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TECHNICAL COMPLETION REPORT

Project Number UCAL-WRC-W-871

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University of California Water Resources Center

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ABSTRACT:

Quarterly monitoring of Upper Newport Bay, a highly eutrophic southern California estuary, has provided conflicting indicators of nutrient limitation for the seasonal macroalgal blooms in this system. Water column N:P ratios were high, up to 370:1, suggesting phosphorous limitation, while sediment N:P ratios were low, (<4:1), suggesting nitrogen limitation. A microcosm experiment was conducted to test whether macroalgal biomass was nitrogen or phosphorous limited in this system. Results indicate that even at high nutrient levels (300uM N/30uM P) macroalgal growth was nitrogen limited. Despite high N loading over the course of the experiment, water column N remained low as it was removed by the increasing algal biomass. The data also indicate that macroalgae used N fluxing from sediments for growth. Nitrogen accumulated in algal tissue while decreasing in microcosm sediments.

These results have important management implications. Winter releases of treated wastewater have been permitted to begin next year in Upper Newport under the assumption that they will not worsen blooms because macroalgae are not present at that time to utilize the increased nutrients. Our study suggests that this assumption is incorrect. This microcosm experiment shows that macroalgae are capable of utilizing sediment nutrient reserves to fuel growth. Our field monitoring data support this finding. Sediment nutrient levels in the bay peak after the spring rainy season, then decline, reaching a minimum in early winter. We hypothesized that this decline was due to utilization of sediment nutrients by the substantial macroalgal blooms (max. of 1.1 kg wet weight/ m2) that occur throughout summer and fall. The microcosm experiment supports this hypothesis and demonstrates that macroalgae are capable of using sediment stores of nutrients to fuel bloom events. These results indicate that winter releases of treated wastewater are likely to exacerbate bloom conditions in Upper Newport Bay by loading sediments and providing a larger source of nutrients to fuel the summer and fall macroalgal blooms.

Key Words: Eutrophication, Algae, Estuaries and estuarine modeling, Nitrate, Phosphorous, Biogeochemical cycling, Biomass, Water quality, Bays, Ecosystems and ecology.

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TITLE: Will Releasing Treated Wastewater Stimulate Algal Blooms in Southern California Estuaries?

INVESTIGATOR(S): Dr. Peggy Fong, Karleen Boyle, and Krista Kamer; Department of Organismic Biology, Ecology, and Evolution, UCLA

KEY WORDS: Eutrophication, Algae, Estuaries and estuarine modeling, Nitrate, Phosphorous, Biogeochemical cycling, Biomass, Water quality, Bays, Ecosystems and ecology.

PROBLEM AND RESEARCH OBJECTIVES:

The objective of this research project was to determine whether releases of treated wastewater will worsen "nuisance" blooms of macroalgae in southern California estuaries. Wet season (October-March) releases of tertiary treated wastewater have been proposed in Upper Newport Bay (UNB), Orange County, CA, one of the largest remaining wetlands in southern California. Upper Newport supports several endangered species and is one of the few marshes in this region with a vascular plant community dominated by the cordgrass *Spartina foliosa*. Upper Newport is characteristic of most southern California estuaries in that it is in close proximity to a heavily urbanized area, and is subject to frequent and intense macroalgal blooms. The primary

source of freshwater flow to this estuary is San Diego Creek, which drains a watershed area of 118 square miles. San Diego Creek and most of the tributaries feeding into it are channelized, and the majority of the watershed is heavily developed. As a result, freshwater flow from the creek is heavily influenced by nonpoint nutrient sources including urban runoff and golf course and nursery drainage. The macroalgal community of Upper Newport is characterized by blooms of the macroalgae *Ulva expansa* and *Enteromorpha intestinalis* which typically begin in June and persist through November. At their height, these blooms cause episodes of water column hypoxia and, occasionally dissolved oxygen minima which may kill fish and invertebrates. Blooms are also aesthetically displeasing to human users of the Bay.

The objective of this work was to couple quarterly field monitoring of conditions in the Bay with a controlled microcosm experiment to increase our understanding of interactions between nutrient loads and macroalgal bloom dynamics in this and similar systems in the region. Quarterly field monitoring allowed us to quantify the components of macroalgal blooms as they occurred in the estuary, while our microcosm experiment allowed us to isolate and test the effects of specific factors affecting these blooms, such as nutrient supply. Our microcosm experiment investigated whether nitrogen or phosphorous was the limiting nutrient for macroalgal growth in Upper Newport Bay.

METHODOLOGY:

Quarterly Field Monitoring

Field sampling was conducted in Upper Newport Bay, Orange County, California. Quarterly sampling began in December 1996, and continued through December 1997. Eight paired sampling stations were established ranging from the head to the middle of the bay (figure 1) and a stratified random sampling design was employed to survey them. At each station, two strata were sampled, one in the upper intertidal and one in the lower intertidal. Parameters measured were water column organic and inorganic nitrogen (N) and phosphorus (P), and salinity, sediment N and P, algal species % cover and biomass, and algal tissue N and P. One additional sampling station (transect 9) was established where San Diego Creek enters Upper Newport Bay. Only water column data were taken at this location.

Experimental Microcosms

All microcosms were assembled using biological materials collected from Upper Newport Bay. Each experimental unit contained estuarine sediment, the macroalgae *Enteromorpha intestinalis* and *Ulva expansa* , and the gastropod *Cerithidea californica* which occurs in high densities in California estuaries and may facilitate algal growth via transfer of N from sediments to the water column (Fong et.al., 1997).

Sediment cores were collected in acrylic cylinders 25cm x 25cm. Cores were taken from the bank of a tidal creek at low tide while the sediment was emmersed. The acrylic cylinders were pushed into the bank to a depth of 8cm, and intact cores were removed using shovels. After collection, the cores remained in these cylinders and were transported to the laboratory where a flat acrylic base was attached to the cylinder and sealed with silicone aquarium sealant. After a four hour drying period, the sealed cores were placed outdoors in a water bath which maintained

their temperature between 21 and 25 degrees C for the duration of the experiment. At this time, 3.5 L of seawater including the experimental nutrient doses was added to each microcosm along with 10 g each of *Ulva expansa* and *Enteromorpha intestinalis*. The seawater used in all experimental solutions, and all macroalgae and snails used in the experiment were collected from Upper Newport Bay, station 1. Initial samples of seawater, algal tissue, and sediment were collected at the field site for comparison with later microcosm data. Finally, 40 individuals of *Cerithidea californica* were added to each experimental unit. The appropriate number of *Cerithidea* for experimental additions was determined by conducting a field count of individuals at the study site. Microcosm densities were then set based on the average number of snails observed in the same area in the field. Throughout the experiment the microcosms were screened with one layer of fiberglass window screening to prevent snail escape. A 30% light reduction resulted from this screening. Because *Enteromorpha* and *Ulva* have relatively low light saturations (300 $\mu E/m^2/sec$) , this light reduction did not significantly affect macroalgal growth.

Experimental Treatments: Microcosms were randomly assigned to four treatment groups: control, nitrogen enriched, phosphorous enriched, and nitrogen and phosphorous enriched. Solutions were made by adding certified A.C.S. sodium nitrate and sodium phosphate, monobasic to seawater collected from Upper Newport Bay. Fresh solutions were made weekly and each batch was analyzed to determine nutrient content. The actual treatment levels achieved after nutrient addition were as follows:

Control = Water from Upper Newport Bay

3.9 uM NO^3 (se = 1.5)

3.7 uM PO^4 (se = 0.84)

$+NO^3$ = 210 uM NO^3 (se = 25.5)

2.3uM PO^4 (se = 0.35)

$+PO^4$ = 3.9 uM NO^3 (se = 1.44)

33 uM PO^4 (se = 1.5)

$+NO^3 + PO^4$ = 270 uM NO^3 (se = 25.5)

29 uM PO^4 (se = 1.4)

These values reflect the average of four solutions, one per week, for each treatment level over the course of the experiment.

Five replicates were run per treatment group. All treatments were flushed at a rate of 10% per day for the duration of the experiment. Flushing consisted of gently stirring each microcosm to break up any stratification, removing 350 mls of water from each unit, replacing it with 350 mls of the appropriate treatment solution, and stirring again to thoroughly mix it. Care was taken not to disturb the sediment surface at all points during solution removals and addition.

Experimental Variables: Algal biomass was sampled weekly during the four weeks of the experiment. All macroalgae present in a microcosm were sorted by species, excess water was

removed by spinning the tissue for one minute in a manual centrifuge, and each sample was weighed on an Ohaus balance. Sediment, water column, and algal tissue nutrients were sampled at the end of the experiment, Day 28. Sediment cores were removed from the center of each microcosm using a 10cc plastic syringe with the tip cut off to isolate and remove a core 5cm deep. Cores were frozen immediately after collection until they could be processed. They were later dried in a drying oven, and ground. Algal tissue was sorted by species, rinsed in distilled water to remove salts, dried in a drying oven, and ground. Water samples were filtered through a 0.45um glass fiber filter and frozen immediately after collection. Sediment samples were analyzed for TKN and Total P. Algal tissue was analyzed for %N total and %P total, and water samples were analyzed for nitrate, phosphate, total phosphorous, TKN, and ammonia.

PRINCIPAL FINDINGS AND SIGNIFICANCE:

Quarterly Field Monitoring:

Our field data indicate that Upper Newport is a highly eutrophic system. Salinity measurements indicate that storm-associated freshwater pulses persisted at all stations (figure 2) during the winter. The estuary is a marine embayment for the remainder of the year, with the exception of sampling stations nearest San Diego Creek which were subject to year round freshwater input.

Large amounts of nitrogen accompanied San Diego Creek's freshwater flow during all seasons (figure 3). Nitrate levels in the bay exceeded 700 μM in the region of freshwater influence near San Diego Creek, but decreased to 15 μM toward the middle of the bay. Nitrogen

levels were highest in spring, following seasonal winter rains. The nitrogen pulse associated with this freshwater was rapidly diluted and removed from the water column by the biotic community. NH_4 values were consistently below 25μM. Phosphorous levels were low relative to nitrogen, and were spatially and temporally variable (figure 4). A comparison of seasonal nitrogen to phosphorous ratios shows a large spring pulse of N which was not accompanied by P, and the selective removal of N (figure 5). These ratios suggest P limitation.

The seasonal pattern of sediment nutrients in Upper Newport Bay reflects nitrogen loading during winter rains. Sediment TKN increased significantly following the winter rainy season, then declined throughout the year (figure 6). We hypothesize that this nitrogen was utilized by the algal community. Sediments in the bay contain a large pool of phosphorous throughout the year (figure7). This large phosphorous pool results in low sediment N:P ratios (figure 8), suggesting nitrogen limitation for the system, in contrast to the measured water column N:P ratios which suggested phosphorous limitation.

Nutrient loading to Upper Newport Bay resulted in tremendous algal blooms. Although nutrient supply varied among seasons, algal tissue nitrogen was extremely high and variable throughout the estuary (figure 9). Algae may remove nitrogen directly from sediments during seasons when water column nitrogen is low. Algal biomass was extremely high during the summer and fall (figure 10). Blooms of this magnitude resulted from both the high nitrogen load from San Diego Creek and the phosphorous stored in the sediments.

The algal community of Upper Newport shifted over the course of the year from dominance of benthic cover by epibenthic diatoms to dense blooms of the green macroalgae

Ulva expansa and *Enteromorpha intestinalis*. During the winter season, diatoms dominated at all sampling stations (figure 11). In the spring, diatoms continued to form a large portion of the algal community but *Enteromorpha* increased in abundance (figure 12). During summer, blooms of *Enteromorpha* and *Ulva* dominated the community and a filamentous red algae (putatively *Ceramium* sp) becomes abundant (figure 13). *Ulva* and this filamentous red alga dominated the algal community during the fall, together often reaching or exceeding 100% cover (figure 14).

Microcosm Experiment:

Our data demonstrate that water column nitrogen concentration is not a good indicator of nitrogen supply to a system. Despite high nitrogen loading over the course of the experiment, water column N remained low (figure 15). At the conclusion of the experiment, no significant accumulation of nitrogen or phosphorous was detected in the water column, despite treatment additions. Water column TKN was elevated in experimental units given nitrogen additions. Water column PO^4 was elevated in microcosms treated with phosphorous, however there was no significant accumulation of TKN, Total P, or PO^4 in the water column. Water column levels of NH_4 and NO^3 were below detection limits .

Data from this experiment indicated that even at high nutrient levels (300 μM N / 30 μM P), macroalgal growth was nitrogen limited. Algae grew rapidly in all treatments. By the end of the experiment, there was a significant treatment effect of nitrogen on biomass ($p= 0.035$) (figure 16). We hypothesize that the algae did not become strongly nitrogen limited during the experiment because they used nitrogen fluxing from sediments for growth. Over the course of the experiment, nitrogen accumulated in algal tissue while decreasing in microcosm sediments (figure

17). All microcosms, regardless of treatment, showed a significant loss of sediment TKN from initial values after 4 weeks. Control treatments lost the most sediment nutrients. Sediment levels of total phosphorous also dropped significantly from initial values in all microcosms. Nitrogen accumulated in Ulva tissue, while phosphorous remained low and did not vary with treatment (figure 18). Our data demonstrate that macroalgae are capable of using sediment stores of nutrients to fuel growth.

CONCLUSIONS

Data from this study have important management implications for Upper Newport Bay. Our findings confirm that this estuary is highly eutrophic and must be managed carefully. Winter releases of 6.5 million gallons per day of tertiary treated wastewater to the estuary have been proposed under the assumption that they will not worsen blooms because macroalgae are not present at that time to utilize the increased nutrients. Our study suggests that this assumption is incorrect. The N vs P limitation microcosm experiment demonstrated that macroalgae are capable of utilizing sediment nutrient reserves to fuel bloom events. Our field monitoring data support this finding. Sediment nutrient levels in the bay peak after the spring rainy season, then decline, reaching a minimum in early winter. We hypothesize that this decline was due to utilization of sediment nutrients by the substantial macroalgal blooms reaching a maximum of 1.1 kg wet weight/m2 that occurred throughout summer and fall. These results indicate that winter releases of treated wastewater are likely to exacerbate bloom conditions in

Upper Newport Bay by loading sediments and providing a larger source of nutrients to fuel the summer and fall macroalgal blooms.

From the beginning of this project, our data have been made available to the Newport Beach Technical Advisory Committee (TAC), a panel composed of state and federal resource and regulatory agencies, private industry, scientists, and citizen representatives. Data from both the field sampling and experimental phase of this work are being employed by the EPA and Regional Water Quality Control Board in the development of regulations for the Total Maximum Daily Load (TMDL) for sediment and nutrients in the Newport Bay/San Diego Creek Watershed. Our data have encouraged permitting agencies to review their decision to allow the wastewater release in Upper Newport, and have provided a scientific basis for management decisions affecting this estuary.

SOURCES CONSULTED

Fong, P., J. Desmond, and J. Zedler. 1997. The effect of a horn snail on Ulva expansa (Chlorophyta): consumer or facilitator of growth? *Journal of Phycology* 33:353-359.

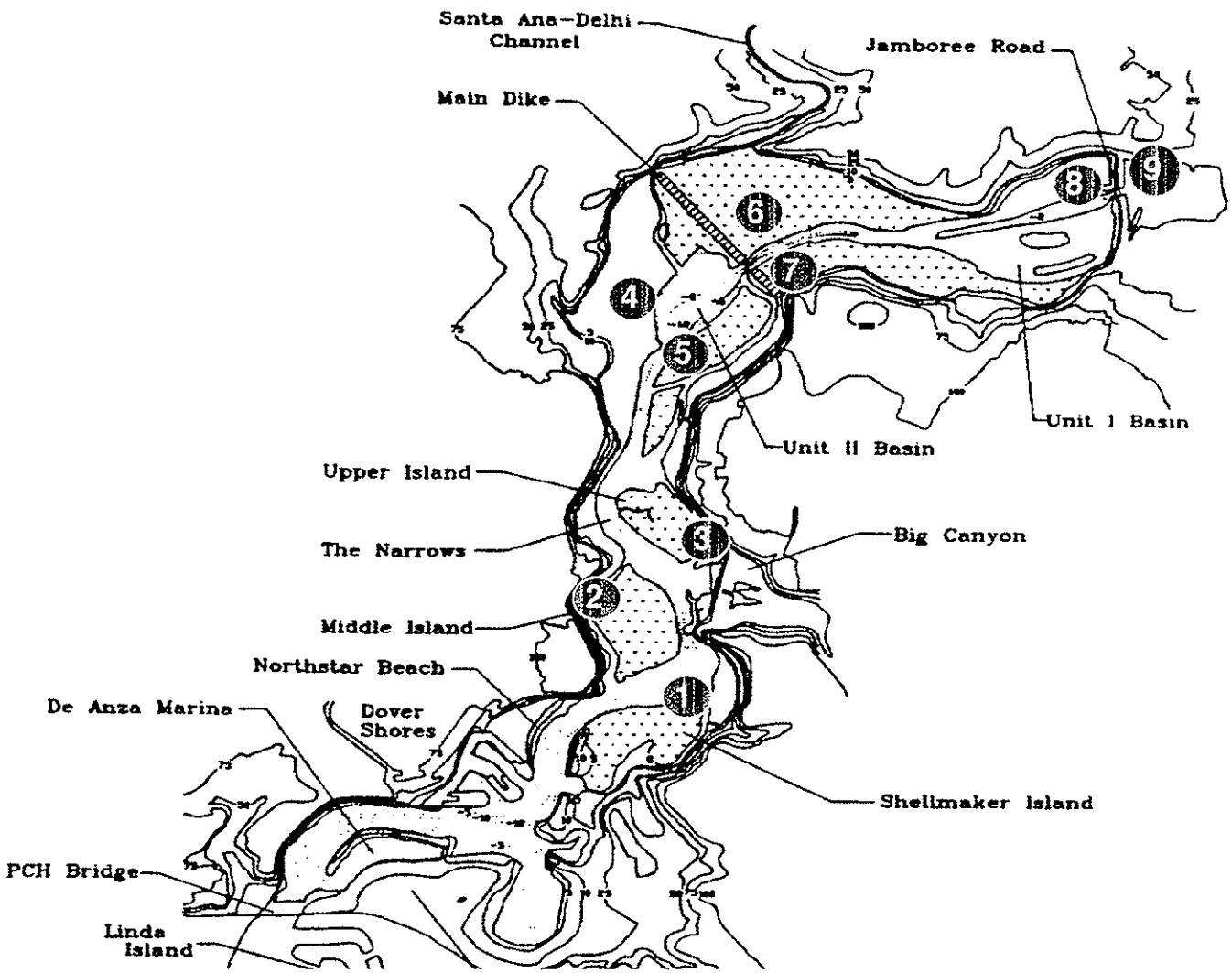

Figure 1. Map of sampling stations in Upper Newport Bay, CA. Data on algal biomass, percent cover, sediment nutrients, algal tissue nutrients, and water column nutrients were collected at sites 1-8. Only water column nutrient data were collected at site 9.

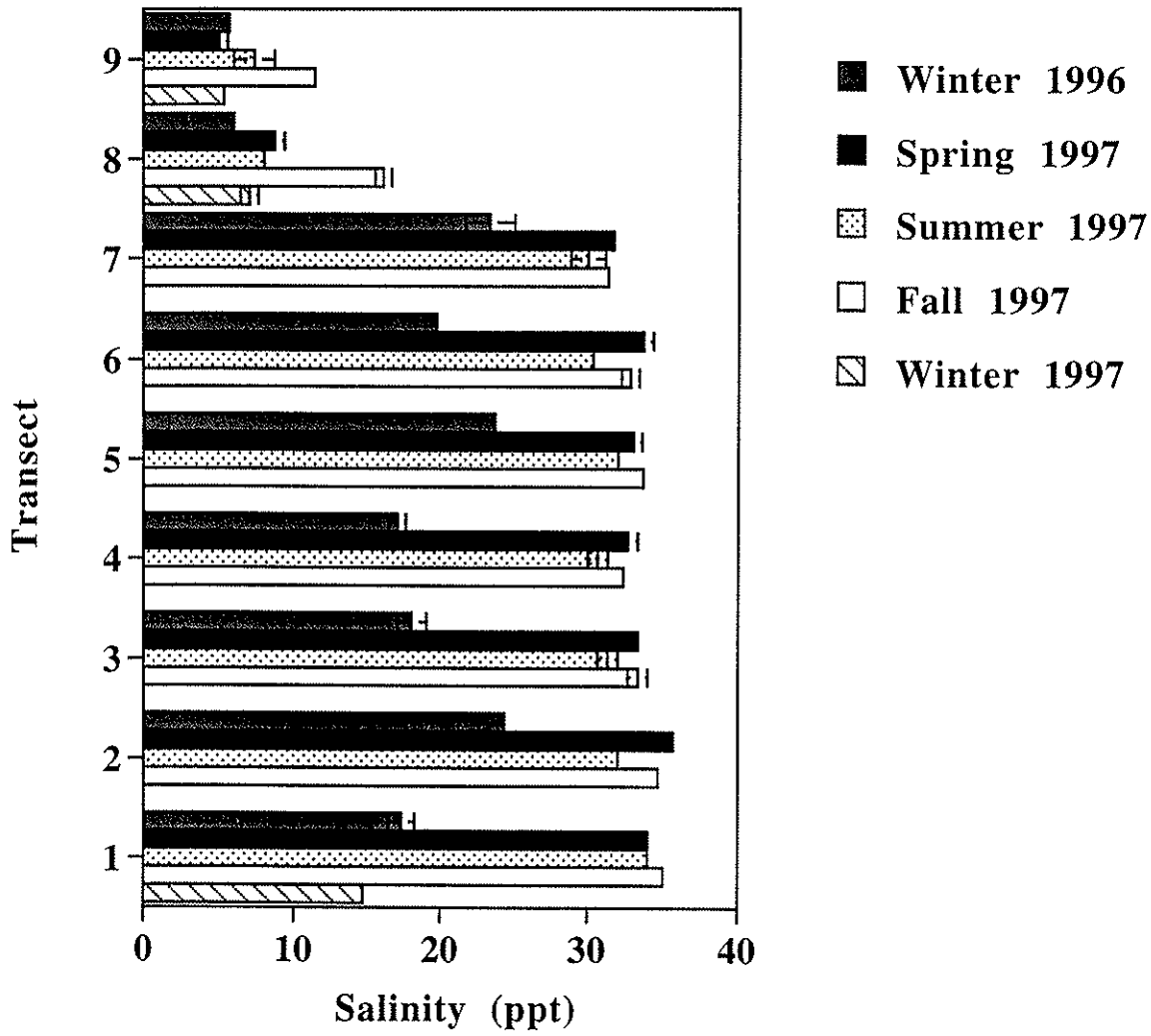

Figure 2. Seasonal variations in salinity at samping sites in Upper Newport Bay. Transect nine is closest to San Diego Creek, transect one is closest to the ocean.

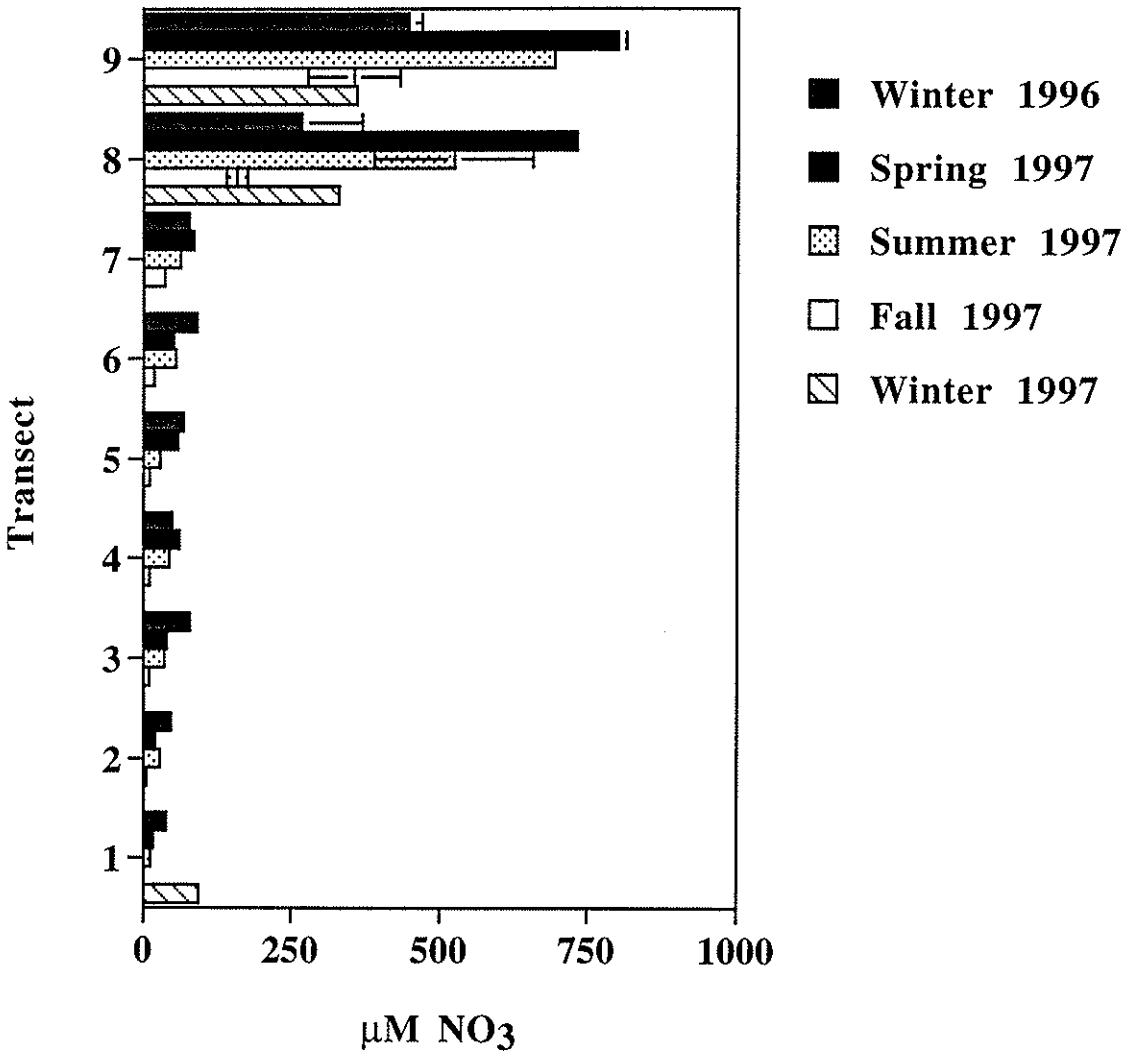

Figure 3. Seasonal variations in water column nitrate levels in Upper Newport Bay.

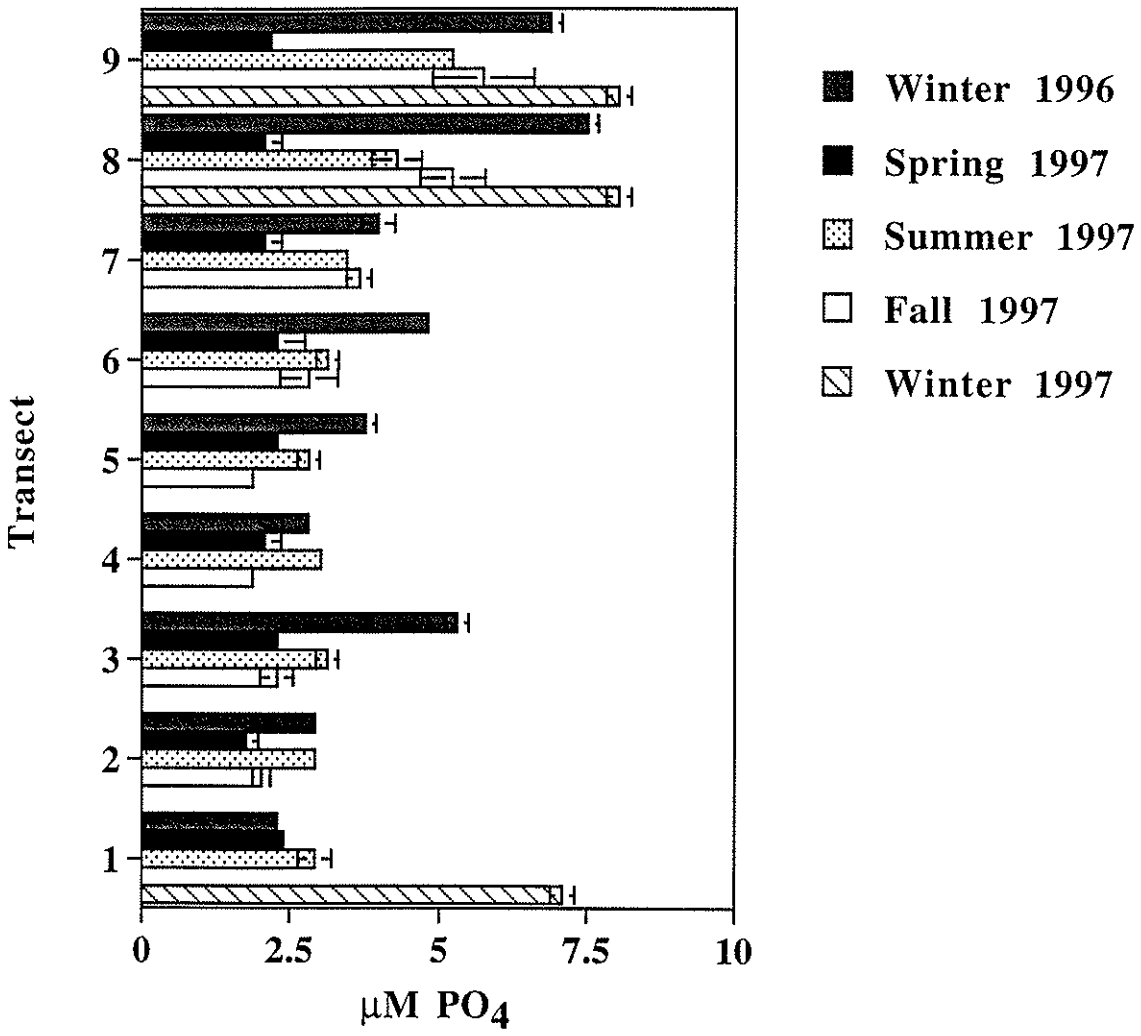

Figure 4. Seasonal variations in water column phosphate in Upper Newport Bay.

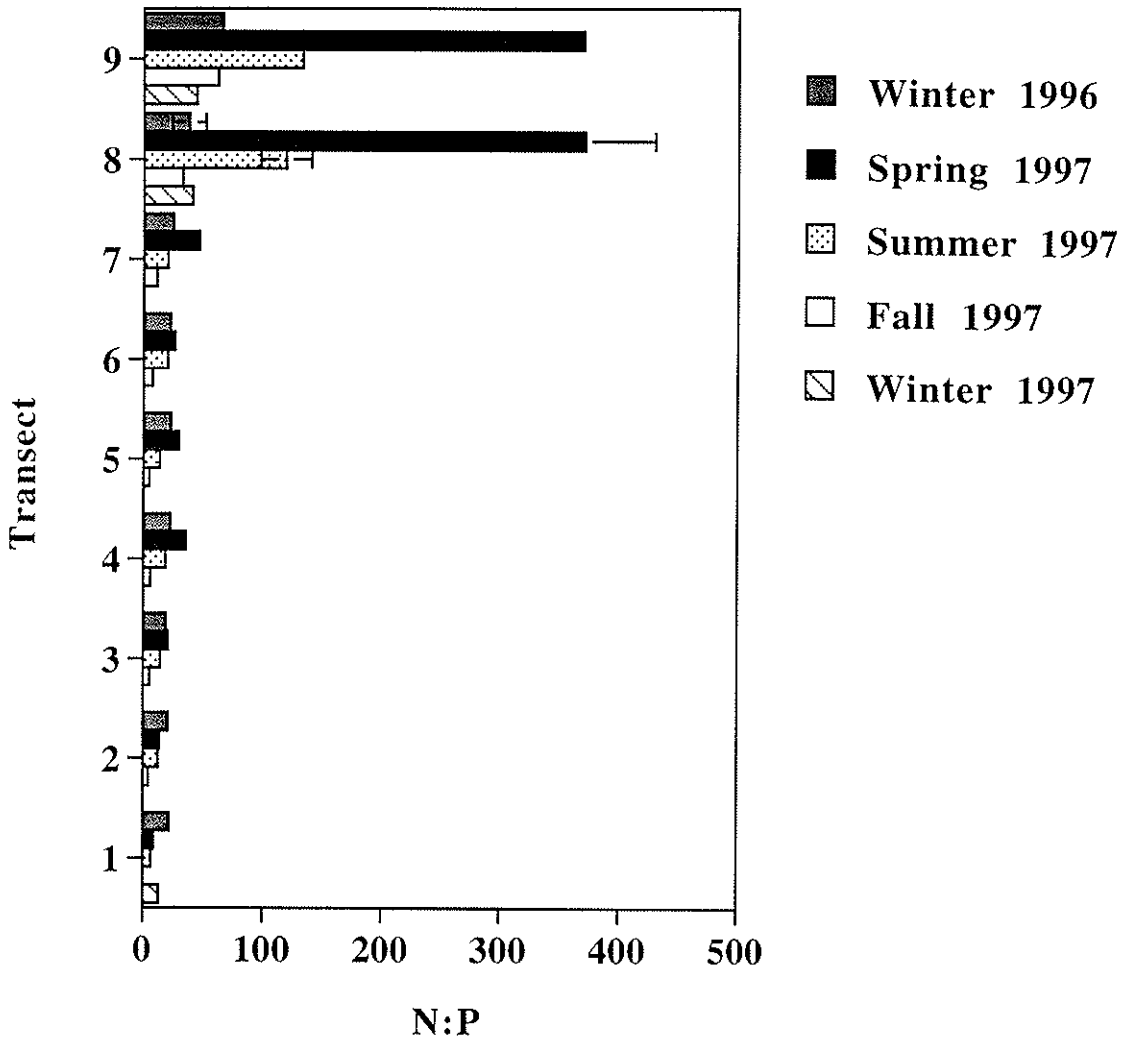

Figure 5. Seasonal variations in water column nitrogen to phosphorous ratios in Upper Newport Bay.

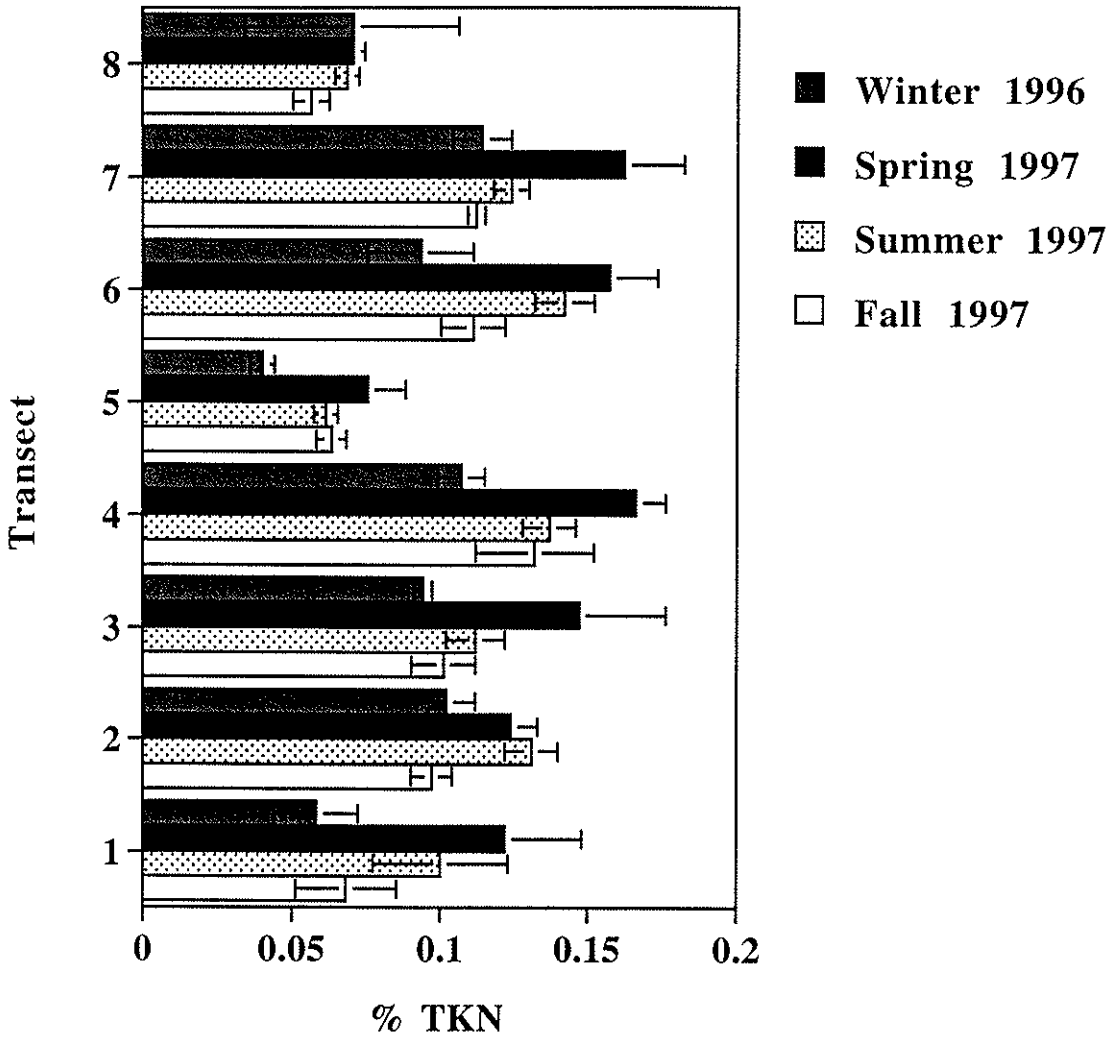

Figure 6. Seasonal variations in sediment levels of Total Kjeldahl Nitrogen (TKN) at sampling stations in Upper Newport Bay.

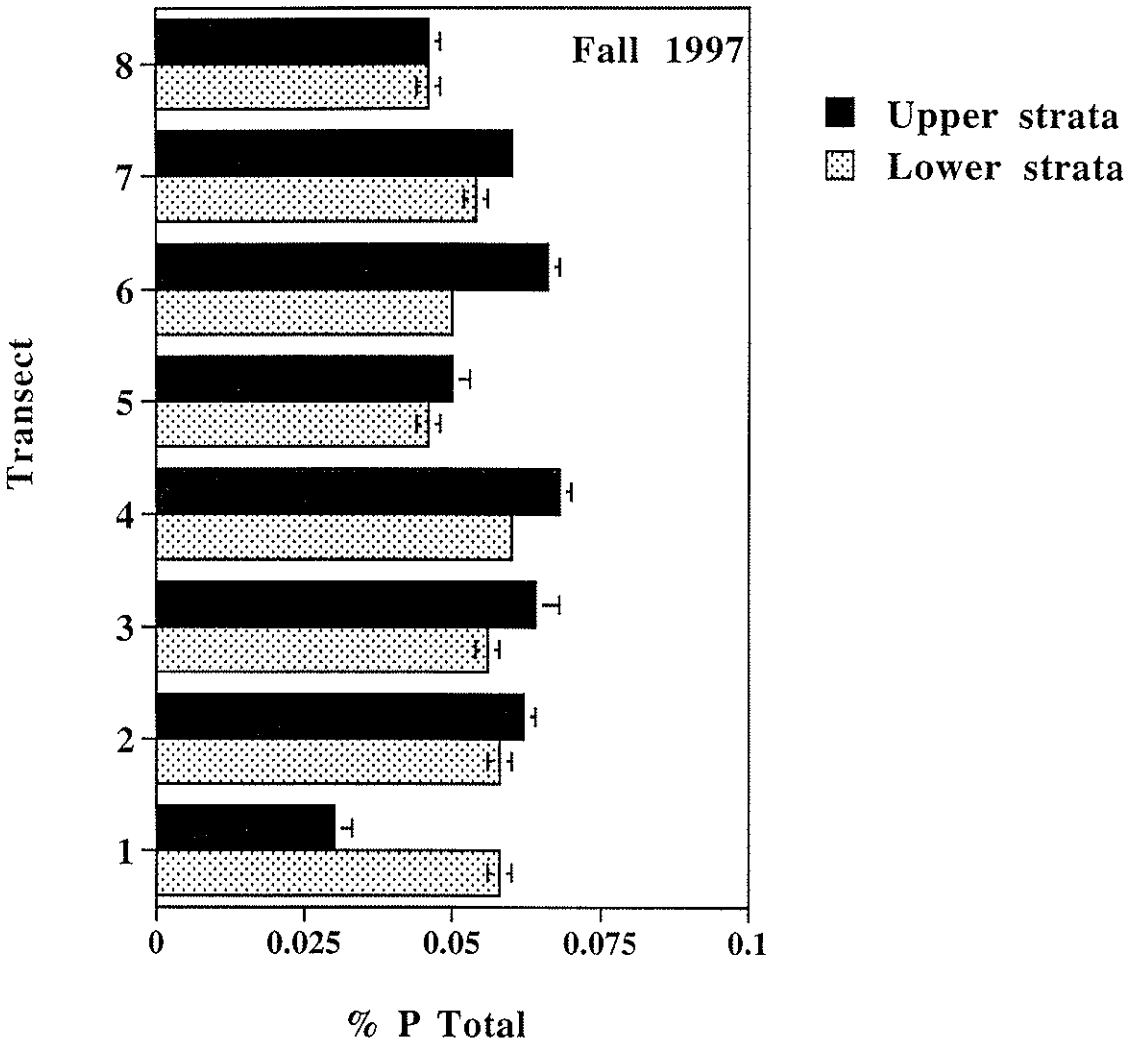

Figure 7. Sediment levels of total phosphate during Fall 1997. A single representative season is shown because no significant seasonal variation was observed in sediment phosphate levels.

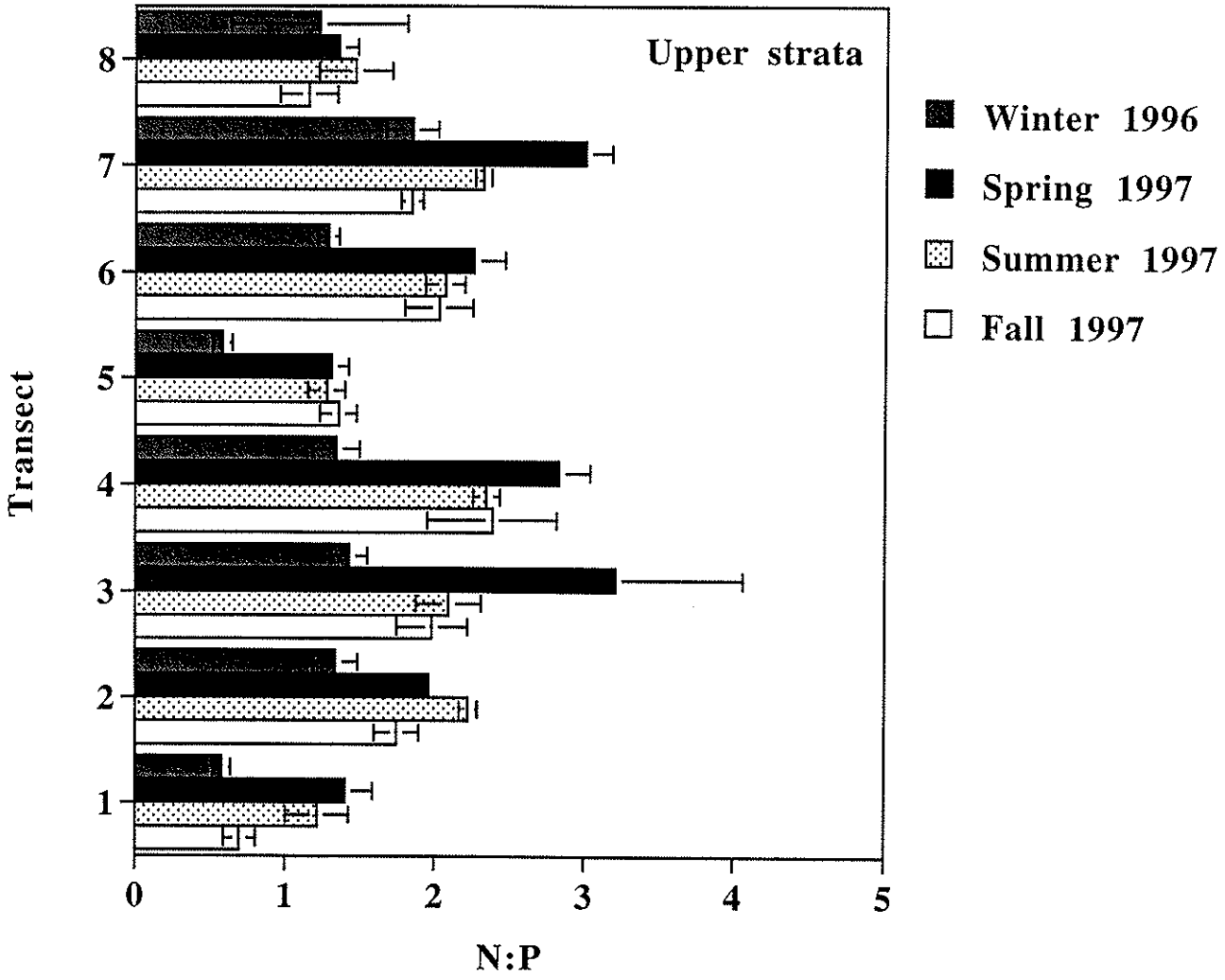

Figure 8. Seasonal variation in sediment nitrogen to phosphorous ratios in Upper Newport Bay.

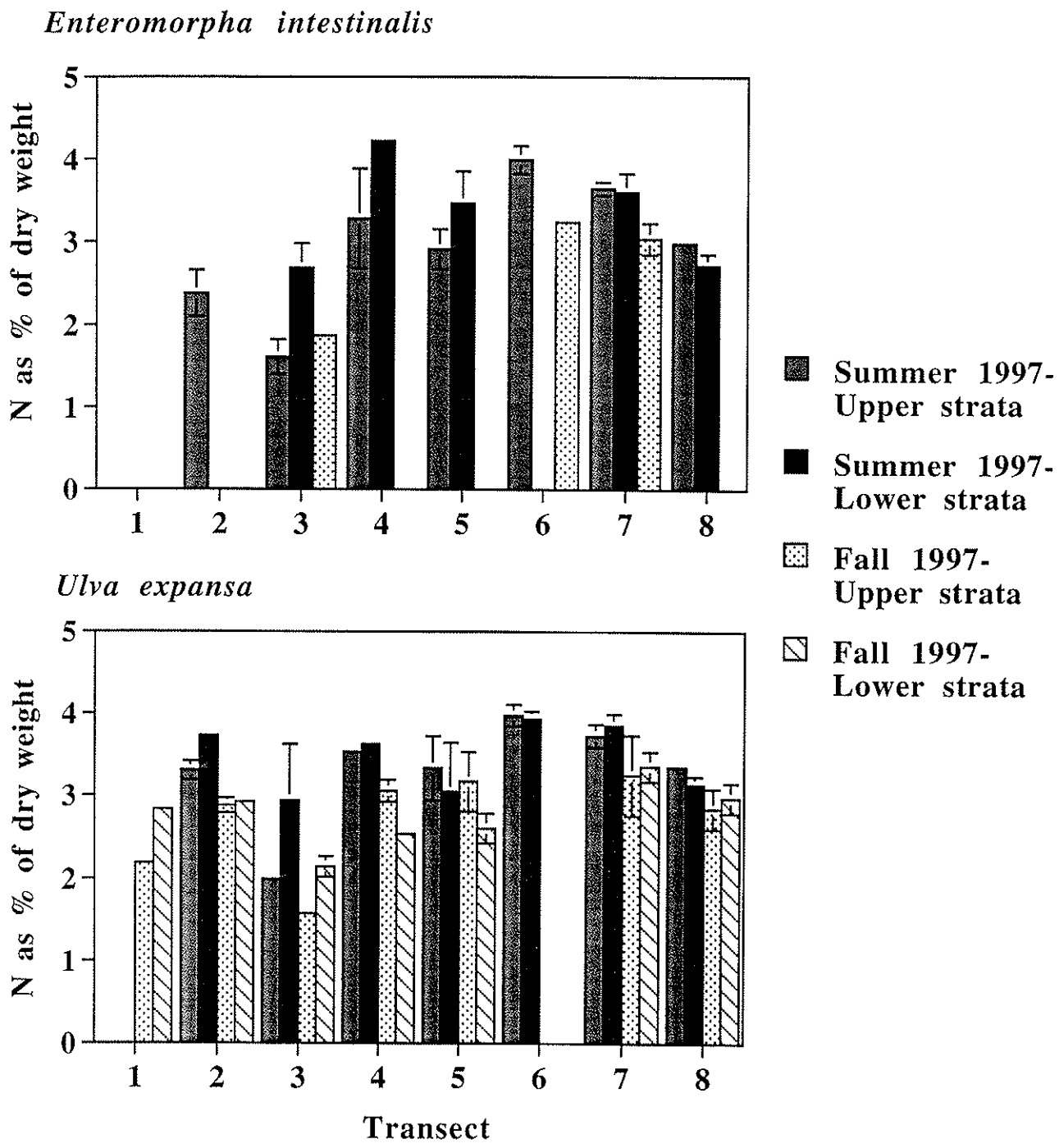

Figure 9. Macroalgal tissue nitrogen levels during summer and fall 1997 in Upper Newport Bay.

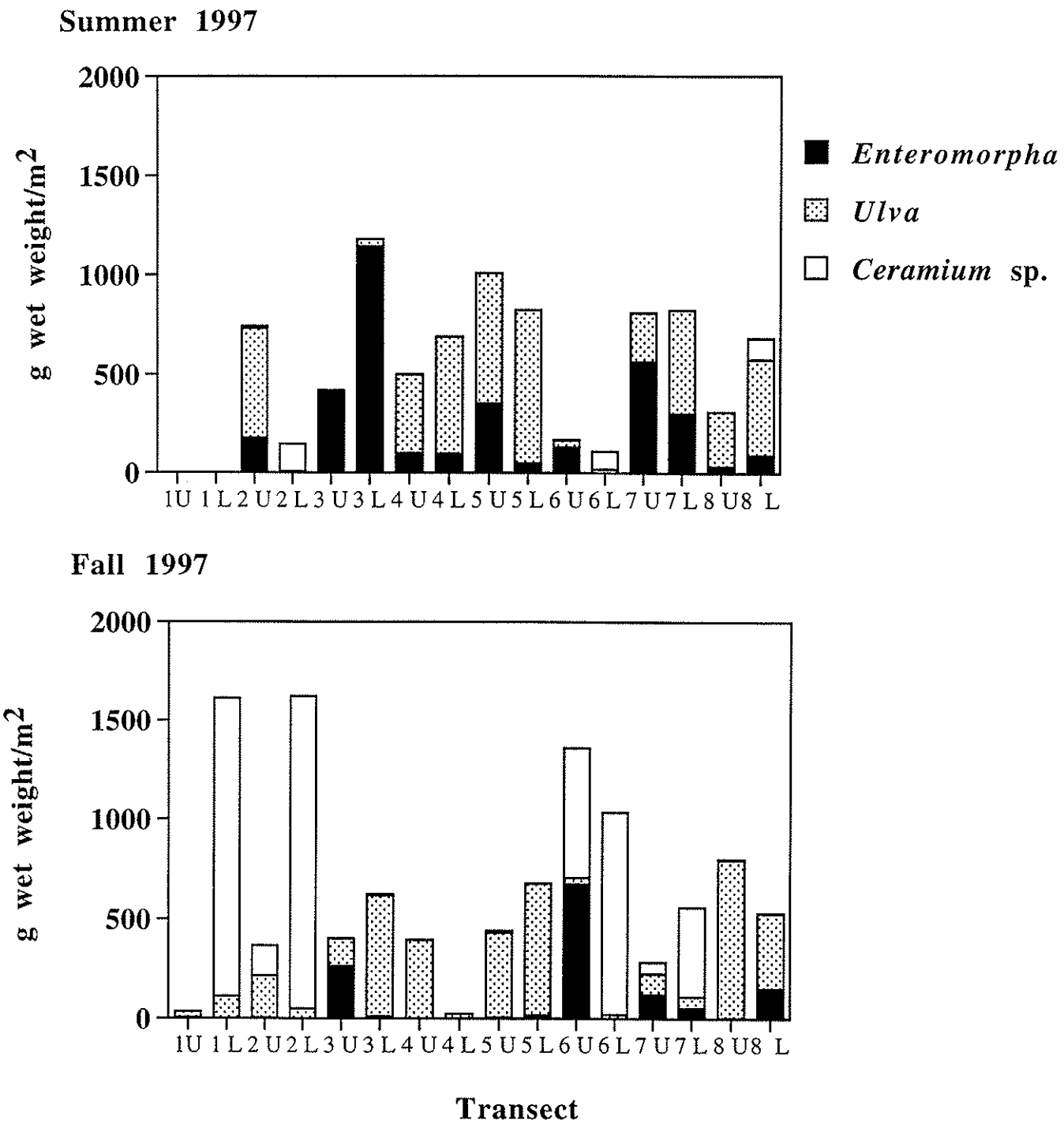

Figure 10. Macroalgal biomass in Upper Newport Bay during summer and fall of 1997.

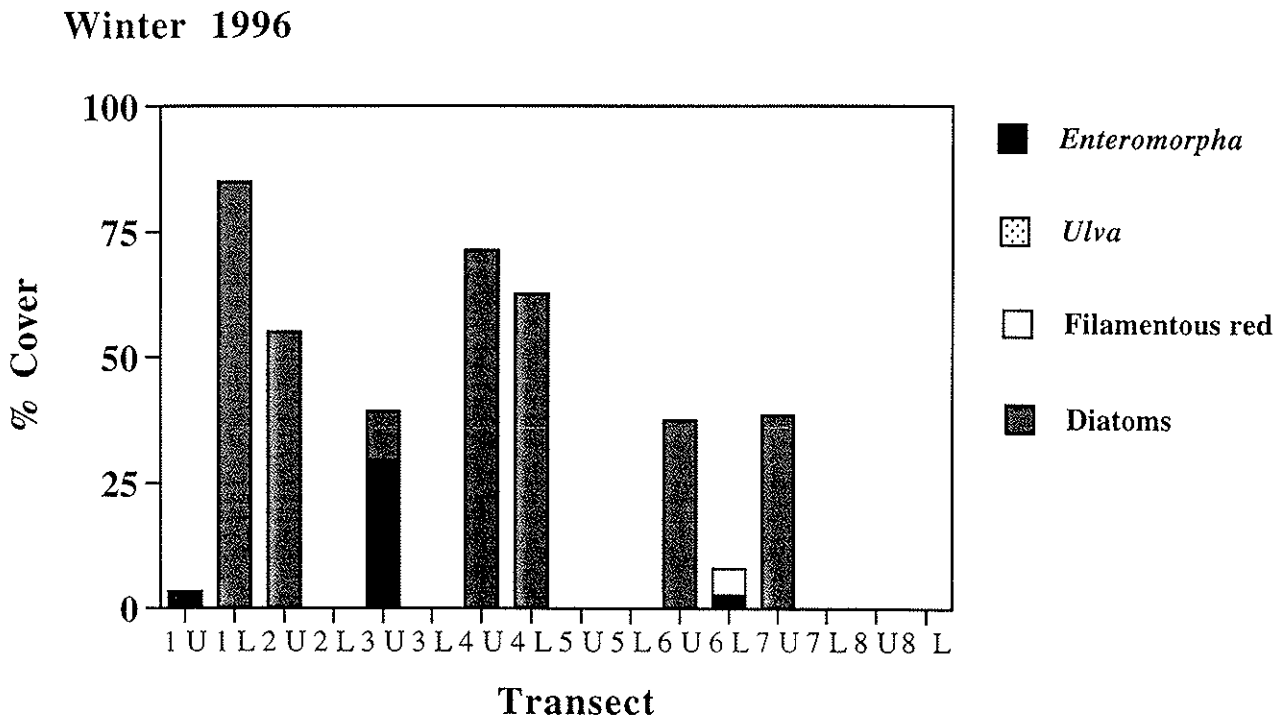

Figure 11. Algal community composition in Upper Newport Bay during winter 1996. Both upper (U) and lower (L) transects are shown.

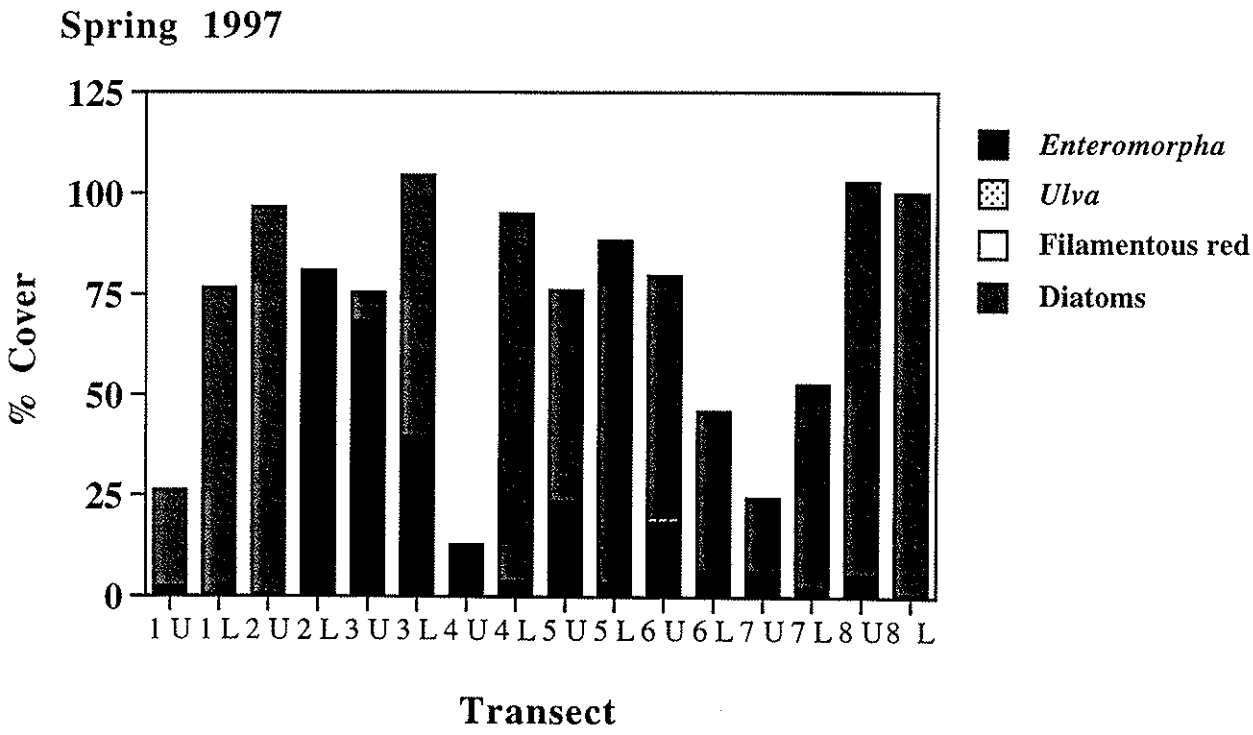

Figure 12. Algal community composition in Upper Newport Bay during spring 1997. Both upper (U) and lower (L) transects are shown.

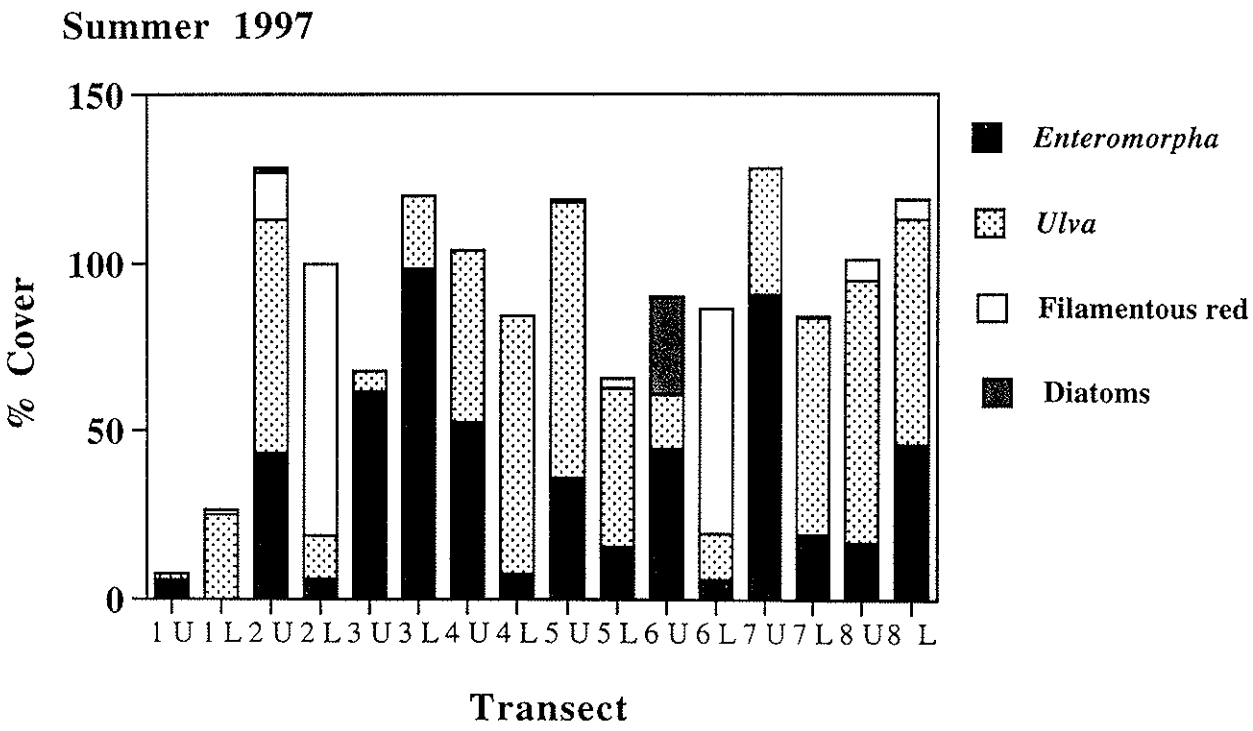

Figure 13. Algal community composition in Upper Newport Bay during the summer of 1997. Both upper (U) and lower (L) transects are shown.

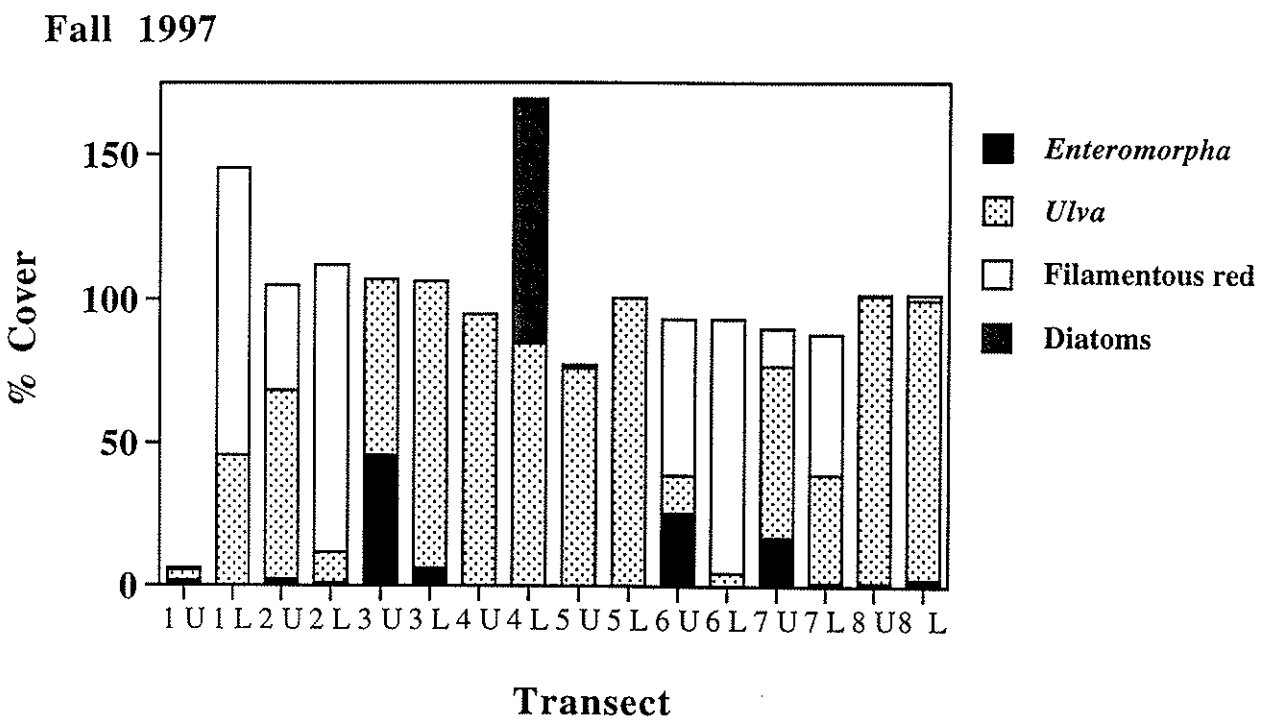

Figure 14. Algal community composition in Upper Newport Bay during fall 1997. Both upper (U) and lower (L) transects are shown.

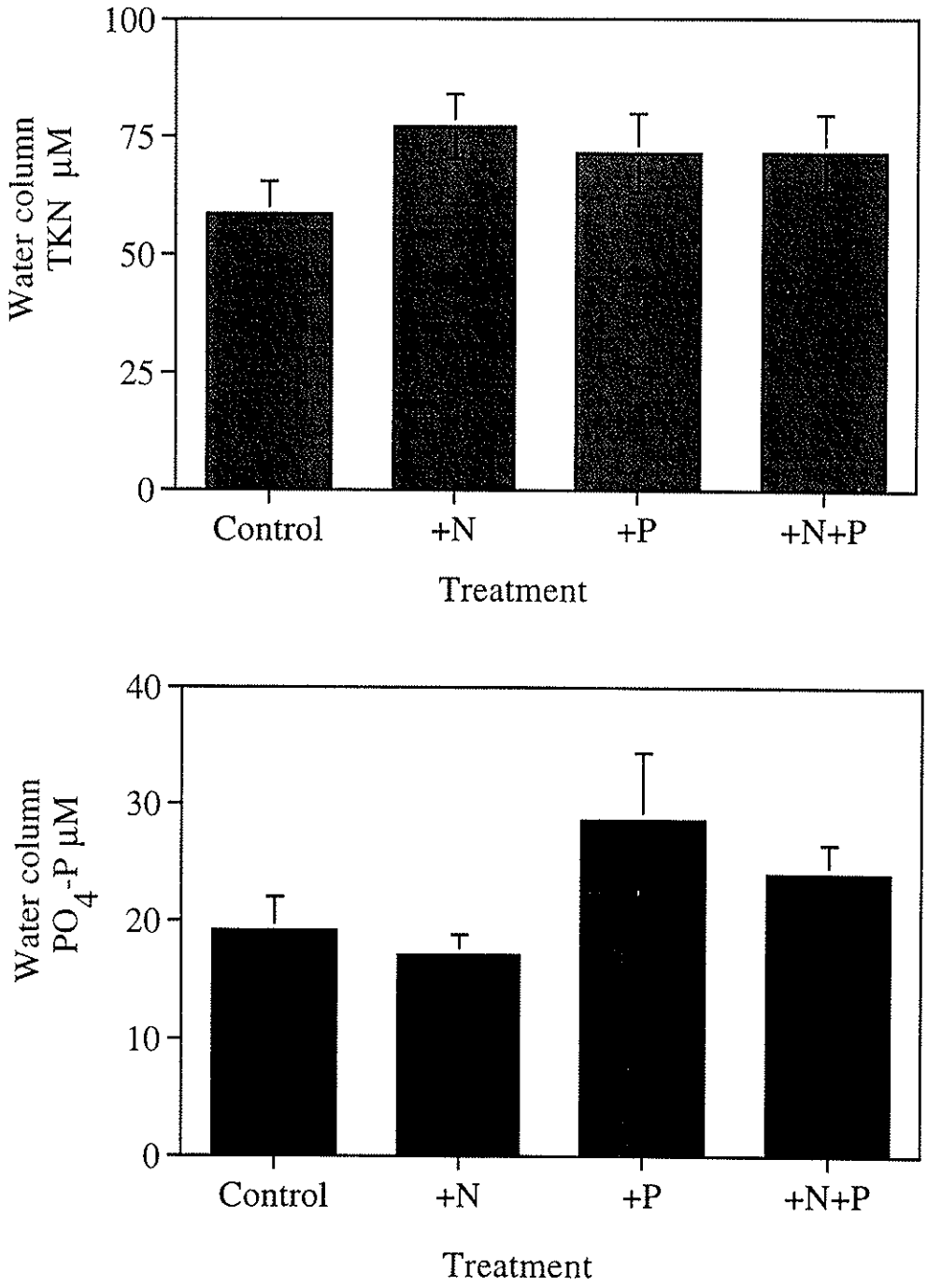

Figure 15. Water column levels of phosphate and total Kjeldahl nitrogen at the conclusion of the experiment. Non-significant treatment effects were observed.

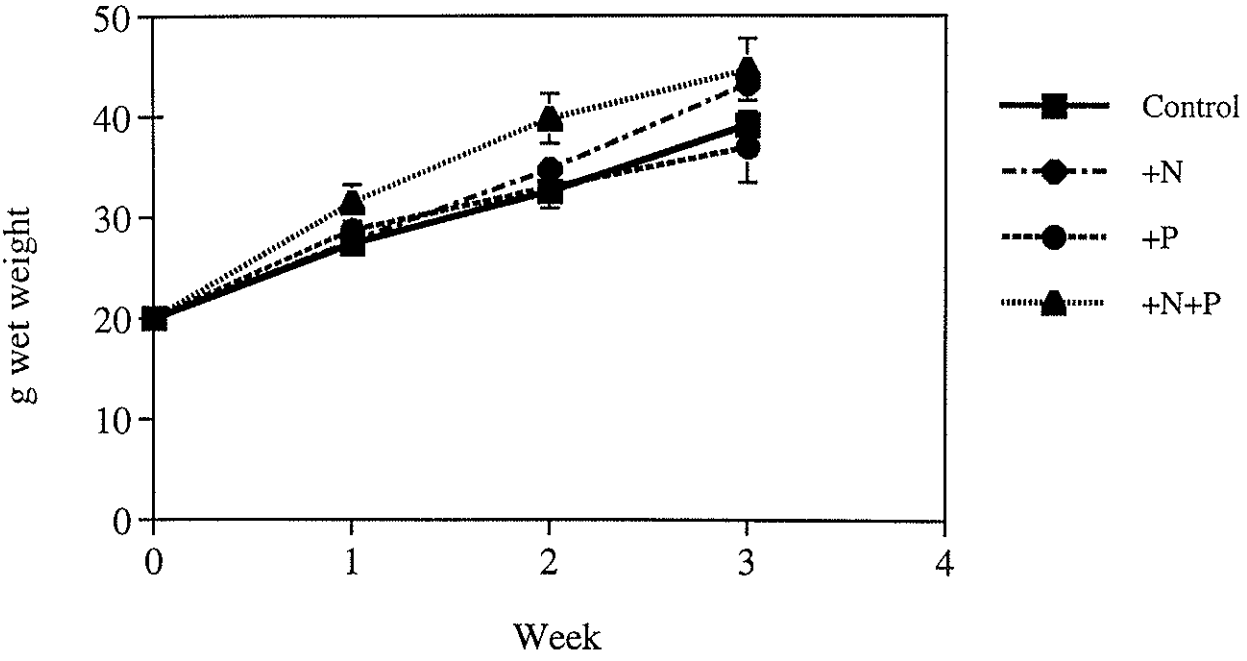

Figure 16. Mean total biomass of *Enteromorpha* and *Ulva* per microcosm after 4 weeks of experimental treatments.

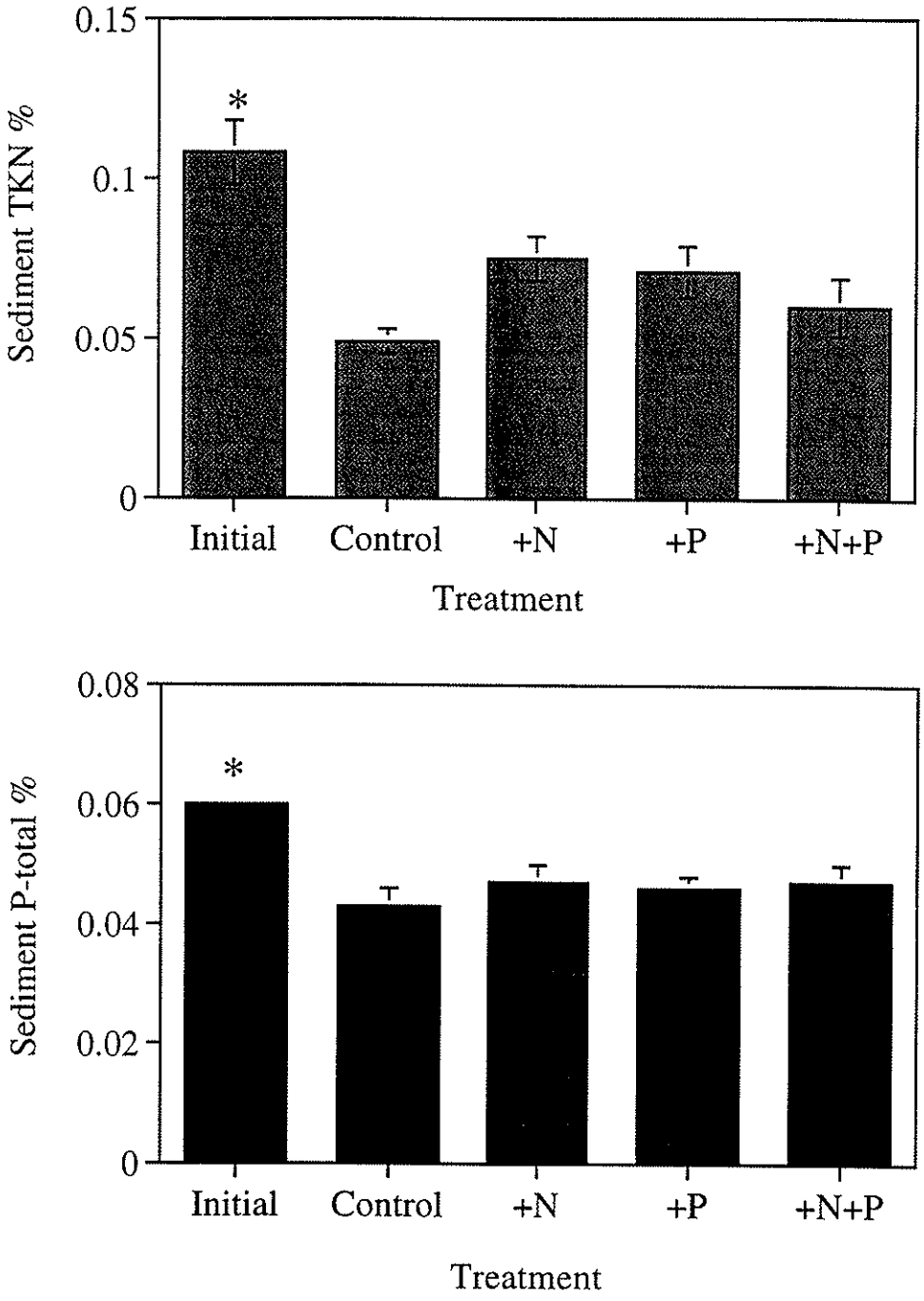

Figure 17. Sediment levels of TKN and total phosphorous after four weeks of experimental treatments. Asterisks indicate statistical significance at $p<0.05$.

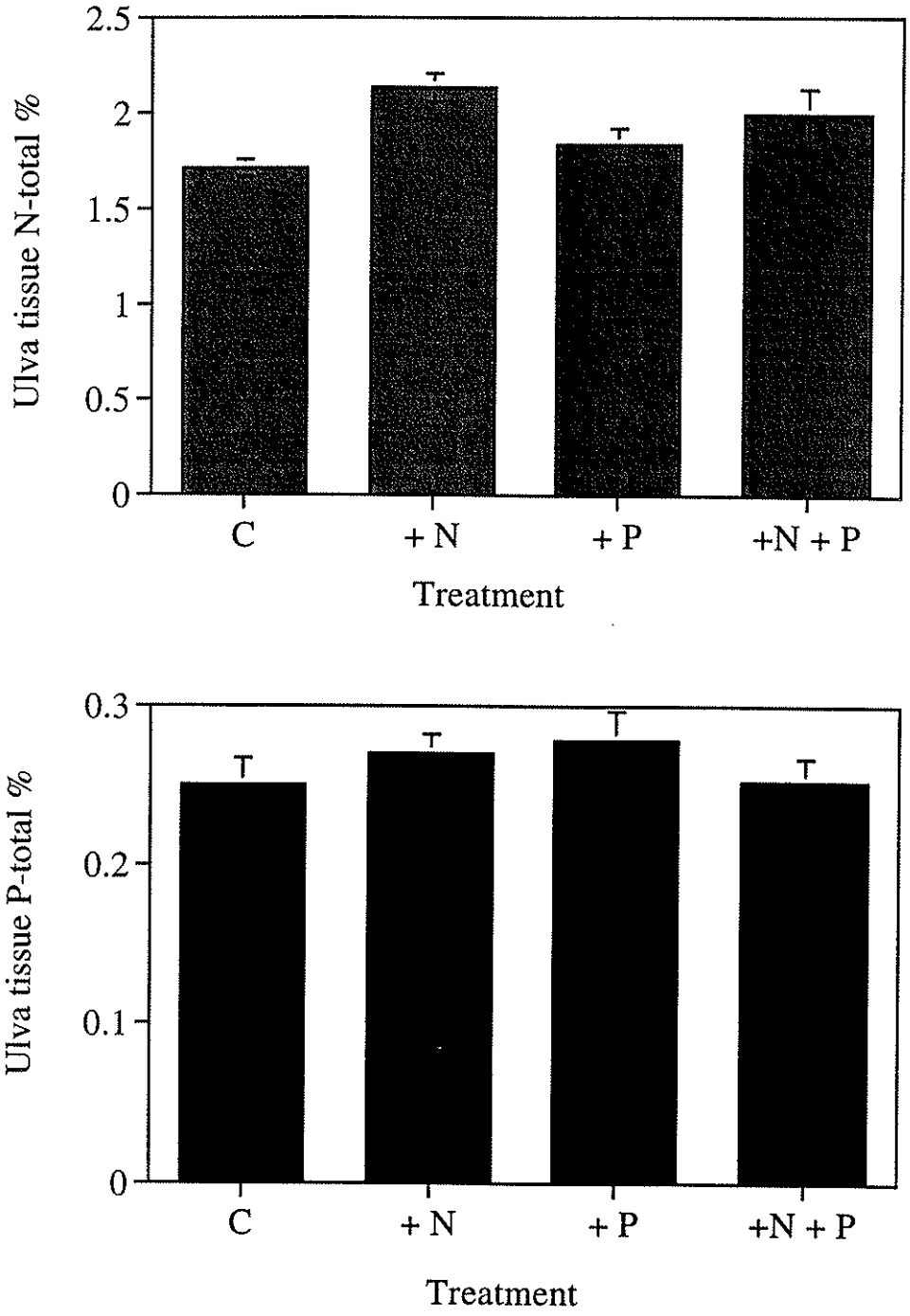

Figure 18. Ulva tissue nutrients after 28 days of experimental treatments.