Limitation of water hyacinth by nitrogen in subtropical lakes of the Paraná floodplain (Argentina)

Abstract—Low (0.1–0.7 μmol liter^{-1}) dissolved inorganic nitrogen concentrations are common in the floodplain lakes of the Paraná River, where the water hyacinth (*Eichhornia crassipes*) is the major primary producer. We tested the hypothesis that N supply could limit production of *E. crassipes* during the growth season by adding NH_4^+ or PO_4^{3-} during 1 month to large in situ enclosures. Those enclosures amended with N produced twice as much biomass as the controls and P-treated enclosures. The low and limiting N content (1.3% AFDW) of the control and P-treated plants provide a first diagnostic value for N deficiency in the field for *E. crassipes*. N limitation in these lakes may be attributable to high P supply from Andean rivers, high losses of macrophyte N during floods, and the unstable nature of floodplain lakes.

The Paraná River drains the second largest watershed in South America. Thousands of lakes occupy the ~40,000-km² floodplain which borders a large part of the river's course. At our study site (near Resistencia, Argentina), 30 km downstream from the confluence of the Paraguay and Paraná Rivers, these lakes are generally small (100–500 m wide, 500–3,000 m long) and shallow (1–4 m). Floods are less pronounced (amplitude, 1–3 m), more irregular, and of shorter duration than for the Amazon or the Orinoco Rivers. In most lakes, indirect hydrological contact with the river and throughflow occurs 1–3 times per year for 1–4-week periods, when the relative level of the Paraná reaches or exceeds the 4.8-m datum at Corrientes. During exceptional floods (1905 and 1983) the entire floodplain may become submerged for several months. A more detailed description of the study site is provided by Carignan and Neiff (1992).

In contrast with the Amazon and Orinoco floodplain lakes, many of the Paraná lakes sustain high biomasses of the floating macrophyte *Eichhornia crassipes* where it is, by far, the major primary producer. The plant forms cohesive and sometimes mobile meadows that may cover <5% to nearly 100% of the available surface at the end of the growth season (August–March). Biomass ranges from 5 to 30 t ha^{-1} DW, and net production has been estimated at 10–15 t ha^{-1} during the growth period (Neiff and Poi de Neiff 1984). Water hyacinth production in the Paraná floodplain lakes appears to be substantially lower than often reported elsewhere (*see* Mitsch 1977; Lorber et al. 1984). In the open waters, planktonic chlorophyll *a* ranges from 10 to 25 mg m³ and primary production from 200 to 1,000 mg C m^{-2} d^{-1} (Bonetto et al. 1984; Carignan and Planas 1994). The phytoplankton is mainly composed of small green cyanobacteria and cryptophycean algae (e.g. *Sphaerocystis schoeteri, Microactinium pusillum, Monoraphidium tortile, Merismopedium tenuissima, Anabaenopsis* sp., *Crucigenia* sp.).

The incomplete coverage of the lakes by *Eichhornia* led us to investigate whether P or N could limit its production. The study was conducted in Lake San Nicolás north (27°27'S, 58°55'W; width, ~150 m; length, ~1 km) and five other surrounding lakes located 1–3 km from the river. Lake San Nicolás belongs to a complex of several similar lakes that are oriented parallel to the river. They are separated by alluvial levees (~50 m wide, 1–2 m high) occupied by gallery forest. On average, the lakes are disconnected from the river ~80% of the time, and nutrient influx from the river appears to be substantial only during extraordinary floods (every 10 yr on average). We use the term "lake" for any permanent water body, large or small, located on the floodplain (Welcomme 1985).

A survey of nutrient concentrations in the

Table 1. Average concentrations of NH_4^+, NO_3^-, and DRP (μmol liter^{-1}) and molar N : P ratios in six floodplain lakes sampled (duplicates) in open waters (O) or under *Eichhornia* (E) between 6 and 13 January 1990 during low-water conditions. The river was sampled on 24 January at the beginning of a flood pulse.

Lake	[NH_4^+]		[NO_3^-]		[DRP]		N : P	
	O	E	O	E	O	E	O	E
San Nicolás north	0.09	—	0.12	—	0.82	—	0.26	—
San Nicolás south	<0.03	0.19	0.04	<0.02	0.70	0.81	0.05	0.23
Baltazar north	<0.03	0.60	0.55	<0.02	1.16	0.77	0.47	0.78
Baltazar south	<0.03	0.11	0.68	<0.02	0.42	0.41	1.62	0.27
Puente north	0.13	0.47	0.17	<0.02	0.85	0.58	0.35	0.81
Puente south	9.64	—	6.39	—	1.30	—	12.3	—
Paraná River	0.23		15.28		0.35		44.3	

six lakes was conducted in December 1989 and January 1990 during low-water conditions. Water samples were collected in open sections of the lakes or within the ~50-cm-thick root mat with plastic tubing and a hand-held vacuum pump, at least 20 m inside the meadows. The samples were preserved on ice for 1–2 h until filtration on prewashed Gelman DM-450 membranes (0.45-μm nominal pore size); NH_4^+ was measured by the indophenol blue method, $NO_3^- + NO_2^-$ by reduction on Cd, and dissolved reactive P (DRP) by the molybdenum blue method (Stainton et al. 1977); total P (TP) was measured after persulfate oxidation. The results (Table 1) revealed undetectable or very low NH_4^+ and NO_3^- and relatively high DRP in all but one of the lakes. Our NH_4^+ and NO_3^- values are orders of magnitude lower than those previously reported during low water for the same systems (Bonetto et al. 1984) and are much lower than those typically used in studies of N uptake by floating macrophytes (e.g. Gossett and Norris 1971; Shiralipour et al. 1981). In these lakes, the ($NH_4^+ + NO_3^-$) : DRP ratio is notably lower than the Redfield molar proportion of 16 : 1 required by living organisms. Low available N concentration or low N : P ratio does not, however, necessarily imply N limitation, as it is the supply not the concentration that is most relevant to production.

To verify whether N could be limiting floating macrophyte production in the floodplain, we deployed six 20-m² enclosures in 155±5 cm of water in L. San Nicolás; the lake supported a 50–60% plant cover at the time of the experiment. The 2-m-high hexagonal enclosures were open to bottom sediments and made of woven polyethylene covered by an impermeable 5-mil polyethylene layer; 1.8 kg m^{-1}

of chain was used at the bottom to ensure good contact with the sediments. The enclosures were supported by six vertical steel pipes driven into the sediment. To simulate natural initial conditions in terms of biomass and nutrient demand, we selected 1,800 middle-sized *E. crassipes* specimens from a homogeneous stand and introduced 300 into each enclosure. The plants thus covered ~60% of the available surface. Biomass and plant-bound nutrients added to the enclosures were estimated within 7% (1 SE) from the mean weight and nutrient content of 72 randomly picked individuals. Two enclosures were fertilized (analytical grade reagents) with 34.6 mmol m^{-2} d^{-1} of NH_4Cl, two others with 4.3 mmol m^{-2} d^{-1} of Na $H_2PO_4 \cdot H_2O$, and two were used as controls. Our apparently high loading rates were calculated to match the expected nutrient demand of the plants. Under optimal conditions, *E. crassipes* can potentially double its biomass in 2–3 weeks.

During the experiment, vertical mixing of the water column was monitored inside and outside the enclosures with thermistor chains connected to LiCor LI-1000 data loggers (Carignan and Neiff 1992). Temperature profiles were recorded every 15 min at 10–20-cm intervals with a precision of 0.01°C and an absolute accuracy of 0.05°C.

Nutrient salts were dissolved in 40 liters of water and dispersed every 3–4 d under the plants and near the sediment–water interface. Nutrient transport to the 40-cm-thick root zone occurred daily during the complete vertical mixing of the waters, usually between 0200 and 0900 hours. Dissolved inorganic nutrients were measured on water samples taken at mid-depth in the morning before each fertilization. DRP concentrations in the enclosures reached

Table 2. Net biomass production and allocation, nutrient, ash, and chlorophyll *a* concentrations in *Eichhornia* after 30 d of fertilization. Elemental concentrations are expressed on an ash-free dry weight basis; t—total plant material; g—green parts; r—roots.

Enclosure	Production (g m⁻² d⁻¹)	Allocation (%)	TC (%)	TN (%)	TP (%)	TK (%)	Ash (%)	Chl *a* (mg m⁻²)
2 control	5.27		45.0(t)	1.33(t)	0.221(t)	2.51(t)	21.7(t)	250±43
		51.2(g)	44.8(g)	1.06(g)	0.198(g)	3.07(g)	12.8(g)	
		48.8(r)	45.3(r)	1.62(r)	0.245(r)	1.93(r)	31.0(r)	
5 control	6.58		46.9(t)	1.29(t)	0.243(t)	1.93(t)	22.6(t)	—
		48.4(g)	47.0(g)	1.04(g)	0.169(g)	3.00(g)	14.0(g)	
		51.6(r)	46.9(r)	1.53(r)	0.312(r)	0.92(r)	30.7(r)	
3 + P	5.94		46.1(t)	1.22(t)	0.340(t)	2.05(t)	20.0(t)	301±84
		46.7(g)	45.3(g)	1.00(g)	0.252(g)	2.72(g)	12.5(g)	
		53.3(r)	46.8(r)	1.42(r)	0.418(r)	1.47(r)	26.5(r)	
6 + P	4.25		48.0(t)	1.36(t)	0.436(t)	1.97(t)	22.8(t)	228±41
		46.8(g)	47.5(g)	1.09(g)	0.327(g)	2.40(g)	12.6(g)	
		53.2(r)	48.6(r)	1.60(r)	0.532(r)	1.59(r)	31.7(r)	
1 + N	12.11		45.7(t)	2.30(t)	0.158(t)	1.95(t)	20.0(t)	660±78
		63.6(g)	45.4(g)	2.33(g)	0.131(g)	2.24(g)	10.9(g)	
		36.4(r)	46.3(r)	2.24(r)	0.205(r)	1.44(r)	36.0(r)	
4 + N	10.74		46.2(t)	2.53(t)	0.194(t)	1.93(t)	18.5(t)	608±38
		64.1(g)	45.6(g)	2.58(g)	0.151(g)	2.25(g)	11.3(g)	
		35.9(r)	47.2(r)	2.45(r)	0.271(r)	1.35(r)	35.9(r)	

20 μmol liter⁻¹ within 2 weeks. Consequently, we decreased P supply, and the average P supply was 2.5 mmol m⁻² d⁻¹. In contrast, dissolved inorganic N (DIN) always decreased to <2 μmol liter⁻¹ within 48 h of fertilization. The experiment was stopped after 30 d at the onset of an unforeseen strong flood pulse. The harvest (100–200 kg FW per enclosure) was air-dried in the shade for 3 d (during which ~80% of the water was lost) and weighed. The plants were shredded by hand, and 0.5–1-kg subsamples were weighed, dried at 105°C, and weighed again to calculate total dry production. Total C (TC) and total N (TN) were measured with a NA-1500 Carlo-Erba analyzer. Ash content was measured after ignition at 550°C. TP was measured after dissolution of the ash in dilute HCl (Anderson 1976). Epiphytic green algae were observed on the superficial roots of the N-treated plants but remained visually absent in other treatments; no attempt was made to separate macrophytes from epiphytes, since epiphytic biomass appeared negligible compared to that of the macrophytes. Leaf Chl *a* was measured spectrophotometrically at 663 nm after maceration (24 h) of freshly collected 1-cm² disks in methanol at 4°C.

The plants responded rapidly to N additions. Within 5 d, the leaves of the N-treated plants developed significantly more (*t*-test, α = 0.05) Chl *a* (397±10 mg m⁻² of leaf surface, *n* = 18, error = SE) than those of the control (322±16 mg m⁻² of leaf, *n* = 17) or P-treated (274±28 mg m⁻², *n* = 18) enclosures. After 30 d (Table 2), leaf Chl *a* had reached an average of 634 mg m⁻² in the N-treated enclosures. The N-treated enclosures produced significantly more plant biomass than the control (ANOVA, $F = 34.4$, $P < 0.05$) or P-treated enclosures ($F = 34.2$, $P < 0.05$). Interestingly, although at least 30 m separated the enclosures from the shore or from floating meadows, we observed that the N-treated enclosures attracted large numbers of grasshoppers. We flushed the insects out of all six enclosures once or twice daily with tree branches in order to avoid excessive grazing. In spite of these efforts, grasshoppers apparently did more damage to the plants of the N-treated enclosures than to those of the control or P-treated enclosures. Thus, the actual production values for the N-treated enclosures may have been slightly higher than reported in Table 2. It can be concluded that inorganic N supply limits the production rate of *E. crassipes* in L. San Nicolás during the growth season. During colder months (April–July), low temperatures rather than N supply may limit growth.

We believe our results are the first in situ

demonstration of N limitation of floating macrophytes in tropical or subtropical floodplain lakes. Previous experimental evidence for N limitation in such waters is limited to bioassays performed on phytoplankton where additions of NH_4^+ or NO_3^- to isolated water samples maintained for days or weeks under favorable light regimes were found to stimulate production or biomass (Setaro and Melack 1984; Zaret et al. 1981; Henry et al. 1984).

The low and limiting average TN content in total plants (1.30% AFDW), green parts (1.05% AFDW), and roots (1.54% AFDW) of the control and P-treated plants provide a first diagnostic value for N deficiency in the field for *E. crassipes*. These values are close to the critical N requirement reported by Gerloff (1970) for submersed aquatic plants. He found reduced growth in batch cultures when TN was <1.3% and TP <0.13%, expressed on a dry weight basis. When expressed on the same basis, the average TN concentrations in *E. crassipes* grown in the control and P-treated enclosures are 1.00% for total plants, 0.92% for green parts, and 1.08% for roots; average TP concentrations in plants grown in the control enclosures are 0.18, 0.16, and 0.19% for total plants, green parts, and roots. Critical tissue N and P concentrations in emergent parts of floating vegetation are expected to be somewhat lower than in submersed vegetation because the latter has relatively less structural tissue.

Plants collected at different times of the year during low-water conditions in San Nicolás and four other lakes systematically showed a low N content ($1.29 \pm 0.18\%$ AFDW, total plants; $n = 25$). N limitation therefore appears to be common for *Eichhornia* in the floodplain. In contrast, the P content of the control plants (0.23%, Table 2) or plants collected in other lakes ($0.29 \pm 0.03\%$ AFDW; $n = 25$) was always nearly twice the critical concentration reported by Gerloff (1970). Note that in the control and P-treated enclosures, which were limited by N supply, more biomass (52% of the total) was allocated to root development than in the N-treated enclosures, where roots represented only 36% of total plant biomass (Table 2).

It is widely accepted that production and biomass in freshwater ecosystems is generally limited by P supply (Schindler 1977, 1978).

Limitation by N is considered improbable because ecosystems can fix atmospheric N_2 and because experimental evidence for N limitation at the ecosystem level is still scarce. Compared to other freshwater systems, the Paraná floodplain (and possibly other floodplains) may be exceptional in terms of the limiting nutrient (N instead of P). There are several possible explanations for this peculiarity. During flood events, nutrients with a very low N : P ratio are supplied to the lakes. We measured an average $(NH_4^+ + NO_3^-)$: DRP molar ratio of 1.4, with DIN concentrations of <0.1–4 μmol liter^{-1} in water flowing through L. San Nicolás during an exceptional flood, while DIN in the main channel was 15–20 μmol liter^{-1} (January 1990; Carignan and Neiff 1992). In addition, a large fraction (~40%) of the main channel total particulate P (~22 μmol g^{-1}) becomes available when subjected to the acidic pH conditions (6–6.6) prevailing in these floodplain lakes (Carignan and Vaithiyanathan unpubl.). Denitrification at the sediment–water interface of the lakes may further lower the N : P ratio.

N limitation and low N : P ratios in the Paraná floodplain lakes may also arise from the resetting effect of floods. Stable freshwater systems tend to build-up and recycle N reserves contained in living biomass and in decaying organic matter; such systems are generally limited by P or by light if P is abundant. In contrast, most floods in the Paraná do not appear to bring large amounts of DIN to the lakes, but they do cause significant losses of macrophytes by stranding. Exceptional floods periodically washout most of the organic N reserve contained in floating macrophytes, but the same floods supply riverine mineral sediments rich in available P. Several years of N supply from external sources and N_2 fixation may be required to re-establish optimal N stocks.

Richard Carignan

INRS-Eau, Université du Québec
C.P. 7500
Sainte-Foy G1V 4C7

Juan José Neiff

Centro de Ecología Aplicada del Litoral
CONICET
3400 Corrientes, Argentina

Dolors Planas

Dept. des sciences biologiques
Université du Québec à Montréal
C.P. 8888, Succ. A
Montréal H3C 3P8

References

ANDERSON, J. M. 1976. An ignition method for determination of total phosphorus in lake sediments. Water Res. **10**: 329–331.

BONETTO, C. A., Y. ZALOCAR, AND H. G. LANCELLE. 1984. A limnological study of an oxbow-lake covered by *Eichhornia crassipes* in the Paraná River. Int. Ver. Theor. Angew. Limnol. Verh. **22**: 1315–1318.

CARIGNAN, R., AND J. J. NEIFF. 1992. Nutrient dynamics in the floodplain ponds of the Paraná River (Argentina) dominated by the water hyacinth *Eichhornia crassipes*. Biogeochemistry **17**: 85–121.

———, AND D. PLANAS. 1994. Recognition of nutrient and light limitation in turbid mixed layers: Three approaches compared in the Paraná floodplain (Argentina). Limnol. Oceanogr. **39**: 580–596.

GERLOFF, C. G. 1970. Evaluating nutrient supplies for the growth of aquatic plants in natural waters, p. 537–555. *In* Eutrophication: Causes, consequences, correctives. Natl. Acad. Sci.

GOSSETT, D. R., AND W. E. NORRIS, JR. 1971. Relationship between nutrient availability and content of nitrogen and phosphorus in tissues of the aquatic macrophyte, *Eichhornia crassipes* (Mart.) Solms. Hydrobiologia **38**: 15–28.

HENRY, R., J. G. TUNDISI, AND P. R. CURI. 1984. Effects of phosphorus and nitrogen enrichment on the phytoplankton in a tropical reservoir (Lobo Reservoir, Brazil). Hydrobiologia **118**: 177–185.

LORBER, M. N., J. W. MISHOE, AND P. R. REDDY. 1984. Modeling and analysis of water hyacinth biomass. Ecol. Model. **24**: 61–77.

MITSCH, W. J. 1977. Water hyacinth (*Eichhornia crassipes*) nutrient uptake and metabolism in a north central Florida marsh. Arch. Hydrobiol. **81**: 188–210.

NEIFF, J. J., AND A. POI DE NEIFF. 1984. Cambios estacionales en la biomasa de *Eichhornia crassipes* (Mart.) Solms y su fauna en una laguna del Chaco (Argentina). Ecosur **11**: 51–60.

SCHINDLER, D. W. 1977. The evolution of phosphorus limitation in lakes. Science **195**: 260–262.

———. 1978. Factors regulating phytoplankton production and standing crop in the world's freshwaters. Limnol. Oceanogr. **23**: 478–486.

SETARO, F. V., AND J. M. MELACK. 1984. Responses of phytoplankton to experimental nutrient enrichment in an Amazon floodplain lake. Limnol. Oceanogr. **29**: 972–984.

SHIRALIPOUR, A., L. A. GARRARD, AND W. T. HALLER. 1981. Nitrogen source, biomass production, and phosphorus uptake in water hyacinth. J. Aquat. Plant Managc. **19**: 40–43.

STAINTON, M. P., M. J. CAPEL, AND F. A. J. ARMSTRONG. 1977. The chemical analysis of fresh water, 2nd ed. Can. Fish. Mar. Serv. Misc. Spec. Publ. 25.

WELCOMME, R. L. 1985. River fisheries. FAO Fish. Tech. Pap. 262.

ZARET, T. M., A. H. DEVOL, AND A. DOS SANTOS. 1981. Nutrient addition experiments in Lago Jacaretinga, Central Amazon Basin, Brazil. Int. Ver. Theor. Angew. Limnol. Verh. **21**: 721–724.

A sound scattering layer in a freshwater reservoir

Abstract—A near-surface sound scattering layer has been observed in a freshwater reservoir with a 200 kHz echosounder over the last 5 yr. Net sampling revealed that *Chaoborus* is the cause of the strong 200-kHz sound scattering in this layer. With 50-kHz sound, this layer cannot be distinguished from the background. Recent acoustic measurements have determined the target strength of a single *Chaoborus* larva and the frequency response of backscattering from *Chaoborus* in the reservoir so that an acoustic estimation of the *Chaoborus* population can be made. This population estimate is compared with simultaneous net-sampling estimates.

Sound scattering layers for frequency ranges of 1–25 kHz in the deep ocean have been reported quite often (e.g. Chapman et al. 1974; Urick 1975), but those at higher frequencies such as 200 kHz have been reported rarely. When the dimensions of a scattering object are much smaller than the wavelength of the incident sound, the object is called a Rayleigh scatterer. The acoustic scattering cross-section of such an object increases as the fourth power