

# **Shedding light on the cryptic underbelly of Lake Tanganyika's benthos: New environmental indicators and potential paleoenvironmental indicators among sponges**

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## **Introduction**

Sponges fill a unique niche in benthic aquatic communities creating trophic links which otherwise may not exist. They are able to quickly and effectively clear the water of organic particles not readily collected by other organisms, including prokaryotes and other small plankton (Reiswig, 1971; Pile, 1997) with filtration rates estimated between 6-20 ml/hr/mg dry mass tissue (Reiswig, 1971). In marine systems alteration of sponge populations has been attributed to changes in both water column chemistry and biology (Butler et al., 1995).

Recent work also suggests that sponges may make significant contributions to net primary production and nutrient regeneration because of the microbial symbionts harbored by many species (Wilkinson & Fay 1979; Wilkinson 1983b; Rutzler 1990; Wilkinson 1992; Diaz 1997; Diaz & Ward 1997). In some North American lakes during later summer and fall sponges have been found to contain chlorophyll equal to that of the entire phytoplankton community (Frost 1978; Frost & Williamson 1980).

In both marine and freshwater systems nutrient availability has been suggested as a key factor in explaining the degree of dependency which sponges have on photosynthetic symbionts (Wilkinson, 1987; Wilkinson & Cheshire, 1990). In some North American lakes it has been recorded that 50-80% of the growth of *Spongilla lacustris* could be attributed to its algal symbionts (Frost & Williamson 1980). Recent studies show that density of symbionts in freshwater habitats varies with availability of light and particulate resources (Frost & Elias 1990).

Silica availability has also been shown to play an important role in sponge morphology. In lakes in northern Wisconsin both the number and morphology of megascleres vary directly with lake silica concentrations. In low silica conditions it has been observed that megasclere width decreases but numbers increase substantially. Indirect responses to silica concentrations have also been observed such as a change in growth form from branching to encrusting in *S. lacustris* when silica availability went from high to low (Thorp & Covich 1991).

This change in megasclere morphology has also been found to be correlated with increased predation. It is thought that spicules provide a mechanical deterrent to predation by irritating the mouths and digestive tracts of predators. Covich observed that snails would not consume *Spongilla lacustris* from high-silica habitats but that they readily consumed *S. lacustris* specimens with smaller, weaker spicules from low silica habitats.

For these reasons sponges can be valuable tools in monitoring aquatic systems, providing insight on both water quality and nutrient availability. Despite their possible functional roles as environmental and paleo-indicators, there is very little known about Lake Tanganyika sponges. Eight species from four genera have been recorded in Lake Tanganyika, all from the Burundi and Democratic Republic of Congo coastlines (Manconi & Pronzato, 2002), and no ecological work has been done to date. Thus I aimed to intensively survey the Kigoma region to make initial estimates on species diversity, habitats, and environmental correlates.

## **Materials & Methods**

Nine sites in total were surveyed for sponges. Six sites of these sites were selected as contrasting in wave energy and turbidity, were quantitatively sampled for sponges and for physical variables. Turbidity was measured using a Hach 2100p Turbidimeter. Results were expressed in Nephelometric Turbid Units (NTU). Water samples for turbidity estimation were collected over two days between 12-1pm and analyzed all together the next day. Doty's (1971) clod card technique was used to measure wave energy. Clod cards were made by mixing 500 grams of Plaster of Paris with 335 ml of water. The mixture was then poured into flexible ice cube trays and allowed to cure for thirty minutes. The cubes were then removed from the trays and placed on the lab bench to dry for four more days. Once the cubes were completely dry they were weighed and sanded to within two grams of each other. The cubes were

then attached to the underside of plastic petri dishes using silicon sealant and allowed to cure for 24 hours. The clod cards were then weighed and the mass noted on the petri dish. The petri dish was then attached to a rock using rubber bands to assure the stability of the clod cards. The clod cards were numbered and placed at sites in water depths of 50 centimeters, 5 meters, or 10 meters. They were left for a full period of 24 hours and then recollected. The cards were dried for four days on the lab bench and then re-weighed. The difference was then assessed to evaluate relative wave energy across different localities and depths by comparing clod degradation rate.

Sponge biodiversity was assessed with 20 minute time-surveys at all sites. Open taxonomic names were given and vouchers collected for morphologically distinguishable groups. Among the six sites quantitatively sampled, surveys were conducted at three depths where possible—50 centimeters, 5 meters, and 10 meters. SCUBA was used for all work below 50 centimeters water depth. Quantitative sampling methods varied depending on the substrate type being sampled. Three replicates were conducted of all samples, transects and quadrats. The two sites on the Luiche platform were sampled using a 40 cm x 40 cm quadrat. The quadrat was first placed on the bottom and photographed; then each specimen within the quadrat was photographed. All large particulate substrate other than silt (i.e. rocks and shells, shell fragments) was removed from the quadrat and placed in whirlpac bags. The specimens were then taken back to the lab and separated by sponge morphotype. Volume was assessed for each sponge using water displacement. All other sites were rocky substrates and were assessed using a 5 meter transect parallel to the shoreline. Ten points along the transect were randomly chosen and all cobbles within 12 centimeters on either side of the point were assessed. The cobbles were turned over (all sponges were on the underside of cobbles in shallower depths) and photographed with a coin for scale. If an unknown morphology was found it was placed in a sample bag and taken back to the lab for examination under a microscope, followed by spicule preparation, and description. Shannon-Weaver Diversity Indexes were calculated for each site (Fowler & Cohen, 1992).

Sponge surface area was analyzed from photographs using Arc GIS 3.2. Using the polygon tool the outline of both the sponge and the scale coin were drawn. Pixels contained within the outlines were recorded and by determining pixels per millimeter in the image surface area of the sponge was approximated.

Sample specimens were collected of each morphology and processed individually by microscopically examining ostia size, external patterning, coloration, spicule morphology, consistency and shape. Ostia size was assessed by examining individual ostia diameter and assigning a given rank between 1 and 4, 1 being the smallest diameter. To examine spicule morphology approximately 0.5 grams of tissue were heated in 50% hydrogen peroxide for 60 minutes. The sample was then centrifuged for ten minutes and rinsed with distilled water three times. The precipitant was then mixed with 2 ml of distilled water and pipetted onto a coverslip. The coverslip was then heated on a hotplate until all of the liquid evaporated. Once the coverslip was completely dry it was fixed to a glass slide using Naphrax™ mounting medium. One sample was taken for DNA analysis stored in 99.6% ethanol, one sample preserved as a type specimen was stored in 70% ethanol, and one sample was taken for spicule and tissue observations which was dried or fixed to a slide.

## Results

A total of 13 morphologies were found during qualitative sampling. Due to the difficulty of correct species identification in freshwater sponges the morphologies were given open taxonomic distinctions of species A-N. Table 1 is a summary of the characteristics and observed distribution of species A-N, photographs are shown in Figs 5-8. During quantitative sampling only 9 of the 13 putative species were found.

The Shannon Weaver Index correlated strongly with turbidity (Figure 1). No correlation between either wave energy or turbidity was found with mean individual sponge size and a very weak correlation ( $R^2=0.385$ ) between wave energy and number of individuals. It appears that the combined effects of wave energy and turbidity can explain number of species found in a given area (Figures 2 & 3).

It is important to note that sponges with photosynthetic symbionts (species D & I) were only found at the sites with highest turbidity—Tafiri Bay (3.94 NTU) and the southern Luiche Platform (7.45 NTU). It was also observed that the only sites with a growth morphology other than encrusting were on the Luiche platform which was dominated by massive and branching growth forms, with species I only occurring at the southern Luiche site.

Interactions between individuals of species H and I were relatively common (one in every 20 shells) on the Luiche platform. Interactions between species B and colonies of phylactolaemate bryozoans were also observed in Tafiri Bay and at Hilltop (see Smith, this volume).

## **Discussion**

For a freshwater system the sponge diversity in Lake Tanganyika seems to be very high, as is diversity in many other taxa within the lake. It is interesting to note the disparity of species representation when comparing qualitative to quantitative sampling methods. It should be considered when planning future research that while a strong emphasis within the scientific community is placed on quantitative data, without qualitative sampling there may be important diversity and species interaction data that would be overlooked.

The strong positive correlations between both the Shannon-Weaver Diversity Index and turbidity and the number of species with turbidity suggests that there is definitely potential for paleoenvironmental information from fossil sponges. The two pairs of sites compared for turbidity investigations were biogeographically very different, but yet still followed a similar pattern of increased diversity with increased turbidity. However, similar patterns of coverage were not observed. From the Tafiri and Hilltop site pair the high turbidity site had double the surface area coverage than the low turbidity site whereas in the Luiche site pair the high turbidity site had a slightly lower volume of sponge tissue than the low turbidity site. This suggests that there may be a filtering threshold which the southern Luiche site has surpassed, seeing as the turbidity there is twice that of Tafiri. The southern Luiche site is also the only site where species I was observed, which is one of two species noted with photosynthetic symbionts. I think that this could be due to the turbidity of the water being of such density that pores may easily become clogged making filter feeding more difficult, in which case photosynthetic symbionts would be necessary to provide the needed energy for growth. Further investigation is required to understand this system.

The lack of correlation between either turbidity or wave energy with number of individuals present provides important data that when studying sponge populations, due to the asexual methods of reproduction such as gemmulescence and fragmentation it is imperative to use more in depth coverage estimates like percent cover and/or volume.

## **Suggestions for Future Research**

More information on the water chemistry across the sample sites such as organic and inorganic matter, silica, and pH could provide useful insight for explanation of relative surface area coverage and the patchy distribution of sponges with photosynthetic symbionts. Secchi disk measurements would also be interesting for assessing depth of light penetration across the Luiche sites. A measure of chlorophyll in the sponges from the southern Luiche Delta and Tafiri sites can also provide insight into the nature of symbiotic relationships with turbidity. It is possible that photosynthesis is much greater at the Luiche Delta site than the Tafiri Beach site, which would provide quantitative data which shows that dependence on photosynthetic symbionts increases with turbidity. It would also be interesting to conduct experiments in which different sponge species are placed in dark conditions and growth rate or time until death is assessed.

A regression curve of species B spicule morphology which is located at several sites across various wave energy and turbidity readings would be useful for understanding changes in spicule morphology within a species across various environmental conditions. Such information would be very useful for interpreting the paleolimnological record.

Studies comparing dry biomass present across the sample sites would be a good method for accurately comparing the Luiche sites to the rest of the sites where only encrusting sponges are present. Measures of filtration rates using dye markers could also provide a method of comparison across sites with encrusting versus branching and ball growth forms.

Creation of slides from different layers and across several individuals of each species would also be useful for identification. No gemmules or gemmulescleres were present in my slides but sponges which appeared to be

undergoing gemmules were observed and gemmulescleres were observed in core samples. Gemmules in some North American freshwater sponges occur only near the basal disk, in which case they would only be seen if the slide preparation was from the correct portion of tissue. Gemmules and gemmulescleres not only provide information for identification but could also be useful for paleolimnological studies and provide information on the biology of Lake Tanganyikan sponges. Historically it was thought that asexual reproduction doesn't occur in the sponges of Lake Tanganyika (Coulter, 1991). However, if gemmulation is occurring then asexual reproduction is in fact present in Lake Tanganyika.

It was also observed that the sponges on the Luiche platform seem to be an example of taphonomic feedback, since without the shell beds there would be no substrate for the sponges. This could have had a major effect on the evolution of sponges in Lake Tanganyika and on many other taxa associated with sponges including but not limited to bryozoans, algae, arthropods, annelids, fishes and mollusks. This is especially true for example with the "mobile sponges" observed on the Luiche platform near Ujiji beach where species K sponges were found growing on live *Paramelania* gastropods. This is an entire ecosystem which may not have otherwise existed if the shell beds weren't present. The sponges also provide stabilization of the bottom which may help perpetuate the existence of the shell beds.

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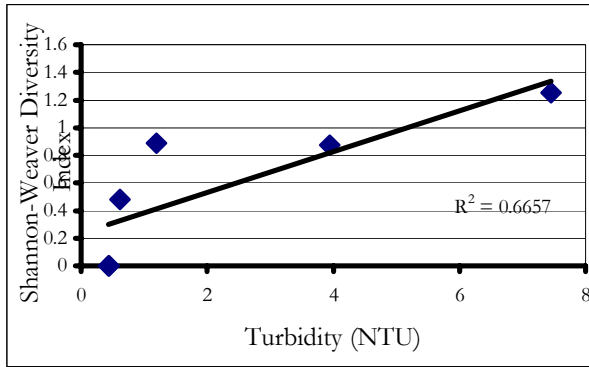
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Table 1- Description of sponge morphotypes

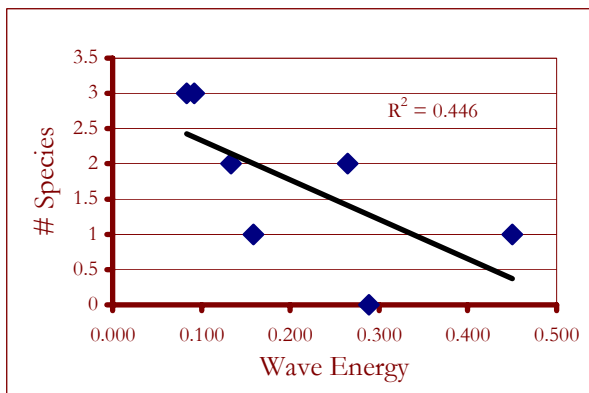
Morphospecies	Location	Color	Ostia Size	Growth Form	Consistency	Substrate	Patterning
A	H	Yellow	2	Ball	2	Gastropod shell	none
B	T, H, M, Kat N&S, Kal N&S	Yellow	1	Encrusting	2	Cobble & bivalve shells	Random stars
C	T, H, Kal S., Kat N&S	Yellow	1	Encrusting	1	Cobble	None
D	Kat N., T, H	Yellow-green	3	Encrusting	2	Cobble & stromatolites	Star edges
E	T, Kal N&S, Kat N&S	Yellow	3	Encrusting	2	Cobble	Stars only around obvious ostia
F	L N&S	Grey-beige	3	Encrusting/branching	2	Gastropod shells	None
G	LS	Grey-beige	3	Encrusting/branching	2	Gastropod shells	More spiculate than F
H	L N&S	Grey	2	Encrust	3	Gastropod shells	Recessed ostia
I	LS	Grey-green	2	Branching	4	Gastropod shells	Woven pinacoderm
J	LS	Yellow	4	Ball	1	Gastropod shells	Highly porous
K	Kat N.	White-brown	1	Encrusting	3	Cobble	Porous interior
L	L S&N	Beige	3	Encrusting/branching	2	Gastropod shells	Regular spiculation in rows
M	Kal S., H, T, Kat N&S	White	1	Encrusting	1	Cobbles, stromatolites, bivalve shell	Grows in crevices

**Site abbreviations:** H=Hilltop T=Tafiri Bay M=Mzungu Kal S.=Kalalangabo South Kal N.=Kalalangabo North Kat N.=Katabe North Kat S.=Katabe South LS=Luiche Platform South LN=Luiche Platform North. Coordinates provided in paper by Smith, this volume.

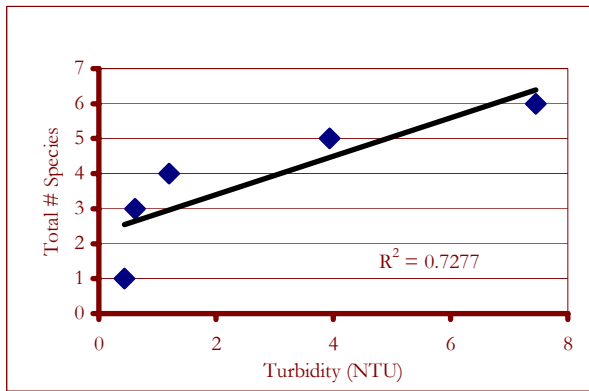
**Figure 2: Shannon-Weaver index as a measure of diversity plotted against turbidity**

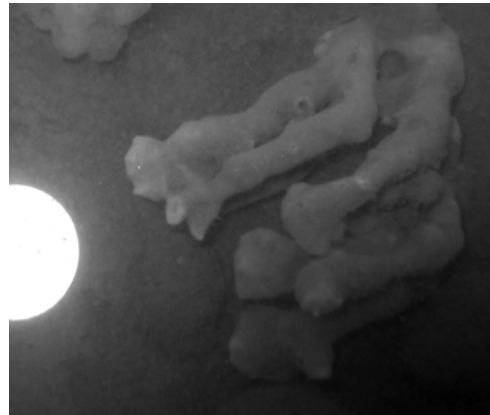
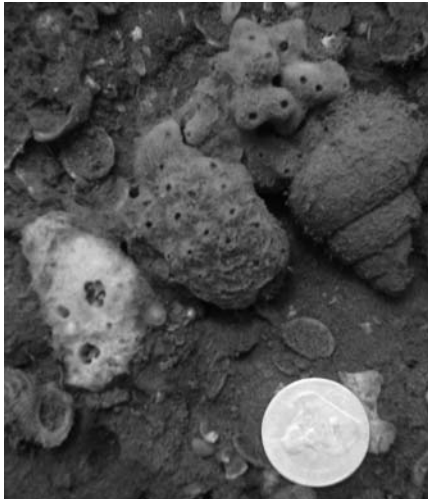
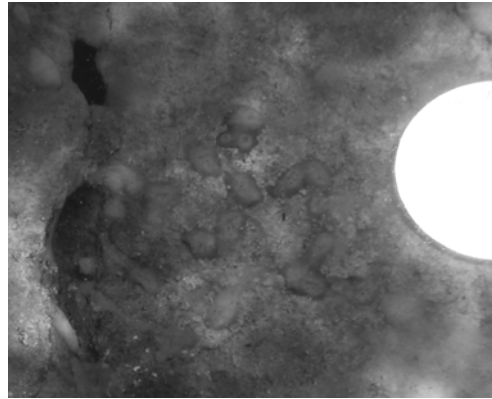


**Figure 3: (Morpho)species richness relationship to wave energy**



**Figure 4: (Morpho)species richness relationship to turbidity**





**Figure 5** (upper left) : Morphospecies E, and example of the encrusting growth form. **Figure 6** (upper right): Possible regrowth after gemmulation. **Figure 7** (lower left): Morphospecies J, F, & G. **Figure 8** (lower right): morphospecies I, and example of the branching growth form