**Extreme dives for rare DNA**

*The exploration of an abandoned cold water mine, for a microbial study, 602 feet below surface.*

The Outaouais region has largely been developed due to heavy mining industry. Since industrial work was deemed not of historical value in Outaouais, there is very little documentation about the mines and how the work activities. The dive team was able to find old pictures and rudimentary maps that no longer reflect the state of the Forsyth mine; documenting the passage was therefore key to the safety of the diving operation to allow the collection of DNA samples.

**History of the Forsyth Mine**

The Gatineau Valley Historical Society believes the discovery of iron ore, in the region, goes back to the first survey of the area in 1801; the survey, done by John MacTaggart, saw the needle of his compass fluctuate in the proximity of what would eventually become the small village of Ironside. In 1854, the rights were sold to an American firm named the Forsyth and Co., of Pittsburgh, Pennsylvania and the mine was renamed as such.

The ore that was extracted from the biggest iron mining deposit of the region was shipped on the Rideau Canal to Kingston and subsequently to Cleveland, Ohio. The mining operation ceased in 1880 and the activities were taken over by Hull Iron Mines, Ltd. In 1957.1 It is at this moment that the shaft was dewatered and the deeper sections of the mine were dug; the haulage tunnel with rails was to reach the depth of 200 feet, the other deep tunnels were going to be connected via an elevator shaft that was later sunk in 1959. This was the last known exploitation of this mine. Maps gathered of future development projects for this site were particularly confusing as we had no idea if they reflected the current state of the mine.

The mine and village that used to be 10 km northwest of Gatineau City is now abandoned. It sits on the boundary of a recent suburb neighborhood. Nature has claimed back its right and vegetation occupy the residual foundation of what used to be houses of Ironside.

**The Minex Project**

Upon realizing that the region had many abandoned flooded mines with great possible depth and mostly unchartered tunnels, we decided to build a project to explore, document and study those unknown environments. The Minex project started two years ago with the exploration of virgin mines in the region.

This year’s exploration goal was Forsyth. As the surrounding aquatic environment was abandoned, since the mine closed, microbial communities have thrived and adapted to the unique undisturbed conditions found in this underground ecosystem. This constituted a unique opportunity to explore and discover new life forms and rare microorganic communities in this atypical environment.

We collaborated with the University of Ottawa and University of Quebec in Montreal. The Explorers Club endorsed this expedition and sent us Flag 101, with the aim to assess microbiota diversity within the flooded mine ecosystem. A research permit was also secured with the proper authorities. The collected data could possibly lead to important changes in the ways in which we understand the tree of life.

Furthermore, the exploration of this underwater mine provided an opportunity to study deep diving in an anthropologic way – that is – what it does to someone’s humanity to be doing deep diving.

As divers, what we observed was fascinating; the formations that resembled long stalactites algae dripping from the ceiling, or white filament spreading like a spider’s web, were surreal. The first thought that came to my mind was that those were algae growing, but after a discussion with the microbiologists, I quickly learnt that this would be impossible since there was no light – it was most likely a bacteria colony. We knew that things that grow without light, in 42 degrees water, deep underground, and with little oxygen would be resilient and possibly unique to this kind of environment. Studying and understanding the resilience of life in an environment that has been exploited by humans and abandoned when profit plummeted are timely. In those harsh conditions, finding new life forms at depth that have different metabolism mechanisms, could be of great importance for science.

With a team of six divers, we started collecting samples in May 2019; this task was particularly challenging due to the cold-water element and the large number of samples required to perform a credible analysis. A total of 34 human dives were conducted to collect samples between 20 feet and 602 feet.

**A dive at 602 feet**

After a full year of preparation for this deep dive, I entered the mine on July 20th, which had no more than six feet of visibility, and followed the intact train track as a guidance. After only about 400 feet of penetration, I passed the first restriction, a small squeeze between an industrial pipe and the silt leading to the elevator room. The main haulage tunnel was clogged by years of accumulation of sediments. That old dual elevator cage, where miners used to lift the mineral, was the only way to access the deeper levels of the mine, which ran every 100 feet.

Earlier in the project, during my first dive in that shaft I was anxious; we thought that the mining company had sealed the elevator shaft from the surface with a concrete slab, but I had no way of testing the stability of the potential underwater land fill. I knew that once I would be returning from the deep, the bubbles from my rebreather would accumulate and create a pressure under the landfill, possibly causing some parts to collapse and seal shut my only way out. In my first descend, some of the wood planks came loose and fell on my head – which was not that critical at the time. If that were to happen in a dive that accumulated 6 hours of decompression in 42 degrees water – a single nail from those planks could rupture my drysuit and put me in a difficult situation having to choose between severe hypothermia or a decompression accident; both could lead to death before exiting the mine. I knew there was no way I could stay in water this cold for over an hour in a leaking suit, even with a heated undergarment. This is the risk that I had to accept to make this dive.

I started my descent into the shaft. Everything that I could plan had been planned, everything that could have been practiced and rehearsed had been so. As I passed the 150 feet mark the visibility improved to about fifty feet. I had previously dove it and installed a line up to 450 feet, but descending another 152 feet would be challenging since the shaft’s unstable structure is filled with debris, rocks and metal cables- objects that could rupture my equipment - creating multiple restrictions along the way. I needed to analyze and memorize every single detail on the way down because I knew that on the way up, purging my suit and rebreather from such depth would create a whirlpool of silt, making it a zero-visibility exit.

Upon passing the 500 feet level, I observed a section of the shaft where the wood structure was caving in, there was no time for debate here since I was accumulating significant decompression time, so I decided to bypass it and continue.

I finally reached my planned depth of 602 feet, the laborious descend took more time than I anticipated due to unforeseen restrictions; I would therefore have only a few minutes at this depth. Everything was dark, the wooden structure appeared to be burned from a fire – or perhaps it was a bacteria that produced this effect. I pulled out the syringes to gather the DNA samples. I could feel the high-pressure neurological syndrome (HPNS), my hands were not steady, and my eyes were having difficulty focusing – this was further complicating the laborious sample collection with my dry mitts.

Shortly after, I started my long ascent in total silence, with only the clicking of my solenoid to reassure me. As predicted, the silt started falling forming a dense cloud and I began my excruciatingly long decompression in the frozen water at around 450 feet of depth—a long way from my safety divers Alain and Gabriel. After a total of 6 hours and 13 minutes, I surfaced with the precious samples without any incident. Drained from all forces, Gabriel helped me walk out of the water.

**Results**

In summary, we have explored the elevator shaft to 602 feet along with the 200’ level and the 300’ level. When the visibility was at its best, we could see the 200’ level that consisted of a big room approximately 50’ by 30’ with a few industrial materials laying around.

Towards the end of the project I explored the 300’ level but because of the difficulty to run a reel with dry mitts and the lack of natural element to tie the string onto, I only made it to about three quarters of my planned penetration; as the research permit was expiring, I could not return and confirm the end of the tunnel although I had collected enough remote samples.

Due to lack of time and logistical complication, the 400’ level, the 500’ level, and 600’ level remains unexplored. There is also one section of the main corridor pass the elevator shaft that was obstructed with silt and we choose not to disturb it. We believe that the exploration dive at 602 feet in 42 degree water sets a new world record for deep diving in cold water mine (autonomous – that is without decompression environment). At this time no further exploration of the mine is planned.

This exploration allowed the researchers to collect a total of 33 water samples and 62 biofilms (gelatinous matrices secreted by microorganisms to favour their survival). Genetic material (DNA) present in the samples were then extracted at the University labs, and these are now awaiting to be sequenced. DNA sequencing allows researchers to precisely identify the family of microorganisms present in each sample, which deepens our understanding of the biodiversity present in the unique ecosystem.

Preliminary results on DNA concentrations are promising. We expect to find well established non-phototrophic (do not use light for energy) microbial communities within the mine. Furthermore, the variety of biofilm forms observed and sampled gives hope for the discovery of new microbial species.

During the next year, biology Ph.D. candidate Francis Comte will bring some of the samples to the University of Texas to perform a metagenomic and bioinformatics analysis. This will allow the researchers to identify all the genes present in a given sample and expose the metabolic diversity of the mine’s ecosystem. Since DNA sequencing can’t identify previously unknown microbes the metagenomic methods will palliate for that.

This exploratory study will advance our understanding of microbial development in underground, aquatic man-made environments, and the discovery of new microbe species might challenge the current tree of life.

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**The following team members contributed to the project and the article:**

Alain Delisle, Ayesha Ratnayake, Cassandre Lazar, Chantal Bergevin, David Caissy, David Jaclin, Francis Comte, Gabriel Benoit-Martin, Guy Allard, Jules Valeur, Kevin Brown, Luc Gilbert, Nicolas Rutherford, Robert Deproy, Serge-Olivier Rondeau, Steve Duplessis, and Steve Doyon.