

Tales of the Mzungu
Episode IV: Anaerobic
The Water Boy for WeDev Water

Carefully placing the lid on the yankee candle; the flame flickered once, twice, then blinked out. "Where did the flame go, Mom?" the little boy asked. "The flame used up all the oxygen and it went out," the mother answered.

Anaerobic incubation. "I know we can kill the cryptosporidium, but how can we prove it? I don't have 3000 euros for an anaerobic incubator, I thought", staring a hole through the ceiling of the plane. Then I knew...

One of the most important parts of improving water quality is detection. How do you know if you've improved the quality if you can't test what the quality is? WeDev Water has been fortunate in that respect because we received water quality detection equipment from Fondation Veolia in cooperation with the SAME Kaiserslautern chapter. But some parameters aren't quite as easy to detect – anaerobic bacteria, for example.



As a part of our work with the Island Mission School on Zinga Island, we received lab data indicating the presence of cryptosporidium, a difficult pathogen to detect, and that is where our adventure begins...

Cryptosporidium is a genus of protists recognized as a major cause of diarrheal illness, contributing significantly to the global burden of gastroenteritis, especially in young children.¹ One of the primary limitations for evaluating the effectiveness of treatment of a cryptosporidium

infection is the detection method. Under laboratory conditions, clostridium perfringens is sometimes used as an indicator organism for cryptosporidium. The difficulty in using this method in fieldwork is the need for anaerobic incubation at 45°C.

In the absence of an anaerobic incubator, which was way beyond our means, we'd need a commercial lab, which is equally cost prohibitive for testing a new treatment method. In addition, because of the distance from Zinga Island to the mainland and limited transport possibilities, delivery of the samples within the time and temperature requirements would be difficult to achieve.

Both Cryptosporidium and Clostridium are chlorine-resistant, so although we had chlorinated the water supply under a previous project, the problem remained. However, much like legionella, water temperatures of 60°C for half of an hour or 70°C for a couple of minutes are sufficient to kill both Cryptosporidium and Clostridium. Our team was developing a solar water heating solution to achieve such temperatures to disinfect the water supply (A future *Tale*)

¹ Betancourt, W. 2019. Cryptosporidium spp. In: J.B. Rose and B. Jiménez-Cisneros, (eds) Global Water Pathogen Project. <http://www.waterpathogens.org>



Our wonderful biology student that year, Valentina Stoll, determined that blood agar would be quite suitable to cultivate the clostridium, if we could manage to incubate at 45°C under anaerobic conditions.

Our field incubator could achieve the temperature, but it wasn't anaerobic, nor was there a practical way to make it so. Sitting at the airport before departure we realized that the same water we were heating to kill the bacteria could be used to cultivate it – we could create a water bath, but could we hold temperature? We decided to buy whatever insulation we could find, wrap it around half a beer barrel of warm water and give it a try.

Keeping the samples dry was easy; just empty a mayonnaise jar. Then, again the unexpected cropped up – How do you keep the samples upright in the jar? Every time we'd put the jar in the water bath, it would tip over. We tried a bunch of things. Glue it to the bottom? The buoyancy was too great. Tie it to a rock? It slipped out of the tie ropes every time. Put a heavy weight at the bottom of the jar? Still too unstable, the jar flipped. Then I considered a hot air balloon. More string is the answer! Still too slippery. The jar flipped. :-/

The solution? A Thermal Suspension Apparatus. Thanks to the wonders of science (and my poor packing technique), I discovered an unused athletic sock in my luggage. Wrap the sock around the jar, tie it to a rock and eureka! A Thermal Suspension Apparatus (aka a sock and a rock).



One of the great limitations of our ventures is time. In consideration of our team's vacation length, we had allotted one week for the work on Zinga Island. One day to ensure we made it across the lake to the airport; one for supplies, and two to set up the solar heat apparatus. We lost a day due to a solar wiring accident. That left one day to test the incubator and one to test the disinfection capabilities of the solar heat apparatus. There was no time for mistakes; no second chances.

Lake water was full of clostrium, that we knew, so Valentina prepped the samples. We warmed the water bath to 53°C to account for a temperature drop in the night, closed the top, wrapped the insulation and waited.

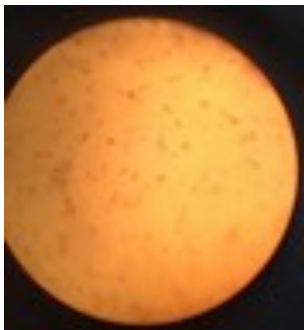
In 18 hours, we would know.



There is an important side note to the sampling procedure for this project. We were looking for a presence/absence test. For drinking water purposes, it was unimportant how many bacteria we had. If we had any, it's bad. If we had none, it's good. The accuracy of plate counts at this stage was irrelevant.

18 hours later, we opened the incubator. 42°C! Our hopes rose. That should be enough for growth!

We carefully opened the mayonnaise jar. Another cute sidelight to our *very* scientific methods. No one had hands small enough to remove the petri dishes from the jar. But... we were at a school; small hands were plenty. 😊



The moment of truth... a joyous cry! The samples were beautiful! The lake samples showed roughly 20 colonies of clostridium. Microscopic analysis showed both the rods and the spores typical of clostridium. Although certain species identification was impossible with the limited microscope capabilities, there is a strong probability, given a known presence of clostridia in the sample, that the analysis was correct.

We won this round against the challenges of Africa.

But we're still missing one essential element, how did we get it anaerobic? The scientific process is often a long one, built of small elements gathered over years. That's where those childhood memories came in. The cleaned mayonnaise jar was large enough for the petri dishes. Then all that was needed was a simple candle. The wax held it in place. Light the candle, close the cover, and "look Mommy!" we have anaerobic!



Stay tuned for the next episode of: *Tales of the Mzungu!*
The Water Boy