.	3,630	16.8	----	6.2	2,070	436	1,250
11	2	32,500	16.1	----	---	816	243	317
12	Surf.	4,010	17.2	----	6.4	1,930	431	1,100
12	2	34,300	14.9	----	---	764	436	478
13	Surf.	6,550	17.5	----	6.4	1,800	406	1,120
13	2	36,700	15.8	----	---	870	472	694
14	Surf.	10,100	18.8	----	6.1	1,750	556	1,280
14	2	38,300	15.7	----	---	1,220	484	667

Hour	Depth (m)	Sp. Conductance at 25° C	Temp. (°C)	pH	D.O. (mg ℓ^{-1})	Kjehldahl-N ($\mu g\ \ell^{-1}$)	NO_2+NO_3-N ($\mu g\ \ell^{-1}$)	NH_4^+-N ($\mu g\ \ell^{-1}$)
15	1	20,200	18.4	----	---	1,020	466	505
15	B + 1 (5.5)	44,300	16.1	----	---	331	280	195
16	1	17,100	22.6	----	---	1,130	550	1,062
16	B + 1 (5.0)	40,300	21.5	----	6.7	464	292	128
17	1	20,700	23.5	----	---	718	370	938
17	B + 1 (6.0)	41,300	20.5	----	7.3	175	289	74
18	1	12,600	23.0	----	---	700	571	774
18	B + 1 (6.0)	43,500	17.8	----	6.9	201	298	34
19	1	17,200	21.0	----	---	1,050	592	1,050
19	B + 1 (5.6)	44,300	17.0	----	---	464	280	47
20	1	15,600	22.7	----	---	1,150	550	1,230
20	B + 1 (6.1)	43,700	18.0	----	---	464	271	74
21	1	12,800	24.2	----	---	1,130	255	1,170
21	B + 1 (7.0)	44,200	17.7	----	---	420	234	74
22	1	14,100	23.5	----	---	937	538	993
22	B + 1 (7.0)	44,300	17.5	----	---	166	268	47
23	1	14,700	22.8	----	---	1,150	523	1,060
23	B + 1 (7.0)	44,000	18.0	----	---	378	295	101

APPENDIX B

Physical and Chemical Parameters Recorded for Stationary Diuranal Survey of October 15-16, 1981, on the Duwamish River Estuary.

Hour	Depth (m)	Sp. Conductance at 25° C µmhos	Temp. (°C)	pH	D.O. ($mg\ \ell^{-1}$)	Kjeldahl-N ($\mu g\ \ell^{-1}$)	$NO_2^- + NO_3^-$-N ($\mu g\ \ell^{-1}$)	NH_4^+-N ($\mu g\ \ell^{-1}$)
0	Surf.	882	10.2	7.09	9.1	1,270	419	914
0	2	883	10.1	6.95	---	1,160	503	979
1	Surf.	230	10.0	7.14	9.6	---	446	764
1	2	250	10.0	7.10	---	937	459	775
2	Surf.	234	10.2	7.10	9.6	972	449	700
2	2	275	10.1	7.09	---	1,000	459	732
3	Surf.	279	11.3	7.14	9.8	922	352	512
3	2	450	10.5	7.14	---	917	1,310	667
4	Surf.	374	11.5	7.30	9.7	1,010	469	689
4	2	13,200	11.0	6.90	---	781	436	710
5	Surf.	835	11.2	7.30	9.4	1,030	446	721
5	2	21,700	12.0	7.30	---	1,000	429	689
5	B + 1 (4.5)	38,900	13.0	7.30	---	665	322	281
6	Surf.	2,180	11.8	7.30	8.9	1,070	449	743
6	2	28,200	13.0	7.30	---	716	443	635
6	B + 1 (5.1)	39,600	13.6	7.30	---	308	4,897	126
7	Surf.	2,480	11.9	7.40	9.7	1,130	439	708
7	2	35,600	13.2	7.37	---	580	345	312
7	B + 1 (5.0)	40,300	13.6	7.40	---	453	251	138
8	Surf.	3,230	11.5	7.32	8.9	982	530	604
8	2	22,800	12.8	7.40	---	1,110	217	534
8	B + 1 (4.0)	40,000	13.7	7.40	---	570	164	138
9	Surf.	6,080	12.0	7.25	9.0	655	728	522
9	2	10,200	12.4	7.30	---	771	453	569
9	B + 1 (4.0)	39,600	13.5	7.35	---	359	338	336
10	Surf.	6,060	12.2	7.20	8.8	907	348	371
10	2	9,690	12.1	7.20	---	746	840	435
11	Surf.	891	10.8	7.23	9.3	1,270	456	778

Hour	Depth (m)	Sp. Conductance at 25° C µmhos	Temp. (°C)	pH	D.O. ($mg\ \ell^{-1}$)	Kjeldahl-N ($\mu g\ \ell^{-1}$)	$NO_2^- + NO_3^-$-N ($\mu g\ \ell^{-1}$)	NH_4^+-N ($\mu g\ \ell^{-1}$)
11	2	889	10.8	7.23	---	1,290	463	743
12	Surf.	300	10.5	7.23	9.7	1,380	443	906
12	2	355	10.5	7.24	---	1,300	537	860
13	Surf.	360	10.5	7.22	9.6	1,450	695	988
14	Surf.	381	10.4	7.15	9.5	1,100	500	743
15	Surf.	541	10.5	7.11	9.6	1,100	513	685
16	Surf.	401	10.4	7.07	9.7	1,110	506	697
16	2	897	10.5	7.07	---	1,100	503	691
17	Surf.	753	10.5	7.14	9.3	1,160	500	743
17	2	2,800	11.3	7.03	---	1,140	493	---
18	Surf.	1,350	11.2	7.13	---	1,200	480	778
18	2	8,680	12.2	6.99	---	989	338	697
19	Surf.	2,150	11.3	7.25	8.8	1,210	523	813
19	2	24,600	13.3	7.25	---	814	453	685
19	B + 1 (3.8)	31,300	14.5	7.40	---	394	258	114
20	Surf.	2,500	11.5	7.30	9.2	1,250	332	639
20	2	31,500	13.0	7.40	---	324	359	260
20	B + 1 (4.2)	33,000	14.2	7.40	---	333	318	149
21	Surf.	3,780	11.6	7.20	9.1	1,090	533	784
21	2	29,900	13.1	7.25	---	613	436	598
21	B + 1 (4.3)	40,300	13.6	7.45	---	359	311	246
22	Surf.	4,560	12.0	7.25	8.9	1,100	396	580
22	2	22,700	13.0	7.30	---	1,020	449	712
22	B + 1 (4.0)	33,100	13.7	7.40	---	420	311	208
23	Surf.	3,870	11.8	7.25	8.8	936	510	769
23	2	20,000	12.7	7.25	---	446	449	626
23	B + 1 (3.6)	39,600	14.1	7.35	---	289	332	310
24	Surf.	4,160	12.0	7.15	9.2	1,140	503	837
24	2	8,180	12.2	7.25	---	770	254	487
24	B + 1 (3.5)	39,300	14.4	7.30	---	446	345	336

APPENDIX C

$NO_2^-+NO_3^-$-N Occurring in Sediment Pore Waters of the Duwamish River Estuary, 1 October 1981.

Sample Station (river km)	Sediment Depth (cm)	$NO_2^-+NO_3^-$-N ($\mu g\ \ell^{-1}$)
5.6	0 - 3	678
5.6	0 - 3	291
5.6	3 - 6	592
5.6	3 - 6	1131
5.6	6 - 9	406
5.6	6 - 9	527
7.7	0 - 3	183
7.7	0 - 3	162
7.7	3 - 6	114
7.7	3 - 6	162
7.7	6 - 9	183
7.7	6 - 9	291
10.0	0 - 3	75
10.0	0 - 3	398
10.0	3 - 6	75
10.0	3 - 6	162
10.0	6 - 9	75
10.0	6 - 9	291
21.0	0 - 3	140
21.0	3 - 6	291
21.0	6 - 9	260