The availability of phosphorus to *Nodularia* blooms in the Peel-Harvey Estuary

Department of Conservation and Environment
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The availability of phosphorus to *Nodularia* blooms in the Peel-Harvey Estuary

by

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A technical report to the Peel-Harvey Estuarine System Study

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ABSTRACT

Nodularia counts and cellular phosphorus concentrations were monitored from 1981 to 1985 in the Peel-Harvey Estuary. In the Harvey Estuary, the mean cellular phosphorus concentration and the maximum observed Nodularia count increased each year but there was no correlation between annual river phosphorus input and the size of the following Nodularia bloom. Since external phosphorus input was minimal during Nodularia blooms, most of the phosphorus contained in the blooms was derived from sediment phosphorus release and recycling. The increased tonnage of phosphorus in Nodularia suggests that the sediment store is becoming enriched with phosphorus since the largest Nodularia bloom (1984-85) followed the lowest annual river P input. The increase in Nodularia cellular phosphorus concentrations from each bloom indicates that phosphorus is now less limiting. Although cellular phosphorus concentrations from the 1984-85 Peel Inlet bloom indicated adequate phosphorus nutrition, a large biomass did not occur. This suggests that Nodularia populations in the Peel Inlet are being limited by something other than phosphorus.

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INTRODUCTION

Since 1981 Nodularia blooms in the Peel-Harvey Estuary have been monitored at six sites for biomass (Nodularia counts). Because phosphorus (P) has been shown to be the limiting nutrient in the Peel-Harvey System (Birch et al., 1981), cellular P concentrations were measured in Nodularia collected at stations 1 and 4 surface water (Fig. 1). Gerloff and Skoog (1954) demonstrated that phytoplankton cellular P levels can indicate the P status of the environment. Laboratory experiments with Nodularia showed that cellular P concentrations of 5.5 mg P (g dry wt)$^{-1}$ or above are indicative of P sufficiency and lower values indicate P deficiency (Hamel & Huber, 1985). By monitoring cellular P concentrations throughout each bloom, it is therefore possible to determine whether or to what extent P is limiting Nodularia growth in each year. Comparison of yearly mean bloom cellular P concentrations can also help to assess whether there is increase or decrease in the P contained in each bloom.

Since there is generally little external P input into the estuary at the time of Nodularia blooms (October to December), most P is obtained from the sediments and recycling. Thus, cellular P concentrations reflect the amount of P being released from sediments during each bloom.

During the 1984-85 bloom a new measure of P demand was added to the sampling program. This procedure (P debt) measured the amount of P taken up by Nodularia during 24 hours in the dark. Generally phytoplankton do not exhibit dark P uptake unless they are P deficient and the greater the amount of P taken up the

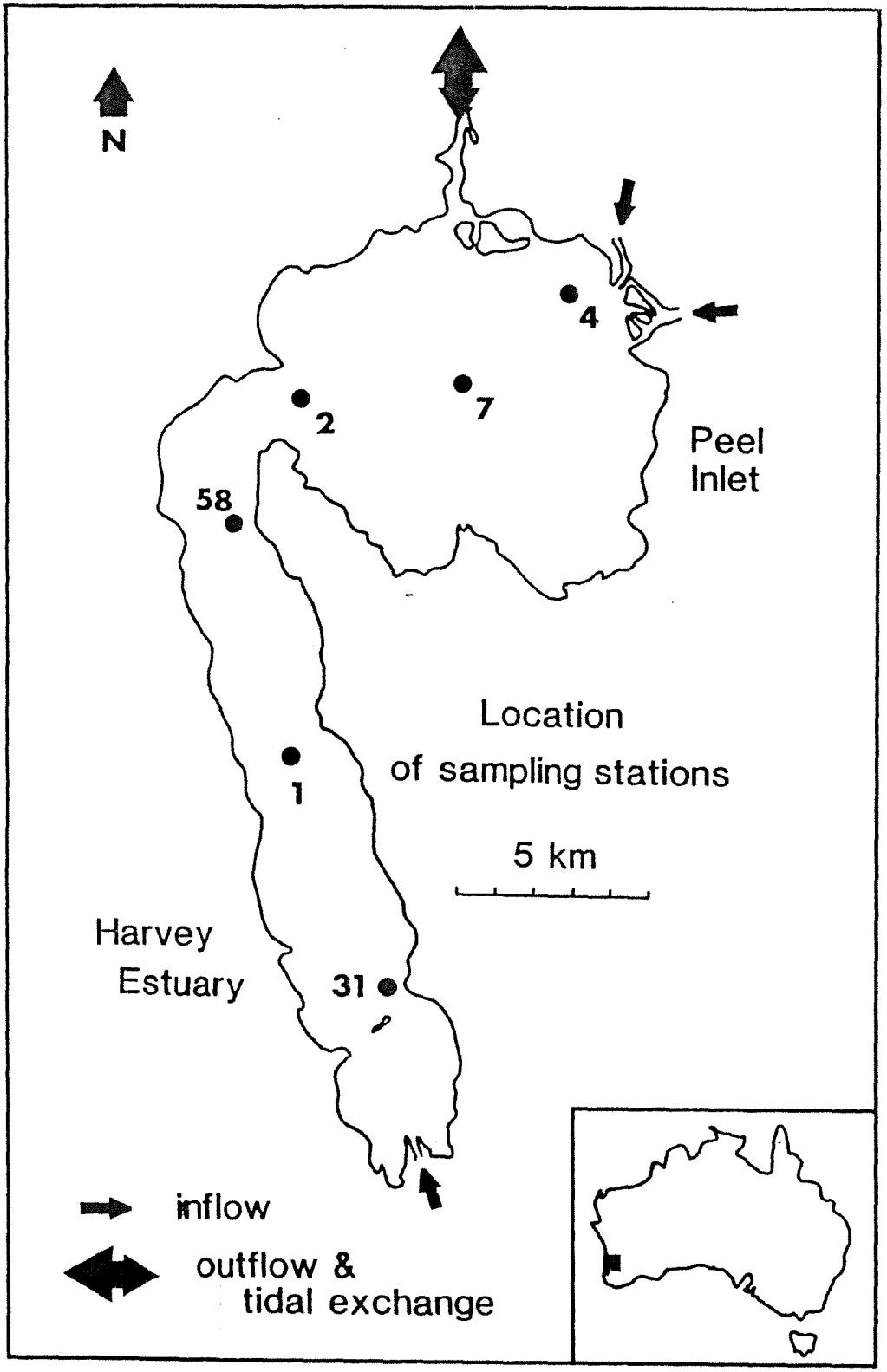

Figure 1. Location of sampling sites in the Peel-Harvey Estuary.

greater the degree of P deficiency (Healey, 1975). P debt is generally thought to be a more sensitive indicator of P deficiency than cellular P concentration and could therefore lead to a more accurate assessment of the P status of Nodularia.

METHODS

Sampling Procedure

Water samples for Nodularia counts were collected at weekly intervals throughout the duration of the Nodularia blooms from Harvey Estuary stations 1, 31, and 58, and Peel Inlet stations 2, 4, and 7 (Fig. 1). Samples were collected in whirlpacs and stored on ice until formalin could be added (2% final concentration). During the 1981-82 bloom, surface and bottom water was collected, but in subsequent years integrated water column samples were taken using weighted plastic tubing.

Surface and bottom water samples were collected for chlorophyll a analysis. These samples were either filtered in the field or returned to the laboratory on ice and in the dark and filtered there (Atkins & McComb, 1978).

Five liters of Station 1 and Station 4 surface water were collected weekly for cellular P determinations. In the 1984-85 bloom this water was also used for P debts.

Nodularia Counts

A known amount of formalinized sample water was filtered onto a 0.45µm gridded millipore filter. The volume of water filtered was varied so that generally 15 or more Nodularia

filaments were present on each grid. After drying, the filter was placed on a microscope slide and cleared with immersion oil. Nodularia filaments from 10 grids were counted and average filament number per grid determined. The length of 10 representative filaments was measured but a constant width per filament was assumed. The length of Nodularia filaments per ml sample was estimated by multiplying the average number of filaments per grid by the mean filament length and the number of grids per filter, and dividing by the sample volume.

Chlorophyll a Determination

An acetone extraction method was used for the determination of chlorophyll a . A detailed method is given in Atkins & McComb, (1973).

Cellular P Determination

The positive buoyancy of Nodularia caused it to concentrate on the surface of the sample container. Nodularia was pippetted from the surface and filtered onto a preweighed GFC Whatman filter. After drying to a constant weight at 70° C and reweighing, the filter was digested for 1 hour at 170-220° C in perchloric acid. After appropriate dilution, the digestrate was analysed for total P using an ammonium molybdate-ascorbic acid method (Murphy & Riley, 1962). Appropriate standards and filter blanks were carried thoughout digestion and analysis. A Nodularia dry weight of greater than 1 mg per filter was needed for accurate cellular P analysis and when Nodularia counts were below 10^4 $\mu m \cdot ml^{-1}$, there was not enough biomass present to analyse for cellular P or P debt.

P Debt

A subsample of floating Nodularia was removed from the surface of either Station 1 or Station 4 water. Filtered (0.45µm millipore) water from the appropriate station was added to the Nodularia to a volume of 450 ml. This gave an approximate dry weight of 20-30 $mg.l^{-1}$ Nodularia in the flask. Phosphate was added (about 2 $mg.l^{-1}$ PO_4-P final concentration), mixed, and an aliquot immediately removed and filtered through a prerinsed 0.45 um millipore filter for soluble reactive P (SRP) analysis. Known volumes of water were filtered onto preweighed GFC Whatman filters for determination of Nodularia dry weight and cellular P. One hundred ml was added to 3, 500ml Erlenmeyer flasks which were stoppered with cotton bungs, and placed in the dark at 22°C for 24 hours. After 24 hours aliquots were removed as previously described for SRP, dry weight, and Nodularia cellular P concentration. The difference in SRP concentration before and after 24 hour dark uptake divided by the dry weight of Nodularia in the flask was expressed as ug P (mg dry wt)$^{-1}$ Nodularia. SRP was analysed using ammonium molybdate-ascorbic acid method (Murphy and Riley, 1962) after filtration through a 0.45 µm millipore filter. Cellular P concentration was determined as described previously.

Data Tabulation

An estimate of Harvey Estuary Nodularia biomass was obtained by averaging the counts from stations 1,31, and 58. Estimates from Peel Inlet were averaged from stations 2,4, and 7. Area under the curve was calculated by plotting the Nodularia counts on a linear scale and weighing the paper. The units are

arbitrary with the largest bloom 100 and the other blooms adjusted accordingly. An average biomass for each bloom was calculated by adding all Nodularia counts 10^5 mm/ml or above and dividing by the number of observations.

RESULTS

Harvey Estuary

Both Nodularia counts and chlorophyll a data gave similar biomass measures in all blooms except for 1981-82 (Fig.2). The area under the curve (Nodularia counts) was greatest in 1984-85. The 1983-84 bloom was 50% the size of the 1984-85 bloom while the 1981-82 and 1982-83 bloom were both about 35% the size of the largest bloom (Table 1). The maximum observed Nodularia count and dry weight progressively increased each year.

Nodularia cellular P concentrations from the 1984-85 bloom were generally above 5.5 mg (g dry wt)$^{-1}$ (the concentration at which P is not limiting growth) while the cellular concentrations of Nodularia from the 1981-82 bloom were never above this value (Fig. 3). In the other two blooms, the values were intermediate. The maximum observed cellular P concentration was similar for 1984-85,1983-84, and 1982-83 blooms but was lower in 1981-82 bloom (Table 2). The average concentration of cellular P increased progressively each year and the tonnage of P contained in the 1984-85 bloom was nearly double that contained in the preceding blooms.

A comparison of station 1 surface P debt with SRP surface water concentrations and station 1 cellular P concentration is

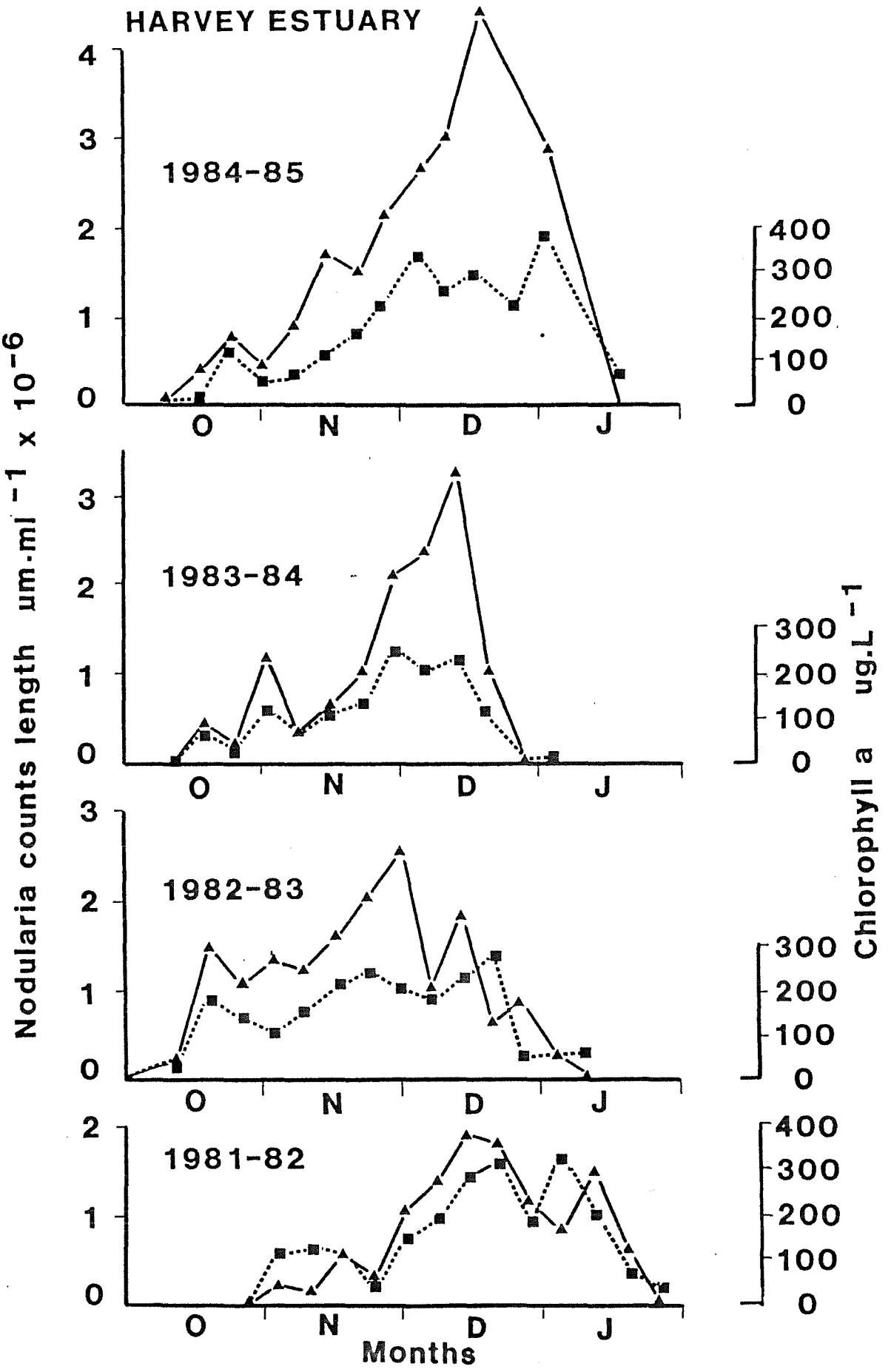

Figure 2. *Nodularia* counts (▲ ——) and chlorophyll *a* concentrations (■ ·····) from 4 Harvey Estuary blooms (average of stations 1, 31 and 58.)

TABLE I
SIZE OF NODULARIA BLOOMS

Comparison of Nodularia blooms in the Harvey Estuary (mean of stations 1,31,& 58) and Peel Inlet (mean of stations 2,4,& 7) as estimated by Nodularia counts.

HARVEY ESTUARY

Year	Maximum observed biomass (length u/ml)	Tonnes dry weight of Nodularia biomass in the estuary at maximum count *	Mean biomass of all observations 10^5 or over during each bloom	Area under curve (by weight)
1984-85	4.42×10^6	7492	1.91×10^6	100
1983-84	3.25×10^6	5509	1.36×10^6	39
1982-83	2.56×10^6	4339	1.24×10^6	50
1981-82 (Oct-26 Jan)	1.91×10^6	3237	9.59×10^5	36

PEEL INLET

1984-85	1.92×10^6	4184	5.56×10^5	28
1983-84	7.99×10^5	1354	4.88×10^5	14
1982-83	1.46×10^6	2475	5.6×10^5	15
1981-82	1.09×10^6	1848	4.66×10^5	15

* Dry weight was converted from Nodularia counts by multiplying length per liter by the number of liters in each estuary and then dividing by a conversion factor of 1 tonne dry weight Nodularia equals 4.13×10^{16} um Nodularia.

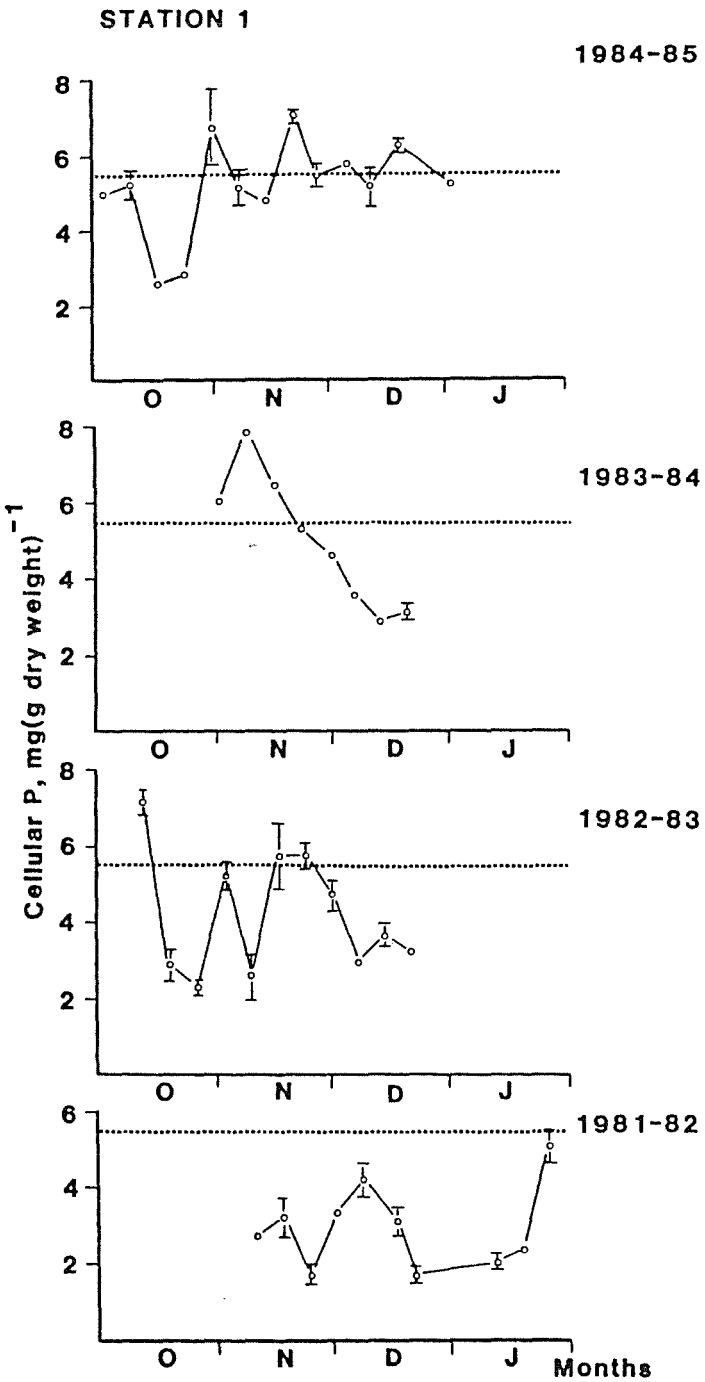

Figure 3. *Nodularia* cellular phosphorus concentrations from 4 Harvey Estuary blooms (station 1). The dotted line represents the "critical concentration" and observations below this line are considered to be P-limited. Standard errors are shown except where the size of the symbol is larger than the error bars.

Table 2

PHOSPHORUS (P) IN *NODULARIA* BLOOMS
Comparison of phosphorus contained in yearly *Nodularia* blooms

HARVEY ESTUARY

Year	Maximum observed cellular P conc. mg/g	Mean concentration of cellular P during each bloom (mg/g)	*Tonnes P calculated using sta. 1 counts	Tonnes P calculated using mean Harvey counts	P loading Harvey R + drains Tonnes
1984-85	7.04±0.2(SE)	5.17±0.4	33.2	33.2	56
1983-84	7.91±0.1	5.00±0.6	18.8	15.6	111
1982-83	7.19±0.3	4.22±0.5	19.9	18.7	90
1981-82	5.13±0.4	2.98±0.3	10.1	8.4	134

PEEL INLET

			calculated using sta. 4 counts	calculated using mean Peel counts
1984-85	15.00±.5	7.04±1.4	3.4	7.8
1983-84	5.27±.04	4.20±0.8	6.7	6.9
1982-83	2.66±.3	1.50±.05	4.2	4.4

*Estimated from 3 weeks of consecutive maximum biomass converted to dry weight and using corresponding cellular P concentration to estimate P tonnage.

shown in Fig. 4. The maximum observed P debt occurred when the Nodularia cellular P concentration and SRP in the water column was low. The lowest SRP, cellular P concentrations and highest P debt were observed early in the bloom and corresponded to exponential growth of Nodularia. An unusually high SRP value of 18 $ug.l^{-1}$ P was associated with a high cellular P concentration and 0 P debt. The high cellular P concentrations which were seen throughout much of the bloom were generally accompanied by P debt less than 10 ug P $(mg\ dry\ wt)^{-1}$. and SRP values around 8 $\mu g.l^{-1}$.

Peel Inlet

Peel Inlet Nodularia counts were much lower than Harvey Estuary counts with the largest bloom in the Peel Inlet being substantially smaller than the smallest bloom in the Harvey Estuary (Fig. 5). The 1984-85 bloom was the largest bloom in the Peel Inlet. (Table 1). Blooms in earlier years were similar in size.

The maximum observed cellular P concentration and tonnage of P in the Inlet increased from 1982 through 1985 (Table 2). During the 1981-82 and 1983-84 blooms, cellular P concentrations were never observed above "critical concentration" while cellular P concentrations from 1984-85 were generally above this concentration (Fig. 6). Some extremely high P concentrations were observed in Nodularia during the 1984-85 bloom but total tonnage of P contained in Peel Inlet blooms was very much lower than the tonnage of P contained in Harvey Estuary blooms.

Because of low Nodularia biomass at station 4, it was possible to do P debt only 4 times during the course of the bloom. Zero P debts were associated with cellular P

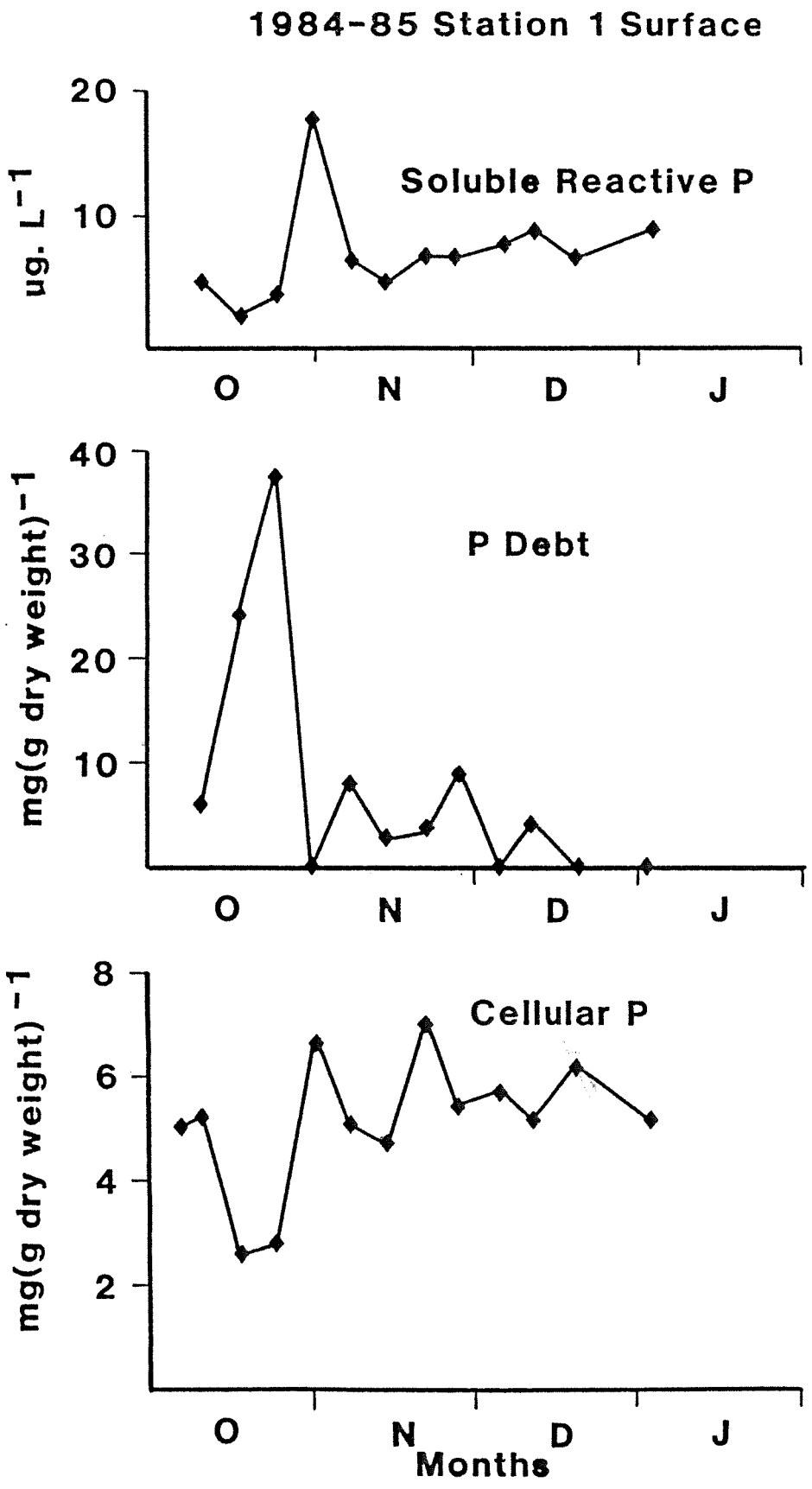

Figure 4. Comparison of 1984-85 station 1 surface soluble reactive phosphorus, phosphorus debt and *Nodularia* cellular phosphorus concentrations.

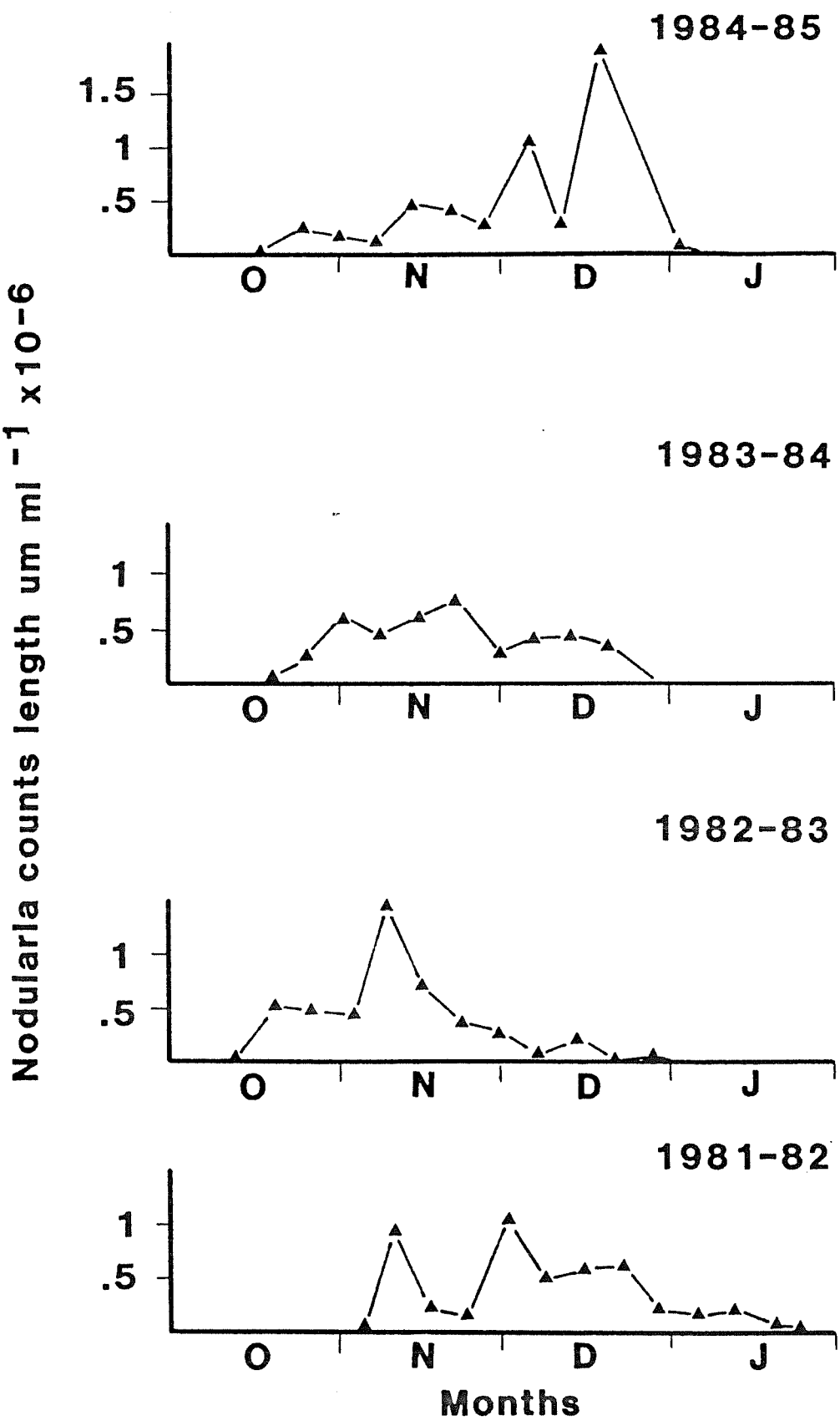

Figure 5. *Nodularia* counts from 4 Peel Inlet blooms (average of stations 2, 4, & 7).

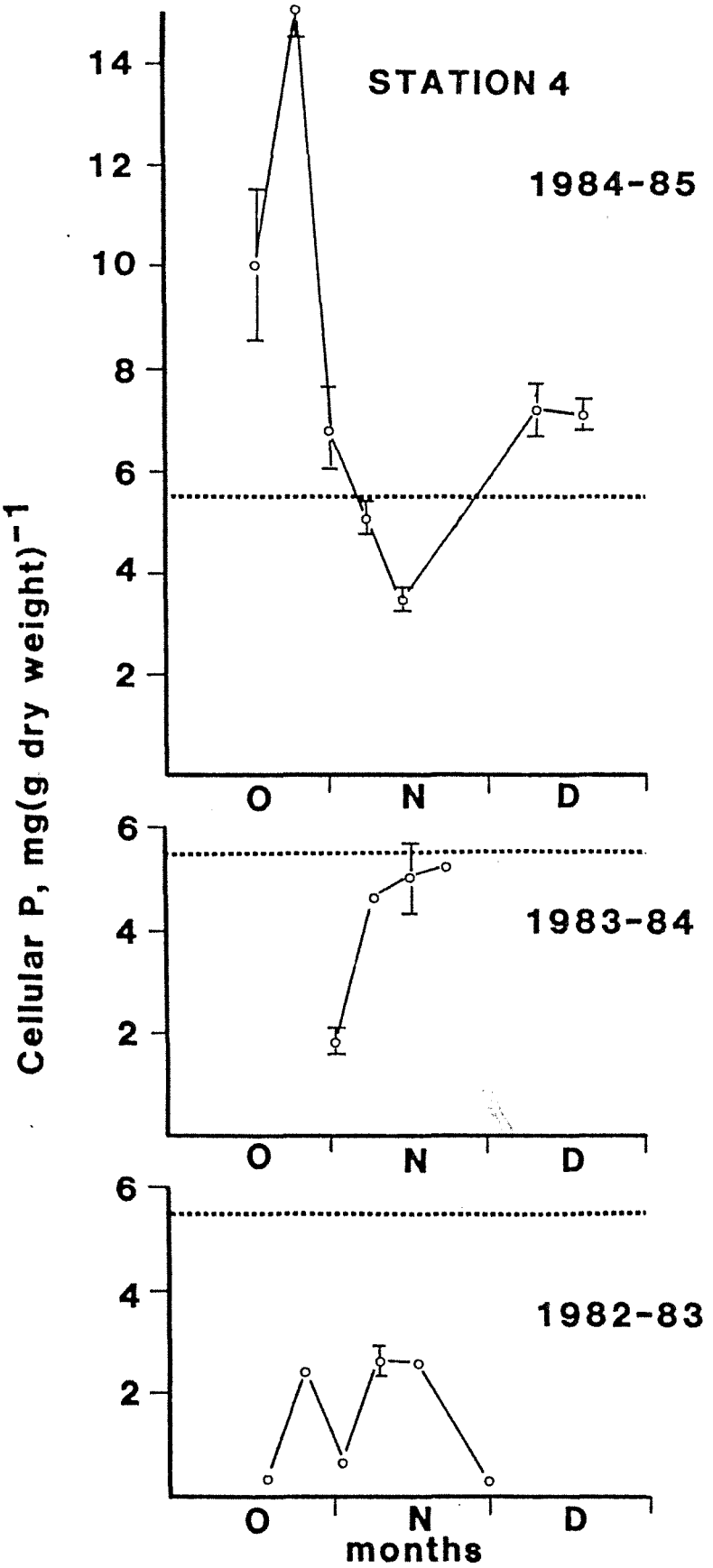

Figure 6. *Nodularia* cellular phosphorus concentrations from 3 Peel Inlet blooms (station 4). The dotted line represents the "critical concentration" and observations below this line are considered to be P-limited. Standard errors are shown except where the size of the symbol is larger than the error bars.

concentrations above the "critical concentration" while P debts greater than 0 corresponded to cellular P values below "critical concentration". Soluble reactive P in the water remained low (data not shown).

DISCUSSION

Harvey Estuary

The mean cellular P concentration of each Nodularia bloom has increased in every year. This indicates that P has become more available for phytoplankton growth in this estuary since 1981. In 1981-82 cellular P was always below the "critical concentration" while during the 1984-85 bloom cellular P was generally above "critical concentration". There was no direct correlation with annual river P load and the mean cellular P concentration of the following Nodularia bloom. Since sediments are the major source of P for Nodularia blooms this suggests that sediments have become progressively enriched with P over time and they are now releasing a greater amount of P to the water column during Nodularia blooms. Therefore even with lower river P loads, large Nodularia blooms can be supported largely from the sediment store.

Cellular P concentrations suggested that the 1984-85 bloom was not P-limited, but the P debt data indicated that some P demand did exist during much of this bloom. However, high P debt values (38 ug p (mg dry wt)$^{-1}$) only occurred early in the bloom during exponential growth when sediment release may have failed to supply the growing Nodularia at a rate fast enough to satisfy the P demand. As growth rate declined, the P debt remained low

(0-9). Although the degree of P deficiency in each bloom decreased from 1981 through 1985, even the 1981-82 bloom had enough cellular P reserve for at least one doubling in biomass. Under optimal conditions, laboratory Nodularia can deplete cellular P stores to approximately 1.4 mg (g dry wt)$^{-1}$ before growth ceases. Since nutrient addition bioassays have shown no nutrient other than P is growth-limiting for Nodularia in the Harvey Estuary (Hamel & Huber, 1985), then Nodularia is probably being limited by physical factors such as light.

It is difficult to decide what is the best estimate of overall bloom size. Maximum standing crop as measured by maximum observed Nodularia count showed an increase each year from 1981-1985, with the 1981-82 bloom being 50% the size of the 1984-85 bloom. However peak biomass failed to take into account the duration of the bloom. Area under the curve can account for both duration and peak biomass and by which measure the 1984-85 bloom was the largest observed.

A comparison of chlorophyll a and Nodularia counts shows that generally these two estimates of biomass are equivalent. However Nodularia counts in 1981-82 bloom appear to have underestimated biomass by about 50%. Based on chlorophyll a, the 1981-82 bloom was similar in size to the 1984-85 bloom. Since the mean cellular P concentration in the 1981-82 bloom was almost half the mean cellular P concentration in 1984-85, the tonnage of P in the 1984-85 bloom was still double that of the 1981-82 bloom.

The ratio of chlorophyll a concentrations to counts decreases when the counts become high (1.5 x 10^6 µm. ml^{-1}) ie

less chlorophyll a per unit biomass. This is not unexpected since Huber (1985) showed that nitrogen fixation decreases during the latter part of the bloom when maximum counts often occur. Since the nitrogen content of phytoplankton is highly correlated with chlorophyll a concentration, the lower nitrogen content of Nodularia may cause a decrease in chlorophyll a concentration per unit biomass.

Since Nodularia decomposes rapidly (55% release of soluble P in 3 days) (Gabrielson & Hamel, 1985), the large tonnage contained in each bloom can be rapidly returned to the water and/or sediment for use by other phytoplankton. Only 13% of Nodularia P is refractory so 87% of all the P in each bloom may be potentially available for follow-on diatom or macroalgal blooms after Nodularia blooms collapse. If tidal flushing carries Nodularia from the esturay to the ocean this can be a mechanism for removal of large tonnages of P from the estuary.

Peel Inlet

The mean concentration of Nodularia cellular P in the Peel Inlet has progressively increased since 1982 suggesting that Nodularia in Peel Inlet has become less P-limited in each bloom year. Unusually high cellular P concentrations measured in October 1984 resulted in a mean cellular P concentration of 7.04 mg (g dry wt)$^{-1}$ for the 1984-85 bloom. Although the mean concentration was above the "critical concentration", there were times during this bloom when cellular P levels fell below this value and P debt data indicated some P demand. Laboratory studies have shown that cellular P concentrations of P-sufficient

exponentially growing Nodularia tend to remain near the "critical concentration". Cellular concentrations above this occur when cells are in P-sufficient conditions but not growing or when P-starved cells are exposed to P. The high cellular P concentrations measured during October 1984 probably indicate that Nodularia was not growing because of other limitations but that availability of P was high and P was therefore accummulated by Nodularia.

The size of the blooms in the Peel Inlet seems to be poorly related to P nutrition of the Nodularia although the best nourished 1984-85 bloom was the largest bloom out of the 4 blooms. However the other blooms were similar in size even though P supply was clearly inadequate in the 1982-83 bloom. Since nutrient addition bioassays showed only P limitation for Nodularia in the Peel Inlet it is likely that physical conditions limit Nodularia in this part of the estuary.

Peel Inlet Nodularia blooms are always of shorter duration and have lower maximum standing crops than Harvey Esturay blooms. Although P appeared to be limiting Nodularia growth in 1982-83, this was not the case in the following two years where cellular P concentrations were adequate for growth in the Peel Inlet. Nodularia blooms will probably never be a problem in the Peel Inlet because physical conditions in this estuary do not favor large blooms. However the progressive increase in mean cellular P concentration shows that P is becoming more available each year. This P could be used by other organisms which are not subject to the same growth limitations as Nodularia.

CONCLUSIONS

The Peel-Harvey estuarine system is being eutrophied with respect to P availability for algal growth. In the past 4 years there was little relationship between annual river P load and the size of the following Nodularia bloom. The 1984-85 Harvey Nodularia bloom was not only the largest bloom, but it also had the highest mean cellular P concentration and the lowest river P input of the 4 Harvey Estuary blooms. Although P has been shown to be the limiting nutrient for Nodularia growth, cellular P concentrations above the "critical concentration" were often observed during the 1984-85 Nodularia bloom and indicate that P was in sufficient supply during much of the bloom. This constrasts with the 1981-82 bloom in which cellular P concentrations were never above "critical concentration". Since Nodularia decomposes rapidly, the tonnage of P contained in the Nodularia bloom can be quickly recycled. Since there is little external P input during Nodularia blooms, the P contained in the blooms was obtained through sediment P release and recycling. The progressive increase in mean cellular P concentration for the past 4 blooms indicates that sediment is becoming more P enriched and/or the right conditions for sediment P release are occurring more often during the Nodularia blooms. The sediments will continue to become P enriched unless there is a net loss of P to the ocean in each year.

RECOMMENDATIONS

(1) *Nodularia* cellular P analysis should be continued.

(2) *Nodularia* counts should be continued at stations 1 and 4, but the counts at other stations could be discontinued since chlorophyll *a* concentrations give similar biomass estimates.

(3) P debt provides backup data to cellular P concentrations and may be a more sensitive indicator of the environmental P status than cellular P. However if only one P monitoring method is employed then *Nodularia* cellular P concentration is preferred.

ACKNOWLEGEMENTS

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