Egg banks of the freshwater systems of Namibia

By

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Certification

This is to certify that this report has been examined and approved for an award of degree of Bachelor of Science in Fisheries and Aquatic Sciences of the University of Namibia.

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Declaration

I hereby declare that this work is the product of my own research efforts, undertaken under the supervision of Mr. Lineekela Kandjengo and Mr Kevin Roberts and has not presented elsewhere for the award of the degree. All the sources have been duly and appropriately acknowledged.

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Abstract

Temporary water bodies like rock pools and mud pools have specific environmental conditions and are characterised with several short hydration periods in a season. This requires a specific set of life-history adaptations of their permanent inhabitants, which affects species occurrence in the water bodies. In Namibia, large branchiopods are the most abundant species found in these temporal variable environments. Branchiopods like other invertebrates rely on banks of resting eggs to survive long periods of adverse environmental conditions and to buffer against the effects of environmental variability. The eggs usually remain dormant in the dry substrate until the next rain falls. Substrate samples were used to hatch invertebrates, in order to test the viability of using these pre collected samples in invertebrates' studies. Substrate samples that had been collected from all around Namibia over many years and stored at the Ministry of Agriculture and Water Affairs were hydrated, in order to observe the hatching of invertebrates. Some eggs from the substrate hatched very fast and reached sexually maturity after five day, however some species took longer to hatch or did not hatch at all as they needed more hydration periods or they remained in a diapausing stage for a very long time. Two species of fairy shrimps were identified: *Streptocephalidae indistinctus* and *S. cafer* and a clamp shrimp *(Leptestheriidae inermis)* from different areas. The aim of the study was achieved as samples as old as 13 years yielded some invertebrates, therefore substrate samples can be used for invertebrate studies.

Keywords: rock pools, mud pools, egg banks, Branchiopods, *Streptocephalidae, Leptestheriidae*

Introduction

Aquatic invertebrates are an important source of food for birds, mammals, amphibians, reptiles, fish, and other invertebrates that depend on the fresh water systems. Changes in terrestrial and aquatic habitats lead to changes in invertebrate assemblages, which in turn changes food supplies for other animals. Invertebrates also play vital functions such as the decomposition of organic debris and release of plant nutrients (Curtis, 1991). Some species are confined to the water during their whole life cycle; others only rely on water during their early life stages.

Aquatic invertebrates includes the floating plankton, swimming nekton, organisms associated with plants (periphyton) and sediments (benthos) and the surface-dwelling neuston (Curtis, 1991). Different habitats have different characteristics, therefore supporting different species. Some of these mud pools and floodplain depressions which only get filled up with rainwater before floods arrive have species that are capable of completing their life cycle rapidly, these taxa include shrimps, tadpole shrimps, fairy shrimps and clam shrimps, all these species survive dry seasons as eggs. The eggs or spores of most invertebrates are retained by the parents or attached to the bottom of the pond, lake or river instead of eggs floating like marine animals (Curtis, 1991) Branchiopod crustaceans rely on banks of resting eggs or cysts to survive long periods of adverse environmental conditions and to buffer against the effects of environmental variability. After the eggs are laid they usually sink to the bottom or attach to plant leaves or sediments. Eggs are usually produced in large quantities from an early stage to create egg banks. The eggs usually remain dormant in the substrate until the pool dries and the next rain falls.

Most invertebrates live in temporary water bodies such as rain pools, which are usually dry for very long periods, while some live in permanent water bodies. In temporary water bodies, the environmental conditions often fluctuate, and only some eggs that are laid hatch during the period when they are hydrated, leaving other eggs to hatch in the subsequent rainy seasons (Brendonck and de Meester, 2002).This adaptation strategy allows generation of egg banks to be viable for decades without being hydrated, therefore ensuring potential species diversity amongst the invertebrates (Brendonck *et al,* 2007).

According to Brendonck *et al*, (2007) the most common invertebrate species are from the Branchiopoda class as these are commonly found in arid to semi-arid regions. This project focuses on the Branchiopoda class that includes the Notostraca, Conchostraca, Anostraca and Cladocerans. These are morphologically diverse groups that play an important ecological role in aquatic environments. These groups are referred to as large branchiopods, and often inhabit seasonal wetlands, such as flood plains, salt pans, rain pools, while others inhabit permanent water bodies such as lakes.

Literature Review

Organisms that inhabit temporary variable aquatic environments rely on the production of resting or dormant egg banks to survive periods of unsuitable physical (temperatures, low oxygen) or biological (limited food, predation, competition) (Brendonck and De Meester, 2002).

According to Brendonck and De Meester (2002), after the eggs are deposited, most of the resting eggs sink to the bottom, while some float along the water body or attach to plants or sediment particles. The resting eggs can survive extreme conditions of temperatures, oxygen salinity or drought and remain dormant until the conditions are favourable after breaking a diapauses period. The conditions necessary for the breaking of diapauses and inducing hatching vary depending on the species and also vary among populations within the same species. Only part of the resting eggs hatch, the other eggs would hatch in another hydration period (Brendonck and De Meester, 2002).

In a natural environment, resting stages that do not receive the necessary stimulus to hatch (e.g. when covered by sediment when photoperiod and light intensity are important stimuli) will also not hatch even when diapause is broken, which results in more eggs being dormant. So the dormant eggs can survive for many seasons, accumulate with stages produced at different episodes and gradually form mixed persistent egg banks. Dormant eggs that are not able to survive for long in a dormant state may form a temporary egg bank. Egg banks can be collected from upper layers of the water body (eggs floating or attached and in the soil surface). Banks of resting stages occur in most ecosystems of the aquatic environment, especially in variable systems like shallow lakes and ephemeral pools (Brendonck and De Meester, 2002).

Delayed hatching of part of the egg bank, extending the average generation time of the organisms, has been observed in several species, and results in the generation of an egg bank with strong generation overlap. In a variable environment, this process can lead to the generation of an egg bank with a high genetic and species diversity (Hairston, 1996).

Many studies have contributed to the knowledge of the role that invertebrates have in aquatic environments. According to these studies the most common invertebrate species are from the Branchiopoda class (Brendonck *et al,* 2007), as these are commonly found in arid to semi-arid regions. Rock and mud pools in arid and semi-arid areas are very specific temporary habitats, because of their often extremely short hydro-period and irregular hydrological cycles, which depends on the size of the temporary water body and the local climate.

Some temporary water bodies hold water more than one year while others remain dry for longer periods; this depends on the amount of rainfall received. Therefore these water bodies differ greatly in size, shape and depth, duration of flooding; hence there is a great diversity of invertebrates found in different habitats (Zacharias *et al*, 2007).

Local pool characteristics, such as the length of hydration, conductivity and temperature, are main factors determining species occurrence in pool. In temporary aquatic habitats, the duration of time that pools contain water is one of the most important variables impacting population and community characteristics. Despite being subjected to time pressure and unpredictability, temporary pools often contain a rather diverse assemblage of permanent and temporary residents (De Roeck *et al,* 2009).

According to Zacharias *et al* (2007), hydrological changes in the water happen naturally and as the water quality changes, so does the vegetation and aquatic invertebrates change in the water body. During hydration periods, limited resources are available and predation is low, however when the water is drying up, there is great competition and predation amongst the inhabitant of the water body.

Zacharias *et al* (2007), further explains that physical and chemical characteristics of temporary water bodies often fluctuate and may reach or exceed biological limits that organisms can tolerate. The nitrates and phosphates vary throughout the year but their concentration levels remain low. Salinity plays an important role in temporary wetlands especially when the ponds are drying up because of the increased concentration of dissolved ions by evaporation. Salinity tolerances vary between different groups of organisms found in temporary ponds. These waters often undergo extreme changes in pH and levels of dissolved carbon dioxide, as well as oxygen. Oxygen levels are constantly changing and are often very low, due to the very high surface to volume ratio of these shallow depressions, mostly experienced when there are extreme changes in temperature.

Branchiopods cope with the time pressure of their habitat by reaching maturity within the first week after hydration and producing almost daily small broods of resting eggs (Brendonck *et al*., 2000). In many parts of the world Streptocephalus species are not known to occur in unpredictable rock-pool habitats with a short hydration period, but do occur in mud pools with a longer hydration period of about three months (De Roeck *et al*, 2009).

Ecological importance of studying aquatic invertebrates

These studies can improve education of communities, and create incentives for community based wetland conservation, in order to increase awareness of the values of Namibian wetlands.

These studies also help describe invertebrate habitats and communities, helping to develop an invertebrate habitat map that may help to conserve such areas.

Key ecological drivers for each habitat can be identified and therefore key vulnerabilities and threats in the community are identified.

Sensitive assessment for each habitat can be developed from these studies, which will help to evaluate current or proposed industrial activities; therefore studying invertebrates is an important component of Environmental Impact Assessments

Fairy shrimps are also useful as indicators of water toxicity, so these species can be used in waste water treatments

Economical importance of invertebrates in Namibia's

The brine shrimps play a very important role in the salt industry as they help clear the water through filter feeding which aids the rapid evaporation in the salt pans (Mitchell, 1989).

In Asia, some species are used for human consumption and some are domesticated and kept as pets (Brendonck, *et al*, 2007)

Some Notostraca species are found in rice fields as pests, however these species can also be used as weed controllers and furthermore, they have been proposed as biological mosquito controllers.

Some triops species are being considered as potential aquaculture feed, because of their fast growth, early maturation and uni-parental reproduction (Brendonck *et al*, 2007)

Objectives of the study

To hatch invertebrates from different substrate samples, in order to test the viability of using substrate samples in invertebrate studies.

Research hypothesis

Substrate samples from different water bodies in Namibia harbours different invertebrate species.

Materials and Methods

A total of 22 samples that were obtained from Ministry of Agriculture, Water and Forestry were analyzed in this study and these were collected from different water bodies in Namibia (see Table 1).

Table 1: Samples used in the study

	Date collected	Co-Ordinates (S)	Co- Ordinates (E)	Location
1		25 58 35	19 07 69	Koes pan
2				Pool gravel plains
3		21 19 40.8	14 44 115	Small pool Brandberg
4		20 44 490	014 14.371	Rock mud pool
5		27 74 64	15.52.15	
6		25 60 06	16.35.07	Southern Namib
7		27 43 840	15.47.44	Boegenfell pan
8		217 89 474	12.55.381	Mud hole
9		27 19 54 5	16.12.06	Koes Pan
10				Kloof Oasis
11				Cheetah pool
12				Namib pool
13				Dam between junction
14				Namib pool
15				Dam between Junction
16				Kloof Oasis
17		17 42 59	12.54.27	Kunene Dry Stock Dam
18				Etendeke mountain pool
19				Small stream pool
20				Dumbo Reeds stalks
21		18 .57.36	19.46.61	West bushmanland Waterhole
22		18. 1709	17.16.905	

This project was carried out in both summer and winter. The substrate samples (see Figure 1) that included sand, stones and dry leaves and stems of aquatic vegetation. About 100 grams of the substrate samples were put in 3litre

Erlenmeyer flasks (Figure 2 & 3) that were filled with 2litres of rain water. These flasks were kept against a glass door or a window to expose the flasks to sunlight.

Figure 1: substrate samples

Figure 2: substrate samples

Rain water was regularly added to the flasks to maintain the water level at 2liters per flasks. The tanks were stirred twice a day, in the morning and before sunset, to mix the substrate well with the rain water. Observations were conducted twice a day, in the mornings and before sunset.

Figure 3: Rain water added to substrate

After 3 days of hydrating the samples some eggs hatched, and after approximately a week, these organisms were considered sexually mature and were identified easily.

In tanks where there was no algae growing, 2grams of soup powder as advised by Mr Roberts (Figure 4) was added to the flasks as feed for the invertebrates. In summer, there were more algae in the flasks, but some flasks did not have any algae growing, so they were supplemented with the soup.

Figure 4: alternative feed for the invertebrates.

After approximately 3 weeks of growing the invertebrates in the tanks, easily collected with a very fine mesh sieve and placed in 50ml tubes where 25ml of 70% ethanol was added for preservation purposes.

The preserved specimen were transferred to a petri dish and carefully examined using a dissecting microscope (Zeiss, model 47 50529901, West Germany) so that they could be identified.

The animal's position was constantly changed with forceps so that all the features can be observed in order to positively identify them. Some specimens had to be dissected with dissecting needles, in order to remove its shell so that the internal body could be examined to identify the species.

Day *et al* (1999) was used to identify the species. The identified specimens are kept in labelled preserved bottles, which were kept for future references.

Results

Experiments that took place in summer were more successful in terms of invertebrates that hatched. Environmental condition in summer was ideal for the eggs to hatch and also to produce algae (which served as feed for invertebrates) which grow best in warmer sunnier months.

According to Peckersky (1990), the development is often rapid in the summer or spring, but can be slowed by unusually low temperatures. Young which have hatched from winter or autumn eggs develop more slowly than those that have hatched from summer eggs.

The invertebrates that were collected from the samples were from the Class Branchiopoda and only from 2 Orders Anostraca (fairy shrimps) and Conchostraca (clamp shrimps) (see Table 2).Most of these species hatched during the summer period, where temperatures were warmer and more algae were present.

Table 2 : Species that hatched from the substrate

	Date collected	Co-Ordinates (S)	Co- Ordinates (E)	Location	Species Collected
1		25 58 35	19 07 69	Koes pan	2 fairy shrimps
2				Pool gravel plains	Nothing
3		21 19 40.8	14 44 115	Small pool Brandberg	Nothing
4		20 44 490	014 14.371	Rock mud pool	Nothing
5		27 74 64	15.52.15		Nothing
6		25 60 06	16.35.07	Southern Namib	Nothing
7		27 43 840	15.47.44	Boegenfell pan	2 fairy shrimps
8		217 89 474	12.55.381	Mud hole	Nothing
9		27 19 54 5	16.12.06	Koes Pan	2 fairy shrimps
10				Kloof Oasis	Nothing
11				Cheetah pool	2 fairy shrimps
12				Namib pool	12 fairy shrimps and 2 clamp shrimps
13				Dam between junction	3 fairy shrimps
14				Namib pool	2 clamp shrimps
15				Dam between Junction	5 fairy shrimps
16				Kloof Oasis	1 fairy shrimp
17		17 42 59	12.54.27	Kunene Dry Stock Dam	Nothing
18				Etendeke mountain pool	Nothing
19				Small stream pool	Nothing
20				Dumbo Reeds stalks	Nothing
21		18 .57.36	19.46.61	West bushmanland Waterhole	Nothing
22		18. 1709	17.16.905		Nothing

Very few species hatched during winter, and if the eggs hatched, they did not live too long, the mortality rate of the invertebrates during winter was also attributed to the lack of food, natural food supply (algae) which was limited. Algae production in winter is quite low, so the invertebrates' diet had to be supplemented with 2grams of powder soup (Figure 4).

During the first three weeks of the project, the invertebrates where not fed, due to a lack of information on the side of the investigator and this resulted in the early mortality of fairy shrimps.

On a specific day, invertebrates that were present in all flasks were found dead in the evening, this was attributed to the very high temperatures that day, most invertebrates tolerate ranges between 13-20 degrees Celsius (De Roeck *et al*, 2010).

Two species of fairy shrimps were identified: male and female *Streptocephalidae indistinctus* and the male and female *S. cafer* and a clamp shrimp *Leptestheriidae inermis* during the study. The fairy shrimps were most abundant amongst the samples.

The *S.indistinctus* species hatched from substrate a sample that was collected from the Ubisis kloof's dry pool on the 17 May 1999; this was one of the oldest samples that had a date of collection recording. This is evident that egg banks do survive without hydration for several years, as this fairy shrimp species' eggs survived for 13 years.

The *S.kafer* species hatched from substrate samples from Kunene Rock pools.

The *L.inermis* species hatched from substrate samples collected from Namibrand Rock Pool.

In the same flask that the *L.inermis* species hatched from, there was anostrachan species that were growing, but they died naturally when the temperatures were high. But few days after they died, the *L.inermis* species was noticed in the flask.

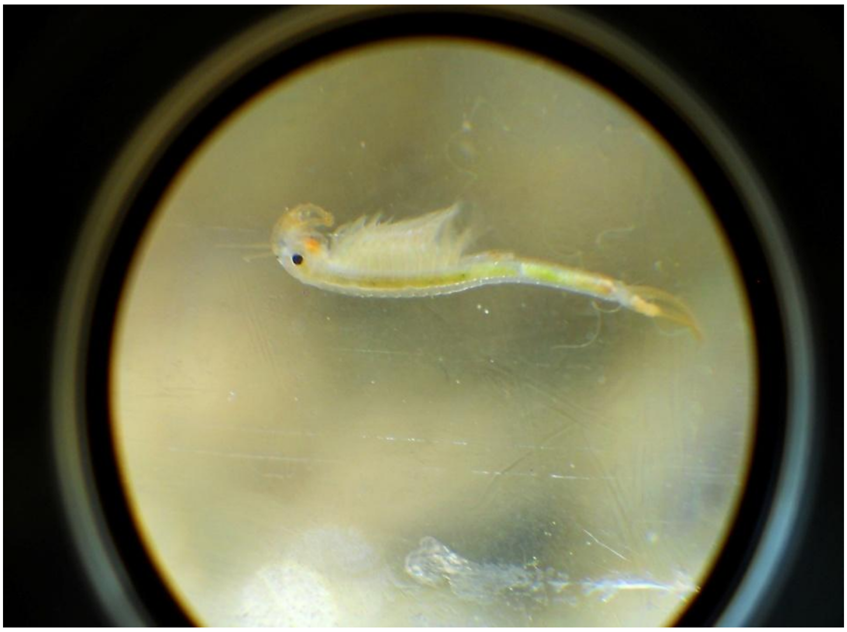

Figure 5: Anostracha species

Figure 6: female and male anostracha species in their typical swimming position

Figure 7: conchostraca swimming near the bottom of the flas

Discussion

The Anostraca were the majority of the species that were identified during this project even though they were not identified to species level.

According to Day *et al* (1999), southern Africa has one of the richest anostracan faunas in the world, of which 80% is endemic to southern Africa. During this study it was found that they have a wide distribution all over the country and they are able to survive dry periods of the habitats in the form of drought resistant eggs, which remained in substrate even in the substrate that was 10 years old.

They exhibit strange life-history characteristics that making them well-adapted to such temporary variable habitats. They hatch and mature fast and deposit an almost daily brood of relatively small resting eggs (De Roeck *et al,* 2009). According to Hammer & Appleton 1991, the Anostracans reach sexual maturity when they are five days old.

Another strategy of the anostracans that might have resulted in more anostracans hatching is that not all the eggs hatch at once, but others only hatch after more than one hydration period so a reserve of eggs is maintained in the substrate. This mechanism ensures that even if a cohort does not reproduce in 1 hydration period, and before the pool dries out, the entire population will not be destroyed by the adverse environmental conditions.

According to Delekto (2002), some eggs from the same cohort did not hatch until their pools had been hydrated eight successive times. If all eggs hatched during the same hydration and the conditions did not allow those organisms to survive, the species would likely face extinction. Differences in hatching times allow many species of fairy shrimp to avoid predation and interspecific competition.

There was another Anostraca species that could not be identified using available literature and identification keys. This species hatched from a mud sample collected from the Kunene Region, west of Okangwati, on the 16 November 2009. These species do not resemble any other species found in Southern Africa; their basal projections do not resemble projections described in available literature.

These fairy shrimps are still to be sent to Mr. Brendonck, an invertebrate specialist who is based at the University of Stellenbosch in South Africa. The species will only be sent to Mr. Brendonck after Mr. Roberts verifies the results of this study.

Only a few of these fairy shrimps were identified to species level , some fairy shrimp species could not be identified to species level, because they were too young and their external features were not fully developed to be successfully identified. Some of the species died naturally at a very young stage, their external features were not fully developed to identify them.

While some species were removed from the tanks, after 3 weeks, but then they could also not be identified even though after 3 weeks they were expected to be fully matured. So their growth may have been impeded by water quality factors such as low oxygen levels, limited carbon dioxide and limited light penetration in the flask, the shape and size of the cylinders may also have affected the growth of the invertebrate. The ideal environment should be entirely self sustaining in terms of natural feed availability and light intensity and gas saturation levels.

According to Brendonck (2008), the cysts of some branchiopods do not hatch immediately after deposition even if the environmental factors are ideal for growth and reproduction. So some substrate samples used in this project that did not hatch any invertebrates, might have had eggs that were in a diapausing stage preventing them from hatching even though environmental factors were favourable. Some species remain in this diapausing stage for several months or even years. Therefore the experiment should have taken place over a longer period to allow more eggs to hatch.

The mud samples might have been collected during a time where there was no reproduction, or drying of the water body occurred before the invertebrates reached sexual maturity, leading to reduced population size.

Various environment factors also play a role in the hatching of the invertebrate eggs. More invertebrates were hatched in summer, because temperatures were favourable during that time and also during summer there was a great deal of natural feed (algae).

However, when the temperatures were too high in the summer, it was a major stressful situation as the oxygen levels in the tanks also decreased and in some cases invertebrates died, some of the species had also died after being fed with the soup. So it is very important to control the environmental factors to successfully grow the invertebrates.

Conclusion

The fairy shrimps were most dominating species that hatched from different samples; one can conclude that fairy shrimps inhabit most temporary wetlands in Namibia. It can be concluded that their eggs can survive long periods without being rain, and they grow quick and also they adapt very well to different adverse environmental conditions. Like in the case of *S.indistinctus* species that hatched from mud sample collected in 1999, which is 13 years ago.

Substrate samples can be used to hatch invertebrate eggs as samples as old as 13 years hatched invertebrates in this project, so substrate samples can be used for invertebrate studies.

It is therefore important to study freshwater invertebrates as they are an important component in the natural environment, so it is important to monitor them to prevent them from becoming extinct. These types of studies are very important to carry out, because identifying species can add to the national and international databases of wetlands and also because invertebrates can be used as environmental indicators so it is important to study the presence of invertebrates in a country.

Recommendations

Season and environmental factors play a major role in this study. During summer there were ideal environmental conditions like temperature, oxygen levels, ph levels, so the eggs hatched faster and invertebrates grew faster. So this type of project to be carried out only in summer because very few invertebrates hatched in winter and as a result a lot of resources, like mud sample and collected water went to waste.

The experiment should take place in a controlled lab, whereby environmental factors such as light intensity and temperature can be controlled to provide ideal conditions for the invertebrates and production of natural algae which serves as feed.

Since the water body in this case the tanks affect the growth of the invertebrates, in future more specialized tanks should be used, these tanks should be big giving the invertebrates enough space to move around.

Such experiments should take place over a long period of time to maximize results, since some resting eggs hatch only after several hydration periods and need to be hydrated for longer periods of time.

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