GROUNDWATER BIOTICA: OCCURRENCE OF STYGOFAUNA AMPHIPODS IN VARIOUS AQUIFER SYSTEMS IN SOUTH AFRICA OF GENUS *STERNOPHYSINX*.

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ABSTRACT

This paper deals with the occurrences of Stygofauna Amphipods of the Genus Sternophysinx discovered in several aquifers systems throughout South Africa. Traditionally, this 5 to 20mm long, cream white amphipod with degenerated or absent eyes, side swimming is more associated with karstic/dolomitic aquifer systems consisting of interconnected subterranean cave systems where movement is obviously not a restriction.

During a groundwater exploration programme in the Northern Bochum District in the Limpopo Province in 1999, several *shrimp-like* organisms were observed swimming in graceless ways in one and later on several bores on the farm Greater Kudu land. Seemingly highly sensitive to the bright, yellow light system on the camera unit, they quickly got hold of the bore side wall and swim-crawl to the safety of a fracture – through which they just disappear.

The life style of stygofauna amphipods in southern African hard rock aquifers is probably a complex association between searching for food, moving through minute fractures and ensuring reproduction.

The now obvious wide spread distribution of stygofauna amphipods in pristine and polluted aquifers puts a new emphasis on their survival skills in the confinements of hard rock aquifers.

Capturing live specimens of those many amphipods observed with the bore camera became a real challenge when having to operate in a narrow bore, several tens of meters below ground zero.

By the luck of nature, one live specimen was accidentally captured in the Beaufort West area during a routine groundwater grab sampling exercise in November 2008. This specimen was lively for about ten seconds and then went into an inert state. It was however sampled and found to be quite lively again after 48hrs. It stayed alive for another ±72 hours when it went for a 'live' photo session. Unfortunately the final session required a 'steady state' shooting and after a 70% alcohol dosing the Beaufort West amphipod was finally put to rest.

The occurrence of *stygofauna* amphipods in aquifer systems can be considered as an indicator of the actual aquifer health. It seems however that in some cases, amphipods are very well adapted to impacted environments. The question that stands therefore, is to what levels of impact can they survive in polluted aquifers and can their presence in aquifers, for example, be used as indicators of regional flow regimes?

1. INTRODUCTION

Aquifers were for long considered lifeless underground water reservoirs. South Africa itself has not reported any indications of subterranean life, in the true sense of aquifer systems. The idea was that only large karst systems with open channel flow systems can support underground life forms. During a routine bore video logging exercise on the Lataba Basalts in the Northern Bochum District, this perception has been found not to be true anymore. Mr. B. L. Venter from the former Directorate: Geohydrology of the Department of Water Affairs and Forestry, whilst video-logging a shallow bore in the upper fractured and weathered portion of the Lataba Basalt on the farm Greater Kudu Land observed a ‘shrimp-like’ animal, later identified by Ms. Sayomi Tasaki from the former Rand Afrikaans University as a South African *stygofauna* amphipod using the bore video images.

The presence of *stygofauna* amphipods in South African fractured hard rock environments came as a surprise as these subterranean animals are known to live in limestone/dolomitic formations, characterised by its large solution channels and cavernous systems. Now they are found in almost hard rock, fractured aquifers all over RSA, varying from fractured/weathered Lataba basalts, to deep seated Ghaap Plateau quartzite’s and lately in weathered/fractured Beaufort siltstones.

Their ability to adapt to various groundwater qualities also came as a surprise as specimens were observed in naturally and artificially polluted aquifer systems. The highest count of amphipods observed in one bore, in the vicinity of a high level waste in Mpumalanga, exceeded 100 individuals.

2. SUBTERRANEAN AMPHIPODS

Various publications worldwide have been reporting the existence of subterranean organisms which include diverse groups as vertebrates, invertebrates and microbial elements. Fish, amphibians, insects, crustaceans, mollusks, protozoans and bacteria are distributed in different groundwater habitats at the vadose (interstitial) and phreatic zones (Wilkens *et al*., 2000).

Understanding the importance of subterranean karsts as ecosystems reached a congruent point during the Ramsar Convention (1971). This congruence derived from the analyses of karst hydrogeology as a study model, the resident fauna depending on subterranean karsts and the recognition of fauna pathways between surface water and groundwater. Movement from these large karst systems into adjoining sedimentary/crystalline rock formations through secondary openings seems to be a natural way of migrating towards distant environments.

Stygofauna encompass all animals related to groundwater which demonstrate different levels of adaptations to sub surface life. Stygofauna groups gather accidentally and obligate subterranean organisms present in various rocks formations at different geographical regions on the globe. Amongst the stygofauna amphipods are reported to be the predominant stygobites (obligate subterranean) inhabiting aquifers in South Africa. Amphipods are malacostracans of the super order Pericardia. The order Amphipoda constitutes the second amongst the two principal groups of pericardia, they are probably descending from a distant evolutionary lineage of Mysidacea. The genus *Sternophysinx* is endemic in South Africa gathering seven described species reported from Limpopo, Mpumalanga, Gauteng and Kwazulu Natal provinces (Tasaki, 2007).

General characteristics:

The great majority of subterranean amphipods seen in the aquifers in southern Africa vary between 15 and 25mm long. The body section consists of seven thoracic segments, with a cephalothorax (head section fused with the first thoracic segment), followed by a three-segmented

abdominal section and finally a tail section or telson. The three abdominal segments each bear paired pleopods (i.e. unsegmented legs) which enhance free water swimming.
One of two pairs of antennae ranging from short (3mm) to medium (7mm) is situated right in front of the head. Some specimens from the US have only one pair, but substantially longer antennae. The mouth parts are relatively small, and compactly arranged and appear to be hidden by the basal sub-segments of the head/first thoracic segment (US specimens). The mouth parts of the southern African (Beaufort West) specimen however, are hidden between two pairs of gnathopods, each with a prominent hook which obviously is used for grasping food particles or prey.
Stygofauna amphipods are fitted with several pairs of appendages which are used for crawling (gnathopods), walking (thoracic legs or pereiopods) swimming (pleopods) and pushing (uropods). Five pairs of thoracic legs are situated along the body and start from the 2nd thoracic segment and consist of five segments (instead of the seven segmented grayfish leg). What is quite noticeable in the southern African specimen, is the enlarged first segment of the third to the fifth leg pairs.
As with most of the subterranean amphipods, eyes are either completely absent or in a process of degeneration. The Beaufort West specimen has no visual eyes, and is probably blind.

Locomotion

The *stygofauna* amphipods are so-called side-swimmers when moving freely in water. Bore camera observation however, has shown that this free water movement is actually bizarre zig-zag paddling, where some of the frontal pereiopods (legs) and the pleopods (abdominal appendages) are used. Figure 5 actually demonstrates the amphipod’s movements whilst swimming and one can clearly see the pleopods in action. Interesting to note is that the rear pereiopods are flapped backwards to probably reduce drag. The pleopods are the chief locomotor appendages for rapid undulatory swimming in free water. Once the animal grasps onto solid matter, i.e. the bore sidewall, it will use its gnathopods, pereiopods (thoracic legs) and uropods to crawl sideways into a fracture.

Feeding, Food

Detritus is a well known form of energy input for amphipods and the ability to feed on matter may be a distinct characteristic shared by several amphipod groups. Although, the character of the food and even preferences in the size of the food particles may be requirements associated to different genera. The relationship between amphipod and the character of food consumed associated with the groundwater habitat profile is an interesting subject for discussion that should be investigated (Gregg and Stednick, 2000; Kostalos and Seymour, 1976).
Stygobite amphipods are capable of tolerating longer starvation periods (food scarcity) but are observed to have an enhanced food finding ability. Obligate subterranean elements demonstrate lower energy demand as a result of reduced metabolic rates and producing fewer eggs when compared with surface forms (Wilkens *et al*., 2000). The ability of blind amphipods species to perpetuate in the aquatic subterranean environment, suggests their close affinity with the subterranean habitat. Recent studies exploring the physiology and behavior of stygobites is becoming a subject of greater interest (Wilkens *et al*., 2000).
The Beaufort West amphipod was however sampled with the remains of a water cricket-like insect. The insect’s internal body was completely gone, thus only the hardened exoskeleton remained. Now, whether the amphipod was actually still nibbling on the skeleton when it was abruptly ‘sampled’, remains a question.

Classification

The history of subterranean fauna derivation evolves from a spectacular point of geological history on earth; some may be descendents from a lineage of survivors during the "Ice Age" (Pleistocene, from 1.8 million to 10,000 years (Barr, 1973).

Amphipod evolution is partly based on the fossil record from which the malacostracan relationship is derived. The study of amphipod derivation has also shown that evolutionary traits of super family groups have been influenced by continental drift and plate tectonics (Bousfield, 1978). It also shows the probable ecological influence in the change of primary food source during two distinct geological periods the Carboniferous and Cretaceous. (Spencer, 1972).
It is commonly believed that stygobite amphipods evolved from aquatic epigean ancestors (freshwater and marine) that had developed some degree of pre-adaptation to the subterranean life prior to permanent invasion of subterranean waters, which may be associated to ecological conditions or *vicariant event*[1], or even both. The subject dealing with the derivation of subterranean amphipods is indeed a matter of extensive debate regarding the various pathways that lead species to inhabit subterranean ecosystems (Barr, 1968; Barr, 1973; Barr and Holsinger; 1985; Botosaneanu and Holsinger, 1991). Furthermore, some evolutionary evidence demonstrates that stygobitic groups might also have evolved from subterranean pre-adapted relatives allied to the Peracarid orders and that amphipoda would then be the product of relatively recent evolution (Bousfield, 1978).

Development, Life Cycle

Although not specifically studied in subterranean *Stygobites* in southern Africa, reproduction takes place by an egg laying process after copulation. Fertilization takes place in a special chamber or marsupium. The number of eggs 'laid' varies amongst the different species from one or two eggs, up to 50 eggs. The newly hatched young will stay with the female in the marsupium and only released to the outside when the female undergoes molting following copulation.

Ecology

The question asose whether the presence of this groundwater related fauna (stygofauna) could be used as an indicator of the health status of an aquifer system. It has been noted by cave researchers that the semi-subterranean species are confined to the most remote parts of these caves, thus indicating they have an aversion to areas where foreign substances may be introduced into their habitat. The cases where the South African *stygofauna* amphipods were located in aquifer systems however indicated that groundwater quality is probably not that much of a controlling factor for their survival. On two occasions, amphipods were found in bores located in a naturally trace metal-polluted aquifer system, and the second case very close to a leaky waste site. The latter one however contained the highest number of subterranean amphipods ever found thus far in South Africa. The groundwater specimens on the contrary, seem to be "attracted" to even impacted water sources.
Studies done on the surface water relatives revealed that they are sensitive to pollutants which end up in water sources. Observations in cave systems reported that colonies moved to the deeper and more pristine parts of the cave systems due to foreign traffic in the cave entrance area.

Specific Characteristics of the Beaufort West Specimen.

Background
The South African blind amphipods are common inhabitants of caves and groundwater eyes (e.g. *Sternophysinx filaris*). In their natural environment they are prone to avoid prolonged periods of total exposure, when disturbed they are predisposed to search for a "refuge". These behavioral characteristics probably contribute to patterns of vertical migration through sub surface voids, which in turn contributed to their adaptation to live in the subterranean environment (Barr, 1973).

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[1] The division of biota ot taxon through the development of a natural barrier.

To date, the sampling of South African blind amphipods have been reported exclusively from headwater streams, springs and caves. Therefore the South African amphipod sampling history changed on 28 January 2009 about 13h30 during a routine "*skim*" sample[2] in a borehole 52m deep, cased down to 6m and 165mm in diameter. At the time E. van Wyk (DWA, Hydrological Services) was sampling groundwater from the top section of water bores for hydrogeochemical correlations with rainwater. The site is an isolated sub-catchment in the Karoo hydrogeological environment, sitting in the extreme head waters of WMA 15 – Fish to Tsitsikama Quaternary Catchment. A specially made *acrylic* sample tube was used, which allows the contents to be actually 'viewed' once retrieved from the bore. The groundwater quality is quite pristine, EC 100mS/m, Ph 7.5 and temperature at 21°Celsius.

The 'Beuafort West Specimen', kept in a plastic sample bottle by pumping fresh air into the water with a syringe and delivered to Me Tagisaki on the 30th, was still alive and swimming. It in fact lasted another 48 hours later.

As was mentioned above, the first amphipod was sampled with the remains of a water cricket. Many attempts have been made to actually catch one alive, it came as a great surprise that during a follow-up groundwater skim sampling exercise at the same site, a second specimen was 'sampled'.

External Anatomy

Blind species encompass aquatic groups which are entirely adapted to subterranean life, exhibiting characteristics that are peculiar to subterranean elements. Their bodies are slightly curved, flattish in profile and slender, with a laterally compressed shape (Fig.1). Another typical characteristic of stygobite amphipods is the absence of melanin, the advanced morphology of the mouth parts with specialized feeding appendages - maxillipedes (Fig.2) and one pair of elongated antennas like the specimens from cave systems (Fig.3). The rather thick-short antennae of the Beaufort West specimen is quite logic as a pair of elongated antennae will not allow free movement through the minute cracks in the aquifer.

The head (cephalothorax) (Fig.3) is proportional to the long body constituted by thoracic, the pereon and abdominal, the pleon and urosome segments (as displayed in Fig.1). The gnathopods (Fig.2) are constituted by strong muscular inner tissue and is linked to the dactyl (hook) which is enlarged in most subterranean species.

Another evolutionary enhancement is the amphipod versatile body movements, they can either walk or swim (Fig 4), demonstrating abilities to grasp, climb and crawl. To the naked eye, subterranean amphipods appear to be "milk white" (albine) (Fig 1), the external reflective appearance in the natural habitat may be attributed to the cuticle transparency of the exoskeleton (Fig 1).

Stygobite amphipods lack in visual organs but are equipped with sensorial appendages along the body which enable them to sense the surrounding environment. Some obligate subterranean species may be equipped with special receptors at the elongated antennae (e.g. *Sternophisinx calceola*) (Holsinger, 1992).

3. AMPHIPODS AS POSSIBLE INDICATORS OF AQUIFER RESILIENCE

Blind amphipods are the most widespread and visible invertebrates in subterranean aquatic habitats, geographically considered ubiquitous as they are distributed from the tropical forests, to arid zones, at high altitudes and at the sea, being the most available stygobite element to sample and study what makes them more vulnerable to human impacts.

Groundwater blind amphipods (stybobites) developed exceptional techniques to cope with the extreme conditions imposed in by the rigour of the subterranean environment, as the lack of photo-producers, permanent darkness and the uncommon rates of low oxygen content (anoxia).

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[2] A 500ml aliquot, representing approximately 19mm from the water table interface in the bore.

In general, the studies investigating the use of groundwater related invertebrates (stygofauna) as biological indicators of the groundwater are increasing (Gibert *et al*., 1994). Recent biogeographical studies are discussing the use of amphipods as a possible geological reference of events that have occurred during the history of Earth. Thereof, studies made within a national scale should consider that prior to envisaging amphipods as environmental record, it is necessary to acknowledge the various types of relationships between these elements and groundwater. Population species distributed within the same geographical range might have different biophysical requirements during their life span, and this might cause the relationship modalities between amphipod populations and their respective groundwater habitat to vary at same point in time. It is then important to highlight that it is only possible to process substantial understanding about relationship-responses between the biological element and groundwater, if the involving parts and concerning institutions are able to provide the necessary time frame to support the development of this young field and that hydrological work may perhaps associate aspects of groundwater ecobiology to groundwater resource assessment and monitoring in South Africa.

4. CONCLUSION.

Stygofauna amphipods occur in many different aquifer systems in southern Africa, and is not confined the those aquifer systems with macro pore spaces like the Chuniespoort dolomites and large fracture systems like the Tshipise Fault Zone in Limpopo. In fact, very few dolomite finds have been noted during DWA's bore camera loggings.
Using *Stygofauna* amphipods as an indicator for aquifer health and resilience is probably not straightforward and requires special identification of the different species; some may be able to adapt/tolerate poorer quality water, whilst others may migrate away from potential pollution sources.
The numbers of amphipods change with time and might be the result of them dying or migrating to different parts of the aquifer. The life cycle, however, could be 150 days or more. During camera logging of the same bore some months later, their numbers were not that consequent, thus indicating that amphipods actually move through the aquifer and are not confined to the bore itself.
Sampling of amphipods in deep, narrow bores is complex and can't be done without the aid of a bore camera and special netting Some specialist research work on the South African *stygofauna* amphipods was initiated through the WRC, which required sampling of these specimens occurring at substantial depths in narrow bores.

5. WAY FORWARD.

Until a few years ago *Stygofauna* amphipods were only observed in cave systems, like the Sterkfontein Cave and surrounding cave systems in the Cradle of Humankind. Bore video logging, however, has proved beyond doubt that they occur just as well in fractured hard rock regions.
Sampling techniques should be tested in practise and not in workshops and laboratories. Bore video logging and special capture nets, as proposed by Prof. Bill Humphreys, should be used instead of sample tubes or similar 'blind sampling' constructions.
Finally, a catalogue of *Stygofauna* amphipod findings and full species description may enlighten a whole new science for aquifer biotica and biomes.

ACCOMPANYING FIGURES.

Figure 1. Reflection of the fused chitinous exoskeleton shelling the Beaufort West amphipod's internal organs/body.

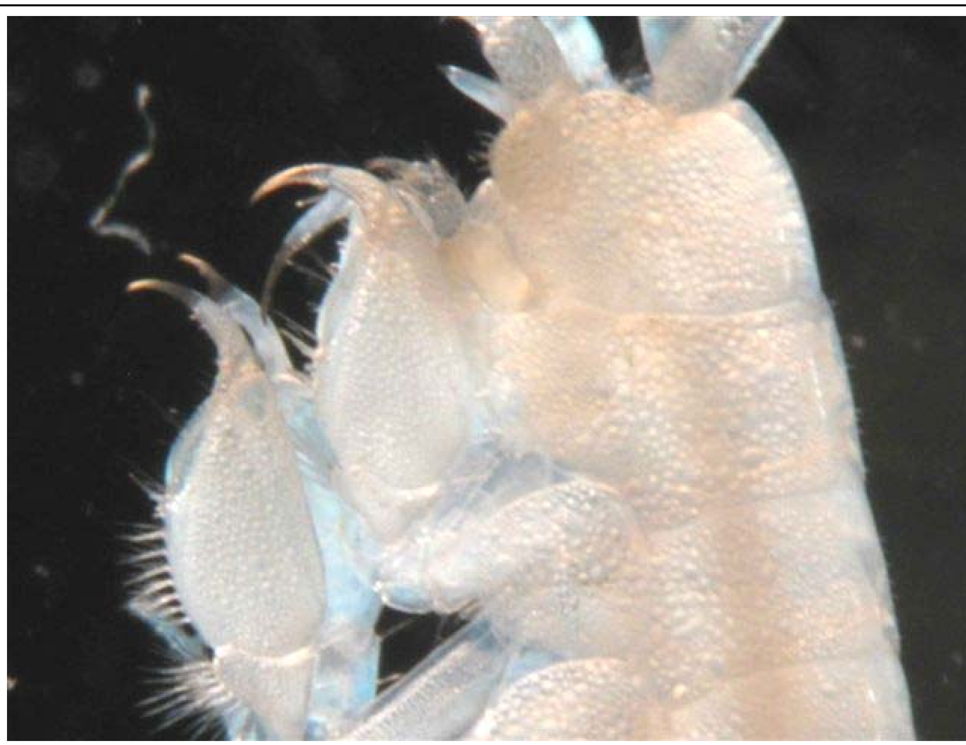

Figure 3. Beaufort West amphipod: Lateral view of the head maxillipede, gnathopods and dactyl (hook). No eyes present in this image.

Figure 3. Beaufort West Amphipod: Lateral view of head and thorax (cephalotorax) and showing gnathopods (claws) constituted by strong muscular inner tissue linked to dactyl (nail)

Figure 4. Beaufort West Amphipod: Swimming. Note three rear pereiopods (thoracic legs) turned backwards and the pleopods actually propelling the body forwards.

Figure 5. Amphipods observed in bore close to waste site area in Mpumalanga.

End of Images.

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