

Spatial variation in productivity and nutrient limitation of benthic algae

Student: Trisha Thoms

Mentor: Pete McIntyre

Introduction

Primary producers play an important role in freshwater systems, acting as chemical modulators and energy sources for the ecosystem food web (Stevenson 1996). Ecologists are often interested in characterizing nutrient and energy flows on an ecosystem-wide scale. Thus, there is a need to determine the relative importance of different ecological factors in regulating primary productivity and the food webs that they support.

Although benthic algal productivity is often of similar magnitude to phytoplankton, scientists tend to focus their studies on the pelagic zone, and a comprehensive understanding of benthic productivity is lacking. (Vadeboncoeur et al. 2002; Lamberti 1996). Large deep lakes tend to be clear and support highly productive periphyton mats that can provide a substantial portion of total lake primary productivity (Vadeboncoeur et al. 2003). Lake Tanganyika fits this description, but little work has been done on littoral productivity, particularly in identifying the limiting nutrients (Bootsma and Hecky 1993). Studies that have addressed this question do not agree: Hecky and Kilham (1993) proposed N limitation based on the stoichiometry of the water; Jarvinen et al. (1999) indicated a co-limitation of nitrogen (N) and phosphorous (P) in the epilimnion, but proposed that P limits primary production based on slight stimulation by P enrichments; and preliminary studies in the Kigoma region show that benthic algae is P-limited (McIntyre, unpublished 2001-2; Olsgard 2002). Additionally, benthic productivity is suspected to be spatially variable based on algal carbon isotope and C/N data (McIntyre 2002, unpublished), triggering the question of whether nutrient limitation also fluctuates through space.

This study investigates four questions to provide insight into the energetic base of the littoral zone: (1) How does productivity vary spatially on natural substrata? (2) Which nutrients limit benthic productivity? (3) Is nutrient limitation spatially variable? (4) Do ambient nutrient concentrations in the water column correspond to nutrient responses by benthic algae? Direct measurements of productivity on natural substrata were used to address our first question, and we employed nutrient diffusing substrates (NDS) enriched with nutrients, namely N and P, to explore the nutrient limitation questions. Finally, a time-series nutrient sampling of the littoral water column, conducted by another student (J. Corman, this volume), was compared with our NDS data.

Methods

Study Sites:

In selecting study sites, our goal was to sample rocky habitat along a productivity gradient based on the preliminary 2002 data (McIntyre unpublished). From south to north,

the following six sites were chosen: (1) *Jakobsen Outer* (04°5.982'S, 29°35.766'E), northwest-facing, low productivity; (2) *Jakobsen Inner* (04°54.982'S, 29°35.766'E), west-facing, high productivity; (3) *Bangwe Point* (04°54.804'S, 29°35.901'E), west-facing, high productivity; (4) *Katabe South*, (04°54.003'S, 29°36.037'E), northeast facing, moderate productivity; (5) *Euphorbia*, (04°50.956'S, 29°36.526'E GPS), southwest-facing, moderate productivity; and (6) *Kalalengabo South*, (04°50.630'S, 29°36.445'E GPS), north-facing, low productivity.

Direct Measurements of Productivity:

Measurements of gross primary production (GPP = NPP – Respiration) and algal biomass were taken from natural rock substrata at all six sites. Productivity was calculated using a spectrophotometric modification of the Winkler titrimetric method for measuring dissolved oxygen (DO) (Wetzel and Likens 1979). On SCUBA, we incubated three replicate light and dark chambers at 2 m depth on large, flat rocks at each site. Chambers (10 cm x 10 cm x 8 cm, 0.8 L) were anchored with circular lead weights placed on top of neoprene skirts attached to the bottoms of the chambers. After initial water samples were taken with a 60 mL syringe, chambers were corked and left to incubate (15 min. light, 40 min. dark). Water inside chambers was homogenized with an internal stirring paddle before the final sample was collected. Samples were immediately fixed in the field with 200 µmL of standard APHA solutions (manganous sulfate and alkaline iodide-azide), following the WAH azide modification of the Winkler method (Hach method 8229), and kept in the dark until reaching the laboratory. Light intensity during incubations was measured using a Li-Cor LI-1925A Quantum underwater flat cosine sensor. In the lab, concentrated H₂SO₄ (200 µmL) was added to each syringe before transferring the solution to BOD bottles and reading the absorbance on a Genesys 20 spectrophotometer at 430 nm. A calibration curve for Lake Tanganyika was established by titrating acidified samples with sodium thiosulfate and starch indicator. The slope and intercept of this curve was applied to the following equations to calculate GPP: $GPP (mg C / m^2 - hour) = (NPP - Respiration) * (0.375 * 1.2)$. $NPP \& Resp (mg O_2 / m^2 - hour) = [Abs_{final} * (10.549 - 0.984) - Abs_{initial} * (10.549 - 0.984)] / hours\ incubated * substrata\ area$

Natural Substrata Biomass:

Concentrations of Chlorophyll *a* (µg/cm²) were spectrophotometrically determined as a measure of algal biomass. Six replicate algae samples were taken at each site, corresponding to each chamber. Periphyton was removed from the rock with a modified 2.7 cm diameter dual-syringe scrubber and kept on ice in the field. In the laboratory, the algal slurry was collected on Whatman GF/F 47 mm glassfiber filters, and stored frozen within aluminum foil until analysis (see methods described in Wetzel and Likens 1979). Filters were placed in 10 mL of 90% pH-buffered ethanol, pulverized with a spatula, and stored in the freezer for 24 hours before running samples on the spectrophotometer (665 and 750 nm). A second reading was taken after acidifying the sample with two drops of 0.1N HCl, to correct for phaeophytin. The equation below was used to calculate concentrations: $Chlorophyll\ a (\mu g / cm^2) = [29.6 * (Abs_{pre} - Abs_{post}) * extract\ vol (mL)] / rock\ area\ scrubbed (cm^2)$

Nutrient Diffusing Substrates (NDS):

To experimentally examine bottom-up limitation of benthic algae, nutrient availability was manipulated using nutrient-diffusing substrates. We filled clay flowerpots with nutrient-rich agar, from which nutrients slowly diffused during the incubation period (19-20 days), promoting algal growth on the outer surface. New pots (10 cm top diameter, 8.5 cm bottom diameter, 2 cm height) were soaked in lake water for 2 days, sealed with shallow plastic dishes and filled with enriched agar (see methods of Fairchild et al. 1985; Scrimgeour and Chambers 1997; Flecker et al. 2002). Four treatments were tested: (1) nitrogen-supplemented (N), (2) phosphorous-supplemented (P), (3) nitrogen and phosphorous supplemented (N+P), and (4) agar only for control (C). The agar (20 g/L H₂O) was mixed with 42.5 g NaNO₃ and 26.75 g NH₄Cl for N enrichment; 8.875 g Na₂HPO₄ for P enrichment; 42.5 g NaNO₃ and 26.75 g NH₄Cl plus 8.875 g NaHPO₄ for N+P enrichment following the Redfield ratio 16 N:1 P (molar). In order to minimize the effects of consumers on algal biomass accrual, pots were covered with plastic mesh (~1.2 cm²). Pots were grouped in complete spatial blocks, secured to a sturdy mesh array with approximately 40 cm between pots, and placed on rocky substrate. Three blocks were deployed per site at roughly 4 m depth. Nutrient release rates were measured from additional pots (2 N+P, 1 C) incubated for 17 days in situ before measurement.

NPP of each NDS was measured as oxygenesis during 15-30 minute incubations at 4 m depth in transparent 2.2 L chambers (water: 1.92 L, NDS: 0.28 L). The mesh exclusions were cut away before incubations, and initial and final DO were recorded using a YSI 95 meter following spinning the chamber to homogenize DO. We did not measure respiration due to time constraints and evidence from previous NDS studies that respiration is unaffected by treatments. Net primary production was calculated as follows: $NPP (mg C / h) = [(DO_{final} - DO_{initial}) * 1.92 L * (0.375 / 1.2)] / \text{hours incubated}$

Following NPP incubations, pots were submerged inside bags of water and transported to the lab for chlorophyll *a* analyses. Periphyton was collected by scrubbing all upward surfaces of the pot (117 cm²) with a wire brush. The volume of the algal slurry was recorded, and a 10 mL sub-sample was collected on a Gelman A/E 25 mm filter and stored on ice. Chlorophyll was extracted as described earlier, and quantified using a Turner Aquafluor ($Chl a (\mu g/cm^2) = [0.003 * (F_{pre} - F_{post}) * \text{extract vol (mL)}] / \text{area (cm}^2)$)

Statistics

Data were analyzed using JMP IN software. Multivariate ANOVAs and one-way ANOVAs were used to determine statistical significance. Tukey's HSD tests were used for *post hoc* treatment comparisons.

Results

Direct Measurements of Productivity:

GPP and algal biomass on natural rock substrata differed significantly among sites (MANOVA: $F_{5,12} = 7.76$, $p = 0.0018$; Fig. 1). Separate ANOVAs indicated significant differences between sites in area-specific GPP ($F_{5,12} = 7.86$, $p = 0.0017$,

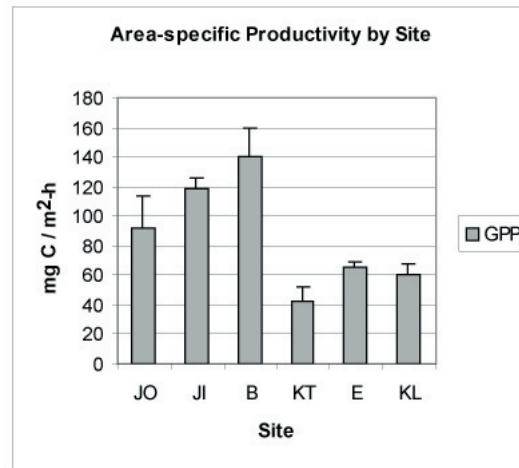


Figure 1: Spatial variability of gross primary production (GPP) (mg C / m² - h) measured on in-situ rock substrata. Sites are presented from south to north and abbreviated as follows: JO = Jakobsen's Outer, JI = Jakobsen's Inner, B = Bangwe Point, KT = Katabe South, E = Euphorbia, and KL = Kalalengabo South

biomass-specific GPP ($F_{5,12} = 7.71$, $p = 0.0019$), and algal biomass ($F_{5,12} = 4.93$, $p = 0.011$). Tukey's HSD test showed that GPP was significantly higher at Jakobsen Inner than Katabe South, and at Bangwe Point than Katabe South, Euphorbia, and Kalalengabo South. Biomass-specific GPP at Jakobsen Inner exceeded that at all other sites. The comparison of biomass across sites indicated that Bangwe Point was significantly higher than Jakobsen Outer, Jakobsen Inner, and Kalalengabo South. Although high algal biomass at Bangwe Point corresponded to high GPP, across all sites there was no significant effect of algal biomass on GPP ($F_{1,16} = 1.49$, $p = 0.24$).

Nutrient Diffusing Substrates:

The algal productivity response to nutrient enrichments varied among sites and was primarily P-limited. NPP and biomass responses to nutrient enrichments resulted in highly significant effects of phosphorous (MANOVA: $F_{1,43} = 11.98$, $p = 0.0012$) and site ($F_{5,43} = 3.10$, $p = 0.017$). Figure 2 illustrates the

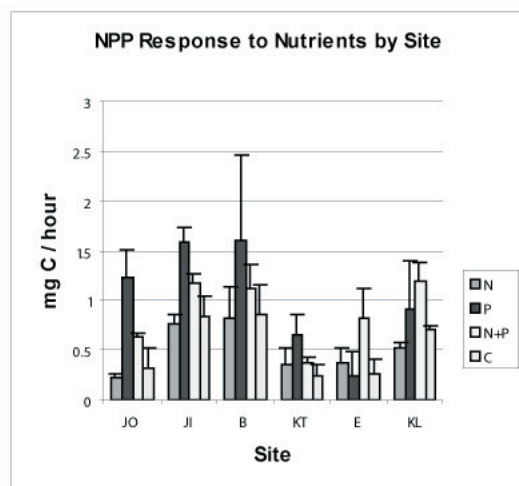


Figure 2: Spatial variability of net primary production (NPP) (mg C / h) on NDS pots in response to nutrient treatments

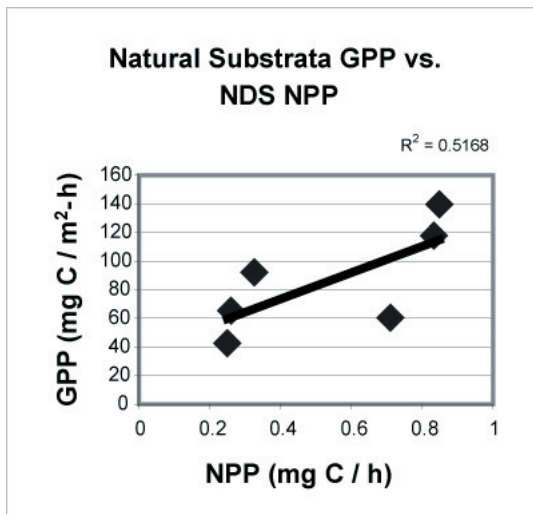


Figure 3: Correlation between GPP on natural rocks and NPP of NDS pots indicates concordant patterns of productivity

mean NPP of each nutrient treatment at all sites, showing a significant trend in the response to P enrichment across sites (one-way ANOVA: $F_{1,45} = 21.36$, $p < 0.001$). Mean NPP (of all treatments) at Bangwe Point and Jakobsen Inner is significantly higher than at Jakobsen Outer, Katabe South and Euphorbia (one-way ANOVA: $F_{5,45} = 7.33$, $p < 0.0001$). Regression of NDS NPP values against natural substrata GPP indicates concordant patterns in site-specific productivity ($F_{1,4} = 4.28$, $p = 0.107$; Fig. 3). Algal biomass also responded strongly to phosphorous ($F_{1,43} = 11.70$, $p = 0.0014$), and differed among sites ($F_{5,43} = 3.03$, $p = 0.012$). NPP and algal biomass on NDS pots were closely related ($F_{1,60} = 174.32$, $p < 0.0001$; Fig. 4), in contrast with the natural substrata regression. Biomass-specific NPP did not differ between treatments or among most sites, indicating the productivity patterns were mediated entirely by differences in algal biomass. There was a hint of a relationship between water-column SRP concentrations and the effect of P-supplementation on NPP of NDS pots (relative to control), however the trend was not significant (Fig. 5).

Discussion

Our results demonstrate that benthic algal productivity in the littoral zone of the Kigoma region is spatially variable. The productivity gradient across sites was evidenced by two independent approaches. Productivity measurements (GPP) from natural substrata paralleled the NPP of control NDS pots at most sites, and algal biomass accrual on pots was consistent with their NPP. Moreover, the apparent productivity gradient matches inferences from preliminary stable isotope data from the same sites.

The NDS experiment indicated that growth of benthic algae at all sites is limited by P availability. This results confirms and expands upon previous results from the Jakobsen's Inner and Hilltop Hotel sites (McIntyre unpublished; Olsgard 2002). Though we do not doubt the validity of the observed patterns, we must note that some algae was detached from the pots during their retrieval and transport. More importantly, many pots had visible snail tracks and chromid cases, suggesting

that invertebrate grazers had removed algal biomass (see also Borchardt 1996). It is also important to note the shortcomings of the NDS method. Algal community composition may be sensitive to artificial conditions (Fairchild et al. 1985), and experimental colonization times are relatively short (Borchardt 1996). Thus, inferences from NDS experiments are most useful when augmented with other methods.

This study did not fully address the factors driving spatial variability of benthic productivity, but there was weak evidence that ambient SRP concentrations may contribute to the observed productivity gradient. Although the pattern in Figure 5 was not statistically significant, the qualitative pattern is suggestive, and we note that the Bangwe Point data was highly variable and skews the regression. In addition to ambient water column nutrients, the algal and grazer community can have an effect on nutrient response. Differences among consumer species can affect the rate at which nutrients are recycled through the community, and algal taxa respond to nutrients differently (Fairchild et al. 1985). Another possible factor is the physical location of sites relative to bathymetry and wave action. Moving water can remove overlying detritus, allowing more sunlight to directly hit the periphyton mat as well as facilitating P uptake in many algae (Borchardt 1996). Further investigation of these areas would contribute to our understanding of the littoral ecosystem.

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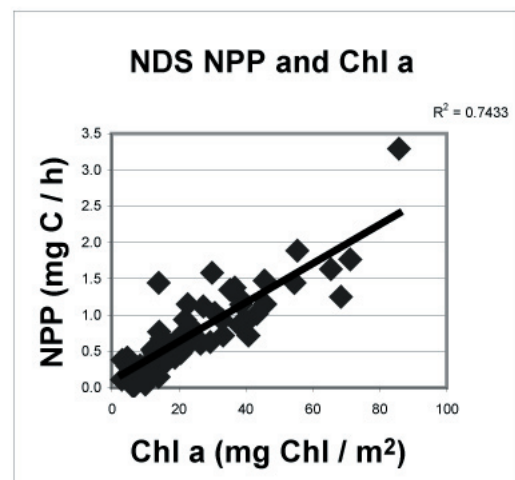


Figure 4: Correlation between NPP and algal biomass in the NDS experiment.

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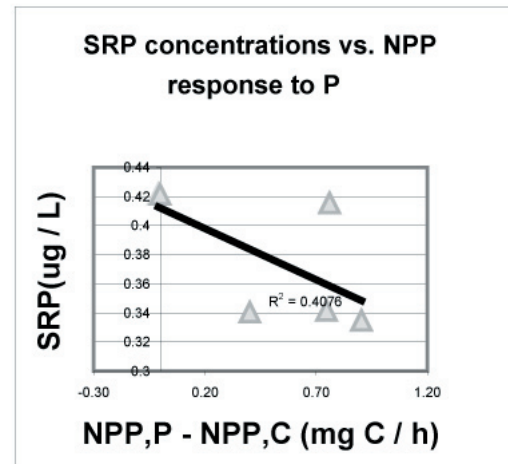


Figure 5: NPP response to P (relative to control pots) plotted against SRP concentration at each site