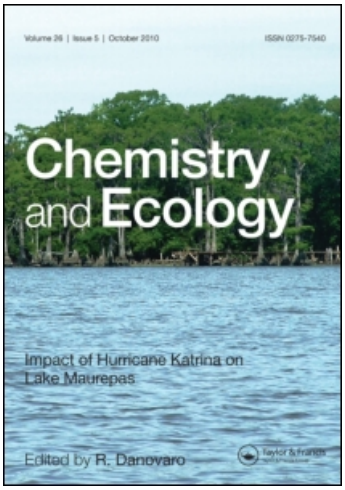

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Effects of Nitrate Nitrogen Additions on Physical, Chemical and Biological Parameters in Lake Enclosures

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The effects of nitrate additions on the physics, chemistry and biology of lake water were studied in 5×10 m polyethylene enclosures installed in Lake Kastoria, a shallow eutrophic lake in Northern Greece. The water physics, chemistry, chlorophyll a and nitrogenase activity were monitored from July 10 till October 17 1985 at 2 week intervals. The experiment included a control enclosure.

Water confinement in the control enclosure resulted in ammonia accumulation, a slight decrease in chlorophyll a, a significant reduction of nitrogenase activity and an increase in phosphorus release from the sediments at the end of the experimental period.

The addition of KNO_3 resulted in higher than the control accumulation of NH_3, chlorophyll reduction, increase in water transparency and reduction of nitrogenase activity. Large losses of nitrogen added were measured which were attributed to denitrification, organic matter sedimentation and ammonia volatilization. Anaerobic but not aerobic phosphorus release from sediments was inhibited at the end of the period. The reduction of nitrogenase activity and of chlorophyll a concentration are attributed to changes in phytoplankton composition from blue-greens to small-sized species grazed by zooplankters.

KEY WORDS Nitrate additions: blue-greens: water chemistry: phosphorus release: sediments:

INTRODUCTION

Water quality deterioration, a result of eutrophication, includes excessive growth of algae, oxygen depletion in the hypolimnion of

stratified lakes, the reduction of water transparency and the dominance of blue-green algae.

Blue–greens appear as a result of nitrogen deficiency (Smith, 1983; Smith, 1986) which they overcome either by fixing elemental nitrogen or by storing nitrogen compounds when nitrogen is abundant (Fogg *et al.*, 1973).

The control of blue–green algae can be achieved by increasing the total nitrogen/total phosphorus ratio as Schindler (1977) suggested. This can be accomplished by reducing the external and internal phosphorus loading by different techniques or by increasing the nitrogen loading. Leonardson and Ripl (1980) have used successfully the addition of nitrate to prevent the development of blue–greens in a hypertrophic pond. However, further experimentation is needed for the nitrate additions to be established as a lake restoration method. The effects of nitrate additions on water chemistry and biology and the phosphorus release from sediments were studied in polyethylene enclosures installed in the shallow eutrophic Lake Kastoria, in Western Macedonia, Greece.

METHODS

Lake description

Lake Kastoria is a shallow dimictic lake in Western Macedonia, Greece, receiving directly or indirectly the sewage from the homonymous city located at the lakeside (Fig. 1). The lake has an area of 30 Km^2 and a mean and maximum depth of 3.9 m and 9 m respectively (summer 1984). The main physical, chemical and biological features of the lake, based mostly on measurements made in 1985 (Tsicritsis and Mourkides, 1985) are shown in Table 1. The release of phosphorus from sediments causes a large increase in total phosphorus concentration during summer and early autumn which supports a bloom of blue–green algae (Tsicritsis and Mourkides, 1985). Among blue–greens the genera *Microcystis* and *Anabaena* have been identified; *Microcystis* forms large scums particularly near the lakeshore (Tsicritsis and Mourkides, 1985).

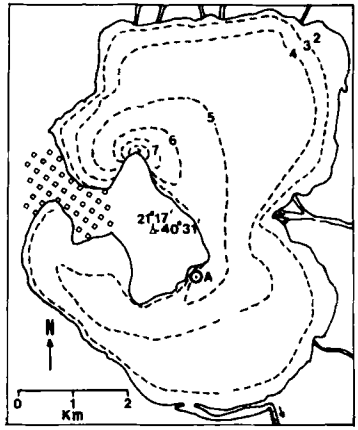

FIGURE 1 Map of Lake Kastoria.

TABLE I
Physical, chemical and biological characteristics of Lake Kastoria (Euphotic zone)

pH (annual range)	8.2–10.1
Transparency (mean annual)	0.84 m.
Total Phosphorus (mean annual)	130 $\mu g\, l^{-1}$
Total Nitrogen (growth period)	0.8–2.0 $mg\, l^{-1}$
Nitrate Nitrogen (spring)	46 $\mu g\, l^{-1}$
Chlorophyll a (mean annual)	29 $\mu g\, l^{-1}$
Nitrogen Fixation (July–October)	170 mmole $C_2H_2\, m^{-2}$

Enclosures-nitrogen additions

Two 5 × 10 m polyethylene enclosures (Fig. 2) were installed on the 10th of July 1985 in site A (Fig. 1) where the depth was 5 m. One of them was used as control while the other received nitrate nitrogen additions in the form of pure KNO_3 according to the following schedule: 7–10–85: 3 mg N/1; 8–1–85: 3 mg N/1; 8–22–85: 6 mg N/1; 9–19–85: 3 mg N/1. The addition of nitrate was made by dissolving the appropriate amount of KNO_3 in a bucket of lake water and distributing the solution uniformly in the surface of the enclosure.

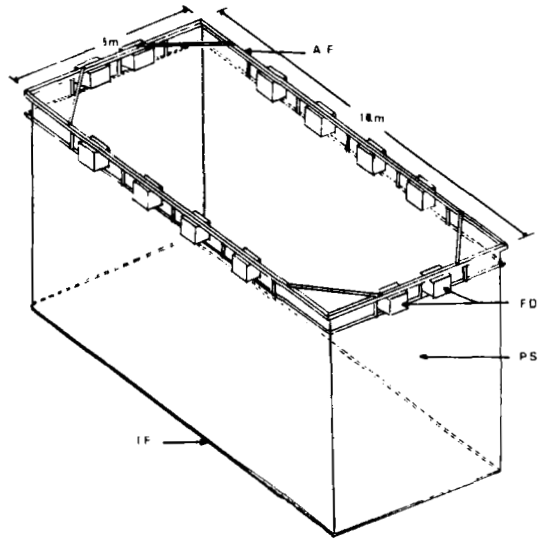

FIGURE 2 Diagram of the enclosure; AF: surface aluminum frame, IF: bottom iron frame, PS: polyethylene sheet, FD floating supports for the surface frame.

Field work

The sampling and measurements of the enclosures and the lake at the site of the enclosures took place every 2 weeks starting on the 10th of July and always before the nitrate additions. All field work except the incubation for nitrogenase activity determination was carried out between 10 a.m. and noon. At each sampling date four samples were taken with a van Dorn sampler from each of the following depths: 0.5 m, 2.5 × Secchi Disc depth and the bottom layer. Subsamples were taken from each sample for nitrate (NO_3—N + NO_2—N), ammonia, total nitrogen (TN), and total phosphorus (TP) determination. Subsamples for chlorophyll a and nitrogenase activity determination were taken only from the samples of the euphotic zone (0.5 m and 2.5 × Secchi disc depth).

In situ measurements included Secchi disc transparency and the measurement of pH, dissolved oxygen, and temperature with the Water Quality Checker, model U-7 of Horiba.

Incubation for nitrogenase activity determination (acetylene reduction) was carried out in the following manner: 50 ml of sample were added in 60 ml serum bottles which were then capped tightly. Ten ml of acetylene under atmospheric pressure were added and the gas phase was brought to atmospheric pressure by removing 10 ml of the mixture. The amount of acetylene added was enough to saturate nitrogenase (Flett, 1976). After mixing, the samples were incubated at the sampling depth in an open box made from transparent plexiglass. Incubation started at noon and lasted two hours after which nitrogenase activity was terminated by adding 0.5 ml of 50% TCA per sample (Carpenter and Price, 1977). TCA was added immediately after acetylene addition in three lake water samples to be used as blanks. All bottles were kept inverted until analysed to exclude any possible gas leakage.

Subsamples for ammonia, TN and TP determination were acidified. Subsamples for nitrate analysis were filtered through Whatmann GF/C filters in the field, kept inside an ice-box until arrival at the laboratory and then frozen until analysed. Subsamples for chlorophyll a determination were filtered through the same filters which were kept in 90% acetone in water until absorbance was measured.

Analyses

Ammonia and nitrates were determined in a Technikon Automatic Analyzer. The indophenol-blue method was used for ammonia and the Cd-Cu reduction coupled with azodye formation for nitrates. Total nitrogen was determined by transformation of all nitrogen forms to nitrate by alkaline persulphate digestion (Smart *et al.*, 1981). Total phosphorus was determined after acid persulphate digestion (APHA, 1975) according to the method of Murphey and Riley (1962).

For chlorophyll determination absorbance was measured in acetone extracts according to the trichromatic equation (Lind, 1974). Chlorophyll concentrations for the 22th and 29th of August were calculated from the corresponding values of water transparency (SD) by using the equation:

$LN(SD) = 3.828/LN(\text{chlorophyll a}) - 1.57$ $(R = 0.84)$ where LN is the natural logarithm. The equation was derived from data for the whole lake.

Ethylene analysis for nitrogenase activity determination was carried out in a Varian 3700 chromatograph with a FID detector and a 2 m × 3 mm column packed with Porapak R(80–100) under the following conditions. Column temperature: 35°C, Detector temperature: 80°C, Carrier gas: nitrogen, 15 ml min^{-1}. Samples were brought to ambient temperature (20°C) and shaken before 1–3 ml of the gas phase was injected. Ethylene solubility in water was taken into account to calculate total ethylene production (Flett, 1976). Nitrogenase activity was expressed per hour after it had been found that there was no significant (5% level) difference between one and two hour incubation.

Statistical comparison of results was made by the t-test at the 5% confidence level.

RESULTS

The vertical distribution of temperature showed that no thermal stratification existed during the experimental period except on the 24th of July (results not shown). Surface temperature ranged between a maximum value of 25.2°C to 26.2°C in late July and a minimum value of 19.1°C to 19.9°C in early October.

The concentration of NO_3—N in the euphotic zone and the bottom layer of both the lake and the control enclosure ranged between 0.10 and 0.20 mg/l^{-1} during most of the experimental period (Fig. 3). No significant differences in nitrate concentration between 0.10 and 0.20 mg l^{-1} during most of the experimental period (Fig. 3). No significant differences in nitrate concentration centration between the euphotic zone and the bottom layer. The concentration increased after each addition, except the last one, which reached a maximum of 4.50 mg N l^{-1} in early September and then decreased to about 2 mg NO_3—N l^{-1}.

The concentration of NH_4—N in the control enclosure increased in the bottom layer first, and then in the euphotic zone, to levels significantly higher than those in the lake and reached a maximum of about 0.5 mg l^{-1} compared to 0.29 mg l^{-1} in the lake (Fig. 4). In the enclosure with added N, the concentration of NH_4—N increased to levels significantly exceeding those of the control enclosure during most of the experimental period and reached a maximum of

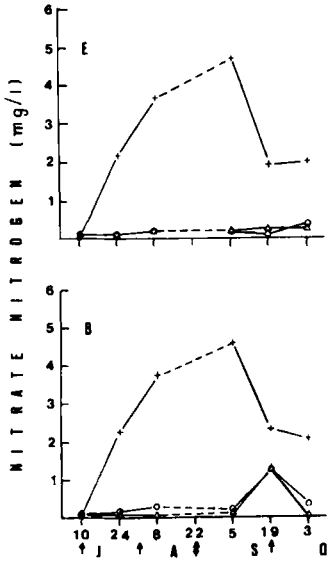

FIGURE 3 Concentration of nitrate nitrogen in the euphotic zone (E) and the bottom layer (B). (+——+): enclosure with added N (○——○): control enclosure; (△——△): lake water. Arrows show the dates of nitrate additions.

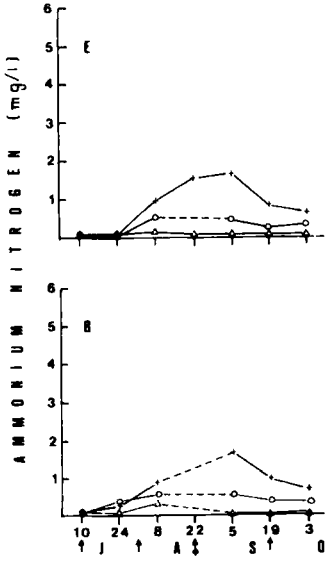

FIGURE 4 Concentration of ammonia nitrogen. (For the symbols see Fig. 3.)

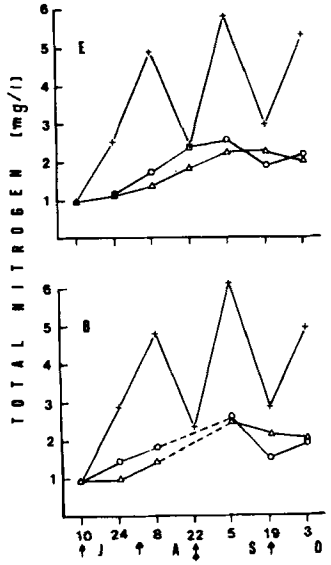

FIGURE 5 Concentration of total nitrogen. (For the symbols see Fig. 3.)

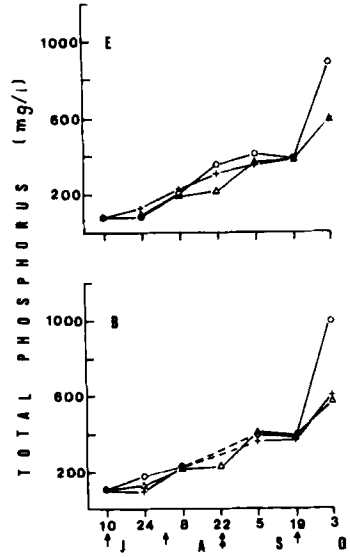

FIGURE 6 Concentration of total phosphorus. (For the symbols see Fig. 3.)

approximately 1.6 mg l^{-1} two months after the start of the experiment. The concentration of NH_4—N in both enclosures started declining in early September.

The concentration of total nitrogen (TN) in the euphotic zone of the control enclosure increased from 0.96 to 2.54 mg l^{-1} in the period from July to early September, then decreased to 1.87 mg l^{-1} in late September (Fig. 5); it was occasionally higher or lower than that in the lake. The concentration of TN in the enclosure with added N increased proportional to, but less than, the amount of KNO_3 added. In addition, it showed a rapid decrease on the 22nd of August and again on the 19th of September.

The concentration of total phosphorus (TP) in the euphotic zone and the bottom layer of the lake increased from about 90 to about 600 μg l^{-1} from early July to early October (Fig. 6). In the control enclosure the increase in TP concentration was parallel to that in the lake during most of the experimental period, except for the period from the 19th of September to the 3rd of October when TP concentration increased to 900 μg l^{-1}. The concentration of TP in the enclosure with added N increased significantly less than that in the control in the last part of the experimental period.

The TN/TP ratio in the lake water decreased from 12.0 to 3.3 from early July to early October (results not shown). In the control enclosure this ratio decreased to 2.5 during the same period. The addition of KNO_3 succeeded in maintaining a high (15) TN/TP ratio during most of the period. However, the ratio decreased to a value of 7.5 for short periods in August and July and it never reached the critical value of 29.

The concentration of chlorophyll **a** in the control enclosure, which increased from 47 to 105 μg l^{-1} from early July to early September and then declined to 66 μg l^{-1} in early October, was lower than that in the lake after the 24th of July (Fig. 7). In the enclosure with added N the concentration of chlorophyll **a** was slightly higher than in the control enclosure during the first month of the experimental period. However, it decreased rapidly to about 10 μg l^{-1} after the 8th of August and it recovered to a level significantly lower than in the control enclosure after the 29th of August.

The water transparency in the control enclosure, which decreased from 0.85 m in early July to 0.50 m in late September, was higher

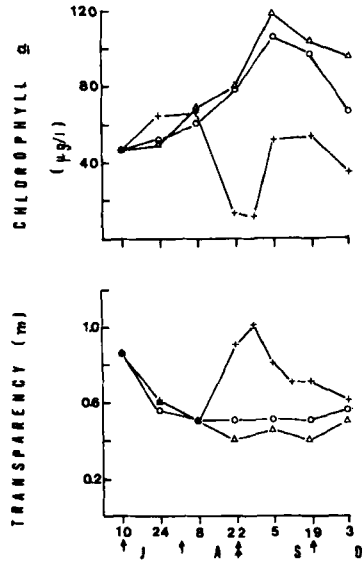

FIGURE 7 Concentration of chlorophyll a in the euphotic zone and water transparency. (For the symbols see Fig. 3.)

than that in the lake from the 8th of August until almost the end of the experimental period (Fig. 7). The water transparency in the enclosure increased rapidly after the 8th of August and reached a maximum of 1.0 m on the 29th of August; then it declined but remained higher than in the control enclosure for the rest of the experimental period.

The concentration of dissolved oxygen (DO) at 0.5 m was always higher in the lake than that in either enclosure (Fig. 8). No consistent differences were observed between the control enclosure and the enclosure with added N. In the bottom layer the DO concentration in the control enclosure was lower than that in the lake and in the enclosure with added N at the end of the experimental period.

The pH value at 0.5 m in the control enclosure ranged between 8.6 and 9.6 (mean value 9.2). The corresponding value in the enclosure with added N was 8.8 to 9.4 (mean value 9.1) and in the lake 8.5 to 9.5 (mean value 9.2). The mean pH values in the bottom layer of the control enclosure, the enclosure with added N and the lake were 8.5, 8.5 and 8.7 respectively.

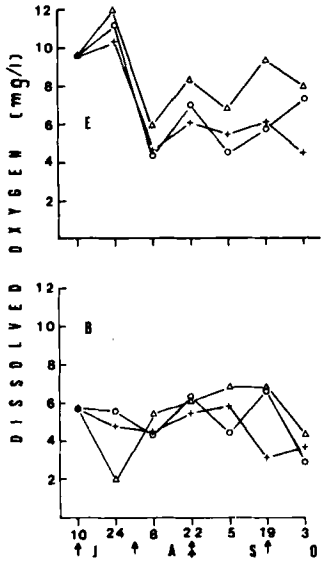

FIGURE 8 Concentration of dissolved oxygen at 0.5 m(E) and in the bottom layer (B). (For the symbols see Fig. 3.)

The activity of nitrogenase in the euphotic zone of the lake peaked in the second half of August (775 nmoles C_2H_2 l-hr^{-1}) and was higher than that in the control enclosure throughout the experimental period (Fig. 9). The activity of nitrogenase in the control enclosure was higher (occasionally significantly higher) than

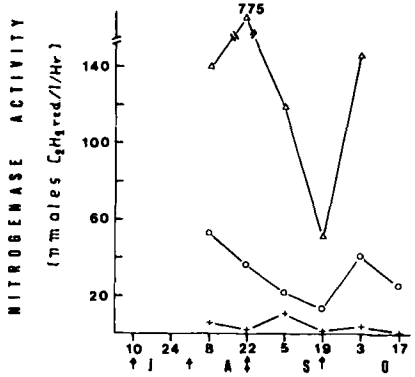

FIGURE 9 Nitrogenase activity in the euphotic zone. (For the symbols see Fig. 3.)

that in the enclosure with added N in which the activity never exceeded 10 nmoles C_2H_2 l-hr^{-1}.

DISCUSSION

The lack of a thermal stratification layer indicates that there was mixing of the water which resulted in an almost uniform distribution of the added nitrate nitrogen throughout the water column within two weeks after its addition. The confinement of the water did not have any effect on nitrate concentration in the control enclosure.

In addition to the rapid decrease of NO_3—N observed in the enclosure with added N in September, a similar decrease must have occurred in mid August as indicated by the simultaneous decrease in TN concentration. Both decreases in NO_3—N are attributed mainly to denitrification; uptake of nitrate probably was not occurring in presence of the high ammonia concentration (Kappers, 1980); reduction of NO_3—N to ammonia cannot explain all the decrease in NO_3—N since the increase in NH_4—N was less than equivalent. Since the total nitrogen increased but chlorophyll decreased, uptake of nitrate nitrogen by bacteria could explain why the nitrate nitrogen did not increase after the last addition (September 19).

Denitrification, sedimentation of dead organic matter, and ammonia volatilization account for the rapid decrease in TN in mid August and September and for the fact that the increase in TN concentration was less than equivalent to the amount of nitrogen added. Such rapid losses of nitrogen were also observed by Leonardson and Ripl (1980) who attributed them to denitrification. Therefore it appears that the system is resistant to a high increase in NO_3—N.

The increase in NH_4—N concentration in both the control and the enclosure with added N are attributed to lower volatilization losses due to decreased agitation. Volatilization causes losses of NH_3 in the order of 20–30 μg N l-hr^{-1} at a pH of 9.0, a wind velocity of 3.9–5.3 m sec^{-1} and a concentration of 700 μg NH_4—N l^{-1} (Murphy and Brownlee, 1981). The fact that the increase of ammonia concentration in the enclosure with added N was higher

than in the control enclosure, is attributed to a higher rate of NO_3—N conversion to NH_3 and/or production of ammonia by zooplankton. However, the increase would be lower if KNO_3 was added in a lake because the rate of loss through volatilization would be higher (Murphy and Brownlee, 1981).

The increases in the TP concentration in the control and the enclosure with added N, which are due to P release from sediments, were equal until late September. The fact that the addition of nitrate nitrogen did not prevent the release of phosphorus, although its concentration was always well above $1\,mg\,N\,l^{-1}$, favours the concept that the release of phosphorus from sediments depends on the sediment type (Bostrom and Pettersson, 1982) and not the NO_3—N concentration (Andersen, 1982). However, in our case the addition of NO_3—N did reduce the release of phosphorus from sediments in the last 15 days of the experiment. Therefore, it may be assumed that anaerobic as well aerobic release of phosphorus may occur in some sediments. The anaerobic release requires a long period, if DO concentrations are not very low in the bottom layer, for the water-sediment interface to establish a concentation of DO of $0.5\,mg\,l^{-1}$, the concentration at which release of phosphorus occurs (Mortimer 1942). Due to the higher DO concentrations the release of phosphorus from the sediments in the open lake was lower than in the control enclosure during the last fifteen days. Release of phosphorus through reductive processes may occur in the deep parts of the lake where stratification in May results in concentrations of DO near zero (Tsicritsis and Mourkides, 1985).

Chlorophyll **a** concentration in the control enclosure was slightly lower than in the lake and resulted in a slightly higher transparency. The initial increase in chlorophyll **a** in the enclosure with added N, accompanied by a decrease in transparency, is probably due to increased inorganic nitrogen levels. The rapid decrease after the 8th of August could be explained by increased grazing by zooplankters. In this case, it must be assumed that a change of composition of phytoplankton had occurred involving replacement of blue-green algae by green algae that were favoured by the increased nitrogen levels. However, the fact that chlorophyll recovered from about $12\,\mu g\,l^{-1}$ to $50\,\mu g\,l^{-1}$ indicates that an undertermined factor enhanced the effect of grazing during the second half of August. The

hypothesis that a change in composition of algal population caused by the high TN/TP occurred, is in accordance with the observed decrease in nitrogenase activity, although the initial decrease in activity (8th of August) must be attributed to suppression of nitrogenase synthesis and heterocyst formation. The possibility that activity per unit enzyme was reduced must be rejected since this is not affected by concentrations of inorganic nitrogen at the levels measured (Stewart *et al.*, 1975).

The fact that ratios of TN/TP less than 29 (the critical value according to Smith (1983)) were able to influence the phytoplankton population at the expense of blue–greens, is probably related (Smith, 1986) to high light intensities which are present in a country like Greece.

CONCLUSIONS

Both aerobic and anaerobic release of phosphorus may occur in some sediments. Additions of nitrate nitrogen cannot inhibit the release of phosphorus in shallow lakes when the bottom layer of the water is oxygenated.

Nitrate additions may improve the water quality by reducing biomass of blue–green algae and increasing transparency. However, in view of the significant losses of nitrogen through denitrification, nitrate additions should be combined with techniques to reduce phosphorus loading so that the amount of nitrate nitrogen required to achieve a high TN/TP ratio can be minimized. A valuable approach to minimize the cost of nitrogen addition is to discharge the domestic sewage into the lakes after it has been treated for phosphorus removal and for transformation of all nitrogen forms to nitrate (Leonardson and Ripl, 1980).

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