Tadpole ecomorphological guilds and gut contents as a proxy for diatom diversity

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ABSTRACT

Tadpole development significantly depends on the availability of water. Metamorphosis will gradually allow tadpoles to inhabit terrestrial environments (Duellman & Trueb, 1986; Duellman & Trueb, 1994; Harvey Pough, 2007). This causes considerable diversity in functional morphology which, in addition to spatial distribution, ecological drivers, and feeding habits are used to establish ecomorphological guilds (Bower & Piller, 2015; Karr, 1975; Sherratt et al., 2018; Williams, 1972). Tadpoles typically feed on algae, of which a large amount is diatoms. Some diatom species are also specific regarding habitat selection (Necchi, 2016; Round et al., 2007). The aim of this study was to assess diatom diversity in the intestinal content of tadpoles from various ecomorphological guilds sampled from different sites in relation to environmental diatom samples. Ecomorphological guilds applied in this study were adapted from Botha (2013). Three study sites were selected from Ukutula Lodge and Conservation Centre, South Africa and eight from Aliwal North, South Africa. Diatoms were isolated from environmental and tadpole samples collected at each study site. For this purpose, tadpoles were euthanized and subsequently identified. Additionally, samples were collected from tadpoles sampled at Ukutula to test for the presence of a parasitic, chytrid fungus referred to as *Batrachochytrium dendrobatidis*. After the excision of intestinal tracts, the diatom content was isolated by means of exposure to caustic chemicals. Microscope slides were made using cleaned material and examined under a light microscope. Diatoms were counted and identified. An extensive literature review was conducted to study these diatom's habitat occurrences in relation to the involved tadpole guild's feeding habits. Ecomorphological tadpole guilds sampled at Ukutula included Rheophilic, Benthic type 2 (Profundal), Suspension feeder, and Lentic Nektonic. Ecomorphological tadpole guilds sampled at Aliwal North also included Rheophilic, Lentic-nektonic, Lentophytophilic, and Lentic-benthic. None of the attempted cultures from Ukutula demonstrated growth that represented *Bd*. The relative abundances of diatom species counted in environmental and diatom samples were used to construct a Detrended Correspondence Analyses (DCA) and calculate Shannon's diversity index, Species Evenness, and Species Richness. The practical and statistically significant differences were also calculated for these samples. One hundred thirty-nine and 178 diatom species were identified from examining the gut content of tadpoles from Ukutula and Aliwal North respectively. Some diatoms were ingested as theoretically expected by tadpoles from the guilds according to existing information on guild-associated feeding habits. Although many seemingly unrelated diatom species were found in the digestive tracts of tadpoles. These species were coincidentally ingested due to resuspension or disturbances in the water

column. There was furthermore no statistical or practical significant difference between diatom samples taken from sites, tadpole species, or guilds. No clusters were discernible between samples and sites when DCA's were analysed as constructed for Ukutula and Aliwal North. The lack of significant differences between tadpoles and environmental diatom samples implies that tadpoles could be used as a method of diatom sampling in the case of insufficient substrata. It additionally gives rise to opportunities to integrate research pertaining to diatoms and zoology.

Keywords: Bacillariophyta *Batrachochytrium dendrobatidis* Diatom habitat variation Ecomorphological guilds Tadpoles

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ABBREVIATIONS AND ACRONYMS

∑ Nsi	Total Number of Species Population
CANOCO	Canonical Community Ordination
DCA	Detrended correspondence analysis
E	Species Evenness
EtOH	Ethanol
H'	Shannon's diversity index
H2O2	hydrogen peroxide
HCL	Concentrated hydrogen chloride
Isi	Total Number of Individual species
KMnO4	Potassium permanganate
LTRF	Labial Tooth Rows Formula
MS-222	Tricaine mesylate
NaHCO3	Sodium bicarbonate
NWIR	National Institute for Water Research
RA	Relative abanduce
rpm	rotations per minute
rt-PCR	real-time Polymerase Chain Reaction
S	Species Richness
Sig.	Significance probability
SiO2	Silicon dioxide
Std. Error	Standard Error
σ^2	Variance

1. INTRODUCTION

Southern Africa is home to an extensive range of amphibians (Du Preez, 2015). Amphibians have unique biological characteristics since tadpole development is often dependant on the availability of a water body. Metamorphosis will bring about biological changes, allowing the tadpoles to gradually inhabit terrestrial environments (Duellman & Trueb, 1986; McDiarmid & Altig, 2000; Kupferberg. 1997).

1.1. An overview of anuran biology

Anurans are intermediate animals; inhabiting terrestrial and aquatic environments (Duellman & Trueb, 1986; McDiarmid & Altig, 2000; Kupferberg. 1997). They have distinct behaviour, biology, and morphology enabling survival in these dual-environments. Most tadpoles, however, spend a considerable amount of their developmental period submerged in an aquatic environment. Different reproduction modes exist among anurans to accommodate aquatic habitat differences during early development.

1.1.1. Anuran reproductive stages

Reproduction modes were initially defined by Salthe and Duellman (1973). It includes the concepts of oviposition site, ovum, clutch characteristics, tadpole development, hatchling development, and involved parental care (Salthe & Duellman, 1973). Anurans represent 29 reproductive modes and exhibits the most reproductive diversity amongst tetrapod vertebrates (Duellman & Trueb, 1986; Haddad & Prado, 2005). Reproduction modes are influenced by water availability and predation (Haddad & Prado, 2005). Temporary ponds are considered drivers for terrestrial reproductive modes (Magnusson & Hero, 1991). An example of such a reproductive mode is the foam nest frogs. Foam nests have several functions, including providing protection against predators, supplying oxygen for the nest, maintaining temperatures and supplying nutrition for development (Dobkin & Gettinger, 1985; Downie, 1990; Seymour & Loveridge, 1994; Tanaka & Nishihira, 1987).

The most prominent, typical reproductive stages of anurans occur when aquatic eggs metamorphose into exotrophic aquatic tadpoles (Duellman & Trueb, 1986; Haddad & Prado, 2005). Exotrophic tadpoles are defined as tadpoles obtaining nutrition from external sources. Whilst endotrophic tadpoles acquire nutrition from a parental source (Haddad & Prado, 2005; Kusrini *et al.*, 2015). Exotrophic tadpoles further lack a feeding stage and are rarely produced from the oviducts (Kusrini *et al.*, 2015). These tadpoles will develop into four-legged terrestrial or semi-terrestrial frogs (Haddad & Prado, 2005).

1.1.2. Tadpole morphology and development

The developmental processes of tadpoles are independent of adult anuran biology (Duellman & Trueb, 1986; Duellman & Trueb, 1994; Harvey Pough, 2007). Although they commonly have a body plan with a short oval shape, tadpoles often have major morphological diversity amongst species (Duellman & Trueb, 1994). They also develop a laterally compressed tail, which can take on several variant forms depending on the stage of development and species. In some cases, the tail is longer than the length of the head.

Tadpoles have lidless eyes and a terminal mouth that, in terms of anatomy, can vary greatly (Duellman & Trueb, 1986; McDiarmid & Altig, 2000; Kupferberg. 1997). Tadpoles have fleshy lips covering a modified beak and keratinized mouthparts. Behind the keratinized mouthparts is a buccal cavity, followed by an esophagus. During primary development, anurans often develop external gills, protected by an opercular skin flap. The gills are used for respiration and to aid the feeding process.

Needless to say, tadpoles are primarily built for feeding and movement (Duellman & Trueb, 1994; Harvey Pough, 2007). Most tadpole species are filter feeders; sieving through the water column whilst collecting smaller particles from the water that passes through their mouth and through the gills. This includes algae and bacteria (Harvey Pough, 2007). Papillae and mucus will move particles from the buccal cavity to the esophagus. Other grazing or predatory tadpoles are equipped with a beak-like structure, used to scrape food from large substrates. Predatory tadpoles are often carnivorous, often feeding on the eggs of other tadpoles.

The structure of the chondrocrania

The chondrocrania of tadpoles, a cartilaginous bone structure containing the organism's brain, is strongly developed (Duellman & Trueb, 1986; McDiarmid & Altig, 2000; Kupferberg. 1997). During metamorphosis, the structure and configuration of the chondrocrania can evolve almost entirely to support mandibular functionality during adulthood. The floor of the chondrocrania also consists of sheets of cartilage and is connected to a singular thin fenestra (Duellman & Trueb, 1994). Three branches of cartilage extend from this surface, providing the brain with protection. A fourth group of cartilage branches extends from this surface, creating the brain's anterior wall. This is known as ethmiodalis pilae. The foramen olfactorium carries the olfactory nerve out of the brain.

The fenestration of the chondrocranium varies between individual tadpoles, depending on the species and the stage of development (Duellman & Trueb, 1986; McDiarmid & Altig, 2000; Kupferberg. 1997). The nasal capsules on the anterior end of the chondrocranium only develop until metamorphosis of the tadpole's mouthparts occurs. The planum trabeculae at the anterior side of the braincase hold the cornua. Cornua's are anterolateral projections. It connects with the anteromedial margin of quadrate cartilage. Ossification of the cranium only occurs once metamorphosis commences.

Gill arches

There is a generous amount of diversification in the branchial arch structure formation in tadpoles (Duellman & Trueb, 1986; McDiarmid & Altig, 2000; Kupferberg. 1997). However, each typically consists of hardy anterior plates and the floor area of the buccal cavity (ceratohyals). The basibranchial cartilage is formed by the fusion of the ceratohyals articulate and the palatoquadrate. The basibranchial cartilage also gives rise to the hypobranchial plates, which have four ceratobranchials on either side. They fuse together, protecting the gill filters in a basket-shaped structure. They are associated with feeding mechanisms utilized by tadpoles.

1.1.3. Tadpole morphology in relation to feeding habits

Since tadpoles lack feeding structures found in other amphibians (functional jaws, teeth, and tongues), it is necessary to have modified feeding mechanisms to compensate for foraging. Tadpoles have mandibular organs that often contain varying amounts of cartilage. They additionally have a coiled gut, and generally feed at the bottom of a water body or in the water column. They are also specialized feeders (Duellman & Trueb, 1986; McDiarmid & Altig, 2000; Kupferberg. 1997).

Tadpoles are often equipped with mechanisms for filter-feeding on particles suspended in the water column (Kenny, 1969; Severtzov, 1969). Their mouthparts are arranged in layers, acting as filters for water content (Duellman & Trueb, 1994; Katz et al., 1981). This allows tadpoles to filter through particles based on size. They implement methods of direct interception and inertial impaction to filter feed from various substrates.

The tadpole's mouth is primarily supported by three cartilage structures (Duellman & Trueb, 1994). The movement of the cartilage is regulated by a collection of intricate ligaments and visceral musculature (Gradwell, 1972a; Gradwell, 1972b). All tadpoles are equipped with keratinized beaks and denticules (with microhylids and pipoids as exceptions). Fleshy labial papillae are also part of the mouth structures. They use their keratinized mouthparts to forage for food, scraping it from various surfaces. Water will typically flow from the mouthparts to the buccal cavity, through the pharynx and over the gills, exiting through the spiracles. This allows respiration to occur whilst trapping tiny food particles for ingestion.

The buccal and pharyngeal cavities form the buccal pump (Duellman & Trueb, 1994). Three valves (mouth, choanae, and ventral velum) control the water flow through the pump. Each choana is equipped with a posterior valve. All tadpoles (except pipids) are equipped with ventral velums, an epithelial flap protruding from the buccal floor. It separates the branchia from the buccal cavity. The hyobranchial structure is also included in this system.

When the ceratohyals cause an expansion in the buccal cavity, it will generate negative pressure (Duellman & Trueb, 1994). This will pull water into the cavity, through the mouth. The tadpole's mouth will close, and the buccal cavity will contract. Water will be forced to move over the ventral velum, and consequently in the branchial baskets located in the pharynx. Lastly, the branchial baskets will contract and force water through the gills and out of the tadpole's system via the gill slits and spiracle.

Tadpoles extract food particles from water that flows into the buccopharyngeal cavities (Duellman & Trueb, 1994). They have this ability because of the presence of secretory epithelium and papillae. Particles are sorted by size in the buccal cavity. Smaller particles will enter the pharynx, over the ventral velum; whilst larger particles enter the esophagus. The gill filters in the ventral velum will filter larger particles, and smaller particles are trapped in the mucus of the branchial food trap. Food particles are moved to the esophagus, through the ciliary groove from the gill filters.

This layered design of tadpole mouthparts allows for efficient filtration of food particals (Duellman & Trueb, 1994; Katz et al., 1981). Other tadpoles will scrape the periphyton with keratinized mouthparts. This will create a concentrated suspension of algae (Duellman & Trueb, 1994). They are equipped with less dense gills and filtering organelles. This enables them to effectively filter the suspended algae. Other tadpoles (from the *Megophrys* and *Microhyla* genera) feeds on large micro-organisms floating on the surface of the waterbody. They lack buccal papillae, but they have ridges used to sort out coarser particles. Some tadpoles even have serrated mouthparts and predate on other tadpole species (like species from the Scaphiopus genus). They demonstrate cannibalistic behaviour as well.

1.1.4. Tadpole morphology in relation to identification

Many of the morphological characteristics discussed in the aforementioned sections (1.1.1 – 1.1.3) are distinct enough to be used to aid species identification of tadpoles (Du Preez, 2015; Duellman & Trueb, 1994). This generally includes the body size, shape and position of physiological and morphological structures. Here follows a brief summary pertaining to these features, as adapted from Du Preez (2015):

- Position of the mouth (anterior, anteroventral, near-ventral or ventral).
- Position of the nostrils (narrowly spaced or widely spaced).
- Position of the excurrent opening from the gill chambers or the spiracle (above, just below, below, well below or paragyrind).
- Position of the vent (supramarginal or marginal).

- Position of the eye (lateral, near-lateral, dorsolateral or dorsal).

- The structure of the jaw sheaths (delicate, moderate or massive).

The labial tooth row formula can additionally be used for species identification of tadpoles (e.g. LTRF: 3(2-3)/4(1-2)). In this formula, 3 indicates that the upper jaw has 3 rows of which rows 2-3 are divided and that the lower jaw has 4 row of which 1-2 are divided (Du Preez, 2015).

During long-term studies, tadpole development stages can be used for identification (Shumway, 1940; Taylor & Kollros, 1946). Shumway (1940) initially tabulated 25 pre-feeding stages of the species *Rana pipens*. Taylor & Kollros (1946) later constructed a table documenting post-feeding stages. Other tables entailed embryonic and ltadpole developmental stages (Gosner & Black, 1958; Volpe & Dobie, 1959). Gosner (1960) adopted this system and incorporated it into a proposed table for identifying tadpole developmental stages. At Gosner stage one fertilization occurs; and stages one to 25 is considered embryonic or pre-feeding stages. The jelly envelope, size and developmental rates will differ to some detail amongst species. Regardless, the table is used as a general guideline to identify developmental stages of tadpoles (Gosner, 1960).

1.2. Tadpole ecomorphological guilds

The developmental modes, internal and external morphological characteristics of anuran species (e.g. characteristics discussed in section 1.1.) can be influenced by ecological factors (Sherratt *et al.*, 2018). This includes the tadpole's body shape, eye position, oral disc structure, the composite configuration of the head and muscular tail (Altig & Johnston, 1989; Both *et al.*, 2011; Sherratt *et al.*, 2018). Additional research found that internal morphology like muscular, buccal and skeletal characteristics is also influenced by ecological drivers (Candioti, 2006; Vera Candioti & Haas, 2004; Vera Candioti *et al.*, 2005). There is a discernible relationship between the functional morphology of organisms and their ecology (Wainwright, 1991). The study of this relationship is defined as 'ecomorphology' (Wainwright, 1991).

Organisms can be grouped together based on the relationship of functional morphology and ecological drivers (Bower & Piller, 2015; Karr, 1975; Sherratt *et al.*, 2018; Williams, 1972). These groups are subsequently referred to as 'ecomorphological guilds' (Bower & Piller, 2015; Karr, 1975; Sherratt *et al.*, 2018; Williams, 1972). Guilds can additionally be linked to specific niches and feeding habits (Altig & Johnston, 1989; Sherratt *et al.*, 2018). Grouping organisms into similar ecological groups is advantageous when applied to studying patterns and behaviour in terms of biogeography, evolution and communities (Wiens, 1989; Williams & Hero, 1998).

Researchers apply this concept to study the effect of the environment on the functional morphology of the involved organisms (Wainwright, 1991). In this setting, the environment is considered the primary driver for the evolution of an organism and hence, the functional morphology of the organism (Wainwright, 1991). The reverse of the aforementioned can also be studied: the effect of functional morphology on the involved organism's ecology (Wainwright, 1991). In this scenario, an organism's behaviour in a habitat is influenced by its functional morphology or build (Wainwright, 1991). The concept of ecomorphological guilds was also applied to studies modelling ecosystems (Bower & Piller, 2015; Sherratt *et al.*, 2018).

Guild classification can furthermore be used to gain a broader understanding of spatial patterns, species richness, and assemblage structure (Williams, 1997; Williams & Hero, 1998). It provides supplementary information regarding spatial patterns, species composition, and richness (Williams, 1997; Williams & Hero, 1998). Studies undertaken on the effect of ecomorphology on an organism's fitness hones in on the ability of said organism to perform essential tasks and consequently obtain environmental resources (Arnold, 1983; Wainwright, 1991).

Species occurring in temporary water bodies, by way of illustration, demonstrates substantial tadpole growth to adapt to rapid changes in water availability (Both *et al.*, 2011; Skelly, 1996). In contrast, species residing in predatory waters tend to be unpalatable (Both *et al.*, 2011; Hero *et al.*, 2001). These adaptations will increase the individual's chances of survival in such an environment. Suspension feeders tadpoles typically have a dorsally positioned oral disc, with lesser tail fins (Sherratt *et al.*, 2018). The nektonic guild, however, displays prominent, well-arched tail fins for swimming freely in open water with anteroventral oral discs (Sherratt *et al.*, 2018).

Amphibians differ considerably in developmental modes and morphology (Altig & McDiarmid, 1999). Tadpole ecomorphological guilds are derived from the development of the tadpoles, external morphology and the microhabitat the tadpole resides in (Altig & Johnston, 1989). These guilds can be applied to studies, as is the intention of this study. It is beneficiary to study the interactions of these guilds with other groupings of organisms, to understand the ecology of a habitat better.

Functional anatomy was studied using observational techniques since the 1950's for ecological studies (Bock, 1994). Ecomorphology primarily relied on examining the functionality of skeletal structures and feeding apparatus (Bock, 1994). Orton (1953) initially introduced four ecomorphological guilds based on oral morphological structures; Type 1 (Xenoanura), Type 2 (Scoptanura), Type 3 (Lemnanura) and Type 4 (Acosmunaru) (Starrett, 1973). These types include frogs from the families Pipids and Rhinophrynids (Type 1), Microhylids (Type 2), Ascaphids and Discoglossids (Type 3) and the remaining families (Type 4) (Orton, 1953; Starrett, 1973). Orton (1953) also classified major groups based on morphological mouthpart adaptations. Examples include arboreal tadpoles, surface-feeding tadpoles, mountain stream, nektonic and carnivorous tadpoles (Orton, 1953). Van Djik (1972) later grouped tadpoles in ecomorphological guilds based on tadpole behaviour and the findings of Orton (1953). Van Djik (1972) applied these principles to specific geographic regions. The ecomorphological guilds he constructed includes:

Pelagic/Hydrophytophilic

Individuals from this guild have lateral eyes, appearing to spend time resting on or attached to vegetative material. Tadpoles also generally spend time between vegetation resulting in a terminal, dorso-ventrally flattened mouth (Van Dijk, 1972).

Hydrophytophilic

Tadpoles in this guild filter-feeds to some extent. They are generally hydrophilic with pointed tails. Some tadpoles are documented to feed on smaller animals like mosquito larvae, pupae and water snails.

Rheophilic

Tadpoles from this guild range from bottom-dwelling, hydrophytophilic forms to torrent-dwelling-hydrophytophilic. For torrent-dwelling tadpoles, the oral disc is expanded into broad suckers, with dorsal eyes and a streamlined head. The restrodonts are reduced, but these tadpoles typically have many keratodonts.

Bottom-dwelling

Tadpoles from this guild is found in streams or ponds with sparse vegetation. They are bottom dwellers with oral discs and long, thin tails. Their tails can be rounded or pointed. Some of these tadpoles are also living in areas exposed to elevated levels of UV radiation. Their pupils are typically protected by an umbraculum.

Gregarious

Tadpoles of this guild tend to swarm and are traditionally black with rounded tails. These tadpoles swarm in muddy water with little to no visibility. Some tadpoles have a flap of skin located behind their eyes, serving as auxiliary gaseous exchange.

Subterranean and other extra-aquatic

This guild includes subterranean tadpoles occurring on top or within humus. These tadpoles usually burrow into the humus of their surroundings. Their behaviour primarily depends on environmental conditions.

Altig and Johnston (1989) also conducted an analytical study based on the results of Van Dijk's (1972) findings. Their study proposed a method to quantify the angular orientation of the oral discs relative to a longitudinal body axis (Altig & Johnston, 1989). Their research also focussed on using oral disc papillae as an aid during guild delineation and identification (Altig & Johnston, 1989). Their study was later revised and led to the construction of new ecomorphological guilds based on genus level appearance and similarities (Altig & McDiarmid, 1999).

In the 1990s, another study involved tadpole ecomorphological guilds (Williams & Hero, 1998). During this study, frogs were grouped in guilds based on the following criteria (Williams & Hero, 1998):

- Habit use and specialization, ranging from generalist to specialist.
- Fecundity measured as the average number of eggs produced per female per clutch.
- Reproductive habitat, ranging from terrestrial to aquatic.
- Adult microhabitat, ranging from terrestrial to arboreal.
- Temporal distribution, ranging from diurnal to nocturnal.
- Average voucher size.

Haas (2003) also studied the contribution of established morphological structures of tadpoles to the understanding of frog phylogeny. The study was based on previous work and literature regarding anuran morphology and phylogeny (Haas, 2003). The study confirmed several clades including *Leptodactylidae, Bufonoidea, Neobatrachia* and *Centrolenidae.* Peltzer and Lajmanovich (2004) additionally conducted a study in which they grouped tadpoles into ecomorphological guilds to describe their distinctive feeding habits. Species were grouped in ecomorphological guilds based on mouthpart structures and microhabitat use (Peltzer & Lajmanovich, 2004). It is evident the usage of ecomorphological guilds will only be beneficial for a study involving ecological interactions. It will grant insight into ecological relations whilst allowing the quantification of data.

1.2.1. Tadpole ecomorphological guilds involved in this study

This study greatly relies on ten guilds delineated from research conducted by Botha (2013). The guilds were constructed based on criteria as taken from Altig and Johnston (1989) and reviewed by Altig and McDiarmid (1999). Other literature consulted for the construction of this criteria includes (Channing et al., 2012; Du Preez, 2015; Lambiris & Board, 1988; Van Dijk, 1972) (Annexure A).

The morphological values used for the construction of the aforementioned criteria includes body length, head shape, tail (length, tip, origin), eye position (dorsal/dorsolateral/lateral), nostrils (size, space between nostrils), oral disc (position, size), jaw sheath (position, size), labial teeth (anterior, posterior), and papillae (position and shape). Ecological variables used includes various habitat parameters (e.g. lentic, lotic, altitude), tadpole behaviour (e.g. gregarious, filter-feeders, bottom-dwelling) and the time it would take a tadpole to complete metamorphosis. The tadpole's position in the water column is equally as critical.

The groups were identified and described according to (Altig & McDiarmid, 1999; Van Dijk, 1972). In some cases, new terminology was constructed. The terminology was based on the variables most descriptive of the guild (Botha, 2013).

Guild 1 (Suspension feeders)

This guild is composed of all species from the *Xenopus* and *Phrynomantis* genera. It additionally includes some species from the *Afrixalu*s genus. These species were previously grouped in the Pelagic (open-water filter feeders) guild by Van Dijk (1972). This is based on the position of these species in the water body. Altig and Johnston (1989) later assigned them to the Suspension Feeder guild based on their feeding habits displayed in open water bodies.

These tadpoles are typically filter feeders, found in the midwater of lentic water bodies (Van Djik. 1972; Altig & Johnston, 1989). Other common relevant characteristics includes wide oral discs, anteriorly positioned mouthparts, keratinized jaw sheaths and absent labial teeth, low tail origins, a sharp flagellum, a strongly depressed head, laterally positioned eyes, small (narrowly spaced) nostrils, laterally positioned eyes, and a moderate developmental rate (35 days to 6 months).

Guild 2 (Nektonic)

This guild includes all species from the *Afrixalus* genus. Van Dijk (1972) initially grouped these tadpoles into a guild named the Pelagic-Hydro Photophilic guild. This is based on their association with aquatic vegetation and their position in the water body. McDiarimid and Altig (1999) later assigned them to a Lentic-benthic guild, despite that they have laterally positioned instead of dorsally positioned eyes. They also do not regularly dwell at the bottom of the water body.

Common relevant characteristics include the ability to move independently of water currents, the presence of depressed heads, laterally positioned eyes, small (narrowly spaced) nostrils, low tail origins, a sharp flagellum, a tail's length double the length of the head, keratinized jaw sheaths and labia, the ability to filter feed in the midwater, association with submerged vegetation, moderate development period (35 days to six months).

Guild 3 (Lentic-nektonic)

This guild contains species from various genera, including *Hyperolius*, *Kassina, Semnodactylus, Hemisus* and *Hildebrandtia.* Species from the genera *Hyperolius, Kassina* and *Hemisus* were initially assigned to the Pelagic-Hydrophytophilic group (Van Dijk, 1972). *Hildebrandtia*, however, was assigned to the Hydrophytophilic group. *Hildebrandtia ornate* is the only predatory species of this group, which is why it was classified under the Hydrophytophilic guild. They may as well hunt and inhabit the pelagic zone of a water body.

Semnodactylus wealii is monotypic and was initially not assigned to any guild (Altig & Johnston, 1989; Van Dijk, 1972). They were later grouped in the Suspension-Rasper guild due to the presence of jaw sheaths, lateral eyes and sharp flagella (Altig & Johnston, 1989). Common relevant characteristics includes keratinized jaw sheaths and labial teeth situated in the upper and lower labium. Tadpoles from this guild are predominantly not filter feeders.

Guild 4 (Benthic type 2/Profundal)

This guild consists of species from the *Ptychadena, Hylarana, and Chiromantis* genera. It additionally includes *Strongylopus grayii.* Altig and Johnston (1989) assigned these genera to a group termed "Benthic type 2". This group primarily consists of benthic-Profundal tadpoles and is also referred to as Profundal. They also move from littoral areas to deeper areas in the water body. They inhabit both lentic and lotic systems.

Van Dijk (1972) assigned *Chiromantis* to the Pelagic-Hydrophytophilic group, despite tadpoles from this genus having dorsolateral eyes. McDiarimid and Altig (1999) grouped the benthic species together in a singular guild. This guild was later separated into a Benthic-Profundal guild (excluding the Bufonidae family) and a Lentic-benthic guild (including the Bufonidae family).

Common relevant characteristics includes having a depressed to globular depressed head, a sharp tail tip, dorsolateral eyes, anteroventrally positioned oral discs, moderately developed jaw sheaths, and a labial tooth row formula of 2 (upper labium)/3 (lower labium).

Guild 5 (Lentic-benthic)

This guild primarily comprises genera from the Bufonidae family, including *Amietophrynus, Poyntonophrynus, Vandijkophrynus, Capensibufo, Schismaderma,* and *Mertensophryne.* Species in this guild are bottom-dwellers, and the majority is located along the shoreline of water bodies. They were subsequentially assigned to benthic or bottom-dwelling groups (Altig & Johnston, 1989; Van Dijk, 1972). Van Dijk (1972) additionally assigned *Schismaderma* to the Gregarious guild since these individuals often aggregate.

Schismaderma carens' tail tip is rounded, and the tadpoles present with a depressed head. It also has dorsolateral eyes with well-developed jaw sheaths. It was subsequently placed in the Lentic-benthic guild (Botha, 2013). Mertensophryne was assigned to Arboreal Type 5 because of its rising head (Altig, 1999). It also closely represents Lentic-benthic forms, despite the tadpoles having elongated form.

Common relevant characteristics includes having shorter bodies (< 40 mm), dorsolateral eyes, properly developed jaw sheath, a labial tooth row formula of 2 (upper labium)/3 (lower labium), depressed to globular depressed heads, rounded tail tip, and near ventral oral discs. They also typically inhabit lentic water bodies without moving into deeper areas. They preferably reside in shallow/weedy areas.

Guild 6 (Rheophilic)

This guild contains species from two genera (*Amietia* and *Strongylopus*). Van Dijk (1972) assigned these genera to the Rheophilic guild based on their preference for lotic habitats. Common relevant characteristics includes a streamlined body shape, with wide oral disks and dorsolateral eyes. Tadpoles from this guild are generally bottom dwellers, that is also found on the margins of lotic habitat. They demonstrate an extended developmental time (at least 6 months) (except *Amietia inyangae* and *Strongylopus fasiatus*).

Guild 7 (Suctorial)

This guild includes species from two genera, namely *Hadromophryne* and *Heleophryne.* Suctorial species were placed in this guild based on their phylogeny and ecological niches (Altig & Johnston, 1989; Altig & McDiarmid, 1999). Common relevant characteristics includes having a streamlined body, low tail origins with rounded tips, dorsal eyes, ventral oral discs with a broad sucker, absent jaw sheaths (except *Hadromophryne natalensis*), numerous labial tooth rows, and small closely spaced sub marginal papillae. They are also fast swimmers, inhabiting the benthic zone in fast-flowing water.

Guild 8 (Excitus-Parageios)

This guild includes species from the genera *Tomopterna* and *Pyxicephalus*. Van Djik (1972) assigned *Tomopterna* to the bottom-dwelling guild and *Pyxicephalus* to the Gregarious guild. McDiarmid and Altig (1999) later assigned both genera to the Lentic-benthic guild. Common relevant characteristics includes having a rounded head, long tails with low origins and rounded tips, dorsolateral eyes, narrowly spaced nostrils, and three rows of lateral papillae. They inhabit shallow temporary lentic habitats and displays rapid development periods.

Guild 9 (Lentophytophilic)

This guild contains species from the genera *Cacosternum, Microbatrachella*, and *Phrynobatrachus.* Van Dijk (1972) classified *Cacosternum* and *Microbatrachella* as Pelagic-Hydrophytophilic tadpoles. *Phrynobatrachus* was assigned to the behavioural mode Hydrophytophilic. Common relevant characteristics includes dorsolateral eyes and ventrally positioned oral discs. They also dwell in the midwater of shallow lentic water bodies and are associated with aquatic vegetation.

Guild 10 (Bentophytophilic)

This guild includes tadpoles from the genera *Leptopelis, Natalobatrachus* and *Poyntonia.* Altig and Johnston (1989) allocated *Leptopelis* to the Lotic Benthic guild. Van Dijk (1972) later assigned *Natalobatrachus* to the *Rheophilic* guild but it was later assigned to a clasping guild. Common relevant characteristics includes having a streamlined body build with longer tails (compared to benthic tadpoles), dorsolateral eyes, and small widely spaced nostrils. They are also bottom dwellers that typically inhabits the benthic zone. They are associated with aquatic vegetation and debris.

1.3. An overview of diatoms

As discussed in the previous section 1.1.3, tadpoles have various adaptations to assist in food acquisition. They habitually ingest algae through filter feeding or grazing. This includes algae from the class Bacillariophyceae, commonly referred to as diatoms (Dalu & Froneman, 2016; Dalu et al., 2014; Stevenson et al., 1996; Taylor et al., 2007).

When Anton van Leeuwenhoek discovered diatoms under his light microscope in 1702, it initiated a change in research pertaining to microorganisms (Lipps & Valentine, 1970). However the study of diatoms only gained notoriety during the early 1900s (Round *et al.*, 1990; Smol & Stoermer, 2010). The following section will briefly discuss the history of diatom research conducted in South Africa.

1.3.1. A summary of history of diatom research in South Africa

Diatom research in South Africa can be recapitulated in five phases (Taylor *et al.*, 2007). The first phase was initiated during the 1800s (Shadbolt, 1854). The second stage commenced in 1910 and concluded in ~1940 (Fritsch *et al.*, 1929). The third stage was initiated when Dr Bela Jeurno Cholnoky commenced his research in South Africa during the 1950s (Taylor *et al.*, 2007). He founded the National Institute for Water Research (NWIR) based in Pretoria, which later became the largest diatom research centre in the Southern Hemisphere (Harding *et al.*, 2004).

After the passing of Cholnoky in 1972, Dr. Archibald and Dr. Schoeman continued growing their research in taxonomy (Taylor *et al.*, 2007). This commenced the fourth stage of diatom research. They published “The Diatom Flora of Southern Africa” ensuing their research which relied on light and electron microscopy (Schoeman & Archibald, 1977). The fourth stage mainly entails two divisions of diatom research (Taylor *et al.*, 2007). One centred on taxonomy. The other involved investigative, qualitative research. Its primary purpose involved improving light/electron microscopic investigative techniques.

The fifth and current phase of diatom research (1990-current) is led by Prof Guy Bate (Nelson Mandela Metropolitan University) (Taylor *et al.*, 2007). This stage’s crux entails assessing water quality by examining the ecological aspects of diatom clusters. This is based on implementing the usage of a South African diatom index based on a European

diatom index. They also produced protocols for sampling and identification of diatoms involved in biomonitoring. This led to numerous studies in diatom taxonomy and ecology throughout South Africa. These studies contributed to academic literature detailing research topics on diatoms, such as their morphological features, community structure, habitat significance and so on. These topics will be discussed in sections 1.3.2 – 1.3.4.

1.3.2. Diatom morphology

Diatoms are unicellular algae that have peculiarities unique to their own class (Dalu & Froneman, 2016; Necchi, 2016; Round *et al.*, 2007; Taylor *et al.*, 2007). They have characteristic cell walls, consisting of opaline silica, referred to as 'frustules' (Necchi, 2016; Round *et al.*, 2007) (Figures 1.4 to 1.6). Two particular types of symmetry are clearly distinguishable: centric diatoms with a circular shape and pennate diatoms with a longitudinal shape (Taylor *et al.*, 2007).

Centric diatoms are usually suspended in the water column; whilst pennate diatoms are adapted to living in benthic habitats, but they can be resuspended in the water body (Taylor *et al.*, 2007). Centric diatoms are divided into three sub-orders (Coscinodiscineae with a marginal ring and no polarity to symmetry, Rhizosoleniinea with no marginal rings and unipolar symmetry, and Biddulphiineae with no marginal ring and bipolar symmetry). Pennate diatoms are also divided into two sub-orders, Fragilariineae (absent raphe) and Bacillariineae (with a raphe).

Another unique feature of diatoms is characteristic photosynthetic pigments like chlorophyll a and c; and additional pigments such as xanthophyll and carotenoids (Necchi, 2016; Taylor *et al.*, 2007). They also store photosynthetic products as oil and/or chrysolaminarin (Taylor *et al.*, 2007). They furthermore attach to various surfaces (rocks, submerged vegetation, soil, manmade structures like wood, paper, plastic) using adhesive pads, a mucilage secretions or fibrillose structures, stalks or tubes (Necchi, 2016; Taylor *et al.*, 2007). They intermittently use this function to form colonies (Taylor *et al.*, 2007).

Diatoms comprise a major part of the microphytobenthos (Dalu & Froneman, 2016; Dalu *et al.*, 2014). Benthic algae act as primary producers in marine and freshwater aquatic systems, consequently establishing them as the main source of nutrition for organisms at higher trophic levels (Dalu & Froneman, 2016; Dalu *et al.*, 2014; Stevenson *et al.*, 1996).

Centred at the base of aquatic food webs, it is evident that the importance of diatoms in limnological systems should not be negated in ecological studies (Taylor *et al.*, 2007).

1.3.3. Drivers of diatom community structure

When attaching to submerged surfaces, diatoms usually become a component of the epilithon (Lock, 1981). This slimy layer covers submerged rocks and habitats which is generally dominated by algae (Lock, 1981). The ability for diatoms to grow in these habitats is determined by an array of environmental factors ranging from hydrological abiotic to biotic components (Stevenson *et al.*, 1996). In actuality, these environmental factors function as a filter influencing biotic community structures (Pan *et al.*, 2000; Poff, 1997; Southwood, 1977).

Multiple studies demonstrate diatoms often respond specifically to various environmental and proximate factors. This include plant nutrients (like phosphates) and the availability of these nutrients. Diatom community structure also depends on habitat characteristics, such as the elevation of the water body, substrate type and availability (Stevenson *et al.*, 1996). Environmental temperatures also influence diatom community structure. For instance, some diatoms, like *Meridion circulare,* are adversely affected by environmental temperatures (Krejci & Lowe, 1987). Several diatoms also respond directly to anthropogenic activities, like pollutants (Archibald, 1972). Other factors diatoms respond to includes salinity, conductivity, pH, exposure to sunlight (Antoniades & Douglas, 2002; Bellinger *et al.*, 2006; Dalu & Froneman, 2016; Jüttner *et al.*, 1996; Krejci & Lowe, 1987; Licursi & Gómez, 2002; Necchi, 2016; Taylor *et al.*, 2007). Because of these responses to environmental changes (natural and/or anthropogenic), diatoms are commonly used as indicators of water quality in bioassays (Korfiatis & Stamou, 1999; Stevenson *et al.*, 1996). In fact, diatoms were used for water quality control since the 1950s (Cholnoky, 1958; Dalu & Froneman, 2016).

1.3.4. Habitat specificity in diatoms

Diatom assemblages can be distinguished based on the aforementioned biotic and abiotic site-specific factors (Pan *et al.*, 1999). Moreover, diatom growth is environmentally distinct in terms of habitat selection (Stevenson, 1984; Stevenson *et al.*, 1996). As discussed in sections 1.3.2., many studies confirm that diatom community structure differs between

microhabitats, depending on substrate type, size, and availability (Antoniades & Douglas, 2002; Krejci & Lowe, 1987; Lim *et al.*, 2001; Ludlam *et al.*, 1996; Reavie & Smol, 1997; Stevenson *et al.*, 1996). For example, diatom communities found on plants differs from diatom communities found on sediment (Soininen & Eloranta, 2004). There is also a variation in the diatom community structure between rock and moss habitats (Antoniades & Douglas, 2002). Two studies, for instance, concluded that diatoms from the *Achnanthes* genus demonstrated a significant affiliation to rocky environments in comparison to moss habitats (Antoniades & Douglas, 2002; Reavie & Smol, 1997). Diatoms from this genus are typically small monoraphids (Roemer *et al.*, 1984). They generally attach to the substratum with their raphe valve, or by subapical mucilaginous stalks (Roemer *et al.*, 1984). Another study involving eleven diatom species found two additional genera (*Navicula* and *Neidium*) strongly associated with sediment substratum (Lim *et al.*, 2001). Many other genera are associated with sediments, but sometimes algal community composition might overlap (Reavie & Smol, 1997).

Current speed affects diatom community structures (Antoniades & Douglas, 2002). Fast- or slow-moving water could impact the availability of substrates for diatom attachment, consequently affecting diatom growth (Antoniades & Douglas, 2002; Whitton, 1975). Other diatoms are planktonic; floating in the water column or near the surface (Necchi, 2016). Motile diatoms are found at sites with loose and fine sediment (Detenbeck *et al.*, 2000; Fore & Grafe, 2002; Kutka & Richards, 1996). Motile diatoms can move through the water column as not to be buried in the sediment (Detenbeck *et al.*, 2000; Fore & Grafe, 2002; Kutka & Richards, 1996). Diatoms confined to the epipelon (*Nitzschia* and *Surirella*) are also motile, since motility is essential for species in epipelic habitats (Pan *et al.*, 1999). Some diatoms are also absent from littoral habitats, e.g. *Hannaea arcus* (Antoniades & Douglas, 2002; Ludlam *et al.*, 1996). Shaded areas might restrict species richness (Pan *et al.*, 2000).

Admittedly, diatom communities might overlap with respect to varied habitats (Ludlam *et al.*, 1996). But some diatom species remain unique to certain habitats (Ludlam *et al.*, 1996). In fact, even when various diatoms were accounted for on numerous substrates, studies discovered multiple indicator species presenting affinities for certain habitat types (Lim *et al.*, 2001; Reavie & Smol, 1997). Species strongly affiliated with aerophilic moss in shallow water include *Cocconeis placentula*, *Achnanthes petersenii* and *Pinnularia balfouriana* (Antoniades & Douglas, 2002; Lim *et al.*, 2001). More species commonly found in moss is *Eunotia arcus* and *Pinnularia balfouriana*, although they are also associated with epiphytic habitats (Douglas & Smol, 1995; Lim *et al.*, 2001). It is evident that some diatoms exhibit clear habitat specificity and can be studied accordingly. Various methods exist for sampling

diatoms from their habitats. It is necessary to have a basic understanding of these sampling and preservation methods to achieve the objectives of this study, which will be discussed in section 1.3.4.

1.3.5. Background on diatom sampling and preparation

Diatoms form colonies and attach to surfaces by secreting mucilage structures (Taylor *et al.*, 2007). They additionally utilize this mechanism to initiate movement. The frustules must be isolated from these materials to be examined under a light microscope for identification.

Diatoms typically grow on solid substrata, exposed moist sediment, submerged vegetative roots, cobblestone and rocks (Taylor *et al.*, 2007). This is subject to the availability substrata and is not limited to the type of stone. Diatoms can also be suspended in the water column as a component of the phytoplankton. They also frequently colonize manmade objects encountered in water bodies, like pipes, plastic, paper, and wood.

Diatoms are usually sampled from four microhabitats: the epipelon (the surface of sediments), epipsammon (between sand particles), epilithon (sediments like gravel, stone, and bedrock), and epiphyton (aquatic plants) (Kelly *et al.*, 1998). They can be sampled from solid substrates, like rocks or pebbles, by vigorously scrubbing the upper surface with a small brush (like a clean toothbrush). Diatoms from submerged vegetation can be sampled by following the same procedure.

Occasionally samples are unintentionally taken from uncolonized substrata, resulting in a smaller yield (Taylor *et al.*, 2007). This can be avoided by inspecting substrata for a slimy texture or for a thin golden film prior to sampling. Diatoms can be sampled from natural or introduced substrata. Man-made substrata can be introduced to a water body and be used to grow diatom communities. However, the material should be submerged for sufficient time to allow growth to occur. This method may have some disadvantages, like causing loss of diatom growth due to the smoothness of artificial surfaces (Taylor *et al.*, 2007). The sample will also be comprised of fast-growing diatoms and would not be an adequate representation of diatoms growing on natural substrata. Artificial substrata may also be lost during sampling while it is colonising in the aquatic environment

When collecting environmental samples, it is essential to collect a sample that is representative of the diatom community (Taylor *et al.*, 2007). This includes sampling from all four aforementioned microhabitats. Samples should also be collected from any available manmade materials, such as piping, tires, bricks, and so on. Submerged aquatic vegetation could also be sampled for diatom communities.

Diatoms close to the sediments can be extracted by using a syringe with plastic tubing at the end. The tubing can be positioned to have oblique contact with the sediment (Taylor *et al.*, 2007). Alternatively, diatoms can be sampled by keeping glass tubing near the sediment of a water body, sealing it with a finger. As pressure is released, the tubing can be moved for approximately one meter over the sediment, collecting diatoms from this area. The tube can be sealed again and removed from the water body. Water can be collected in a two-litre container for phytoplankton sampling. The container should be left undisturbed for some time, allowing the material to settle out (Taylor *et al.*, 2007). Alternatively, a plankton net (maximum mesh size of 25 µm) can be used. The net should be dragged back and forth below the water body's surface.

Soil diatoms can be sampled by collecting six sub-samples (~5 cm2) within a 10 m radius. They should be sampled at a depth of 1 cm, and the sample should be ~200 grams. Soil can be stored in envelopes to minimize the build-up of moisture and prevent fungal growth. In flowing water bodies, samples should preferably be taken after water washed over stones and substrates (Taylor *et al.*, 2007). In deep, slow-moving rivers diatoms can be sampled from cobbles and stones at the edge of the riverbank.

Sample preservation

If the samples will be examined as live material, they should be kept in a fridge for no longer than 24 hours (Taylor *et al.*, 2007). To fix samples for the short term, it could be fixed in Lugol's iodine. This can be prepared by dissolving two g potassium iodine and one g iodine crystals in 300 ml distilled water. For long-term preservation, samples can be fixed in ethanol. This, however, will destroy the chloroplasts. Fixing samples in formalin is not recommended because of its carcinogenicity. Formalin can also damage fragile diatom structures.

Variables used for quality control

When assessing water quality, it is advantageous to consider environmental variables. Although these principally depend on the nature and outcome of the study, it is beneficial to measure as much as is feasible (Taylor *et al.*, 2007). Such variables include hydrological characteristics (channel depth, breadth, and velocity), physical variables (water temperature, turbidity), physio-chemical variables (pH, conductivity, total dissolved solids), nutrients (orthophosphate-phosphorus, phosphates), nitrogen, cation/anion content, oxygen, and organic matter (oxygen saturation, chemical oxygen demand, and biological oxygen demand).

1.4. Chytridiomycosis

As mentioned earlier, an extensive range of anurans are found in Southern Africa (Du Preez, 2015). It is unfortunate that amphibian species are declining on a local and global scale (Skerratt *et al.*, 2007). Habitat loss, destruction, fragmentation, disruption, infectious diseases, pollution, climate change, and predatory hazards are the most prominent threats leading to these declines (Weldon *et al.*, 2008). A parasitic chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*) is one of the key causes of declines and extinctions, and is the cause of the disease chytridiomycosis (Kriger & Hero, 2007; Rizzo, 2005).

Chytridiomycosis can rapidly infect several individual tadpoles (Berger *et al.*, 1999). The infection can transmit via physical contact or with water polluted with infective zoospores (Berger *et al.*, 1999). Clinically healthy frogs can also act as carriers (Berger *et al.*, 1999). It is speculated that cold-blooded, non-amphibian hosts with keratinized surfaces could act as reservoirs or carriers of the disease (Berger *et al.*, 1999). Anthropogenic disturbance also seems to increase the risk of infection (Pauza *et al.*, 2010).

The trade of live animals also contributes to the spread of chytrid (Fisher & Garner, 2007). This includes the pet trade, relocating zoo animals, the usage of bio controllers, involving animals in conservation, and trade in salamanders as fish bait (Fisher & Garner, 2007; Schloegel *et al.*, 2009). The most-traded frogs include *Xenopus laevis, Rana catesbeiana*, and *Bufo marinus*; with *X. laevis* being the most profound carrier of chytrid (Fisher & Garner, 2007).

A pregnancy essay involving *X. laevis* caused it to gain popularity (Shapiro & Zwarenstein, 1934). More females to males (1:1.6) were traded pertaining to this pregnancy test (Weldon *et al.*, 2007). *Xenopus laevis* therefore became a favoured laboratory animal, surpassing *Rana* (Major & Wassersug, 1998). It is additionally used recreationally, for example, in angling. It has also been exported/imported into the food industry (Steyn, 1984). In fact, a study done by (Weldon *et al.*, 2007) concluded that frogs were traded worldwide from South Africa to 32 facilities in 30 different countries; albeit most of the trade occurred between South Africa, France, Germany and the USA.

1.4.1. Pathogenesis

The protection of amphibian skin is significant for the health of the tadpole *(Enciso et al., 2008).* Amphibian skin is water-permeable since it is active in many vital physiological processes; such as ion transportation and gaseous diffusion during respiration (Enciso *et al.*, 2008; Voyles *et al.*, 2011). The preservation of homeostasis, ions, water and electrolytes are other features of amphibian skin (Enciso *et al.*, 2008; Voyles *et al.*, 2011). The fungus will cause chytridiomycosis and consequently hinders the skin's physiological performance (Kriger & Hero, 2007).

The transmission of the disease can additionally destroy epidermal cells causing necrosis; disrupting anurans' respiration and osmoregulation (Berger *et al.*, 1998; Kriger & Hero, 2007). This will potentially cause hyperkeratosis and subsequent death of the animal (Berger *et al.*, 1998; Kriger & Hero, 2007).

A variety of clinical symptoms may be caused by chytridiomycosis including; fatigue, irregular body posture, redness of the ventral skin, seizures, and peeled epidermis with intermittent lesions (Berger *et al.*, 1998; Voyles *et al.*, 2011). Amphibians can also appear asymptomatic; acting as carriers or reservoirs for the disease (Soto-Azat *et al.*, 2010). Chytrid can, in some instances, cause the extinction of an entire population (Fisher & Garner, 2007).

1.4.2. Distribution

The Novel Pathogen Hypothesis (NPH) and the Endemic Pathogen Hypothesis (EPH) are two hypothesises used to explain the distribution of *Bd* (Fisher & Garner, 2007). The NPH notes that the distribution of Bd is influenced by human behaviour. The EPH, on the other hand, notes that due to environmental changes, amphibians have become more vulnerable to pre-existing modes of infection. Data confirms these theories, suggesting that chytrid propagation may be powered by both humans and climate change.

Chytrid outbreaks are usually panzootic of nature (James *et al.*, 2009). This notion is supported by the wavelike emergence of the fungus in the Neo-tropics (Lips *et al.*, 2008). The proliferation of the disease is excelled by anthropogenic activities, such as the pet trade (James *et al.*, 2009). The cause of the epidemic, however, remained a topic of discussion (James *et al.*, 2009).

There's currently four *Bd* linages: *Bd*ASIA-1, *Bd*ASIA-2/*Bd*BRAZIL, *Bd*GPL and *Bd*CAPE (O'hanlon *et al.*, 2018). *Bd*GPL and *Bd*ASIA-1 are more infectious in tadpoles than *Bd*CAPE and *Bach* (O'hanlon *et al.*, 2018). In metamorphs, however, *Bd*GPL is significantly more infectious (O'hanlon *et al.*, 2018). It is estimated that *Bd*GPL originated between 120 and 50 years ago (O'hanlon *et al.*, 2018). *Bd* and *Bsal* both originated from Asia (O'hanlon *et al.*, 2018).

By breeding and distributing *R. catesbeiana* for use and human consumption, Brazil also catalysed the spread (Schloegel *et al.*, 2010). This frog is effective for breeding and exportation because of its growth rate, fecundity and adaptive abilities (Schloegel *et al.*, 2010). Despite *Bd*'s prevalence in South America, mortalities are still to be recorded because of infection (Schloegel *et al.*, 2010). The Brazilian bullfrog agriculture industry could be threatened if exposed to a novel linage (Schloegel *et al.*, 2010).

The inclination of spores to survive long-distance transport and their asexual reproduction methods are factors adding to the success rate of the global monopoly of *Bd* (Rizzo, 2005). When introduced to a new environment, the selective pressures the fungus will experience could serve as evolutionary drivers, increasing their chances of adaptation and survival (Rizzo, 2005). The way *Bd* interacts with amphibian populations may also be impacted by climate change, anthropogenic disturbances, habitat destruction, and degradation.(James *et al.*, 2009; Voyles *et al.*, 2011). In some cases, these disturbances will increase a population's susceptibility to the disease.

1.4.3. Diagnosis and identification

Chytridiomycosis can be diagnosed with molecular tools or histopathology. Histopathology, microscopic and morphological methods are enough for detecting *Bd*, since *Bd* often cause lesions in the skin of adult frogs, but compromises sensitivity of the test when compared with molecular methods (Boyle *et al.*, 2007; Weldon *et al.*, 2007).

Research done on *Bd* involves the isolation and culturing the fungus (Fisher *et al.*, 2018). Isolating chytrid from tadpoles is customarily the most effective and accurate means of data collection. To isolate and cultivate the fungus, it is necessary to drag infected leg tissue over nutrient agar. Unfortunately, this method often necessitates euthanizing anurans. An adjusted method was therefore developed, entailing clipping the 4th hind toe. This method was further improved, relying on non-invasive swabbing. It was well-received, as it did not rely on euthanasia. Another technique involves raising and screening tadpoles in captivity after sourcing chytrid from a wild, infected tadpole (Fisher *et al.*, 2018).

Tadpoles from greater clutches and an increased lifespan and should preferentially be sampled (Fisher *et al.*, 2018). Sampling methodology and toe clipping should be modified to minimize strain and trauma. Unethical sampling methods could significantly hinder the tadpole's survival rates (Fisher *et al.*, 2018). Tadpoles could be inspected for visual signs of infections, followed by buccal swabs (Fisher *et al.*, 2018). Unfortunately this method also entails euthanasia (Fisher *et al.*, 2018).

1.5. Aims and objectives

Diatoms are often used to assess water quality (Korfiatis & Stamou, 1999; Stevenson *et al.*, 1996). Therefore, a lot of research is pursued on environmental parameters driving diatom community structures (Cholnoky, 1958; Dalu & Froneman, 2016). However, in some cases natural substrata may not be available for environmental diatom sampling. (Kelly *et al.,* 1998; Taylor *et al.,* 2007). In such instances, artificial substrata may be introduced for diatom sampling. This method may have some disadvantages, since it often selects for opportunistic, fast-growing diatoms (Taylor *et al.,* 2007). Artificial substrata may also be lost during sampling. Using anuran ecomorphological guilds as a proxy for diatom diversity would, therefore, circumvent the issue of insufficient availability of natural substrata for the sampling of environmental diatoms.

This problem can be addressed through the integration of research on tadpole feeding habits in relation to diatom diversity. There exists ample opportunity for future research in this context. It can especially provide more insight on specific feeding habits of tadpoles from ecomorphological guilds. This can additionally be used to gain a better understanding of the mechanisms of algae ingestion. This can further be employed to assess the spatial distribution of tadpoles. Very little research is done on the integration of these two fields: particularly tadpole feeding habits in relation to environmental diatom diversity. The aim of this study is to examine the usage of diatoms from ecomorphological guilds as a proxy for environmental diatom diversity. This requires sampling of tadpoles and diatoms from various sites at two locations. The first location of the study is Ukutula Lodge and Conservation Center, located 10 Km outside Brits, South Africa. The second location is Aliwal North, situated in the Eastern Cape of South Africa. Tadpoles from the same sites will be sampled, and their associated guilds will be identified.

This study involves the following objectives:

- To determine the ecomorphological tadpole guilds in the area of interest. This is accomplished by counting and identifying tadpole species sampled in the areas of interest. Guild delineation from a previous study conducted by Botha (2014) will be used for tadpole ecomorphological

- To determine the diatom diversity of the area of interest. This is accomplished by identifying and counting the diatom species from environmental samples taken in the areas of interest. Completing this objective is essential for the completion of the subsequent objectives.

- To compare environmental diatom samples with community samples within sites. This is accomplished by counting and identifying diatoms in environmental samples taken at each site. The information gathered in the aforementioned objective will be used to determine practical or statistical significant difference of data gathered on environmental diatom diversity within study sites.

- To compare the diatom data gathered on different ecomorphological tadpole guilds within individual sites. This is achieved by examining the intestinal diatom diversity of the different guilds. After collecting raw data, further statistical analyses can be executed to determine the similarity between guilds.

- To compare tadpole species within and between sites by examining the intestinal diatom diversity of the tadpole species sampled. After collecting raw data, various statistical analyses can be executed to determine the similarity between tadpole species.

Furthermore, tadpole vouchers will be collected and assessed for the presence of *Bd*. This is a supplementary addition to this study. The information gathered from this will provide insight on the transmission of chytrid mediated by ecomorphological tadpole guild specific feeding habits. By testing for chytrid infection and correlating infection with ecomorphological tadpole guilds, we can establish whether tadpole ecomorphological guilds are an indicator of the risk of transmitting chytrid from the environment.

By accomplishing these objectives, it is possible to establish if a practical or significant difference exists between diatom diversity found in the environmental samples and the intestines of tadpole species and guilds. Even though a lot of research is invested in the responses of diatoms to anthropogenic disturbances (Fore & Grafe, 2002; Hill *et al.*, 2000; Whitton & Kelly, 1995), there exist opportunities for further research when integrating diatoms with zoological fields of study. The results of this study will identify such opportunities.

2. METHODS

2.1. Guild delineation

A desktop survey was conducted to identify all potential anuran species in the areas of interest. The species were then grouped in ecomorphological guilds as identified and described by Botha (2013). The ten guilds described includes: Guild 1 (Suspension Feeders), Guild 2 (Nektonic), Guild 3 (Lentic-nektonic), Guild 4 (Benthic type 2/Profundal), Guild 5 (Lentic-benthic), Guild 6 (Rheophilic), Guild 7 (Suctorial), Guild 8 (Exitus Parageios), Guild 9 (Lentophytophilic), Guild 10 (Bentophytophilic) (Annexure A).

2.2. Field site selection

Sites were selected based on comprehensive tadpole diversity and guild composition. Accessibility and population heterogeneity were also considered during site selection. The first study location was at Ukutula Lodge and Conservation Center, located 10 Km outside Brits, South Africa. Three sites were selected based on overall biodiversity and species abundance: site 1 (-25.518500, 27.661530), site 2 (-25.517770, 27.664370), and site 3 (-25.520140, 27.673550) (Figures 2.1 to 2.3). Sites 1 and 3 were both isolated ponds. Site 1 was surrounded by terrestrial vegetation, subsequently casting shade over most of the water body. There was also substantial aquatic vegetation present. Sites 2 and 3 had considerably less aquatic vegetation and shade. Site 2 was a large, deep river, with sandy substrata and minimal to no water flow.

2.1

2.2

2.3

Figure 2.1: A photograph of site 1 located at Ukutula (coordinates: -25.518500, 27.661530). **Figure 2.2:** A photograph of site 2 located at Ukutula (coordinates: -25.517770, 27.664370). **Figure 2.3:** A photograph of site 3 located at Ukutula (coordinates: -25.520140, 27.673550).

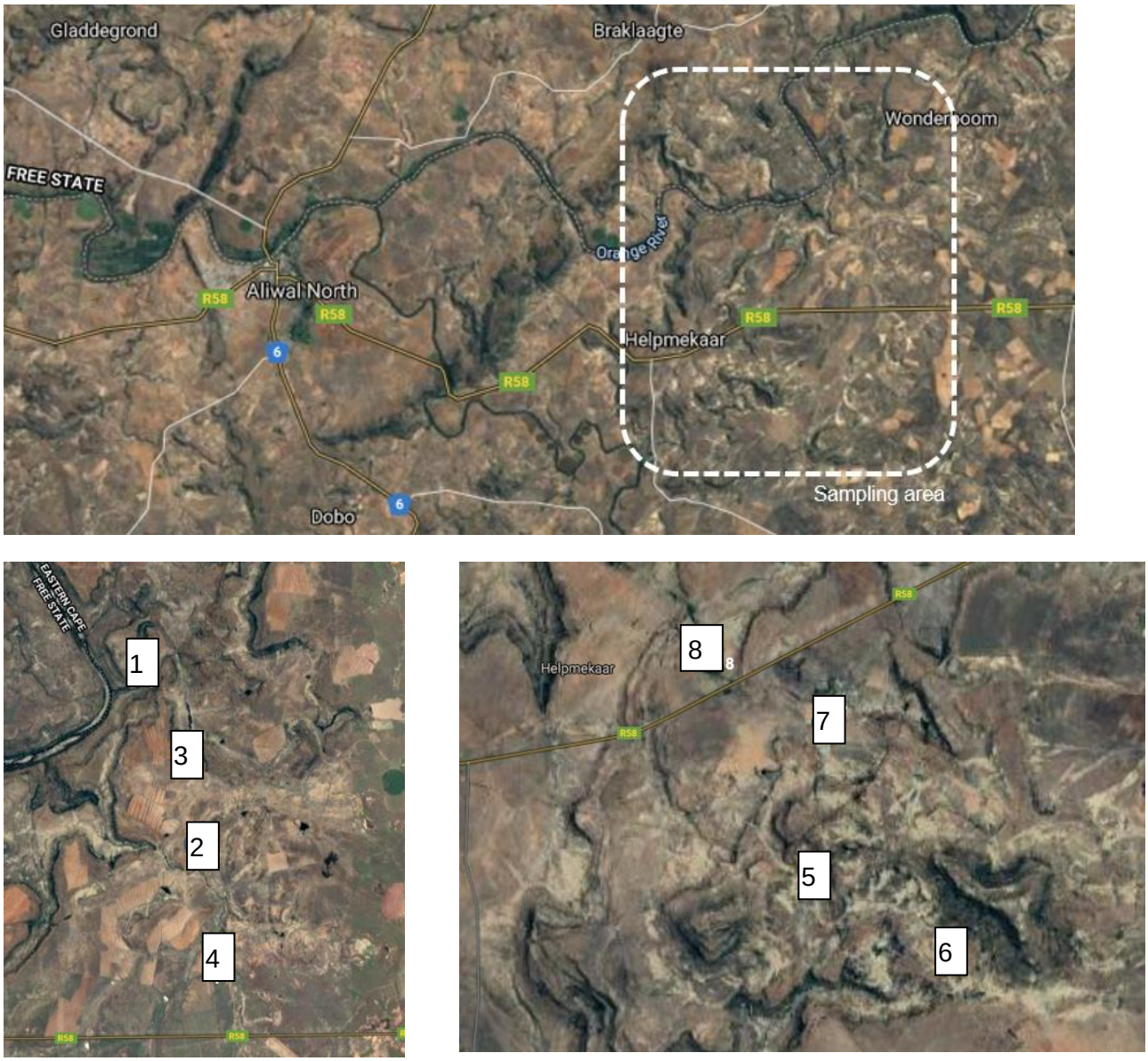


Figure 2.4: Satellite images showing the study sites at Aliwal North situated in the Eastern Cape of South Africa; 1: Site 1 (coordinates: -30.65200, 1014 026.95576). 2: Site 2 (coordinates: -30.67950, 026.96444) 3: Site 3 (coordinates: -30.66553; 026.96233) 4: Site 4 (coordinates: -30.69550, 1015 026.96849). 5: Site 5 (coordinates: -30.72255, 026.90648) 6: Site 6 (coordinates: -30.73452, 026.90525) 7: Site 7 (coordinates: -30.74161, 1016 026.91824) 8: Site 8 (coordinates: -30.715983, 026.895633).

2.3. Environmental diatom sampling

Various substrata were inspected for the presence of diatoms. This included available rocks, submerged vegetation, boulders, and manmade materials like paper, plastic, piping and so on. A slimy texture or a visible golden-brown coating typically indicated the presence of diatoms (Taylor et al., 2007).

These substrata were scraped with a clean toothbrush for diatoms and collected in 15 ml centrifuge tubes. The toothbrush was rinsed with distilled water and 70% ethanol (EtOH) between each site to prevent cross-contamination. Water from the site (7 ml) was added to the tubes, assuring sampling of suspended diatoms (Taylor et al., 2007). The samples were fixed by filling the tubes with 70% EtOH and stored in a cool, dry place.

2.4. Tadpole sampling and examination

An ongoing liaison was established with the Ukutula office to assure occupational safety and to prevent congestion or interference with frequent activities and lodge operations. Tadpoles required vouchers for the excision of mouthparts and the harvesting of gut contents for diatom analysis. Tadpoles were collected with dip nets; sweeping movements in the water created a current that drew in material. Tadpoles were handpicked from the nets while wearing disposable gloves and transferred to plastic bags containing water from the source of collection. Additionally, the presence of any adult frogs was also recorded. This allowed for better insight into overall anuran diversity.

Tadpoles were euthanized prior to any tissue sampling procedures. Tricaine mesylate (MS-222) was administered by immersing a tadpole to allow absorption of chemicals through the skin. Tadpoles were placed inside a small plastic tub (500 ml) containing MS-222 solution (300 mg/l buffered with sodium bicarbonate (NaHCO3) for 2-5 minutes, depending on the response of the species) (SANS, 2008). Tadpole carcasses underwent a series of procedures in the following order: 1) microscopic confirmation of species identity, 2) excision of mouthparts, 3) excision of gut contents and 4) fixing of carcasses. Microscopic examination of diagnostic features included the tail shape, proportionate tail length and mouthpart characteristics according to identification keys from literature (Du Preez, 2015; Wager, 1986).

The mouthparts, including labial papilla, keratodonts and restrodonts were carefully dissected out with a pair of surgical scissors and placed in cryovials containing ethanol (with a 7:3 ratio to distilled water), which are then stored at -20 °C. An incision of 5 mm was made laterally through the skin of the abdomen, and slight pressure was applied from the opposite side of the abdomen. This forced the intestinal tract to protrude from the abdominal cavity. The intestinal tract was then excised by cutting the dorsal and ventral attachments and placed inside a 1.5 ml Eppendorf tube filled with 70% ethanol. The remaining carcass was fixed in a 15 ml Falcon tube filled with 70% ethanol.

2.5. Terrapin sampling

As a supplementary addition to this study, diatoms were sampled from the carapace of available terrapins found in Ukutula for a second, unrelated study pertaining to reptiles. While diatoms on marine turtles have been studied extensively (Azari et al., 2020; Majewska et al., 2015a; Majewska et al., 2015b; Robinson et al., 2016), this is not the case for terrapins. It was, therefore, worth assessing the diatom diversity of samples taken from the carapace of the terrapin in terms of environmental diatom samples; in addition to the diatom samples taken from the intestinal tadpole content.

Baited funnel traps were used to capture terrapins from ponds or rivers. Store-bought chicken liver was used as bait and placed in a perforated plastic container. The smell emitted from the bait lured nearby terrapins into the trap. The funnels of the traps allowed for terrapins to enter safely but prevented exit. Traps were placed a few centimetres above water level to allow the terrapins to surface for air.

The traps were set during late evenings and checked during early mornings. After the terrapins were captured, their carapaces were scrubbed with a toothbrush to collect algae. The samples were collected in 15 ml Falcon tubes and filled with 70% ethanol. The terrapins were released promptly after sampling.

2.6. Chytrid fungus diagnosis

As an addition to this study, samples were taken from Ukutula qnd examined to determine the presence of chytridiomycosis. This was part of an ongoing, unrelated microbial ecology study from Ukutula. *Bd* swabs were incubated on nutrient agar between 15 °C and 23 °C. Plates were inspected after three – ten days for any visible zoospores or growth resembling *Bd*. *Bd* DNA was isolated from the mouthparts of tadpoles according to the PrepMan Ultra protocol (Applied BiosystemsTM, Foster City, CA). The presence and concentration of the fungus were determined by using a real-time Polymerase Chain Reaction (rt-PCR) TaqMan standard curve assay (Boyle *et al.*, 2007).

The StepOnePlusTM real-time PCR system from Applied BiosystemsTM is utilized for the TaqMan assay. *Bd* isolates CW36 isolated from an *Amietia* from Van Staden's Bridge, Eastern Cape, South Africa in 2004, was used as a positive control. Non-template controls were additionally included to ensure the absence of contamination. All samples and controls were analysed in duplicates.

2.7. Diatom isolation and slide preparation

2.7.1. Environmental diatom samples

To eliminate organic material from environmental samples, it was necessary to treat the samples with various caustic chemicals (Taylor *et al.*, 2007). The SiO_2 frustules are resistant to oxidation and were thus able to survive these processes, allowing for later microscopic identification.

Tubes containing the organic material and 70% EtOH fixative were left to settle. The supernatant was discarded into a hazardous waste container (Taylor *et al.,*2007). The remaining sample was transferred into clean test tubes and labelled accordingly. Potassium permanganate ($KMnO_4$) was added to the test tubes in a 1:2 ratio. The test tubes were left at room temperature in a fume hood for ~24 hours. This ensured complete oxidation of the organic material.

The samples were inspected after the 24 hours had elapsed. If the content of the test tubes appeared brown, oxidation occurred and the reaction was complete. If the contents of the

test tubes appeared blue/purple, the reaction was not complete. Incomplete oxidation is usually attributed to insufficient organic material or dilute $KMnO_4$. In the case of insufficient sample material, supplemental material was added to the test tube. Alternatively, if the KMnO4 is too dilute, extra $KMnO_4$ was added to the sample.

Upon completion of the reaction, the test tubes were vortexed to resuspend the samples. Concentrated hydrochloric acid (HCl) was added to the sample in a 1:2 ratio to complete oxidation of the organic material. The added HCl also neutralized the remaining $KMnO_4$. A water bath was prepared at 100 °C. The test tubes were placed in the water bath and left at boiling point for ~20 minutes. The added heat catalysed the reaction.

The contents were heated up until the organic material dissolved. The remaining sample collected at the bottom of the test tube. Once the brown contents of the test tube became translucent or transparent, the reaction was complete. Cloudy or brown material could be indicative of undissolved organic matter. In this case, a few drops of hydrogen peroxide (H_2O_2) were added to the sample. This produced an exothermic reaction, dissolving the remaining organic material. Once all the reactions were completed, the test tubes were taken out of the water bath and left in the fume hood to cool down to room temperature. The water in the water bath was discarded in a hazardous waste container, in the event that acidic chemicals might have spilled from the test tube into the water bath.

After the tubes cooled, the supernatant was discarded in a hazardous waste container. The remaining diatom samples were transferred to sterile 15 ml centrifuge tubes and labelled accordingly. Tubes were filled to the 5 ml mark with distilled water. The samples were then centrifuged at 3 000 rotations per minute (rpm) for 10 minutes. The supernatant was discarded into a hazardous waste container. The tubes were re-refilled to the 5 ml mark with distilled water. The pellets were vortexed to resuspend the sample material. This process was repeated three more times. Lastly, two drops of ammonium chloride were added to each tube containing the clean diatom samples. The ammonium chloride neutralized any charges from sediment, preventing diatom and sediment aggregation. The tubes were vortexed for resuspension.

2.7.2. Tadpole diatom samples

Tadpole material stored in 1.5 ml Eppendorf tubes was transferred to clean test tubes and labelled accordingly. A glass rod was used to crush the intestinal content into smaller pieces. The subsequent procedure followed the same methodology as set out in section 2.6.1.

At the end of the cleaning process, 0.60 µl of each individual tadpole sample from their corresponding site and species were taken and transferred to a single sterile 15 ml centrifuge tube. The sample was suspended and renamed accordingly.

2.7.3. Slide preparation and microscopy

Clean coverslips of 18 mm in diameter were prepared on a clean surface area in a fume hood. The surface was cleaned with 70% EtOH. A pipette was used to measure 0.35 µl of the sample, placing it on the coverslip. The coverslips were left to dry at room temperature for 24 hours

After the coverslips had dried, they were fixed with Pleurax with a refraction index of 1.73 on a hotplate (maximum temperature of 280 °C) (Taylor *et al.,*2007).. After fixing the slides, they were left to harden for 24 hours on a clean surface in the fume hood. This process was replicated for environmental and tadpole samples.

Once hardened, the slides were examined for diatoms under a Nikon 80i light microscope equipped with a Nikon DS-Fi1 5MP digital camera and phase contrast objectives. A minimum of 300 diatoms were counted per sample. Literature was consulted to identify diatoms genus level and species were possible (Cox & Cox, 1996; Diatoms Of North America, 2020; Krammer, 2000; Necchi, 2016; Taylor *et al.*, 2007; Tornés & Sabater, 2010).

2.8. Diatom habitat usage summary

An extensive literature survey was completed on the diatom species encountered during microscope slide examination for environmental and tadpole samples. The various habitat preferences included attachment (unattached, and attached), motility (moderately motile,

and highly motile), relevant habitat types (benthic, periphytic, epipelic, epilithic, littoral zone, planktonic, periodic desiccation), and substrate types (habitat substrate, organic detritus, moss, dry moss, plants, soil, moist soil, wood). This information was then used to construct a table summarizing habitat usages (Antoniades & Douglas, 2002; Cantonati *et al.*, 2009; Cox & Cox, 1996; Detenbeck *et al.*, 2000; Diatoms Of North America, 2020; Fore & Grafe, 2002; Hall & Smol, 2010; Kutka & Richards, 1996; Lim *et al.*, 2001; Marquardt *et al.*, 2017; Round *et al.*, 2007; Soininen & Eloranta, 2004; Stevenson *et al.*, 1996; Taboada *et al.*, 2017; Taylor *et al.*, 2007; Tornés & Sabater, 2010). It was specified if diatoms sampled commonly attached to substrates (Table 3.7). It was also documented if diatoms found were typically associated with motility (moderately motile/highly motile). Occasionally, however, the literature would not specify a particular substrate type. In those cases, diatoms were classified under "habitat substrate", indicating they do, in fact, attach to the available substrate in their immediate habitat.

2.9. Statistical analyses

The statistical analyses discussed in this section were performed on the datasets obtained from Ukutula and Aliwal North's environmental and tadpole diatom samples.

2.9.1. Transforming raw data

Raw data were collected by counting and identifying diatom species from environmental and tadpole samples. This was transformed for statistical processing by calculating relative abundance values (RA) for diatom data. Relative abundance values were calculated by dividing the Total Number of Individual species (Isi) by Total Number of Species Population (∑ Nsi) multiplied by one hundred (100).

2.9.2. Calculated variables and parameters

Descriptive statistics were used to quantify and summarize data obtained from examining diatom samples collected from tadpoles and sites. This study made use of Shannon's

diversity index (H′), Species Evenness (E), and Species Richness (S) to construct scatterplots and ranked abundance graphs.

Shannon's diversity index, Species Evenness, and Species Richness

The transformed data were imported to CANOCO (Canonical Community Ordination) for Windows 4.5. The relevant statistics (H′, E, and S) were executed and exported to MICROSOFT EXCEL 2017 for Windows 10. The graph features were used to construct scatter plots of H vs E and H vs S.

Ranked abundance

Ranked Abundance was illustrated by constructing bar graphs depicting RA values of diatom species found in environmental samples at the different sampling locations. This was performed in MICROSOFT EXCEL 2017 for Windows 10.

The subsequent analyses required the removal of statistical noise from the RA data. This was performed by removing diatom species from the database that occurred in less than one percent of samples.

Detrended correspondence analysis (DCA)

A DCA was performed by importing data to CANOCO for Windows 4.5. This was an effective tool used to reflect the change in community composition along with multiple community variables. Simple ordination plots of species and sample data were constructed for Ukutula and Aliwal North's data. The distance between the sites and species in the diagram approximates the dissimilarity of distribution of relative abundance of those species across the samples, measured by their Chi-square distance.

Sig.

Mixed models were used for comparative analysis because of the dependency within the data. The site dependency is considered by adding it as a fixed effect into the model. The estimated means of the involved samples (environmental, tadpole, terrapin) were calculated to determine if there were any differences between the mean overall RA values of these groups. Significant probability (Sig.) was calculated to estimate the significant differences between these groups. It is also referred to as the p-value. To test if a parameter estimate

has a statistically significant effect on the results, it should have a p-value < 0.05. The software used to complete these analyses included IBM SPSS STATISTICS VERSION 27 (Copyright© IBM Corporation).

Sample covariance

Covariance parameters are estimated as an indication of the variance within the data. It indicates differences or errors that are not explained by the regression line. Sample covariance is statistics computed from a sample of data on one or more random variables. This included calculating an estimate of residual. This indicated the difference between observed and mean values. It additionally included calculating the Standard Error (Std. Error) for the samples. The software used to complete these analyses included IBM SPSS STATISTICS VERSION 27 (Copyright© IBM Corporation).

Effect sizes

The effect sizes of the samples were also calculated. This indicated the differences and practical significant differences between groups. It is an effective tool for comparative ecological studies. As a guideline, an effect size of 0.2 is indicative of a small effect with no practically significant difference. An effect size of 0.5 is indicative of a medium effect with a practically visible difference, and 0.8 is indicative of a large effect with a practically significant difference. The software used to complete these analyses included IBM SPSS STATISTICS VERSION 27 (Copyright© IBM Corporation).

2.10. Ethics and clearance

Ethical approval was obtained from AnimCare (NWU-00060-19-A5). Specific procedures considered for their risk to animals include the collection, temporary constraint, and euthanasia of tadpoles. Usual field safety precautions were also taken, including working with a local field guide, having emergency numbers on hand, snake removal kit and first aid kits.

To reduce the number of tadpoles sampled during this study, no more than 20 individuals per species per site were sampled. Upon capture, the tadpoles were kept in large containers in a shaded, cool area. They were transported back to the laboratories immediately after

capture and kept under cool conditions, with water taken from their natural environments. They were euthanized via submergence in MS-222 according to ethical guidelines and standards (AACRG, 2014). After buccal swabs were taken and the gastrointestinal tract is removed, the remaining carcasses were frozen. Leftover MS222 is discarded in a biohazard container.

A collection permit was obtained from the North West Department of Rural, Environmental and Agricultural Development, Cnr. Dr. James Moroka Drive & Stadium Road, Mmabatho. Permit no.: NW 7650/02/2019. The permit allowed for 20 specimens per species per locality and includes all frog species found in the North-West Province. A section 20 permit was obtained from the Department of Agriculture, Forestry and Fisheries, because we were be working with a wildlife disease.

3. RESULTS

3.1. Tadpole diversity

It was important to interpret the diatom data in the context of anuran diversity since we tested for tadpole taxon or guild related patterns in diatom composition from their gut contents. The following anurans were encountered at Ukutula and Aliwal North, identified, counted and recorded.

Ukutula

Ecomorphological tadpole guilds sampled from Ukutula included Rheophilic, Benthic type 2 (Profundal), Lentophytophilic and Suspension feeder. A total of 175 tadpoles were sampled from all three study locations at Ukutula collectively (Table 3.1). Nine anuran species were recorded at site 1 (*Amietia delalandii, Chiromantis xerampelina, Kassina senegalensis, Phrynomantis natalensis, Phrynomantis bifasciatus, Ptychadena anchietae, Ptychadena mossambica, Strongylopus grayii,* and *Xenopus laevis*). Subsequently, site 1 had the highest anuran diversity in terms of species richness and ecomorphological guilds.

A total of 36 individual tadpoles were sampled at site 2 and 36 individual tadpoles were also sampled at site 3. Only two species were sampled at these sites (*C. xerampelina* and *K. senegalensis*). *Strongylopus grayii* was additionally sampled from site 2 and *P. bifasciatus* was sampled at site 3. Diatoms were sampled from the carapace of a terrapin (*Pelomedusa galiata*) sampled from Ukutula for an unrelated study.

Table 3.1: Anuran tadpoles collected from Ukutula for diatom analysis.

Anuran species	Guild	Site 1	Site 2	Site 3	Total
Amietia delalandii	Rheophilic	7	0	0	7
Chiromantis xerampelina	Benthic type 2 (Profundal);	7	1	7	15
Kassina senegalensis	Lentic nektonic	23	15	5	43
Phrynobatrachus natalensis	Lentophytophilic	1	0	0	1
Phrynomantis bifasciatus	Suspension feeders	21	0	23	44
Ptychadena anchietae	Benthic type 2 (Profundal)	4	0		4
Ptychadena mossambica	Benthic type 2 (Profundal)	21	0	0	21
Strongylopus grayii	Benthic type 2 (Profundal)	18	20	1	39
Xenopus laevis	Suspension feeder	1	0	0	1
	Totals	103	36	36	175

Aliwal North

Ecomorphological tadpole guilds sampled from Aliwal North included Lentic benthic, Rheophilic, Lentophytophilic and Lentic nektonic. Adult anurans from the Suspension feeder guild were also recorded. Since diatoms were only sampled from tadpoles, no statistical analyses were performed on the Suspension feeder guild. Both tadpoles and adult anurans were recorded from all the sites at Aliwal North, except for site 3 where only tadpoles were found and sampled for diatoms (Table 3.2). A combined total of 172 tadpoles and adult anurans were sampled at Aliwal North. Only five species were recorded across the various sites (*Amietophrynus rangeri, A. delalandii, Cacosternum boettgeri, K. senegalensis, and X. laevis*).

All five species were present at site 1. Subsequently, site 1 had the highest anuran diversity in terms of species richness and ecomorphological guilds. In contrast, *K. senegalensis* was the only recorded species at site 3. Consequently, site 3 had the lowest anuran diversity amongst the sampling locations.

Most individuals were recorded at site 8 (34) and the site was mostly dominated by *A. delalandii*. Five *K. senegalensis* tadpoles were documented at site 3, subsequently making it the site with the lowest number of individuals recorded.

Table 3.2: Anurans collected from Aliwal North. Only tadpoles were sampled for diatom analysis.

Anuran species	Guild	Site 1		Site 2		Site 3		Site 4		Site 5		Site 6		Site 7		Site 8		Totals
		Adult	Tadpole	Adult	Tadpole	Adult	Tadpole	Adult	Tadpole	Adult	Tadpole	Adult	Tadpole	Adult	Tadpole	Adult	Tadpole	
Amietophrynus rangeri	Lentic benthic	1	0	0	0	0	0	0	0	0	0	0	0	2	0	0	5	8
Amietia delalandii	Rheophilic	6	0	16	0	0	0	13	2	4	0	5	0	0	5	28	1	52
Cacosternum boettgeri	Lentophytophilic	0	1	0	10	0	0	0	7	1	3	0	9	0	5	0	0	36
Kassina senegalensis	Lentic nektonic	0	5	0	12	0	5	0	3	0	6	0	5	0	5	0	0	41
Xenopus laevis	Suspension feeder	2	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	7
	Totals	9	6	16	22	0	5	13	12	5	9	10	14	2	15	28	6	172

Chytrid results

None of the isolates or attempted cultures from site 1 (108), site 2 (36) or site 3 (36) showed any growth that resembled *Batrachochytrium dendrobatidis* (*Bd*). This implies that the sites at Ukutula is *Bd* negative.

3.2. Environmental diatom diversity

The next section documents diatoms that were examined and identified from environmental samples taken from sites at Ukutula and Aliwal North.

Diatoms from Ukutula sites

Diatom samples were collected at three sites from Ukutula Lodge and Conservation Centre. They were counted and identified to genus level, and where possible, to species level (Table 3.3, Annexures B and C). Thirty-five diatom species were identified for the first site, 22 species for site 2 and 41 species for site 3.

Statistical analyses can also be employed to describe diatom diversity. This includes calculating H', E, and S values. In terms of environmental diatom diversity, site 3 returned the highest H' value (2.84) (Table 3.4), as well as the highest E value (34). In comparison, the environmental sample from site 2 had the lowest H' (2.22) value, and the lowest S value (20). Site 3 also has a relatively high E value (0.81) based on the environmental sample.

Relative abundance values were also used to describe environmental diatom diversity and were particularly useful when plotted according to ranked abundances (Figures 3.1-3.3). *Stauroneis sp.* was the most abundant diatom species sampled from site 3. *Stauroneis kootenai* was the most abundant diatom species sampled from site 1, while *Gomphonema gracile* was the most abundant species sampled from site 2.

Table 3.3: All relative abundance diatom data (prior to data clean-up) sampled from the three sites at Ukutula. Rows highlighted in green indicates samples removed when statistical noise was removed. See Annexure B for diatom codes. Env: Environmental sample taken at site 1; Env2: Environmental sample taken at site 2; Env3: Environmental sample taken at site 3. Tadpole guild samples are indicated by blue spots (Ter: Terrapin (*Pelomedusa galiata*) sample taken at site 1; G1-1s1: *Phrynomantis bifasciatus* (Guild 1, Suspension feeder) sampled at site 1; G4-1s1: *Ptychadena mossambica* (Guild 4, Benthic type 2) sampled at site 1; G4-2s1: *Strongylopus grayii* (Guild 4, Benthic type 2), and G6-1s1: *Amietia delalandii* (Guild 6, Rheophilic) sampled at site 1; G3-1s2: *Kassina senegalensis* (Guild 3, Lentic-nektonic) sampled at site 2; G6-2s2: *Amietia delalandii* (Guild 6, Rheophilic) sampled at site 2; G3-1s3: *Kassina senegalensis* (Guild 3, Lentic-nektonic) sampled at site 3; U3-1s3: *Phrynomantis bifasciatus (*Guild 1, suspension feeder) sampled at site 3; G6-2s3: *Amietia delalandii* (Guild 6, Rheophilic) sampled at site 3.

Sites and samples →	**Ukutula site 1**						**Ukutula site 2**			**Ukutula site 3**			
Code	**Env**	**Ter**	**G6-1s1**	**G1-1s1**	**G4-1s1**	**G4-2s1**	**Env2**	**G3-1s2**	**G4-2s2**	**Env3**	**G3-1s3**	**G1-1s3**	**G4-2s3**
ACHD1	0,00	0,00	0,00	0,00	0,00	1,24	0,00	0,00	0,00	0,00	0,15	0,00	0,00
ACHD2	0,07	0,00	0,00	5,86	0,16	0,46	0,00	0,00	0,00	0,49	4,22	0,00	0,00
ACHD3	6,45	0,00	0,00	2,35	0,00	0,00	0,00	0,00	0,00	0,82	0,00	0,00	0,00
ACHD4	0,00	0,00	0,00	0,17	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
ACHD5	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	7,35	0,00	0,00	0,00
ACHD6	0,00	0,00	0,00	0,00	0,00	0,46	0,00	0,00	0,00	0,00	0,00	0,00	0,00
ACHD7	0,00	0,00	0,00	0,17	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
ASWA	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,16	0,00	0,00	0,00
ADCR	0,00	0,00	0,00	0,00	0,64	1,55	0,00	0,00	0,00	0,33	0,30	0,00	0,00
BNEO	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,16	0,00	0,00	0,00
CCRU	0,00	0,14	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
CPTG	0,00	0,00	0,20	0,17	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
CAMB	0,00	0,00	0,00	1,84	4,78	0,00	0,00	8,95	0,00	0,00	0,00	0,00	0,00
CRBU	0,00	0,00	0,00	5,86	0,96	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
CRPE	0,00	0,00	0,00	0,00	0,00	4,79	0,00	0,00	16,36	0,00	0,00	0,00	0,00
CRAT1	0,00	1,72	0,00	0,00	0,00	0,00	1,45	0,00	0,00	0,00	0,00	0,00	0,00
CRAT2	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,93	0,00	2,11	1,55	1,65

Table 3.3: (Continued)

Sites and samples →	Ukutula site 1						Ukutula site 2			Ukutula site 3			
Code	Env	Ter	G6-1s1	G1-1s1	G4-1s1	G4-2s1	Env2	G3-1s2	G4-2s2	Env3	G3-1s3	G1-1s3	G4-2s3
CRAT3	0,00	0,00	3,23	0,00	0,00	0,00	0,00	0,00	0,00	1,31	0,00	0,00	0,00
CRAT4	0,00	0,86	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
CRAT5	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,80	0,00	0,00	0,00	0,00	0,00
CRAT6	1,38	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
CVIX	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	4,67	0,15	1,65
CAGI	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,15	0,00	0,00
CNIS	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,98	0,00	0,00	0,00
CYMB1	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,49	0,00	0,00	0,00
CSLP	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,15	0,00
CTUM	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,14	0,00	0,00	0,00
DOOV	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,82	0,00	0,00	0,00
ENCY1	0,43	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
ENLE	0,00	0,14	0,00	0,00	0,00	0,00	0,00	0,96	0,00	0,16	0,00	0,00	0,00
ENVE	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,16	0,00	0,00	0,00	0,00	0,00
EOMI	0,00	1,29	0,00	0,00	0,32	0,00	0,00	0,00	0,00	0,00	0,15	0,00	0,13
EBIL	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,60	0,00	0,00
EFOR	1,01	0,72	10,69	0,00	5,42	0,15	0,00	0,00	0,23	0,49	0,00	1,70	0,13
EMIN	0,00	0,00	0,00	1,17	0,32	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
EUNO1	0,00	0,00	0,00	0,17	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
EUNO2	0,00	0,00	0,00	0,00	0,00	0,15	0,48	1,12	0,00	0,16	0,00	0,00	0,00
EUNO3	0,00	0,00	0,00	0,00	0,32	0,00	0,00	0,00	0,00	0,00	0,15	0,00	0,13
EUNO4	1,09	0,00	0,00	0,00	0,00	0,62	0,00	0,00	0,00	0,00	0,60	0,00	0,13
EUNO5	1,16	1,29	0,00	0,00	0,16	0,00	0,00	4,79	5,14	0,00	0,00	0,31	0,00
EUNO6	3,41	0,00	6,45	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
FANC	0,00	0,00	0,00	0,17	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00

Table 3.3: (Continued)

Sites and samples →	Ukutula site 1						Ukutula site 2			Ukutula site 3			
Code	Env	Ter	G6-1s1	G1-1s1	G4-1s1	G4-2s1	Env2	G3-1s2	G4-2s2	Env3	G3-1s3	G1-1s3	G4-2s3
FBCP	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,13
FRAG1	0,00	0,00	2,22	0,00	0,00	0,00	0,00	0,00	0,00	0,33	0,00	0,00	0,00
FRAG2	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,08	0,00
FRUS1	0,07	0,00	6,05	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
GACU	0,00	0,00	0,81	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
GAFF	0,80	0,57	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
GADI	0,00	0,00	0,00	0,00	0,64	0,00	0,00	0,00	0,00	0,00	0,15	0,00	0,00
GGRA	0,00	7,17	0,00	1,17	0,00	13,29	29,40	3,51	2,80	7,84	1,20	0,00	1,01
GLTC	0,00	0,29	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
GPAR	0,00	0,72	0,00	3,35	5,26	0,00	0,00	0,64	0,70	2,45	0,00	0,00	0,00
GPUM	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	3,27	0,00	0,00	0,00
GOMP1	1,01	0,00	0,00	1,68	10,05	8,81	21,65	4,63	0,00	0,98	1,36	0,00	0,00
GOMP2	0,00	3,16	0,00	0,00	0,00	0,15	0,00	0,00	2,10	2,78	0,00	0,00	0,00
GOMP3	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,13
GOMP4	0,00	5,16	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
GOMP5	0,29	0,86	0,20	0,17	0,64	0,00	0,00	0,96	0,00	0,00	0,00	0,00	0,00
GOMP6	0,00	0,00	0,00	0,00	5,26	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
GVNU	0,00	0,57	0,00	0,00	0,00	1,70	0,00	0,00	0,00	1,63	0,00	0,00	0,00
HAFC	0,00	1,58	1,81	1,17	1,59	3,25	4,68	2,88	7,24	3,92	1,66	1,24	4,44
HELO	0,00	0,00	1,21	0,00	3,99	0,46	0,16	0,00	0,70	0,65	0,00	0,00	0,00
HANT1	10,29	1,58	0,00	0,00	0,00	6,80	4,20	3,67	5,37	0,00	0,00	0,00	0,00
HANT2	0,00	7,89	1,21	0,00	1,28	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
HANT3	1,30	0,00	0,00	1,68	0,00	7,11	0,00	0,64	3,74	0,00	0,00	0,00	0,00
HANT4	0,87	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
LEMN1	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,28	0,00	0,00	0,00	0,00	0,00

Table 3.3: (Continued)

Sites and samples →	Ukutula site 1						Ukutula site 2			Ukutula site 3			
Code	Env	Ter	G6-1s1	G1-1s1	G4-1s1	G4-2s1	Env2	G3-1s2	G4-2s2	Env3	G3-1s3	G1-1s3	G4-2s3
LKOT	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,30	0,00	0,00
LMUT	1,45	1,58	2,02	1,51	1,75	0,93	4,36	2,08	2,34	4,41	1,66	0,31	1,90
LUTI1	0,07	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,47	0,00	0,60	0,00	0,00
LUTI2	0,58	0,00	0,00	0,17	0,00	0,00	0,00	0,00	0,00	0,00	3,01	0,00	0,00
LUTI3	0,72	0,00	0,00	0,17	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
MAAT	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,32	0,00	0,00	0,00	0,00	0,00
MCCT1	0,00	0,00	0,20	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
NCRY	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,16	0,00	0,00	0,00
NNIV	0,00	0,00	0,60	0,00	0,48	0,00	0,00	0,16	0,93	1,14	0,15	0,15	0,38
NRCS	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,38
NAVI1	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,46	0,00
NAVI2	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	2,45	0,00	0,00	0,00
NAVI3	2,32	0,00	0,00	0,00	0,80	0,46	0,00	0,00	0,00	0,00	0,00	0,00	0,00
NSYM	0,00	0,00	0,00	0,17	0,00	0,00	0,00	0,00	0,00	0,16	0,00	0,00	0,00
NVTA	0,00	0,14	0,00	0,00	0,00	0,46	0,00	0,00	0,00	0,00	0,00	0,00	0,00
NEAF	0,65	1,15	0,00	0,00	0,32	1,24	0,00	0,00	0,00	0,00	0,15	0,00	0,00
NEID1	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,45	0,00	0,00
NEID2	0,00	0,00	0,00	0,00	0,00	0,46	0,00	0,00	0,00	0,00	0,00	0,00	0,00
NEID3	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,45	0,00	4,94
NEID4	0,00	0,00	0,00	0,00	0,00	0,77	0,00	0,00	0,00	0,00	0,00	0,00	0,00
NATG	0,00	0,00	0,00	0,17	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
NCLA	0,00	0,29	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
NPAL	0,00	2,01	0,00	35,34	13,40	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
NITZ1	7,17	0,00	0,00	7,37	0,00	0,00	0,00	0,00	0,00	5,72	10,54	5,41	6,21
NITZ2	0,72	0,14	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	8,73	0,00	0,00

Table 3.3: (Continued)

Sites and samples →	Ukutula site 1						Ukutula site 2			Ukutula site 3			
Code	Env	Ter	G6-1s1	G1-1s1	G4-1s1	G4-2s1	Env2	G3-1s2	G4-2s2	Env3	G3-1s3	G1-1s3	G4-2s3
NITZ3	0,00	0,00	0,00	0,00	0,00	0,00	0,00	2,08	8,88	0,00	0,00	0,15	0,00
NITZ4	1,16	0,14	0,00	0,00	0,80	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
NITZ5	0,00	0,00	0,00	0,00	0,00	0,00	0,48	2,24	0,00	0,00	0,00	0,00	0,00
NITZ6	1,30	2,58	0,00	0,00	0,00	2,32	1,45	0,00	0,00	0,00	0,60	0,93	0,00
NITZ7	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,33	0,00	0,00	0,00
NITZ8	0,00	0,00	7,06	0,00	0,00	0,00	1,62	8,95	0,70	0,00	0,00	0,00	0,00
NITZ9	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,23	0,00	0,00	0,00	0,00
NVLC	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	6,54	0,00	0,00	0,00
PBRT	2,61	6,17	3,02	0,17	3,99	5,10	5,01	7,03	16,82	3,92	2,71	1,70	3,42
PDML	10,14	10,04	0,00	2,35	0,00	2,63	0,81	8,31	8,18	2,78	0,00	10,97	8,11
PDSL	0,00	7,75	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
PGIB	0,00	0,00	0,00	0,00	0,00	0,00	6,46	0,00	0,00	0,00	0,00	0,00	3,04
PMRO	0,00	0,00	0,00	0,00	0,32	3,71	0,00	0,00	6,54	0,00	10,69	0,00	0,00
PINU1	0,00	1,72	0,00	0,00	0,00	0,00	0,00	0,96	0,00	0,00	0,00	0,00	0,00
PINU2	5,58	0,57	14,52	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
PINU3	1,45	2,87	0,00	0,00	0,00	0,00	5,65	0,80	0,70	1,31	1,81	1,85	8,49
PINU4	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,31	0,00	0,00	0,00
PINU5	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	5,27	0,00	0,00
PINU6	0,00	0,00	0,00	1,17	0,00	0,00	0,00	5,43	0,70	0,00	0,00	0,31	2,15
PINU7	1,38	0,00	0,00	1,17	0,00	0,15	0,00	0,00	0,00	0,65	0,00	0,00	0,00
PINU8	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,13
PSBV	0,00	0,00	0,00	12,90	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
PSCA	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,15	0,00	0,00
PVIF	0,00	0,00	0,00	0,00	0,00	0,00	0,16	0,00	0,00	0,00	0,00	0,00	0,00
PLFR	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,15	0,00	0,00

Table 3.3: (Continued)

Sites and samples →	Ukutula site 1						Ukutula site 2			Ukutula site 3			
Code	Env	Ter	G6-1s1	G1-1s1	G4-1s1	G4-2s1	Env2	G3-1s2	G4-2s2	Env3	G3-1s3	G1-1s3	G4-2s3
PLTD1	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,16	0,00	0,00	0,00	0,00	0,00
PLTD2	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,49	0,00	0,00	0,00
RABB	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,80	0,00	0,00	0,00	0,00	0,00
SELL1	0,87	0,00	0,00	0,00	0,00	0,15	0,00	0,00	0,00	0,00	0,00	0,00	0,00
SSTM	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,47	0,00	0,00	0,00	0,00
STAN	0,00	13,06	0,00	0,00	0,00	0,00	9,05	25,40	3,50	0,00	31,63	0,00	0,00
SBOR	0,00	0,00	0,60	0,34	0,00	0,00	0,16	0,00	0,00	5,72	0,30	1,55	2,03
SGRC	7,25	13,49	0,00	0,00	0,00	25,66	0,00	0,00	0,00	0,00	0,00	0,00	0,00
SKOO	18,19	0,00	0,00	8,54	29,82	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
STNN	0,00	0,00	0,00	0,00	0,00	0,00	1,62	0,00	0,00	0,00	0,60	0,00	0,25
SSOF	0,00	0,00	0,00	0,00	0,00	0,00	0,32	0,00	0,47	0,82	0,75	0,00	1,27
STAU1	0,00	0,00	0,00	0,00	0,16	0,00	0,48	0,32	0,47	6,05	1,66	1,39	0,00
STAU2	6,01	0,00	37,90	0,00	0,00	4,95	0,00	0,00	3,04	17,32	0,00	66,31	47,78
STAU3	0,00	0,57	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
STAU4	0,00	0,00	0,00	0,17	5,90	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
STAU5	0,00	0,00	0,00	0,00	0,32	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
STDR	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	2,32	0,00
SSRU	0,00	0,00	0,00	1,17	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
SOVI	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,15	0,00	0,00
TCOA	0,00	0,00	0,00	0,00	0,00	0,00	0,32	0,00	0,00	0,00	0,00	0,00	0,00
TLIT	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,23	0,00	0,00	0,00	0,00
TRYB1	0,72	0,00	0,00	0,00	0,16	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00

Table 3.4: Shannon's diversity index (H'), species evenness (E), and species richness (S) values calculated for samples taken at Ukutula. Env = environmental samples, G6 = Guild 6 (Rheophilic), G1 = Guild 1 (Suspension feeder), G3 = Guild 3 (Lentic-nektonic), G4-*Pmos* = Guild 4 (Benthic type 2(profundal) for *Ptychadena mossambica*), G4*Sgray* = Guild 4 (Benthic type 2(profundal) for *Strongylopus grayii*), and G6 = Guild 6 (Rheophilic).

Variables	**Samples for Ukutula site 1**						**Samples for Ukutula site 2**			**Samples for Ukutula site 3**			
	Env	**Terrapin**	**G6**	**G1**	**G4-*Pmos***	**G4-*Sgray***	**Env**	**G3**	**G6**	**Env**	**G3**	**G1**	**G6**
H'	2,5866	2,7122	2,1179	2,3009	2,3375	2,4813	2,2167	2,4559	2,5922	2,8442	2,3145	1,3157	1,9759
E	0,8368	0,8229	1,3678	0,7062	0,7262	0,7808	0,7399	0,7945	0,8267	0,8066	0,6805	0,4644	0,6392
S	22	27	19	26	25	24	20	22	23	34	30	17	22

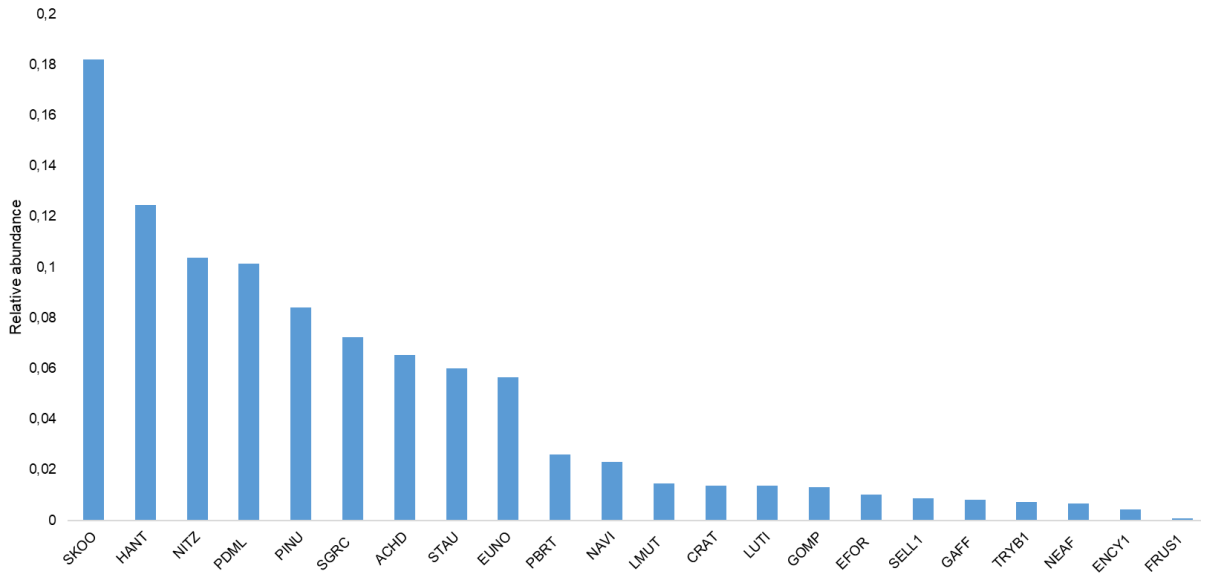


Figure 3.1: Ranked abundances of diatom species identified in the environmental samples taken at Ukutula site 1. See Annexure B for diatom codes.

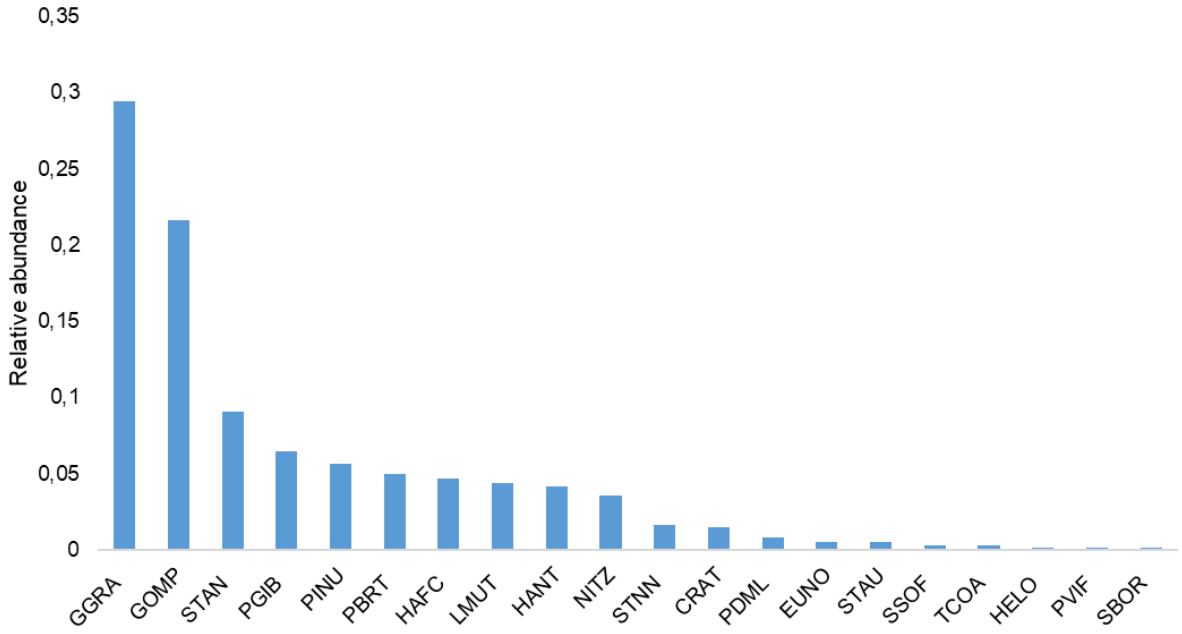


Figure 3.2: Ranked abundances of diatom species identified in the environmental samples taken at Ukutula site 2. See Annexure B for diatom codes.

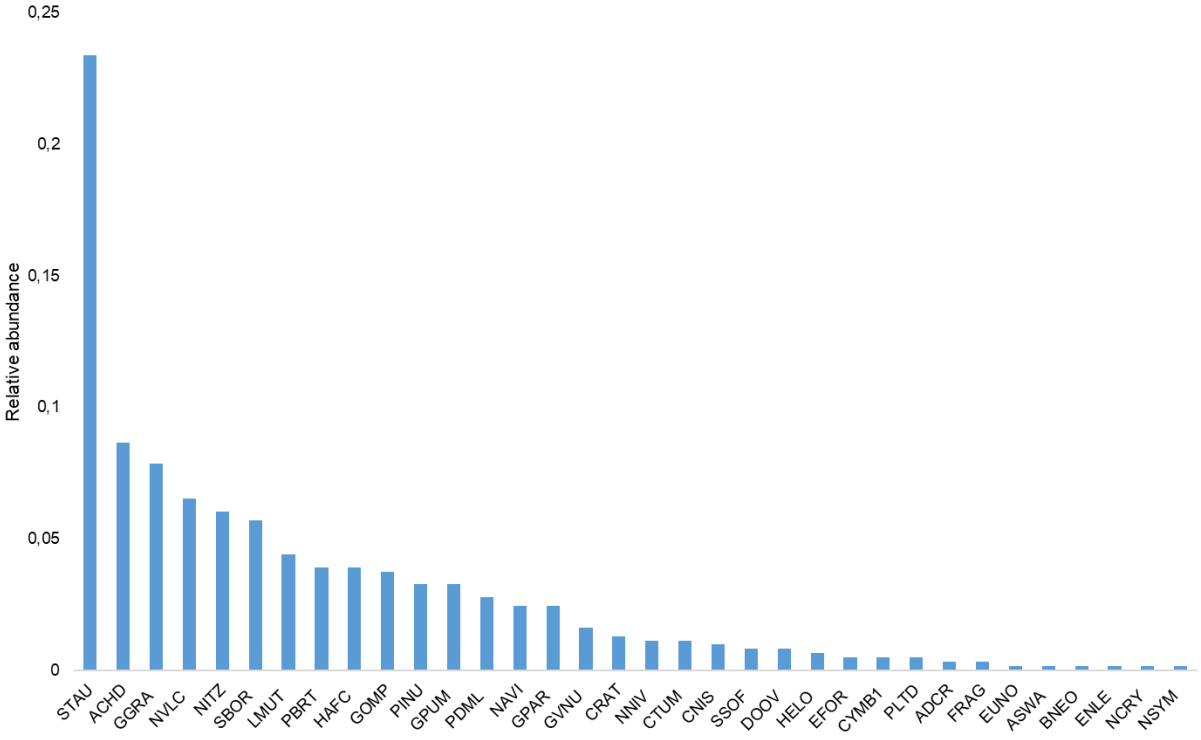


Figure 3.3: Ranked abundances of diatom species identified in the environmental samples taken at Ukutula site 3. See Annexure B for diatom codes.

Diatoms from Aliwal North sites

Total diatom species counted for the Aliwal North environmental samples included 28, 24, 36, 21, 13, 9, 11, and 25 for sites 1-8 respectively. Diatoms were counted and identified to genus level, and where possible, to species level (Table 3.5, Annexures B and C).

Statistical values (H', E, and S) were also calculated for the samples obtained from Aliwal North (Table 3.6). In terms of environmental diatom diversity, site 3 returned the highest H' value (2.58), as well as the highest S value (30). The E value was relatively low (0.769). Site 1 and 2 had low E values (0.777 and 0.750 respectively). In contrast, site 7 had the lowest H' value (1.33) and had the second-lowest S value (11). Site 6 had the lowest S value in the sample pool (10). The E value (0.577) for site 7 was also low. Site 6 had an even lower E value (0.557) and site 5 had the lowest E value in the sample pool (0.518).

Examining the graphs depicting RA values (Figures 3.4 to 3.11), it was evident that *Fragilaria* sp. and *Nitzschia* sp. were the most dominant diatom species in the environmental sample of site one. Site two's most dominant diatom species was *Eunotia formica*, followed

by *Nitzschia palea*. *Gomphonema* sp. and *Fragilaria* sp. were in addition the two most dominant species found in the environmental sample of site 3. *Gomphonema parvulum* and *Nitzschia* sp. dominated site 4. *Gomphonema parvulum* dominated both sites 5 and 6. *Eunotia formica* is the most dominant diatom species in the environmental sample of site 7 and the second most dominant in site six. It is apparent that sites from Aliwal North are commonly dominated by diatoms from the *Fragilaria, Nitzschia*, and *Gomphonema* genera. Site 8 however, is dominated by *Pinnularia subcapitata* and *Denticula* sp.

Table 3.5: All relative abundance diatoms data (prior to data clean-up) sampled from all eight sites at Aliwal North. Rows highlighted in green indicates samples removed when statistical noise was removed. See Annexure B for diatom species codes. Environmental samples includes EnvFE1, EnvFE2, EnvFE3, EnvFE7, EnvTN1B, EnvTN2, EnvTN4, and EnvX taken at sites 1, 2, 3, 4, 5, 6, 7, and site 8 respectively. Samples includes G9-1sFE1; *Cacosternum boettgeri* from guild 9 (Lentophytophilic) taken at site 1, G5-1sFE1; *Amietophrynus rangeri* from guild 5 (Lentic-benthic) taken at site 1, G3-1sFE2; *Kassina senegalensis* from guild 3 (Lentic-nektonic) taken at site 2, G3-1sFE3; *Kassina senegalensis* from guild 3 (Lentic-nektonic) taken at site 3, G3-1sFE7; *Kassina senegalensis* from guild 3 (Lentic-nektonic) taken at site 4, G9-1sFE7; *Cacosternum boettgeri* from guild 9 (Lentophytophilic) taken at site 4, G3-1sTN1B; *Kassina senegalensis* from guild 3 (Lentic-nektonic) taken at site 5, G9-1sTN1B; *Cacosternum boettgeri* from guild 9 (Lentophytophilic) taken at site 5, G3-1sTN2; *Kassina senegalensis* from guild 3 (Lentic-nektonic) taken at site 6, G9-1sTN2; *Cacosternum boettgeri* from guild 9 (Lentophytophilic) taken at site 6, G3-1sTN4; *Kassina senegalensis* from guild 3 (Lentic-nektonic) taken at site 6, G9-1sTN4; *Cacosternum boettgeri* from guild 9 (Lentophytophilic) taken at site 7, and G6-1sX; *Amietia delalandii* from guild 6 (Rheophilic) taken at Site 8.

Sites and samples →	Site 1			Site 2		Site 3		Site 4			Site 5			Site 6			Site 7			Site 8	
Code	EnvFE1	G9-1sFE1	G5-1sFE1	EnvFE2	G3-1sFE2	EnvFE3	G3-1sFE3	EnvFE7	G3-1sFE7	G9-1sFE7	EnvTN1B	G3-1sTN1B	G9-1sTN1B	EnvTN2	G3-1sTN2	G9-1sTN2	EnvTN4	G3-1sTN4	G9-1sTN4	EnvX	G6-1sX
ACHN1	0,06	0,01	0,01	0,02	0,00	0,00	0,02	0,02	0,01	0,02	0,00	0,01	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,01	0,04
CALO	0,01	0,00	0,06	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,01	0,00	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
CPTG	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
COCO1	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
CAMB	0,00	0,03	0,00	0,00	0,00	0,04	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
CRAT	0,00	0,02	0,03	0,03	0,09	0,01	0,02	0,05	0,00	0,03	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,02	0,00
CYCL	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,06	0,02	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
CAGI	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
CCIS	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
CSLE	0,02	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,01
CYMB2	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
CYMB3	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,02	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
CTUM	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
DENT1	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,19	0,00
DIAM1	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00

Table 3.5: (Continued)

Sites and samples →	Site 1			Site 2		Site 3		Site 4			Site 5			Site 6			Site 7			Site 8	
Code	EnvFE1	G9-1sFE1	G5-1sFE1	EnvFE2	G3-1sFE2	EnvFE3	G3-1sFE3	EnvFE7	G3-1sFE7	G9-1sFE7	EnvTN1B	G3-1sTN1B	G9-1sTN1B	EnvTN2	G3-1sTN2	G9-1sTN2	EnvTN4	G3-1sTN4	G9-1sTN4	EnvX	G6-1sX
ENLE	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
ENCY	0,00	0,00	0,02	0,00	0,00	0,00	0,00	0,01	0,00	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
ENVE	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,12	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
EADN	0,06	0,00	0,00	0,00	0,00	0,03	0,00	0,00	0,00	0,00	0,00	0,00	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,03	0,22
ESOR	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
EBIL	0,00	0,00	0,00	0,00	0,03	0,01	0,02	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,01	0,00	0,01	0,02	0,00	0,00
EFOR	0,00	0,00	0,00	0,35	0,06	0,01	0,02	0,10	0,00	0,01	0,00	0,15	0,09	0,16	0,03	0,03	0,60	0,19	0,20	0,00	0,00
EMIN	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,01	0,00	0,00	0,03	0,00	0,00	0,00	0,00
EPEH	0,00	0,05	0,00	0,06	0,03	0,06	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
EUNO	0,00	0,01	0,00	0,03	0,01	0,01	0,00	0,01	0,00	0,01	0,00	0,10	0,04	0,00	0,04	0,01	0,00	0,14	0,20	0,00	0,00
FANC	0,00	0,00	0,00	0,00	0,00	0,00	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
FBCP	0,02	0,00	0,05	0,00	0,00	0,02	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,06	0,00	0,00
FRAG	0,26	0,00	0,00	0,05	0,00	0,10	0,02	0,07	0,00	0,00	0,08	0,00	0,00	0,13	0,00	0,00	0,04	0,00	0,00	0,02	0,00
GSDC	0,00	0,00	0,00	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
GAFF	0,00	0,00	0,00	0,00	0,00	0,02	0,00	0,02	0,00	0,00	0,00	0,00	0,00	0,09	0,00	0,01	0,04	0,01	0,00	0,00	0,00
GGRA	0,00	0,00	0,01	0,07	0,00	0,02	0,06	0,07	0,00	0,01	0,00	0,00	0,00	0,00	0,02	0,00	0,19	0,02	0,04	0,03	0,00
GLGN	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
GLTC	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,02
GPAR	0,09	0,04	0,00	0,02	0,01	0,08	0,16	0,24	0,06	0,06	0,62	0,00	0,01	0,59	0,50	0,05	0,00	0,18	0,00	0,02	0,00
GPUM	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,01	0,00
GOMP	0,08	0,08	0,00	0,00	0,02	0,32	0,41	0,02	0,00	0,13	0,00	0,00	0,03	0,00	0,25	0,82	0,06	0,12	0,10	0,00	0,04
GVNU	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,15	0,00	0,00	0,11	0,00	0,00	0,00
GYRO1	0,01	0,00	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
HAFC	0,02	0,07	0,01	0,01	0,00	0,02	0,03	0,00	0,00	0,00	0,00	0,01	0,02	0,00	0,00	0,03	0,00	0,00	0,04	0,00	0,01
HELO	0,00	0,03	0,08	0,00	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00

Table 3.5: (Continued)

Sites and samples →	Site 1			Site 2		Site 3		Site 4			Site 5			Site 6			Site 7			Site 8	
Code	EnvFE1	G9-1sFE1	G5-1sFE1	EnvFE2	G3-1sFE2	EnvFE3	G3-1sFE3	EnvFE7	G3-1sFE7	G9-1sFE7	EnvTN1B	G3-1sTN1B	G9-1sTN1B	EnvTN2	G3-1sTN2	G9-1sTN2	EnvTN4	G3-1sTN4	G9-1sTN4	EnvX	G6-1sX
HANT	0,00	0,00	0,00	0,00	0,03	0,00	0,01	0,00	0,00	0,01	0,00	0,00	0,02	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
LEMN1	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00
LMUT	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,01	0,00	0,00	0,00	0,00	0,01	0,03	0,01	0,00
MAAT	0,00	0,00	0,00	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
MAYA1	0,00	0,00	0,16	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
NNIV	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
NRCS	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,09	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,03	0,00
NAVI	0,07	0,09	0,25	0,01	0,00	0,01	0,00	0,02	0,00	0,49	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,14	0,05
NSYM	0,01	0,00	0,00	0,00	0,00	0,00	0,01	0,11	0,00	0,00	0,00	0,00	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
NEAF	0,00	0,00	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,02	0,00	0,00
NATG	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,05
NMIC	0,00	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
NPAL	0,00	0,00	0,00	0,18	0,00	0,00	0,06	0,00	0,78	0,02	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
NITZ	0,14	0,26	0,26	0,01	0,37	0,03	0,01	0,22	0,04	0,15	0,06	0,03	0,19	0,00	0,02	0,02	0,02	0,02	0,02	0,09	0,23
PAUN	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,01	0,00	0,00	0,39	0,03	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
PBRT	0,00	0,02	0,00	0,00	0,01	0,00	0,01	0,00	0,00	0,00	0,00	0,18	0,02	0,00	0,00	0,00	0,00	0,02	0,04	0,00	0,00
PDML	0,00	0,08	0,01	0,05	0,17	0,04	0,05	0,01	0,03	0,00	0,00	0,01	0,06	0,00	0,00	0,00	0,02	0,00	0,09	0,00	0,01
PGIB	0,00	0,00	0,00	0,01	0,02	0,00	0,00	0,00	0,00	0,01	0,00	0,00	0,07	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
PMRO	0,00	0,00	0,00	0,01	0,00	0,01	0,03	0,00	0,00	0,00	0,00	0,00	0,02	0,00	0,00	0,00	0,00	0,03	0,05	0,00	0,00
PINU	0,00	0,05	0,00	0,00	0,01	0,06	0,01	0,00	0,01	0,00	0,00	0,01	0,14	0,00	0,01	0,00	0,00	0,04	0,00	0,02	0,01
PSBV	0,00	0,00	0,00	0,00	0,00	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,04	0,00	0,00
PSCA	0,00	0,00	0,00	0,00	0,00	0,01	0,00	0,01	0,00	0,00	0,00	0,02	0,00	0,00	0,00	0,01	0,01	0,07	0,00	0,38	0,00
PVIF	0,01	0,01	0,00	0,00	0,00	0,01	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,01	0,00	0,00
RGIB	0,10	0,00	0,00	0,00	0,00	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,30
SPUP	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,01	0,00

Table 3.5: (Continued)

Sites and samples →	Site 1			Site 2		Site 3		Site 4			Site 5			Site 6			Site 7			Site 8	
Code	EnvFE1	G9-1sFE1	G5-1sFE1	EnvFE2	G3-1sFE2	EnvFE3	G3-1sFE3	EnvFE7	G3-1sFE7	G9-1sFE7	EnvTN1B	G3-1sTN1B	G9-1sTN1B	EnvTN2	G3-1sTN2	G9-1sTN2	EnvTN4	G3-1sTN4	G9-1sTN4	EnvX	G6-1sX
SELL2	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
SMNA1	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,02	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
STAN	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,08	0,00	0,00	0,00	0,00	0,01	0,00	0,00	0,00
SGRC	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,08	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
SSOF	0,00	0,00	0,00	0,00	0,03	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,02	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
STAU	0,00	0,11	0,00	0,02	0,08	0,05	0,04	0,00	0,00	0,00	0,01	0,08	0,03	0,00	0,00	0,00	0,00	0,01	0,03	0,00	0,00
SCBI	0,00	0,01	0,02	0,03	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
TRYB	0,00	0,01	0,02	0,03	0,01	0,00	0,00	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00

Table 3.6: Shannon's diversity index (H'), species evenness (E), and species richness (S) values calculated for samples taken at Aliwal North. Env = environmental samples, G3 = Guild 3 (Lentic-nektonic), G5 = Guild 5 (Lentic-benthic), G6 = Guild 6 (Rheophilic), and G9 = Lentophytophilic.

Variables	Site 1			Site 2		Site 3		Site 4			Site 5			Site 6			Site 7			Site 8	
	Env	G9	G5	Env	G3	Env	G3	Env	G3	G9	Env	G3	G9	Env	G3	G9	Env	G3	G9	Env	G6
H'	2,3972	2,5280	2,1631	2,2456	2,1985	2,5878	2,1945	2,2816	0,9395	1,7677	1,2877	1,7993	2,7065	1,2256	1,3831	0,8288	1,3291	2,3522	2,4880	1,9775	1,9381
E	0,775529	0,80624	0,699781	0,749609	0,701162	0,76851	0,690637	0,774881	0,366296	0,600347	0,518202	0,681798	0,851624	0,557789	0,6295	0,3335	0,5772	0,7726	0,8049	0,6842	0,6705
S	21	23	21	20	24	30	24	19	13	19	12	14	23	10	10	13	11	22	23	18	18

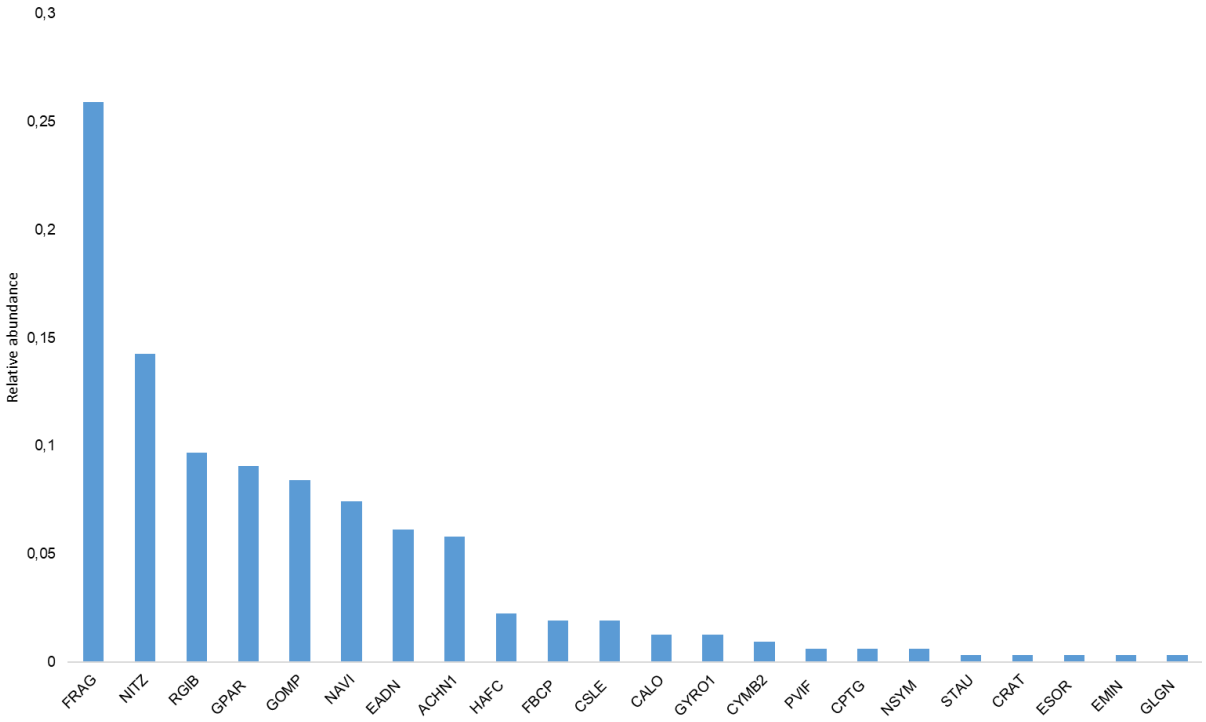


Figure 3.4: Ranked abundances of diatom species identified in the environmental samples taken at site 1 from Aliwal North. See Annexure B for diatom codes.

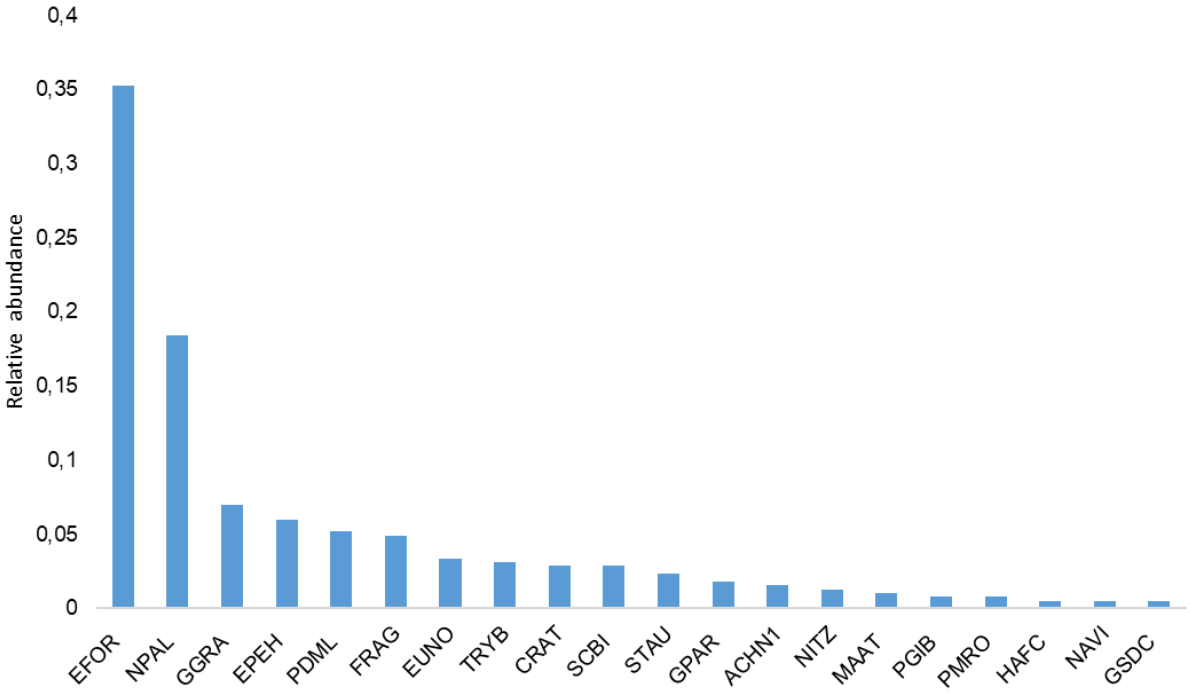


Figure 3.5: Ranked abundances of diatom species identified in the environmental samples taken at site 2 from Aliwal North. See Annexure B for diatom codes.

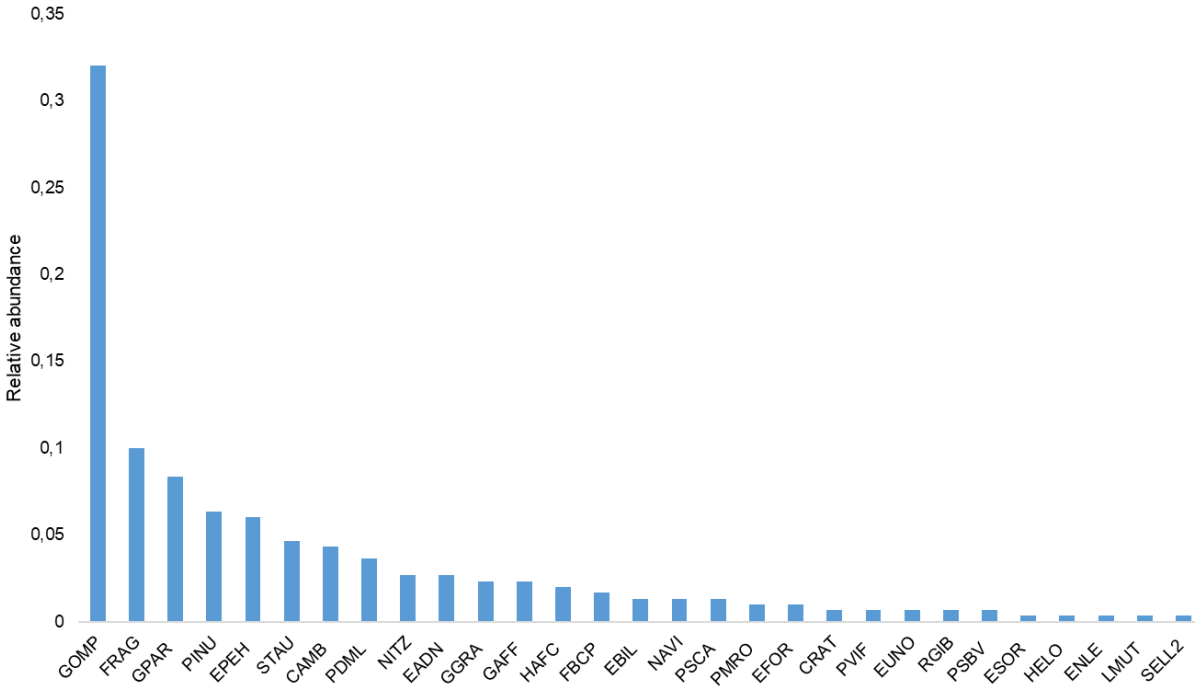


Figure 3.6: Ranked abundances of diatom species identified in the environmental samples taken at site 3 from Aliwal North. See Annexure B for diatom codes.

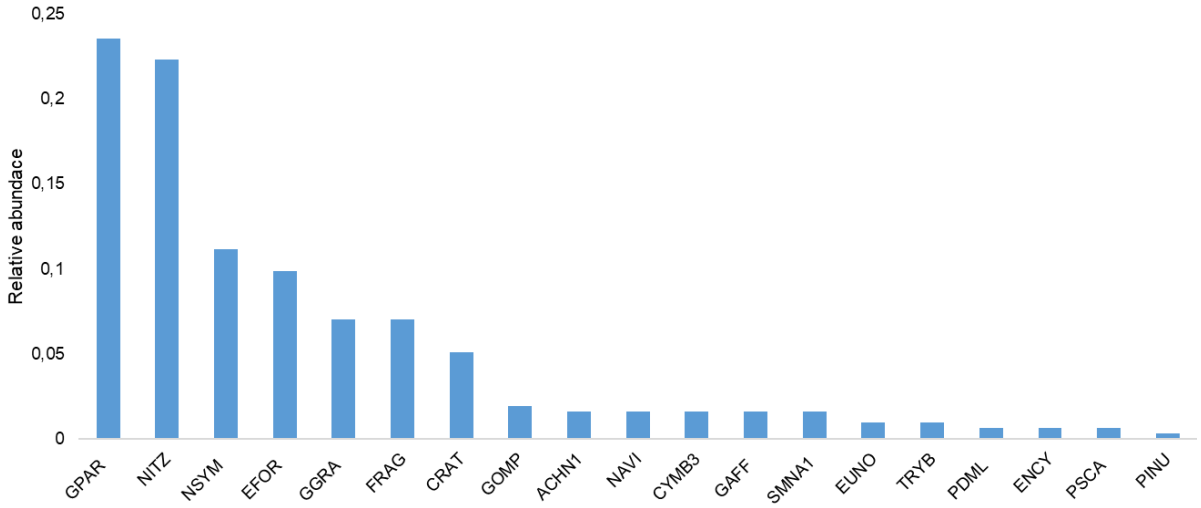


Figure 3.7: Ranked abundances of diatom species identified in the environmental samples taken at site 4 from Aliwal North. See Annexure B for diatom codes.

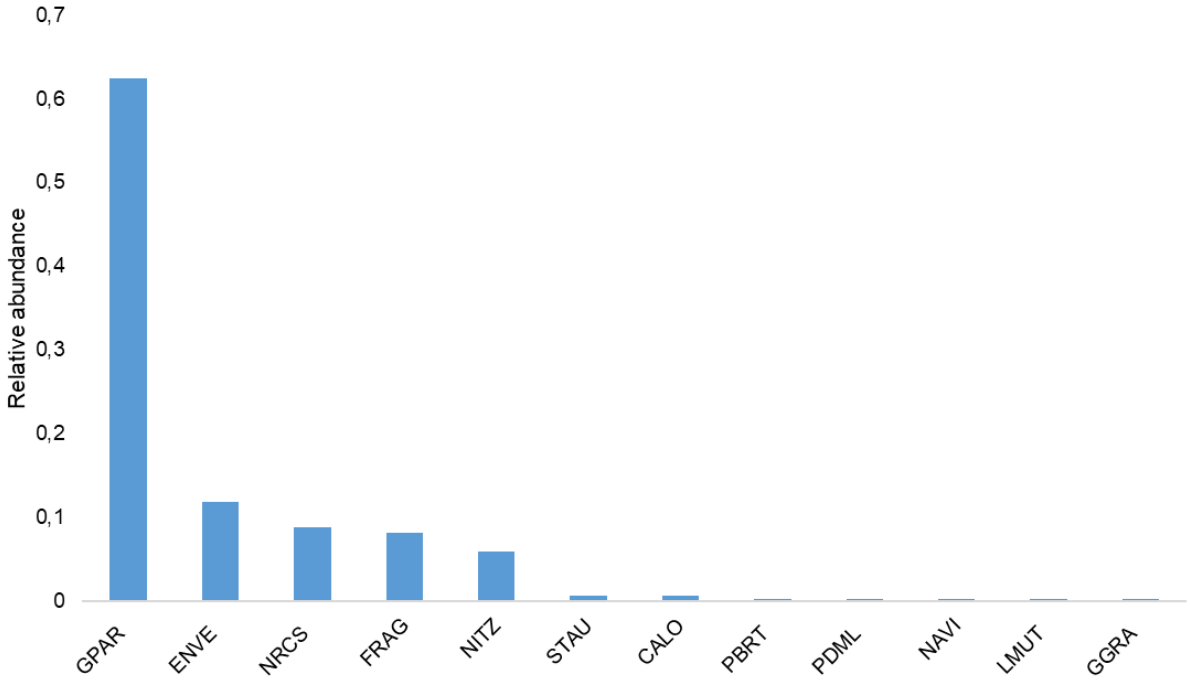


Figure 3.8: Ranked abundances of diatom species identified in the environmental samples taken at site 5 from Aliwal North. See Annexure B for diatom codes.

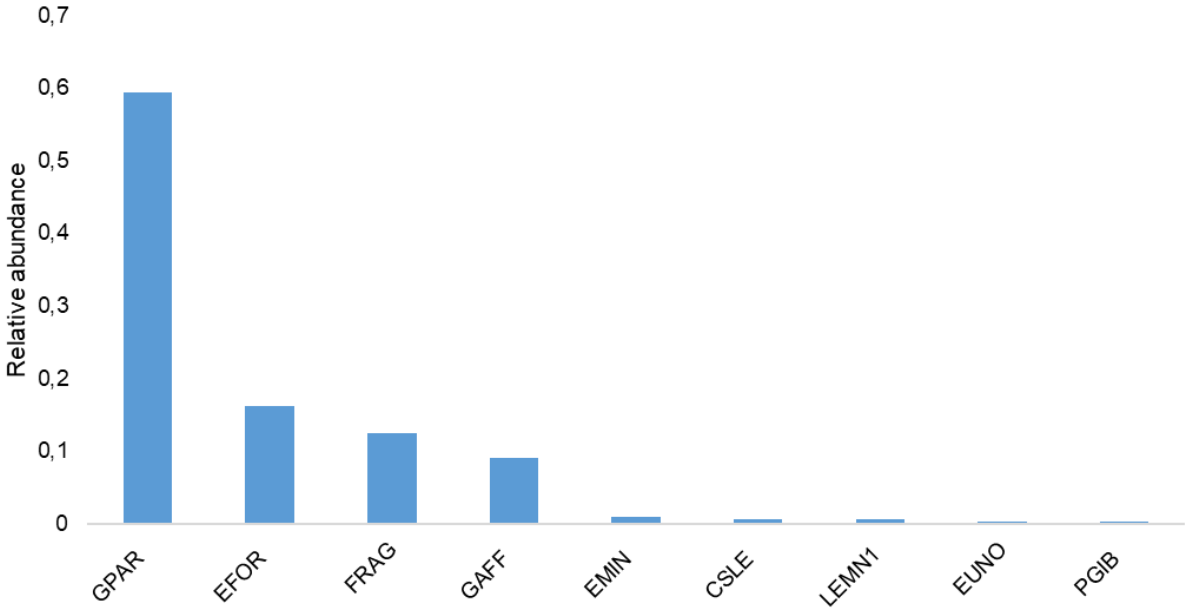


Figure 3.9: Ranked abundances of diatom species identified in the environmental samples taken at site 6 from Aliwal North. See Annexure B for diatom codes.

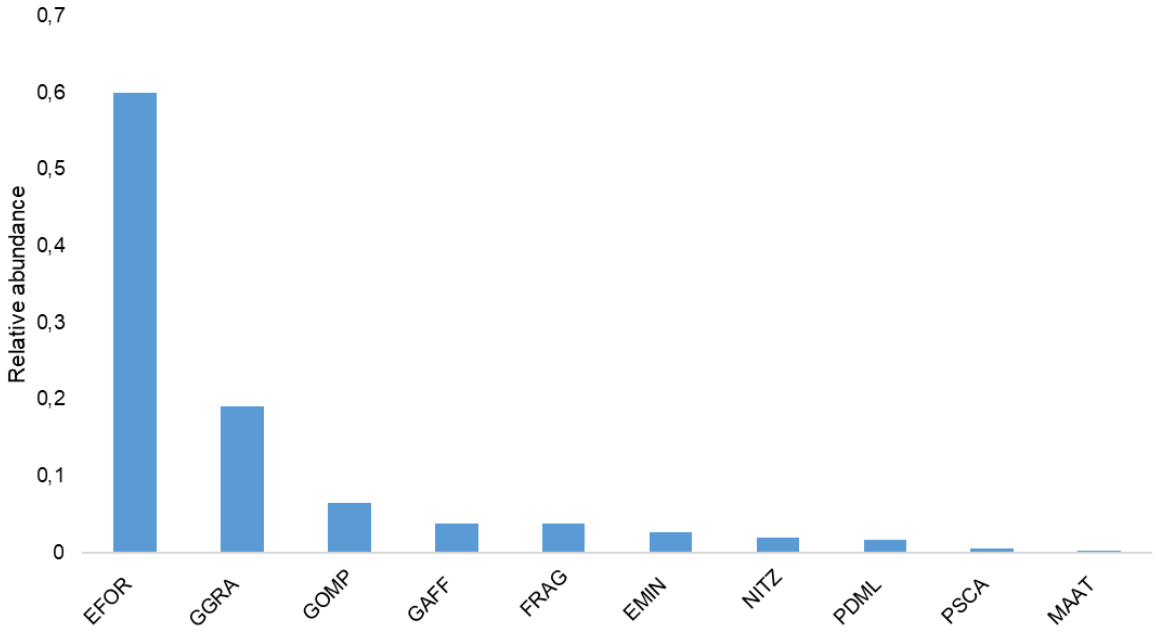


Figure 3.10: Ranked abundances of diatom species identified in the environmental samples taken at site 7 from Aliwal North. See Annexure B for diatom codes.

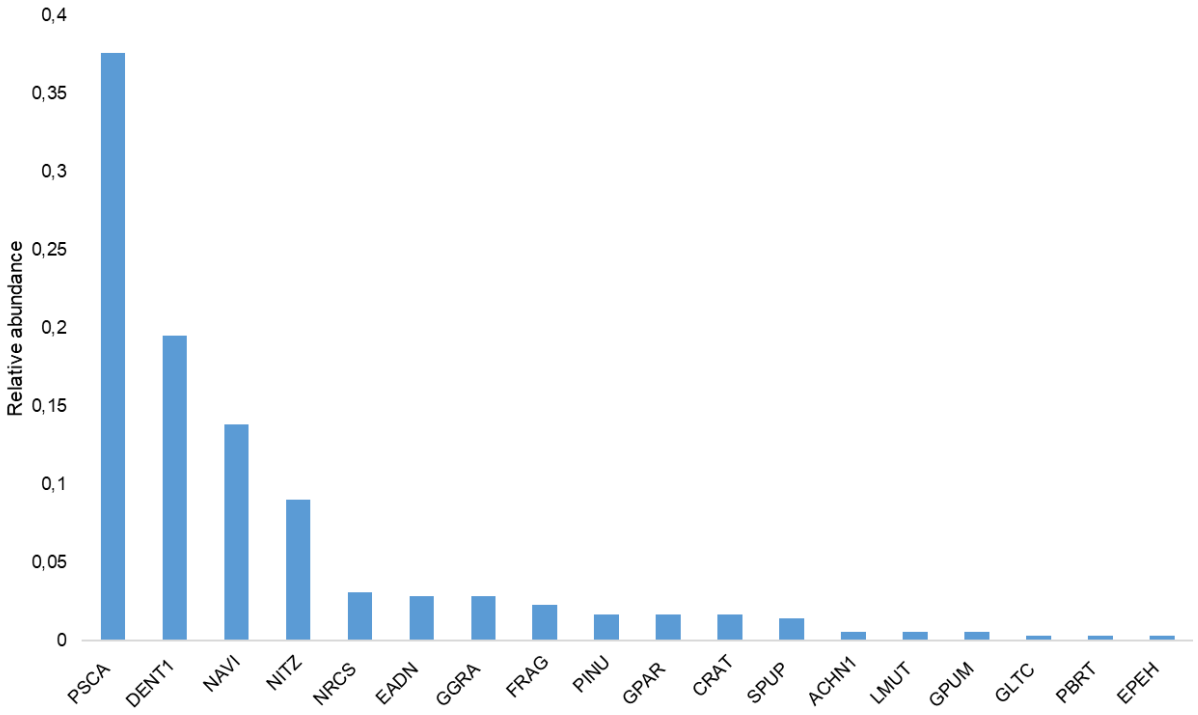


Figure 3.11: Ranked abundances of diatom species identified in the environmental samples taken at site 8 from Aliwal North. See Annexure B for diatom codes.

3.3. Diatom diversity from tadpole gut content

Ukutula samples

The digestive tracts of tadpoles sampled from Ukutula were examined for their diatom content. Diatoms were counted from the intestinal tracts examined from *A. delalandii* (n = 19)*, P. bifasciatus* (n = 32)*, P. mossambica* (n = 30) and *S. grayii* (n = 30) sampled at site one (Table 3.3, Annexure C). Examining diatoms sampled from the carapace of *P. galiata* returned 35 diatom species.

Inspecting the intestinal tracts of *K. senegalensis* and *S. grayii,* returned 29 and 28 diatom species respectively for site two (Table 3.3, Annexure C). Diatoms species were also counted and identified from examining the intestinal tracts of *K. senegalensis* (n = 37), *P bifasciatus* (n = 21) and *S. grayii* (n = 25).

H', E, and S values were also calculated and documented for diatoms counted and identified from the intestinal tracts of tadpoles (Table 3.4). The Benthic type 2 (Profundal) guild at site 1 returned the highest H' value after examining intestinal diatom content (2.48). But its S value (24) is surpassed by the Suspension Feeder guild (26) from the same site. The sample with the lowest H' value (2.12) was obtained from the Rheophilic guild, which additionally had the lowest S value (19). Samples collected from the Rheophilic guild at site 2 had the highest H' (2.59), E (0.827) and S values (23). The sample collected from the Lentic-nektonic Lentic-nektonic guild, however, had a somewhat lower H' value (2.46) and a slightly lower S (20) and E values (0.740). The sample obtained from the Lentic-nektonic guild from site 3 had the highest H' value (2.31). It also had the highest S (30) and E values (0.681). In contrast, the sample collected from the Suspension Feeder guild had the lowest H' (1.32), E (0.464) and S (17) values.

The collective values from Table 3.4 were then used to construct scatterplots (H' vs E, and H' vs S) (Figures 3.12 and 3.13). An apparent positive correlation between the H' and E samples was distinguishable; with the sample collected from the Rheophilic guild as a pronounced outlier. In contrast, there appears to be no apparent correlation between S and H' values.

Ukutula's cleaned relative abundance data is used to construct a DCA (Figure 3.14). No apparent visible clusters were distinguishable between sites and samples. The relative abundance data used to construct the DCA was cleaned and used for further statistical

processing. Ukutula's sample pool has an estimated residual value of 62.89 (± 6.18 SE). Additionally, P values were calculated for ecomorphological tadpole guilds (p = 0.434), species (p = 0.982), and environmental samples (p = 0.954) (Annexure D).

There were no practical differences between terrapin/environmental and tadpole/environmental samples, as the effect sizes calculated were all below 0.2 (Annexure D). However, there was a minor practical difference between the tadpoles and terrapin sample (p = 0.22)

The effect sizes for tadpole species (*P. bifasciatus, K. senegalensis, P. mossambica, S. grayii*, and *A. delalandii*), and ecomorphological tadpole guilds (Suspension feeder, Lentic-nektonic, Benthic type 2 (Profundal), Rheophilic) were also calculated and recorded (Annexure D). Nonetheless, no practical significant difference was recorded ($p < 0.2$).

Aliwal North samples

The intestinal content of tadpoles sampled by Aliwal North's sites was also documented (Table 3.5, Annexure B and C). Diatom species were counted from the intestinal tracts of *C. boettgeri* (26) and *A. rangeri* (24) for site one. Thirty-two diatom species were recorded from the intestinal tract of *K. senegalensis* for site two. However, 29 diatom species were noted for *K. senegalensis* from site 3. Diatom species were also counted from the intestinal tracts of *K. senegalensis* (14) and *C. boettgeri* (26) for site 4. The same tadpole species were also inspected at site 5, returning 18 and 32 diatom species respectively. Fewer diatom species were counted from the intestinal tracts of *K. senegalensis* (11) and *C. boettgeri* (14) from site 6. The same tadpoles were examined at site 7 and returned 25 and 27 diatom species respectively. For site 8, studying the intestinal tract of *A. delalandii* returned 22 diatom species.

H', E, and S values were also calculated and documented for diatoms counted and identified from the intestinal tracts of tadpoles (Table 3.6). In contrast to Ukutula's data, some sites at Aliwal North only had one tadpole species to sample from. Therefore, this dataset was considered in its entirety.

Diatoms counted from the Lentophytophilic guild had the highest H' value (2.71) in the entire dataset (Table 3.6). It also had relatively high E (0.852) and S values (23). The second highest H' value was from the Lentophytophilic guild at site 1 (2.53). This guild also had relatively high E (0.806) and S values (23). The sample with the highest S value, however,

was taken from the Lentic-nektonic Lentic-nektonic guild at site 3 (24). Samples with the lowest H' values included samples collected from the Lentophytophilic (0.82) and Lentic-nektonic Lentic-nektonic (0.94) guilds from site 6 and 4 respectively. Site 6 and 4 have relatively low E (0.344 and 0.366) and S values (13 and 13). However, the sample from the Lentophytophilic guild in site 6 had the lowest S value (10) amongst samples obtained from guilds.

The collective values from Table 3.6 were then used to construct scatterplots (H' vs E, and H' vs S) (Figures 3.15 and 3.16). An apparent positive correlation between the H' and E samples were distinguishable. In contrast, there appeared to be no apparent correlation between S and H' values. Aliwal North's cleaned relative abundance data was used to construct a DCA (Figure 3.17). No apparent visible clusters could be distinguished between sites and samples.The relative abundance data used to construct the DCA was cleaned and used for further statistical processing. Aliwal North's sample pool had an estimated residual value of 123.98(± 10.58 SE).For the rest of the statistical analysis, Aliwal North's tadpole and guild samples was considered as one sample. This was because each tadpole guild comprised a singular tadpole species. P values were therefore calculated for species/guilds ($p = 0.755$) and environmental samples ($p = 0.376$) (Annexure D).

There were no practical differences between species/guilds and environmental samples, as the effect sizes calculated were all below the values of 0.2 (Annexure D). Effect sizes were calculated for sample type (Environment and ecomorphological tadpole guilds/species) and tadpole species/guilds *K. senegalensis* (Lentic-nectonic), *A. rangeri* (Lentic-benthic), A*. delalandii* (Rehophilic), and *C. boettgeri* (Lentophytophilic) (Annexure D). Nonetheless, no practical significant difference was recorded, because of the small P values returned by calculations ($p < 0.2$).

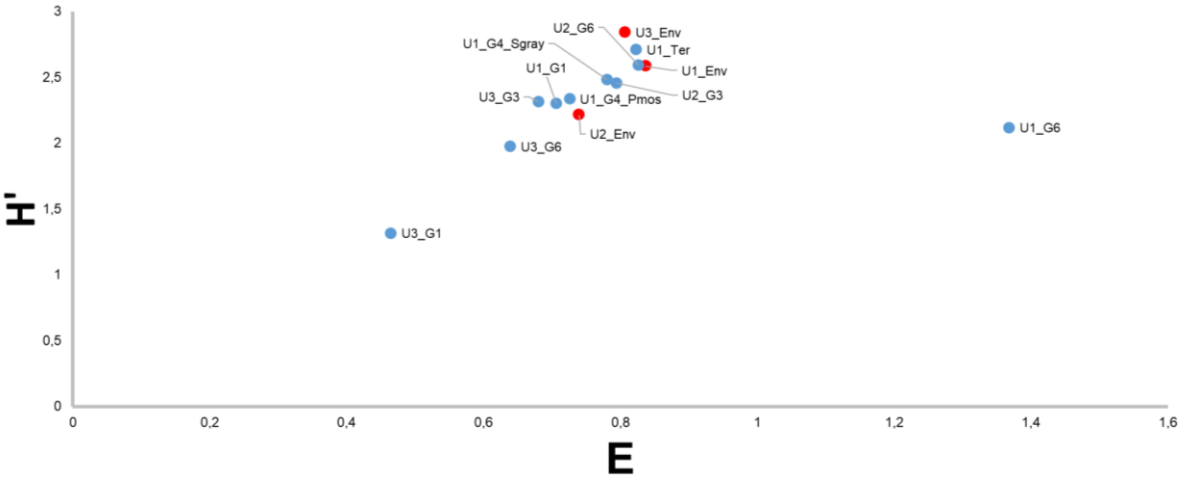


Figure 3.12: Scatter plot of Shannon diversity index (H') against evenness (E) for samples taken at Ukutula. Environmental samples are indicated by red points (U1_Env: Environmental sample taken at site 1; U2_Env: Environmental sample taken at site 2; U3_Env: Environmental sample taken at site 3. Tadpole guild samples are indicated by blue spots (U1_Ter: Terrapin (*Pomatomus galiata*) sample taken at site 1; U1_G1: *Phrynomantis bifasciatus* (Guild 1, Suspension feeder) sampled at site 1; U1_G4_Pmos: *Ptychadena mossambica* (Guild 4, Benthic type 2) sampled at site 1; U1_G4_Sgray: *Strongylopus grayii* (Guild 4, Benthic type 2), and U1_G6 *Amietia delalandii* (Guild 6, Rheophilic) sampled at site 1; U2_G3*: Kassina senegalensis* (Guild 3, Lentic-nektonic) sampled at site 2; U2_G6: *Amietia delalandii* (Guild 6, Rheophilic) sampled at site 2; U3_G3: *Kassina senegalensis* (Guild 3, Lentic-nektonic) sampled at site 3; U3_G1: *Phrynomantis bifasciatus* (Guild 1, suspension feeder) sampled at site 3; U3_G6: *A. delalandii* (Guild 6, Rheophilic) sampled at site 3.

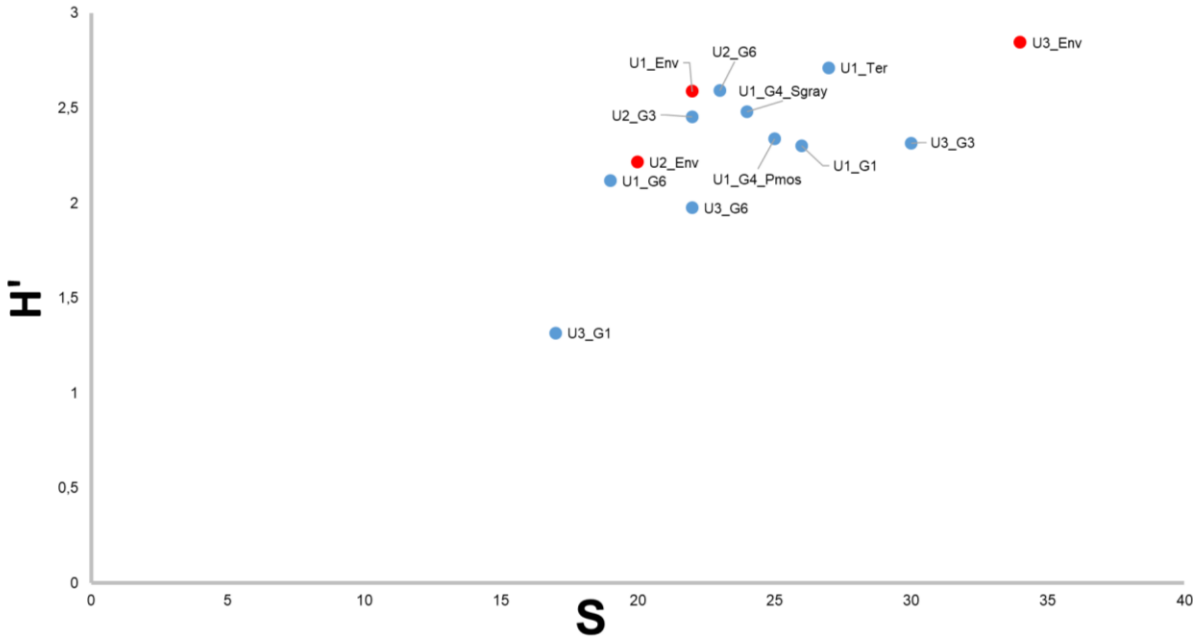


Figure 3.13: Scatter plot of Shannon diversity index (H') against species richness (S) for samples taken at Ukutula. Environmental samples are indicated by red points (U1_Env: Environmental sample taken at site 1; U2_Env: Environmental sample taken at site 2; U3_Env: Environmental sample taken at site 3. Tadpole guild samples are indicated by blue spots (U1_Ter: Terrapin (*Pomatomus galiata*) sample taken at site 1; U1_G1: *Phrynomantis bifasciatus* (Guild 1, Suspension feeder) sampled at site 1; U1_G4_Pmos: *Ptychadena mossambica* (Guild 4, Benthic type 2) sampled at site 1; U1_G4_Sgray: *Strongylopus grayii* (Guild 4, Benthic type 2), and U1_G6 *Amietia delalandii* (Guild 6, Rheophilic) sampled at site 1; U2_G3*: Kassina senegalensis* (Guild 3, Lentic-nektonic) sampled at site 2; U2_G6: *Amietia delalandii* (Guild 6, Rheophilic) sampled at site 2; U3_G3: *Kassina senegalensis* (Guild 3, Lentic-nektonic) sampled at site 3; U3_G1: *Phrynomantis bifasciatus* (Guild 1, suspension feeder) sampled at site 3; U3_G6: *A. delalandii* (Guild 6, Rheophilic) sampled at site 3.

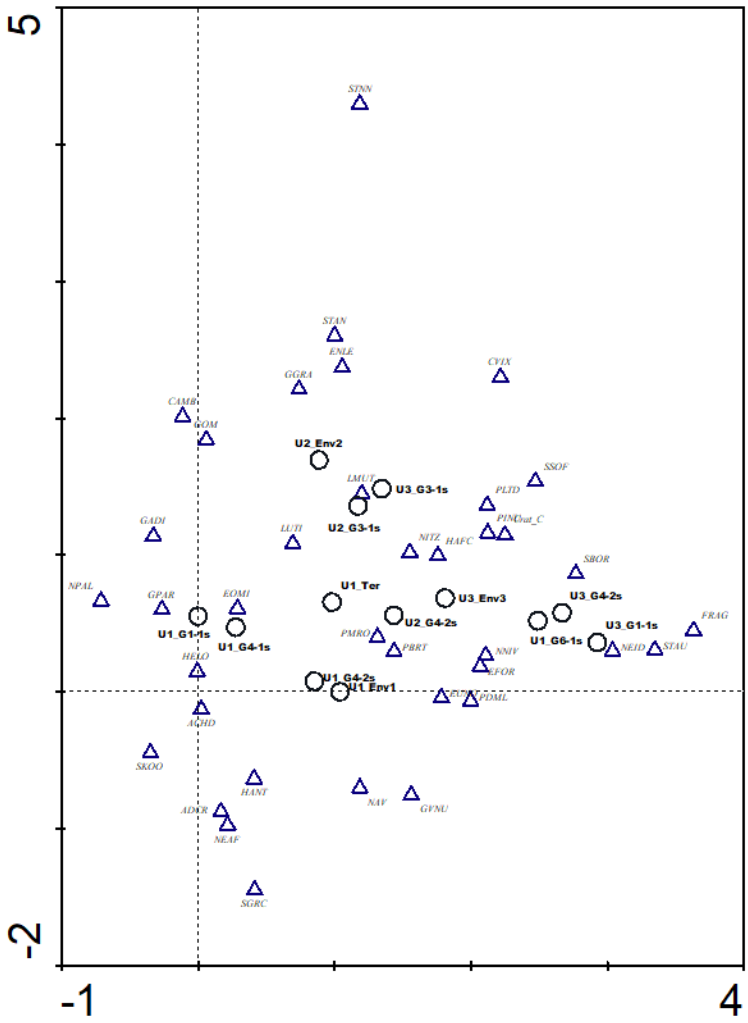


Figure 3.14: A Detrended Correspondence Analysis (DCA) representing diatom species (triangles) in relation to samples (circles) taken at Ukutula. Samples include U1_Env_1 (Environmental sample taken at site 1), U1_Ter_1 (Terrapin sampled at site 1) U1_A del (Rheophilic guild sampled at site 1), U1_G1,1_1 (Suspension feeder guild taken at site 1), U1_P mos (Benthic type 2(profundal) guild sampled at site 1), U1_G6,2_1 (Rheophilic guild sampled at site 1), U2_Env_2 (Environmental sample taken at site 2), U2_G3,3_2 (Lentic-nektonic guild sampled at site 2), U2_G6,2_2 (Rheophilic guild sampled at site 2), U3_Env_3 (Environmental sample taken at site 3), U3_G3,3_3 (Lentic-nektonic guild sampled at site 3), U3_G1,1_2 (Suspension feeder guild sampled at site 3), and U3_G6,2_3 (Rheophilic guild sampled at site 3). See Annexure B for diatom species code.

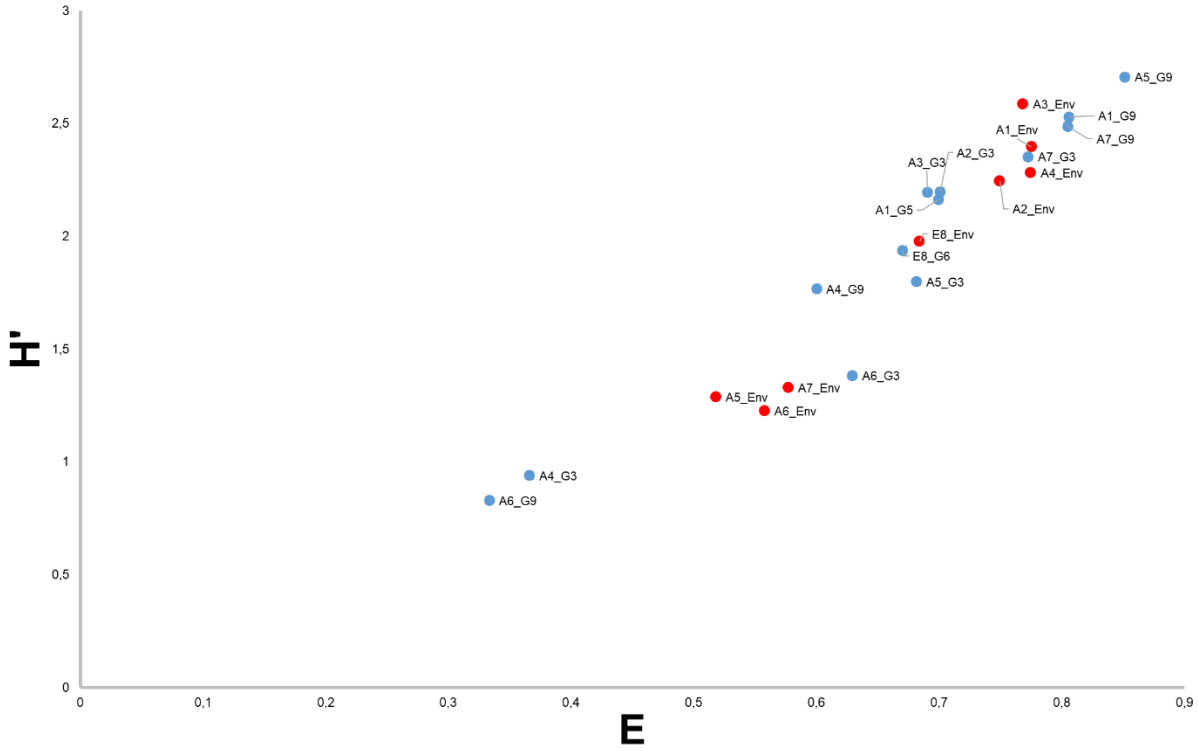


Figure 3.15: Scatter plot of Shannon diversity index (H') against evenness (E) for samples taken at Aliwal North. Environmental samples are indicated by red points and includes sites 1 -9 labelled as sites A1_Env – A9_Env. Samples includes A1_G9; *Cacosternum boettgeri* from guild 9 (Lentophytophilic) taken at site 1, A2_G5; *Amietophrynus rangeri* from guild 5 (Lentic-benthic) taken at site one, A2_G3; *Kassina senegalensis* from guild 3 (Lentic-nektonic) taken at site 2, A3_G3; *Kassina senegalensis* from guild 3 (Lentic-nektonic) taken at site 3, A4_G3; *Kassina senegalensis* from guild 3 (Lentic-nektonic) taken at site 4, A4_G9; *Cacosternum boettgeri* from guild 9 (Lentophytophilic) taken at site 4, A5_G3; *Kassina senegalensis* from guild 3 (Lentic-nektonic) taken at site 3, A5_G9*; Cacosternum boettgeri* from guild 9 (Lentophytophilic) taken at site 5, A6_G3; *Kassina senegalensis* from guild 3 (Lentic-nektonic) taken at site 6, A6_G9; *Cacosternum boettgeri* from guild 9 (Lentophytophilic) taken at site 5, A7_G3*; Kassina senegalensis* from guild 3 (Lentic-nektonic) taken at site 7, A7_G9; *Cacosternum boettgeri* from guild 9 (Lentophytophilic) taken at site 7, and A8_G6; *Amietia delalandii* from guild 6 (Rheophilic) taken at site 8.

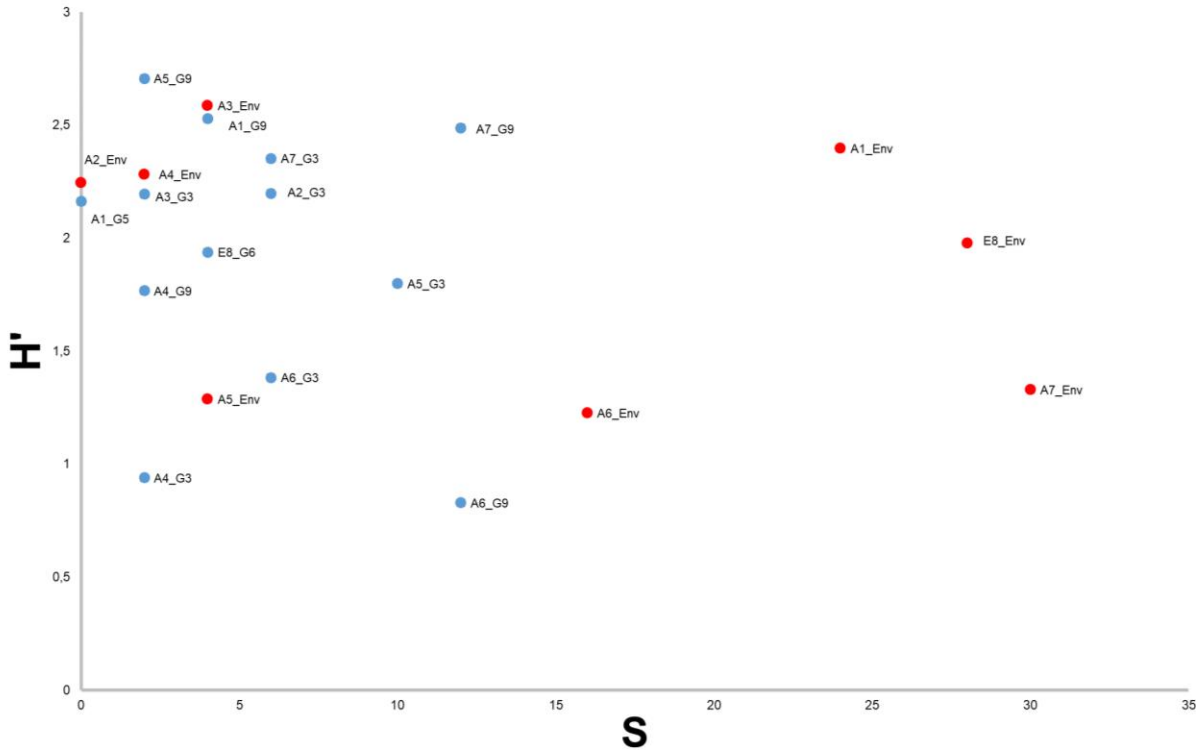


Figure 3.16: Scatter plot of Shannon diversity index (H') against richness (S) for samples taken at Aliwal North. Environmental samples are indicated by red points and includes sites 1 -9 labelled as sites A1_Env – A9_Env. Samples includes A1_G9; *Cacosternum boettgeri* from guild 9 (Lentophytophilic) taken at site 1, A2_G5; *Amietophrynus rangeri* from guild 5 (Lentic-benthic) taken at site one, A2_G3; *Kassina senegalensis* from guild 3 (Lentic-nektonic) taken at site 2, A3_G3; *Kassina senegalensis* from guild 3 (Lentic-nektonic) taken at site 3, A4_G3; *Kassina senegalensis* from guild 3 (Lentic-nektonic) taken at site 4, A4_G9; *Cacosternum boettgeri* from guild 9 (Lentophytophilic) taken at site 4, A5_G3; *Kassina senegalensis* from guild 3 (Lentic-nektonic) taken at site 3, A5_G9*; Cacosternum boettgeri* from guild 9 (Lentophytophilic) taken at site 5, A6_G3; *Kassina senegalensis* from guild 3 (Lentic-nektonic) taken at site 6, A6_G9; *Cacosternum boettgeri* from guild 9 (Lentophytophilic) taken at site 5, A7_G3*; Kassina senegalensis* from guild 3 (Lentic-nektonic) taken at site 7, A7_G9; *Cacosternum boettgeri* from guild 9 (Lentophytophilic) taken at site 7, and A8_G6; *Amietia delalandii* from guild 6 (Rheophilic) taken at site 8.

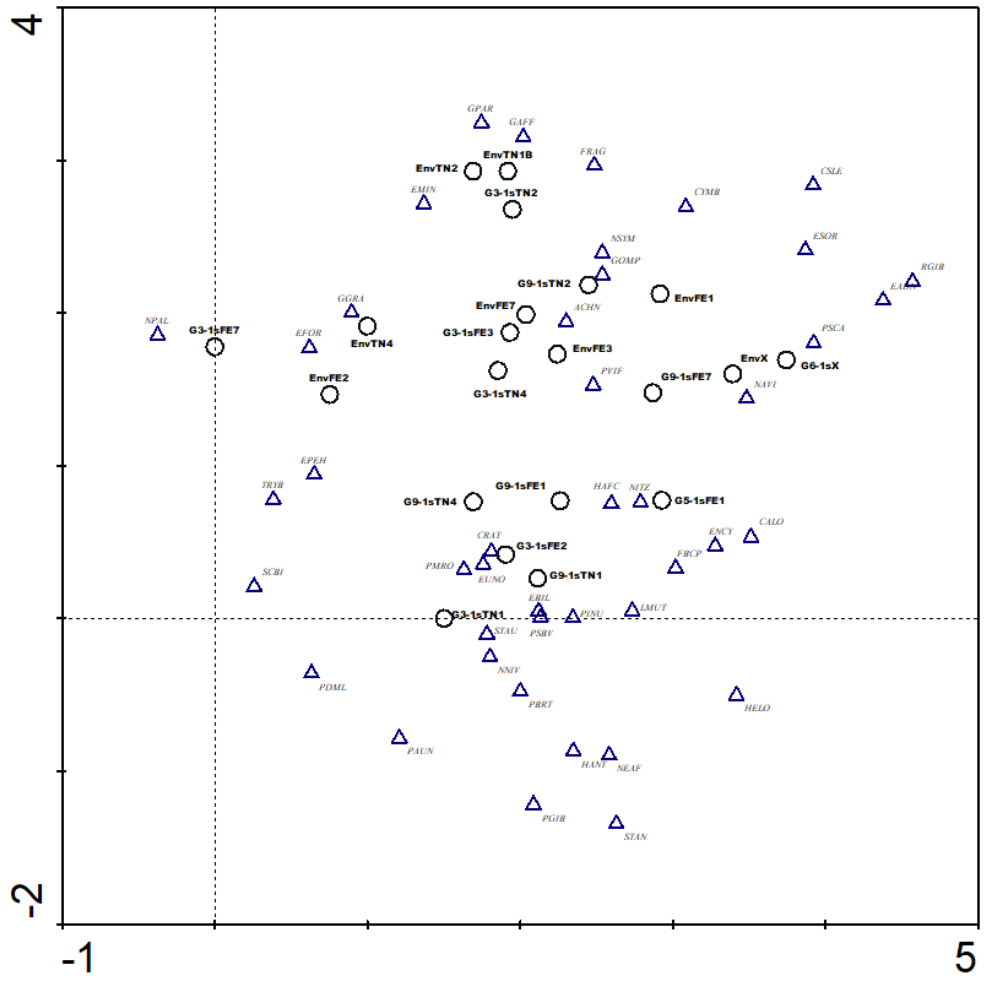


Figure 3.17: A Detrended Correspondence Analysis (DCA) representing diatom species (triangles) in relation to samples (circles) taken at Aliwal North. Environmental samples include EnvFE1, EnvFE2, EnvFE3, EnvFE7, EnvTN1B, EnvTN2, EnvTN4, and EnvX taken at sites 1, 2, 3, 4, 5, 6, 7, and site 8 respectively. Samples includes G9-1sFE1; *Cacosternum boettgeri* from guild 9 (Lentophytophilic) taken at site 1, G5-1sFE1; *Amietophrynus rangeri* from guild 5 (Lentic-benthic) taken at site 1, G3-1sFE2; *Kassina senegalensis* from guild 3 (Lentic-nektonic) taken at site 2, G3-1sFE3 ; *Kassina senegalensis* from guild 3 (Lentic-nektonic) taken at site 3, G3-1sFE7; *Kassina senegalensis* from guild 3 (Lentic-nektonic) taken at site 4, G9-1sFE7; *Cacosternum boettgeri* from guild 9 (Lentophytophilic) taken at site 4, G3-1sTN1B; *Kassina senegalensis* from guild 3 (Lentic-nektonic) taken at site 5, G9-1sTN1B; *Cacosternum boettgeri* from guild 9 (Lentophytophilic) taken at site 5, G3-1sTN2; *Kassina senegalensis* from guild 3 (Lentic-nektonic) taken at site 6, G9-1sTN2; *Cacosternum boettgeri* from guild 9 (Lentophytophilic) taken at site 6, G3-1sTN4; *Kassina senegalensis* from guild 3 (Lentic-nektonic) taken at site 6, G9-1sTN4; *Cacosternum boettgeri* from guild 9 (Lentophytophilic) taken at site 7, and G6-1sX; *Amietia delalandii* from guild 6 (Rheophilic) taken as Site 8. See Annexure B for diatom species codes.

3.4. Diatoms habitat summary

Habitat usages of the 91 diatom species and genera were studied and summarized after consulting scientific literature. This summary collectively entailed diatoms from the study locations from Ukutula and Aliwal North. Diatom characteristics related to habitat locality included; attachment (unattached, attached), motility (motile, highly motile); relevant habitat types (benthic, periphytic, epipelic, epilithic, epiphytic, littoral zone, planktonic, and habitats prone to periodic desiccation), and substrate type (habitat substrate, organic detritus, moss, dry moss, plants, soil, moist soil, and wood).

Epilithic diatoms grow on the surface of stone and rocks (Diatoms Of North America, 2020). These diatoms grow or live on sediments such as mud, clay and slit. Periphytic diatoms live in benthic habitats, attached to solid substrates. Epiphytic diatoms grow on plants or algae. Epidendron diatoms predominantly grow on wood (also known as epixylic diatoms). Planktonic diatoms float on the surface of the water column. This information was used to construct Table 3.7, summarizing habitat occurrences.

Genera and species excluded from this summary due to lack of information, includes *Cyclotella*, *Denticula, Diadesmis*, *Encyonema*, *Frustulia, Geissleria*, *Gyrosigma*, *Mayamaea*, *Neidium*, *Achnanthes swazi, Brachysira neoexilis, Craticula perrotettii, Craticula vixnegligenda, Cymbella cistula, Cymbella neocistula, Diploneis oblongella, Encyonema ventricosum, Encyonopsis leei, Epithemia adnata, Gomphonema lagenula, Gomphonema venusta, Hantzschia elongata, Luticola kotschyi, Luticola mutica, Mayamaea atomus, Nitzschia microcephala, Pinnularia acrosphaeria, Pinnularia divergens* var. *sublinearis, Pinnularia microstauron* var. *rostrata, Pinnularia subbrevistriata, Planothidium frequentissimum, Sellaphora pupula, Stauroneis sofia, Stauroneis dracomontana,* and *Tryblionella littoralis.*

Table 3.7: Diatom habitat usage summary. See Annexure B for diatom species codes.

Diatom codes	Attachment		Motility		Relevant Habitat types							Substrate type							
	Unattached	Attached	Moderately motile	Highly motile	Benthic	Epipelic	Epilithic	Epiphytic	Littoral zone	Planktonic	Periodic desiccation	Habitat substrate	Organic detritus	Moss	Dry moss	Plants	Soil	Moist soil	Wood
ACHD					x	x													
ACHN						x	x							x		x			
ADCR			x		x														
CALO					x	x								x					
CCRU	x		x																
COCO					x														
CPTG			x		x		x									x			x
CRAT					x														
CAMB	x		x			x													
CRBU	x		x		x														
CAGI			x		x	x	x												
CSLE		x	x		x														
CYMB						x													
CSLP		x	x		x														
CTUM									x										
EOMI			x										x						
ESOR		x	x		x														
EUNO												x							
EBIL					x														
EFOR	x		x																
EMIN							x												
EPEH							x	x											x
FRAG		x																	
FANC		x																	
FBCP						x													
GOMP		x										x							
GACU						x													
GAFF					x														

GADI					x							x							
GGRA					x	x													
GLTC						x													
GPAR					x														
GPUM						x													
HANT																	x		
HAFC																	x		
LEMN								x											
LUTI4														x			x		
MCCT																		x	
NCRY	x		x		x														
NRCS	x				x														
NAVI					x	x													
NSYM	x				x														
NVTA	x		x																
NEAF						x	x	x											
NATG			x		x														
NCLA	x		x		x														
NPAL					x					x									
NITZ						x				x									
NVLC			x		x														
PBRT					x		x										x		
PDML					x	x													
PGIB					x														
PINU1						x					x							x	
PSCA											x	x							
PVIF						x	x												
PLTD1												x							
RABB		x	x		x														
RGIB		x	x		x														
SELL							x												
SMNA					x														
STAN	x		x		x														
SBOR																	x		
SGRC	x				x										x		x		
SKOO	x		x		x														
STAU					x									x			x		
SSRU	x		x		x														

SCBI						x				x										
SOVI	x				x															
TCOA					x															
TRYB						x														

3.5. Micrographs

A total of 165 images were collected of various diatom species (Figure 3.18), with the exceptions of *Achnanthes* sp. 1, A. s*wazi*, *B. neoexilis, Caloneis* sp. 4, *Capartogramma crucicula, Cocconeis* sp. 1*, Craticula ambigua, Craticula* sp. 1*, Craticula* sp. 2*, Craticula* sp. 4, *Craticula* sp. 10, *C. vixnegligenda, Cyclotella* sp. 3*, Cymbella* sp. 3*, Denticula* sp. 1, *Diadesmis* sp. 1, *Encyonema* sp. 1*, Encyonema* sp. 2*, Encyonema* sp. 4*, E. adnata, E. formica, Eunotia pectinalis, Eunotia* sp. 4-6*, Eunotia* sp. 8-10, *Eunotia* sp. 12, *Eunotia* sp. 14, *Eunotia* sp. 16, *Fragilaria* sp. 6, *Fragilaria* sp. 7, *Fragilaria* sp. 9, *Fragilaria* sp. 10, *Frustulia* sp. 1*, Gomphonema affine, G. lagenula, Gomphonema* sp. 4, *Gomphonema* sp. 6-9, *Gomphonema* sp. 13*, Gomphonema* sp. 14*, Hantzschia* sp. 2, *Hantzschia* sp. 4*, Luticola* sp. 2, *Luticola* sp. 4, *Luticola* sp. 5*, Mayamaea* sp. 1*, Navicula* sp. 3*, Navicula* sp. 5-10, *Neidium* sp. 4, *Nitzschia clausii, N. microcephala, Nitzschia* sp. 4, *Nitzschia* sp. 11, *Nitzschia* sp. 13-15, *Pinnularia* sp. 5, *Pinnularia* sp. 8, *Pinnularia* sp. 10-16, *Pinnularia* sp. 18*, Pinnularia* sp. 20, *Planothidium* sp. 1, *Sellaphora* sp. 2, *Stauroneis* sp. 4, *Stauroneis* sp. 5*, Stauroneis* sp. 7, *Stauroneis* sp. 10, *Stauroneis* sp. 11*, Stauroneis* sp. 14, and *Tryblionella* sp. 1. These exceptions were attributable to inadequate positioning of frustules in the field of view, broken frustules or visual obstructions.

4. DISCUSSION

4.1. Diatom habitat utilization

An abundant number of diatoms were observed during the course of this study (Tables 3.3 and 3.5). Diatoms have several features which would allow them to survive an array of habitat types. For example, motile diatoms are characteristic of sites with loose and fine sediment (Detenbeck *et al.*, 2000; Fore & Grafe, 2002; Kutka & Richards, 1996). They can move through the water column and avoid being buried in the sediment. Some diatoms can attach to artificial and/or natural surfaces (Whitton, 1975). Other diatoms are planktonic, floating in the water column or near the surface (Necchi, 2016).

Environmental, spatial and temporal factors often function as filters, influencing biotic community structures (Heino *et al.*, 2012; Heino *et al.*, 2014; Jyrkänkallio-Mikkola *et al.*, 2018; Pan *et al.*, 2000; Poff, 1997; Potapova & Charles, 2002; Soininen *et al.*, 2016; Southwood, 1977; Verleyen *et al.*, 2009). Diatoms often respond specifically to salinity, pH, nutritional content, and saprobity of a water body (Bellinger *et al.*, 2006). They are also strongly affiliated with eutrophication and sodium ion levels (Licursi & Gómez, 2002). Substrate type, size, and availability can also lead to differences in community composition (Stevenson *et al.*, 1996).

These habitats and environmental factors were summarized in Table 3.7. It was further used to document diatom species found collectively from environmental and tadpole samples taken at all study locations from Aliwal North and Ukutula.

According to the literature, some unattached diatoms seem to exhibit a degree of motility (examples found included *Capartogramma crucicula* and *E. formica*) (Diatoms Of North America, 2020; Ross, 1963; Taylor *et al.*, 2007). However, some attached diatoms are also motile (examples found included *Cymbella subleptoceros* and *Cymbella silesiaca*) (Diatoms Of North America, 2020; Li *et al.*, 2007; Taylor *et al.*, 2007).

Most of the diatoms found during this study were benthic diatoms. Examples included *Cocconeis* sp., *Cocconeis placentula*, and *Craticula* sp. (Caljon & Cocquyt, 1992; Diatoms Of North America, 2020; Stevenson *et al.*, 1996). The abundance of benthos diatoms is consequential to resuspension in the water column. Numerous benthic diatoms recognized during this study are also motile. Examples of such diatoms included *Epithemia sorex*,

Cymbella sp., and *Craticula buderi*. The epipelic zone exists between the interface of water and sediment. Examples of epipelic diatoms found included *Caloneis* sp., *Achnanthidium* sp., and *Cymbella aspera* (Hall & Smol, 2010; Kociolek & Spaulding, 2003; Ward *et al.*, 2005).

Diatoms residing in epilithic habitats include those that typically grow on the surface of rocks or stone. Some epilithic diatoms found in this study included *Eunotia minor*, *Eunotia pectinalis* and *Gomphonema laticollum.* Epiphytic diatoms typically grow on the surface of aquatic plants or other algae. It is also referred to as the periphyton. Such diatoms found included *Staurosira construens, Surirella ovalis*, and *Tryblionell*a sp motile (Diatoms Of North America, 2020; Hill *et al.*, 2001; Taylor *et al.*, 2007). The littoral zone is a section of the water body that is closest to the shore (*Cymbella tumida*) (Venkatachalapathy & Karthikeyan, 2013). Planktonic diatoms are frequently found throughout the water column, This included *Nitzschia* sp,, *Nitzschia palea,* and *S. construens.* Species known to survive periodic desiccation found included *Pinnularia* sp., *Pinnularia subcapitata,* and *S. construens*. These are hardy species that often survive in environments with minimal to no moisture (Riato *et al.*, 2014; Silva-Lehmkuhl *et al.*, 2019).

Various diatom species typically distinguishing between substrate types (organic detritus, moss, dry moss, plants, soil, moist soil, and wood), were also recorded in Table 3.7.Examples of such species found during this study included *Achnanthidium* sp (moss), *Stauroneis gracilis* (dry moss), *C. placentula* (plants), *Hantzschia* sp. (soil), *Microcostatus* sp. (moist soil), and *E. pectinalis* (wood) (Diatoms Of North America, 2020; Taylor *et al.*, 2007). Here follows a more comprehensive outline of the habitat utilization regarding diatom species found during this study. The genera and species discussed follow the same order as listed in Table 3.7. The descriptions include diatom characteristics involved in habitat selection, such as attachment, motility, relevant habitat types, and substrate type. The corresponding figures (Figures 3.18.1 to 3.18.165) are listed alongside the descriptions, where available. Some descriptions, however, are brief due to the limited extent of available literature.

Genera excluded from this summary include; *Denticula* sp.*, Diadesmis* sp.*, Frustulia* sp*, Mayamaea* sp*., Craticula vixnegligenda*, *Cymbella cistula*, *Encyonopsis leei Krammer* var. *leei*, *Gomphonema lagenula*, H*antzschia elongate*, *Luticola mutica*, *Nitzschia microcephala*, *Pinnularia acrosphaeria*, *Pinnularia divergens* var. *sublinearis*, and *Stauroneis dracomontana.* These exclusions are attributed to fragmented information in literature pertaining to habitat specifications.

Genus: *Achanthidium*

The genus *Achanthidium* (Figures 3.18.1 to 3.18.11) has more than 200 taxa and are monoraphid, freshwater diatoms (Fourtanier & Kociolek, 2009). They are often used as indicators. Identifying these diatoms to species level remains a challenge due to the lack of comprehensive descriptions and their small valves (Marquardt *et al.*, 2017).

Achanthidium are mostly benthic and are often found in both unimpacted and polluted waters. In fact, some *Achanthidium* species (*Achanthidium lanceolata*) are found in depressions and crevices of sand grains; this protects them from vigorous water movement (Biggs, 1996). *Achanthidium minutissimum* attaches to substrates in rapidly flowing streams (Wehr, 2003).

Achanthidium crassum (Figure 3.18.10) is found in alkaline streams and slow-moving water (Taylor *et al.*, 2007). They are often part of the benthic community structure (Diatoms Of North America, 2020; Potapova & Ponader, 2004). They are slightly motile and most prominent in rivers (Diatoms Of North America, 2020).

Genus: *Achnanthes*

Diatoms from the *Achnanthes* genus are generally habitat-specific; and some members demonstrate a high affinity to stone, plant, and/or sediment substrata (Lim *et al.*, 2001; Soininen & Eloranta, 2004). In fact, one study found a large amount of *Achnanthes* diatoms in moss samples (Antoniades & Douglas, 2002).

It's furthermore considered an early colonizer (Soininen & Eloranta, 2004). It was also discovered in fast- and slow-moving water, and its presence is attributed to the availability of substrates for attachment (Antoniades & Douglas, 2002). *Achnanthes swazi* is endemic to South Africa and can be found in clean, well-oxygenated oligotrophic freshwater (Taylor *et al.*, 2007).

Brachysira neoexilis

This is a cosmopolitan species, inhabiting clean, oligo-to mesotrophic water (Figure 3.18.12). But it additionally occurs in acidic and electrolyte poor water. It can also be found in weakly alkaline habitats (Taylor *et al.*, 2007). Diatoms from the *Brachysira* genus are predominantly located in the mid-depth zone of a water body (Cantonati *et al.*, 2009).

Genus: *Caloneis*

Diatoms from the *Caloneis* genus are common in alkaline, brackish, and even marine water (Diatoms Of North America, 2020) (Figures 3.18.13 to 3.18.15). They are also found in the benthos, soils, and moss of water bodies (Diatoms Of North America, 2020).

Capartogramma crucicula

This is a tropical or subtropical species (Taylor *et al.*, 2007) (Figure 3.18.16). It is found in fresh and somewhat brackish water (Diatoms Of North America, 2020; Ross, 1963). It does not attach to surfaces and is moderately motile (Diatoms Of North America, 2020).

Genus: *Cocconeis*

Diatoms from the *Cocconeis* genus are generally low-growing (Stevenson *et al.*, 1996). Low-growing diatoms are typically found in the benthos of aquatic habitats.

Cocconeis placentula can tolerate various levels of alkalinity (Caljon & Cocquyt, 1992) (Figure 3.18.17. They attach to surfaces in benthic habitats, like plants, wood, and stone (Diatoms Of North America, 2020; Taylor *et al.*, 2007). They are also weakly motile (Diatoms Of North America, 2020).

Genus: *Craticula*

Diatoms from the *Craticula* genus typically inhabit the benthic zone of freshwater or brackish water (Round *et al.*, 2007) (Figures 3.18.21 to 3.18.25). They are ordinarily observed on top of the sediment layer in the benthos. Some *Craticula* species are remarkably tolerant of organic pollution (Levkov *et al.*, 2016). Some are so versatile, being able to survive temporary water bodies and dry periods (Levkov *et al.*, 2016). Other species are even documented in Antarctica (Van de Vijver *et al.*, 2010).

Craticula ambigua is a cosmopolitan species typically inhabiting the epipelic zone of benthic environments, albeit they might occur in a range of diverse habitats (Diatoms Of North America, 2020; Levkov *et al.*, 2016; Taylor *et al.*, 2007) (Figure 3.18.18). They are moderately motile, unattached diatoms. They are also frequently in organic material or fine sediments (Diatoms Of North America, 2020; Levkov *et al.*, 2016).

Craticula buderi is a freshwater diatom inhabits water with higher electrolyte content (Taylor *et al.*, 2007) (Figure 3.18.19). It can also tolerate impacted conditions such as mine waste (Taylor *et al.*, 2007). It is a moderately motile diatom, typically found in benthic habitats. It does not attach to any substrates (Diatoms Of North America, 2020). *Craticula perrotettii* typically inhabits oligotrophic environments in fresh or slightly brackish water (Taboada *et al.*, 2017) (Figure 3.18.20).

Genus: *Cyclotella*

Diatoms from this genus form filaments when attaching to each other via marginal spines or faces. They may also be solitary cells or occur in pairs (Cox & Cox, 1996) (Figures 3.18.26 and 3.18.27).

Genus: *Cymbella*

Diatoms from the *Cymbella* genus are mostly periphytic (Hall & Smol, 2010; Ward *et al.*, 2005) (Figures 3.18.30 and 3.18.33). These diatoms are stalked and likely to survive in high UVR habitats (Hall & Smol, 2010). Diatoms from this genus are also the most diverse of the Cymbellaceae family (Kociolek & Spaulding, 2003).

Cymbella silesiaca is slightly motile (Diatoms Of North America, 2020). They are also found in benthic habitat of freshwater systems (Diatoms Of North America, 2020) (Figure 3.18.29). *Cymbella aspera* is a cosmopolitan species found in oligotrophic waters. It attaches to vertically substrata via dichotomous mucilage stalks (Diatoms Of North America, 2020; Taylor *et al.*, 2007) (Figure 3.18.31). It's slightly motile and occurs in the benthic region of the habitat (Diatoms Of North America, 2020). In some studies, this species was found in epilithic, epipelic, and epilithic habitats (Yildirim & Cetin, 2009).

Cymbella neocistula is a cosmopolitan species found in epiphytic and epilithic habitats. It also inhabits mesotrophic water (Taylor et al., 2007) (Figure 3.18.32). It is moderately motile and attaches vertically to benthic habitat surfaces (Diatoms Of North America, 2020; Vishnyakov *et al.*, 2015). *Cymbella tumida* typically inhabits oligotrophic or mesotrophic water, and lives in the littoral zone of flowing or standing water (Figure 3.18.34). It also tolerates low pollution levels (Venkatachalapathy & Karthikeyan, 2013). *Cymbella subleptoceros* is a cosmopolitan species found in mesotrophic water (Taylor *et al.*, 2007). It is slightly motile and attaches vertically to a benthic habitat (Diatoms Of North America, 2020). It typically inhabits an environment with a pH of 5-10 (Li *et al.*, 2007).

Diploneis oblongella

This diatom inhabits well-aerated, unimpacted and in some cases, mildly polluted water (Lukavský *et al.*, 2006; Taylor *et al.*, 2007) (Figure 3.18.35). It typically occupies the epiphytic section of the aquatic habitat (Alakananda *et al.*, 2012).

Genus: *Encyonema*

Diatoms from this genus can form colonies within mucilaginous tubes (Figure 3.18.38). They typically inhabit the benthos of aquatic water bodies (Diatoms Of North America, 2020).

Encyonema ventricosum is a cosmopolitan species found in alkaline, well-oxygenated water (Taylor *et al.*, 2007). It is also associated with an oligotrophic environment (Burfeid Castellanos, 2018) (Figure 3.18.37). They characteristically inhabit mucilage tubes and are able to move within it (Jyrkänkallio-Mikkola *et al.*, 2018). They cannot; however, withstand mechanical stress (Burfeid Castellanos, 2018).

Eolimna minima

This is a cosmopolitan species found in an extensive range of waters, even in heavily polluted environments (Figure 3.18.39). It may be associated with organic detritus or eutrophic water (Rimet *et al.*, 2009; Taylor *et al.*, 2007). They can also occur in wet and humid environments (Rimet *et al.*, 2009). They are motile diatoms, regarded as pioneers and can adapt fairly well to change (Rimet *et al.*, 2009).

Epithemia sorex

This diatom is moderately motile, and attaches to benthic habitats via the formation of prostrate structures (Diatoms Of North America, 2020) (Figure 3.18.40).

Genus: *Eunotia*

Eunotia diatoms are usually solitary or attached to one another. In some cases, they will attach to solid substrates via mucilage pads (Cox & Cox, 1996) (Figures 3.18.43 to 3.18.49). Eunotia are most found in clean, acidic to dystrophic water (Costa *et al.*, 2018)

Eunotia bilunaris typically inhabits weakly acidic, lentic or lotic water (Taylor *et al.*, 2007) (Figure 3.18.41). They attach to their habitat by forming prostate structures (mucilage pads). It is mostly a solitary diatom but can also form occasional colonies. It inhabits most habits, but favours benthic habitats (Diatoms Of North America, 2020). *Eunotia minor* inhabits a variety of environments, including pools and springs (Taylor *et al.*, 2007) (Figure 3.18.42). It typically inhabits the epilithic zone of these habitats (Kim & Lee, 2017). It is generally found in acidic water (Ortiz-Lerín & Cambra, 2007).

Eunotia pectinalis is an acidophilic and cosmopolitan species (Foged, 1981) (Figure 3.18.50). It ranges in distribution; from rare to abundant (Camburn & Charles, 2000; Siver, 2005). It also grows in the epiphyton, epidendron, epilithon, and metaphyton of freshwater bodies (Siver, 2005). They are weakly motile and will form colonies occasionally, but are mostly solitary (Diatoms Of North America, 2020). *E. formica* is found in standing or slow flowing water. Cells aggregate in colonies, joined by their valve faces (Taylor *et al.*, 2007). The colonies are usually unattached and slightly motile. If they attach, they do so by forming a prostate structure. It also inhabits moist environments, the benthic zone, and sediments (Diatoms Of North America, 2020).

Genus: *Fragilaria*

Diatoms from this genus are pennate, and they form chains linked by mucilage pads or spines. They also use these structures to attach to surfaces (Cox & Cox, 1996) (Figures 3.18.56 to 3.18.56). *Fragilaria biceps*

Fragilaria biceps is a cosmopolitan species found in the benthos of water bodies (Figure 3.18.51). It's easily suspended in the water column due to its large surface area. They are typically attached to a substrate (Taylor *et al.*, 2007). They are abundant in sandy habitats (Tornés & Sabater, 2010). This species has an erect form and cells are usually part of filamentous colonies, attaching to substrata via mucilage pads (Romagnoli *et al.*, 2014). It forms rosette colonies and they also use mucilage pads to attach to substrates. They often colonize sand grains in this way (Tornés & Sabater, 2010). The mucilage pads allow them to attach to substrates by providing large surface areas for attachment (Steinaman & McIntire, 1986).

Genus: *Geissleria*

Diatoms from this genus inhabits oligotrophic to eutrophic water, but they are more habitat specific on species level (Diatoms Of North America, 2020) (Figure 3.18.57).

Genus: *Gomphonema*

Diatoms from this genus are generally described as a bottom dwellers (Villac *et al.*, 2016) (Figures 3.18.64 to 3.18.70). They will sometimes attach to the substratum via mucilage pads or stalks. They are generally more likely to survive in habitats with high UVR levels (Hall & Smol, 2010).

Gomphonema acuminatum is typically found in the benthos (Caljon & Cocquyt, 1992) (Figure 3.18.58). They attach to benthic substrates by forming a prostate or ventral mucilage stalk (Diatoms Of North America, 2020; Taylor *et al.*, 2007). *Gomphonema affine* is a tropical/sub-tropical, benthic organism (Caljon & Cocquyt, 1992; Taylor *et al.*, 2007).

Gomphonema angustatum is a cosmopolitan, benthic tropical diatom; found predominantly in oligotrophic water (Caljon & Cocquyt, 1992; Taylor *et al.*, 2007) (Figure 3.18.59). One study states that this species aslo grows on artificial or solid habitats (Bere, 2010). *Gomphonema gracile* is a benthic, cosmopolitan tropical organism (Caljon & Cocquyt, 1992; Taylor *et al.*, 2007) (Figure 3.18.60). It can be found in electrolyte-rich environments (Taylor *et al.*, 2007). One study found this diatom predominantly in sand (Bere, 2010).

Gomphonema laticollum occurs in the benthos of an aquatic habitat (Caljon & Cocquyt, 1992) (Figure 3.18.61). It attaches to substrates via dichotomous mucilage stalks (Taylor *et al.*, 2007). *Gomphonema parvulum* is a benthic and euryhaline freshwater diatom (Caljon & Cocquyt, 1992) (Figure 3.18.62). It is also a cosmopolitan species found in a range of aquatic conditions and can be tolerant of elevated levels of pollution (Taylor *et al.*, 2007). They are found in habitats with low to excessive levels of metal ions (Ivorra *et al.*, 2002). They also occupy eutrophic environments (Lane & Brown, 2007).

Gomphonema pumilum is cosmopolitan diatom and found in meso- to eutrophic waters (Taylor *et al.*, 2007) (Figure 3.18.63). It is also documented in the mud of unpolluted waters, and in fresh to brackish waters (Wojtal, 2003). *Gomphonema venusta* inhabits weakly alkaline, oligo- to mesotrophic waters (Taylor *et al.*, 2007) (Figure 3.18.71).

Genus: *Gyrosigma*

Diatoms from this genus are typically found in epipelic and endopelic habitats (Diatoms Of North America, 2020) (Figure 3.18.72). They are also found in freshwater and brackish waters, but little else is known about them.

Genus: *Hantzschia*

Diatoms from the *Hantzschia* genus typically inhabits soil (Taylor *et al.*, 2007) (Figures 3.18.75 to 3.18.78). *Hantzschia amphioxys* is a common soil diatom (Jahn *et al.*, 2014) (Figure 3.18.73). It is also a cosmopolitan species found in periodically dry habitats such as crevices in rocks or soil and is often washed in from the surrounding areas (Taylor *et al.*, 2007).

Genus: *Lemnicola*

Cells from the *Lemnicola* genus grow on epithetic habitats on aquatic endosperms (Taylor *et al.*, 2007) (Figure 3.18.79).

Genus: *Luticola*

Diatoms from this genus are generally aerophilic and are typically found in soil and moss habitats (Diatoms Of North America, 2020) (Figures 3.18.82 and 3.18.83). They are also found in small puddles (Noga & Rybak, 2019). *Luticola kotschyi* is often found in thermal waters with elevated electrolyte content (Taylor *et al.*, 2007) (Figure 3.18.80). They also prefer elevated levels of nitrogen, especially organically bound nitrogen (Negadi *et al.*, 2018).

Mayamaea atomus

This species is usually associated with poor water quality (Kalyoncu & Serbetci, 2013) (Figure 3.18.84). They also indicate high nutrient levels of enrichment (Soeprobowati *et al.*, 2012).

Genus: *Microcostatus*

Diatoms from this genus are usually found in moist habitats such as soils or walls (Taylor *et al.*, 2007; Taylor *et al.*, 2010) (Figure 3.18.85).

Genus: *Navicula*

Diatoms from this genus are typically part of the benthic community (Blocksom & Johnson, 2009) (Figures 3.18.87 to 3.18.93). They are, more specifically, epipelic microphytobenthos biraphid diatoms (Steele *et al.*, 2009). They often dominate the benthos (Carter & Resh, 2013). *Navicula cryptocephala* is a benthic diatom, that's moderately motile and is unattached (Diatoms Of North America, 2020) (Figure 3.18.86). It occurs in weakly acidic to weakly alkaline water, and is tolerant to extreme pollution levels (Taylor *et al.*, 2007)

Navicula nivalis is a cosmopolitan and aerophilic species (Krammer & Lange-Bertalot, 1988) (Figure 3.18.87). It is also found in caves, in fresh water, and brackish water (Czerwik-Marcinkowska & Mrozińska, 2011). *Navicula recens* is a cosmopolitan species found in eutrophic waters (Figure 3.18.88). It's also found in brackish water. It is tolerant of elevated levels of pollution and is a free-living, unattached diatom (Diatoms Of North America, 2020; Taylor *et al.*, 2007). They typically inhabit the benthos of freshwater bodies (Diatoms Of North America, 2020).

Navicula symmetrica is a cosmopolitan species found in eutrophic waters and is tolerant to strongly polluted conditions (Taylor *et al.*, 2007) (Figure 3.18.94). It is a benthic, unattached diatom which is moderately motile (Diatoms Of North America, 2020). *Navicula veneta* is a cosmopolitan species, found in heavily eutrophic water. It is also found in brackish water and is highly tolerant of pollution (Taylor *et al.*, 2007) (Figure 3.18.95). It is an unattached, diatom inhabiting the benthos of freshwater ecosystems (Diatoms Of North America, 2020).

Genus: *Neidium*

Diatoms from the Neidium genus are not abundant, but they exemplify a broad distribution and often grow in acidic waters (Diatoms Of North America, 2020) (Figures 3.18.97 to 3.18.100). *Neidium affine* is a cosmopolitan species found in generally clean water (Taylor *et al.*, 2007) (Figure 3.18.96). It inhabits epipelic, epilithic, and epiphytic habitats (Cetin, 2008).

Genus: *Nitzschia*

Diatoms from the *Nitzschia* genus are often used as indicator species due to their physiology or performance in ecosystems (Stevenson *et al.*, 2010) (Figures 3.18.103 to 3.18.112). They are also tolerant of an array of pollutants (Stevenson *et al.*, 2010; Wang *et al.*, 2005).

They are considered low growing diatoms and are typically motile (Hill *et al.*, 2001; Stevenson *et al.*, 1996). They also grow on substrates in various habitats (epilithic, epipelic, rocks) (Stevenson, 1984).They are ordinarily epipelic or planktonic (Villac *et al.*, 2016).

Nitzschia valdecostata is a cosmopolitan species found in water with high concentrations of sulphates and carbonates (Taylor *et al.*, 2007) (Figure 3.18.113). It is also moderately motile in a benthic habitat (Diatoms Of North America, 2020). *Nitzschia clausii* is also a cosmopolitan species found in brackish water. It's also tolerant of extremely polluted waters (Taylor *et al.*, 2007). It is a moderately motile diatom, living unattached in a benthic habitat (Diatoms Of North America, 2020).

Nitzschia amphibia is found in eutrophic water (Taylor *et al.*, 2007) (Figure 3.18.101). It is motile and attaches to benthic habitats by forming a prostate structure (Diatoms Of North America, 2020). *Nitzschia palea* is a planktonic, benthic species (Figure 3.18.102). It is also classified as a euryhaline limnobiont (Caljon & Cocquyt, 1992). It is a cosmopolitan, abundant species found in eutrophic waters. It is also tolerant of extremely polluted environments (Taylor *et al.*, 2007). It is a motile diatom (Diatoms Of North America, 2020).

Genus: *Pinnularia*

Diatoms from the *Pinnularia* genus are mostly found in ponds and moist soil (Figure 3.18.122 – 3.18.132). They are also found in the sediment of the ocean and are mostly epipelic (Benke & Cushing, 2011; Diatoms Of North America, 2020).

Pinnularia borealis is a cosmopolitan benthic diatom (Caljon & Cocquyt, 1992) (Figures 3.18.115 and 3.18.116). It is typically found on rocks, soils, moss and in habitats prone to episodic desiccation (Riato *et al.*, 2014; Taylor *et al.*, 2007). It is also moderately motile (Diatoms Of North America, 2020).

Pinnularia divergens occurs in acidic, oligotrophic, electrolyte poor water (Taylor *et al.*, 2007) (Figures 3.18.117 and 3.18.118). One study found that diatoms from this genus occurred

exclusively in epipelic habitat samples (Yildirim & Cetin, 2009). Another study encountered it mostly on aquatic vegetation (Bere, 2010). It is established that diatoms from this genus are mostly epiphytic and benthic of nature (Nascimento *et al.*, 2010).

Pinnularia gibba is a cosmopolitan species, found mostly in the benthos of springs and small streams (Caljon & Cocquyt, 1992; Taylor *et al.*, 2007) (Figure 3.18.120). In one study, it was found on the vegetation at a single site (Bere, 2010). *Pinnularia microstauron* var. *rostrata* is a cosmopolitan species found in clean water with low electrolyte content (Taylor *et al.*, 2007) (Figure 3.18.121). *Pinnularia viridiformis* is a cosmopolitan species is typically found on substrates such as sand, stones and mud (Noga *et al.*, 2014) (Figure 3.18.133).

Pinnularia subcapitata is a cosmopolitan and found in oligotrophic electrolyte poor waters (Figure 3.18.134). It is also found on natural substrates and in habitats prone to episodic desiccation and droughts (Riato *et al.*, 2014; Silva-Lehmkuhl *et al.*, 2019). *Pinnularia subbrevistriata* is a widespread tropical to sub-tropical species diatom occurring in polluted water (Krammer, 2000; Taylor *et al.*, 2007).

Planothidium frequentissimum

This is a common species found in lotic and lentic water and is also capable of tolerating pollution in a wide variety of habitats (Olszyński *et al.*, 2019) (Figure 3.18.135).

Genus: *Planothidium*

Diatoms from this genus attach to substrates by forming an adnate structure near the raphe valve (Diatoms Of North America, 2020) (Figure 3.18.136).

Rhoicosphenia abbreviata

This is a benthic diatom and an euryhaline limnobiont (Caljon & Cocquyt, 1992) (Figure 3.18.167). This species typically inhabits electrolyte-rich and brackish waters. It is also tolerant of extremely high levels of pollution. The cells typically attach vertically to the substratum via mucilage stalks at the basal pole (Taylor *et al.*, 2007). Additionally, they are weakly motile (Diatoms Of North America, 2020).

Rhopalodia gibba

These diatoms typically grow in the benthos as an epiphyte (Diatoms Of North America, 2020) (Figures 3.18.139 and 3.18.140). It also the hosts endosymbiotic cyanobacteria, allowing the ability to survive in habitats with low nitrogen concentrations. They are also motile and attached to benthic habitats through the formation of prostate structures (Diatoms Of North America, 2020).

Genus: *Sellaphora*

Diatoms from the *Sellaphora* genus are widespread in alkaline and brackish water with a neutral pH (Diatoms Of North America, 2020) (Figure 3.18.142). One study found Sellaphora diatoms in epilithic habitats (Heudre *et al.*, 2018). *Sellaphora stroemii* is a cosmopolitan species found in cold, electrolyte rich water (Taylor *et al.*, 2007). It is also tolerant to a wide range of environmental factors, including humidity and dissolved oxygen (Falasco *et al.*, 2018).

Seminavis

This is a benthic diatom found (Stock *et al.*, 2020) (Figure 3.18.144).

Genus: *Stauroneis*

Diatoms from the *Stauroneis* genus are diverse and found largely in the benthos, soils and moss of wetlands and small lakes and ponds (Diatoms Of North America, 2020) (Figures 3.18.152 to 3.18.161). *Stauroneis biceps* is a moderately motile and unattached diatom (Figure 3.18.145). It lives in the benthos as a solitary diatom (Caljon & Cocquyt, 1992; Diatoms Of North America, 2020).

Stauroneis borrichii is found in several soil and wet moss samples (Van de Vijver *et al.*, 2004) (Figure 3.18.146). *Stauroneis gracilis* is a solitary, unattached diatom living in the benthos of the aquatic environments (Figure 3.18.147). But it's less frequent in soils and dry mosses (Diatoms Of North America, 2020; Van de Vijver *et al.*, 2004). *Stauroneis sofia* is present in small, nutrient deficient pools (Van de Vijver *et al.*, 2004) (Figure 3.18.148).

Staurosira construens is a benthic diatom (Figure 3.18.149). It attaches face-to-face, with the terminal cell attaching to the substrates (Diatoms Of North America, 2020). *Stauroneis*

kootenai is an unattached, motile diatom. It is found in moist habitats and in the benthos of the water body (Diatoms Of North America, 2020) (Figure 3.18.150). *Stauroneis superkuelbsii* is a motile, unattached diatom typically inhabiting benthic habitats (Diatoms Of North America, 2020) (Figure 3.18.162).

Surirella ovalis

This is a cosmopolitan species found in water with high electrolyte content (Taylor *et al.*, 2007) (Figure 3.18.163). It occurs in the benthic region of aquatic habitat (Diatoms Of North America, 2020). It does not attach to any substrates (Diatoms Of North America, 2020; Hill *et al.*, 2001).

Genus: *Tryblionella*

Diatoms from this genus are usually epipelic and found in freshwater and marine habitats (Diatoms Of North America, 2020). *Tryblionella coarctata* is a cosmopolitan species found in the benthos of brackish and fresh water (Park *et al.*, 2012; Ryabushko *et al.*, 2020; Taylor *et al.*, 2007) (Figure 3.18.164). *Tryblionella littoralis* is a cosmopolitan species found in brackish water (Taylor *et al.*, 2007) (Figure 3.18.165).

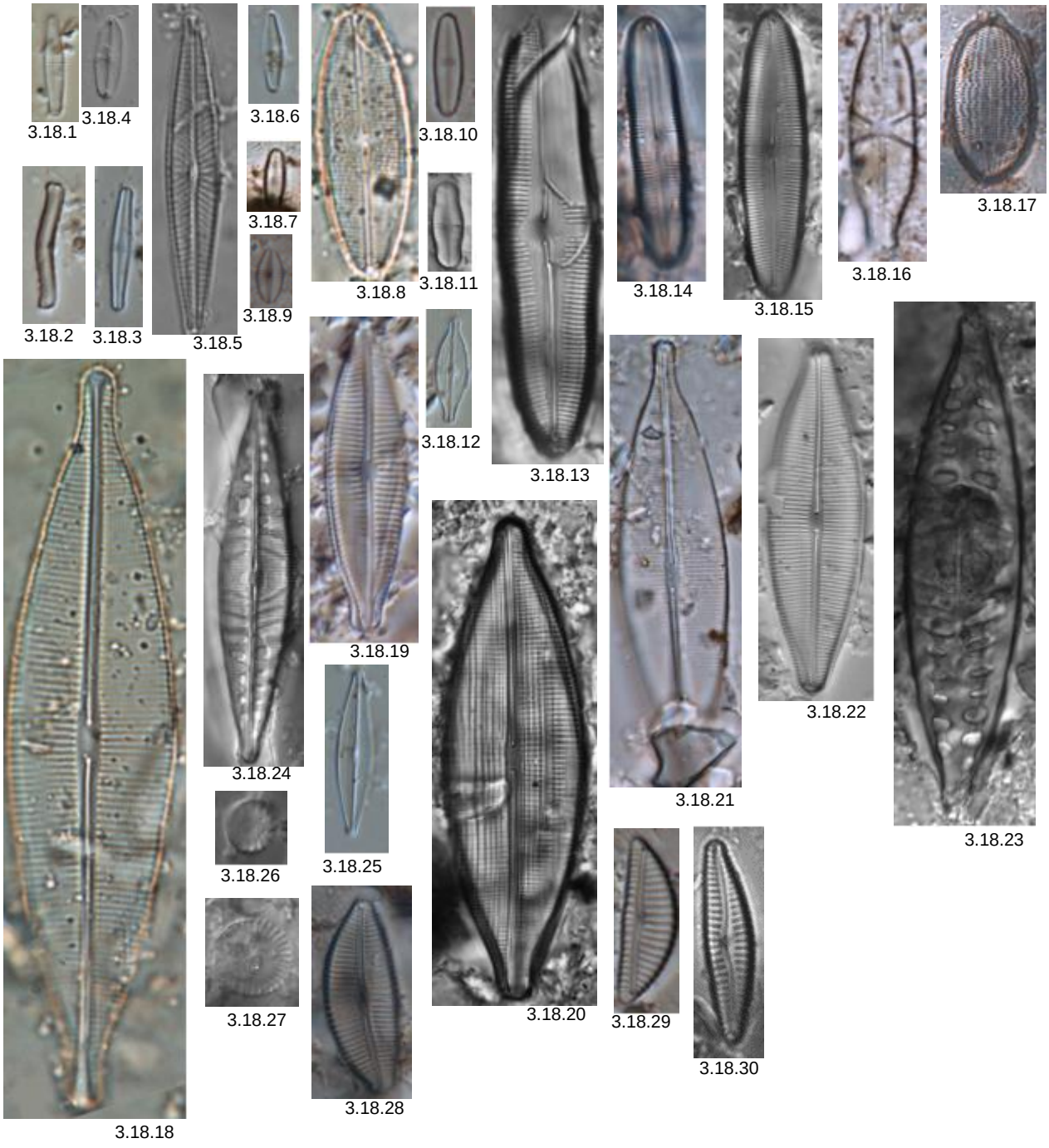
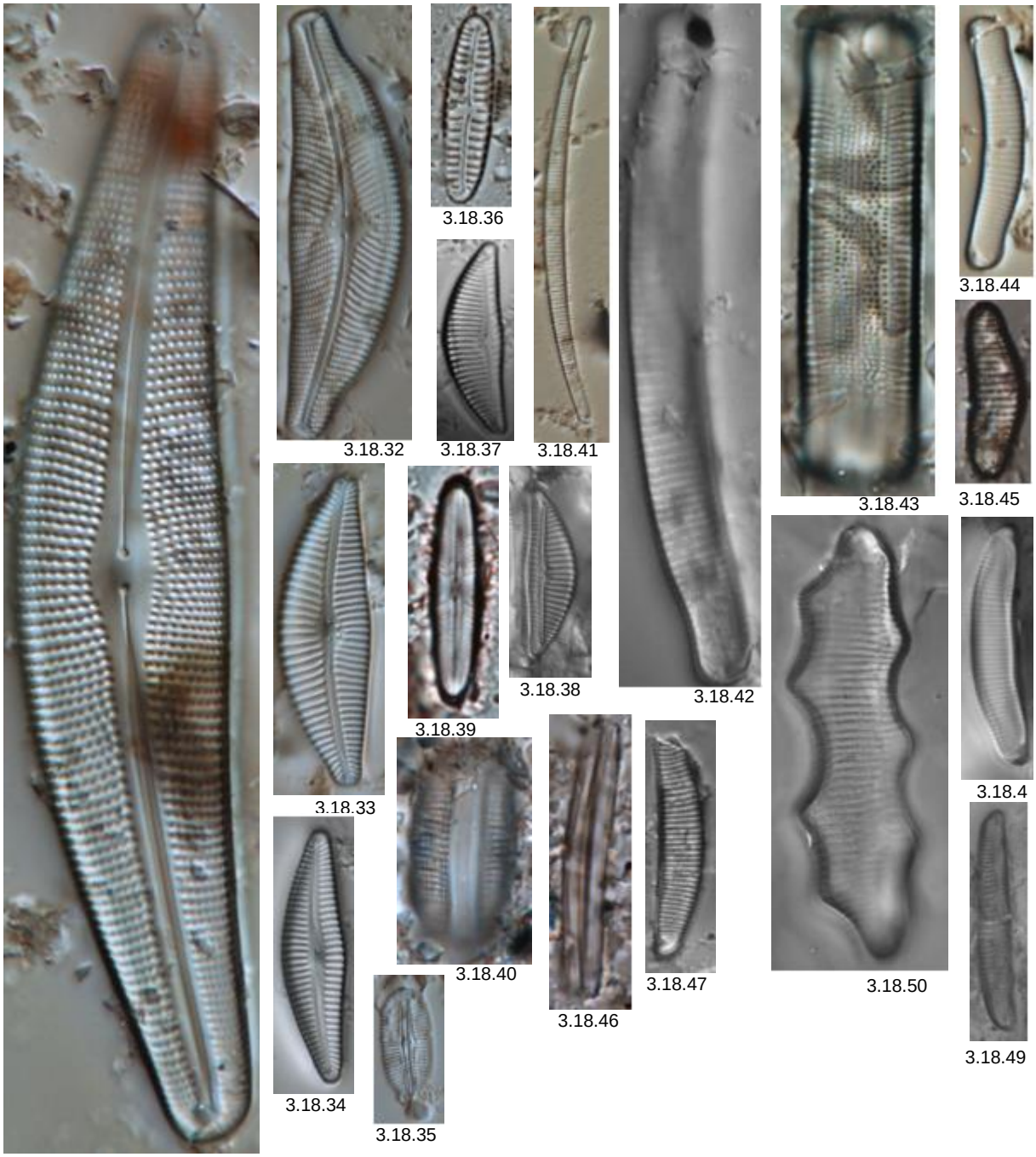
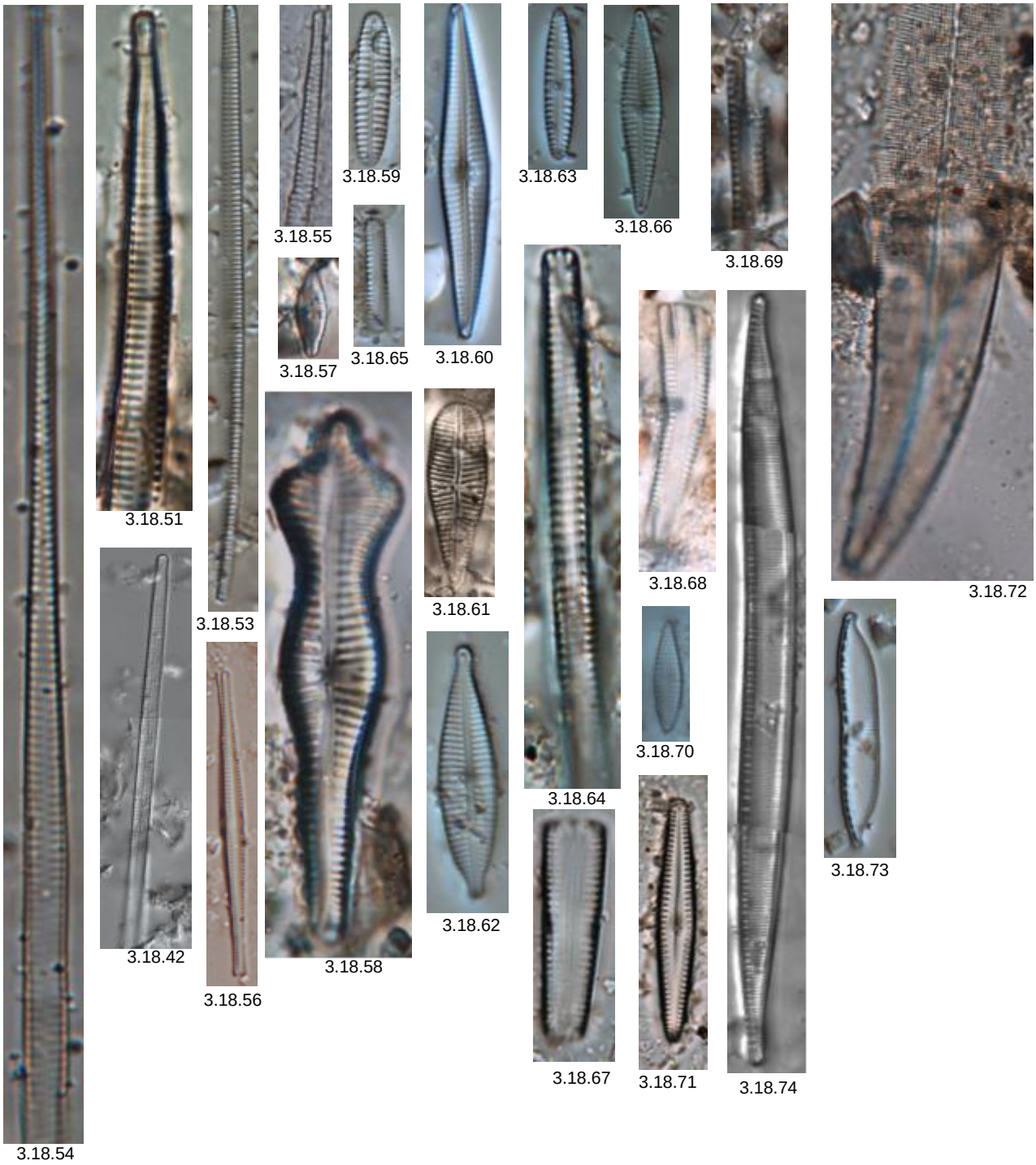


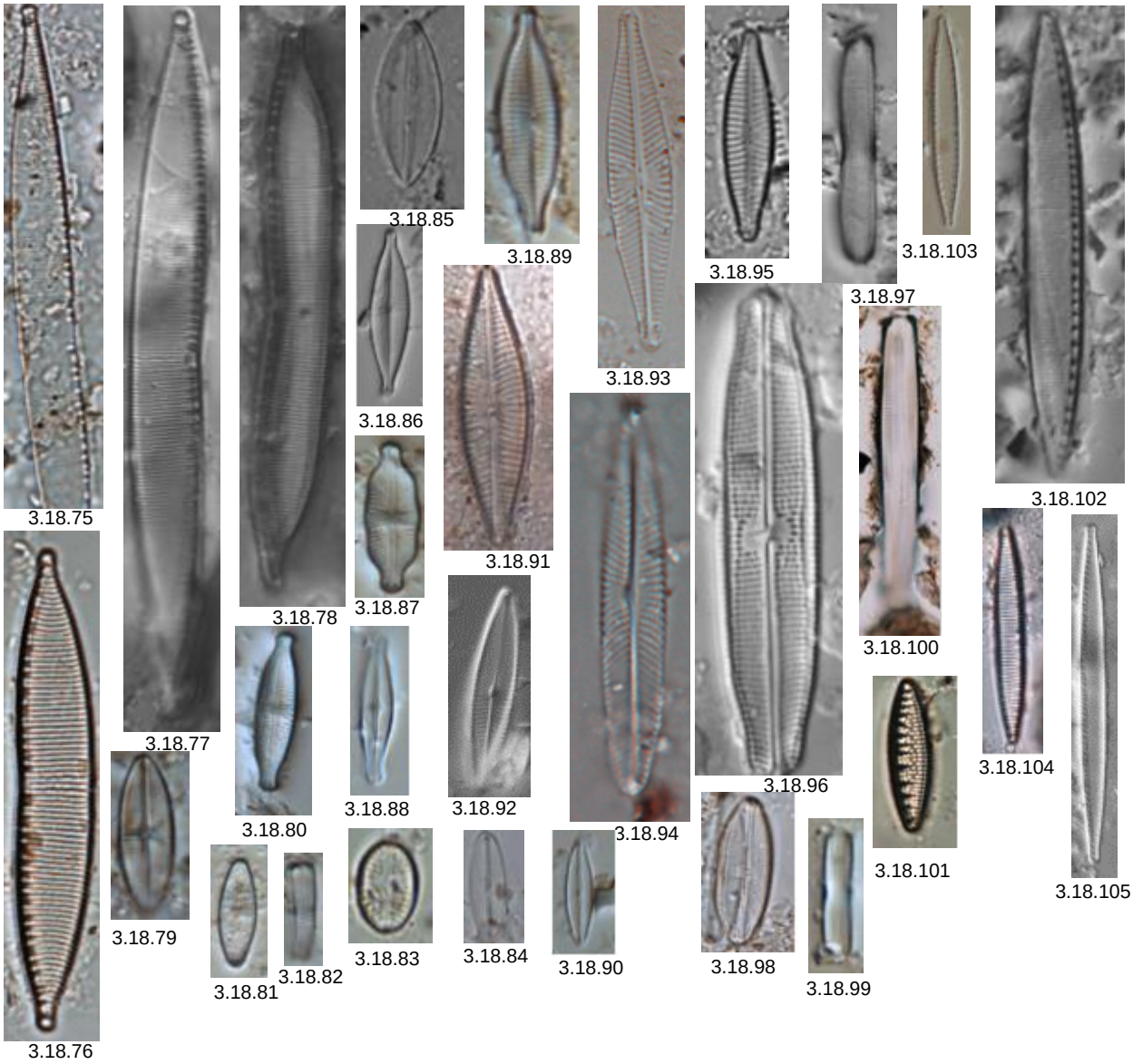
Figure 3.18: Micrographs taken for the duration of this study: 3.18.1: *Achanthidium* sp. 1 3.18.2,3: *Achanthidium* sp. 2 3.18.4: *Achanthidium* sp. 3 3.18.5: *Achanthidium* sp. 4 3.18.6: *Achanthidium* sp. 5 3.18.7: *Achanthidium* sp. 6 3.18.8: *Achanthidium* sp. 7 3.18.9: *Achanthidium* sp. 8 3.18.10: *Achanthidium* sp. 9 3.18.11: *Achanthidium* sp. 10 3.18.10: *Achanthidium* crassum 3.18.12: *Brachysira neoexilis* 3.18.13: Caloneis sp. 1 3.18.14: Caloneis sp. 2 3.18.15: Caloneis sp. 3 3.18.16: *Capartogramma crucicula* 3.18.17: *Cocconeis placentula* 3.18.18: *Craticula ambigua* 3.18.19:*Craticula buderi* 3.18.20: *Craticula perrotettii* 3.18.21: *Craticula* sp. 1 3.18.22: *Craticula* sp. 23.18.23: *Craticula* sp. 4 3.18.24: *Craticula* sp. 10 3.18.25: *Craticula vixnegligenda* 3.18.26: Cyclotella sp. 1 3.18.27 Cyclotella *sp. 2a* 3.18.28: *Cymbella cistula* 3.18.29: *Cymbella silesiaca* 3.18.30: *Cymbella* sp.1



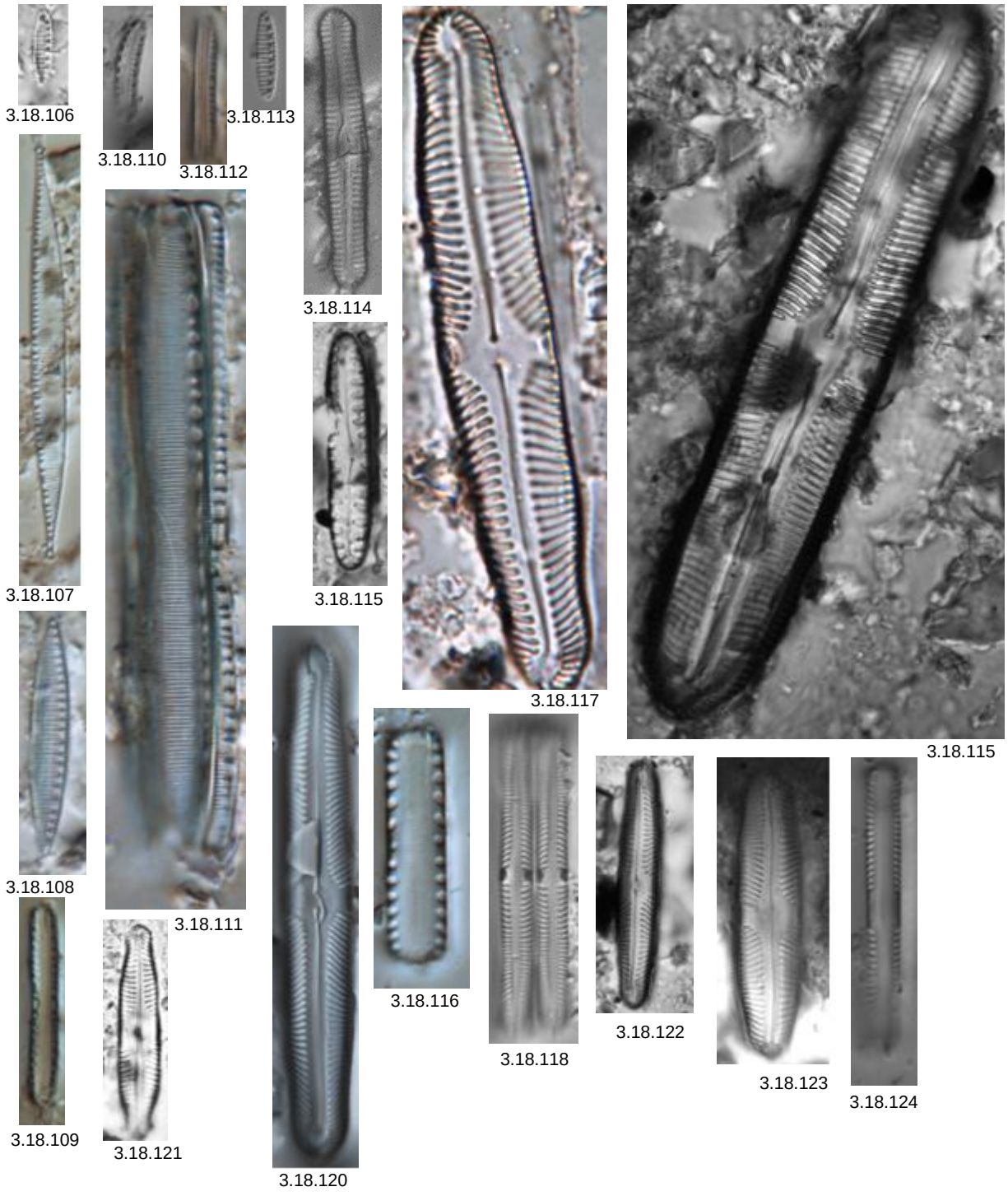
3.18.31: *Cymbella asp. era* 3.18.32: *Cymbella neocistula* 3.18.33: *Cymbella* sp. 2 3.18.34: *Cymbella tumida* 3.18.35: *Diploneis oblongella* 3.18.36: *Encyonema leei* 3.18.37: *Encyonema ventricosum* 3.18.38: *Encyonema* sp. 3 3.18.39: *Eolimna minima* 3.18.40 Epithemia sorex 3.18.41 *Eunotia bilunaris* 3.18.42: *Eunotia minor* 3.18.43: *Eunotia* sp. 1 3.18.44: *Eunotia* sp. 2 3.18.45: *Eunotia* sp. 3 3.18.46: *Eunotia* sp. 7 3.18.47: *Eunotia* sp. 11 3.18.48: *Eunotia* sp. 13 3.18.49: *Eunotia* sp. 15 3.18.50: *Eunotia pectinalis*



3.18.51 and 3.18.52: *Fragilaria biceps* 3.18.53: *Fragilaria* sp. 1 3.18.54: *Fragilaria* sp. 2 3.18.55: *Fragilaria* sp. 3 3.18.56: *Fragilaria* sp. 8 3.18.57: *Geissleria* sp. 1 3.18.58: *Gomphonema acuminatum* 3.18.59: *Gomphonema angustatum* 3.18.60: *Gomphonema gracile* 3.18.61: *Gomphonema laticollum* 3.18.62: *Gomphonema parvulum* 3.18.63: *Gomphonema pumilum* 3.18.64: *Gomphonema* sp. 1 3.18.65: *Gomphonema* sp. 2 3.18.66: *Gomphonema* sp. 3 3.18.67: *Gomphonema* sp. 5 3.18.68: *Rhoicosphaenia abbreviata* 3.18.69: *Gomphonema* sp. 11 3.18.70: *Gomphonema* sp. 12 3.18.71: *Gomphonema venusta* 3.18.72: *Gyrosigma* sp. 1 3.18.73: *Hantzschia amphioxys* 3.18.74: *Hantzschia elongata*

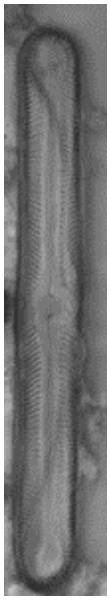


3.18.75: *Hantzschia* sp. 1 3.18.76: *Hantzschia* sp. 3 3.18.77: *Hantzschia* sp. 5 3.18.78: *Hantzschia* sp.6 3.18.79: *Lemnicola hungarica* 3.18.80: *Luticola kotschyi* 3.18.81: *Luticola mutica* 3.18.82: *Luticola* sp. 1 3.18.83: *Luticola* sp. 3 3.18.84: *Mayamaea atomus* 3.18.85: *Microcostatus* sp. 1 3.18.86: *Navicula cryptocephala* 3.18.87: *Navicula nivalis* 3.18.88: *Navicula recens* 3.18.89: *Navicula* sp. 1 3.18.90: *Navicula* sp. 2 3.18.91: *Navicula* sp. 4 3.18.92: *Navicula* sp. 11 3.18.93: *Navicula* sp. 12 3.18.94: *Navicula symmetrica* 3.18.95: *Navicula veneta* 3.18.96: *Neidium affine* 3.18.97: *Neidium* sp. 1 3.18.98: *Neidium* sp. 2 3.18.99: *Neidium* sp. 3 3.18.100: *Neidium* sp. 6. 3.18.101: *Nitzschia amphibia* 3.18.102: *Nitzschia palea* 3.18.103: *Nitzschia* sp. 1 3.18.104: *Nitzschia* sp. 10 3.18.105: *Nitzschia* sp. 12

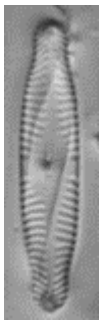


3.18.106: *Nitzschia* sp. 2 3.18.107: *Nitzschia* sp. 3 3.18.108: *Nitzschia* sp. 5 3.18.109: *Nitzschia* sp. 6 3.18.110: *Nitzschia* sp. 7 3.18.111: *Nitzschia* sp. 8 3.18.112: *Nitzschia* sp. 9 3.18.113: *Nitzschia valdecostata* 3.18.114*:* *Pinnularia acrosphaeria* 3.18.115 and 3.18.116: *Pinnularia borealis* 3.18.117 and 3.18.118: *Pinnularia divergens* 3.18.119: *Pinnularia divergens* var. *sublinearis* 3.18.120: *Pinnularia gibba* 3.18.121: *Pinnularia microstauron* var. *rostrata* 3.18.122: *Pinnularia* sp. 1 3.18.123: *Pinnularia* sp. 2 3.18.124: *Pinnularia* sp. 3

3.18.130

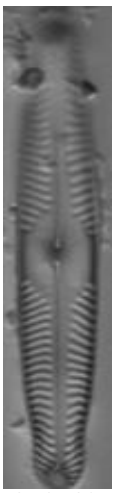


3.18.131

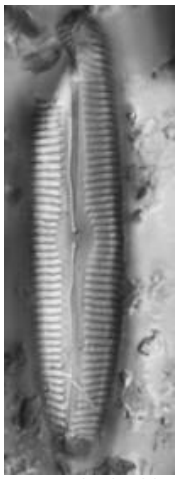


3.18.125

3.18.127



3.18.126



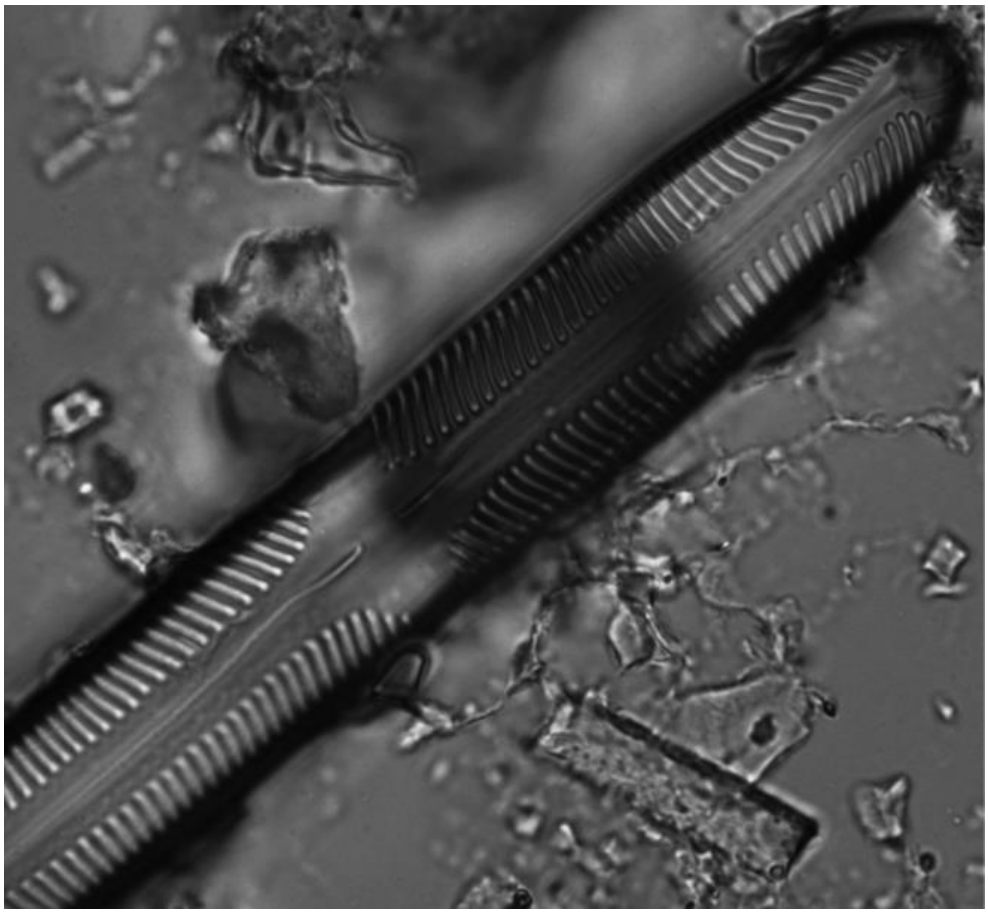
3.18.133

3.18.134

1.18.135

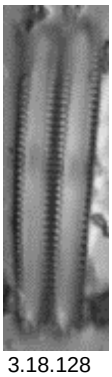
1.18.136

3.18.137



1.18.132

3.18.129

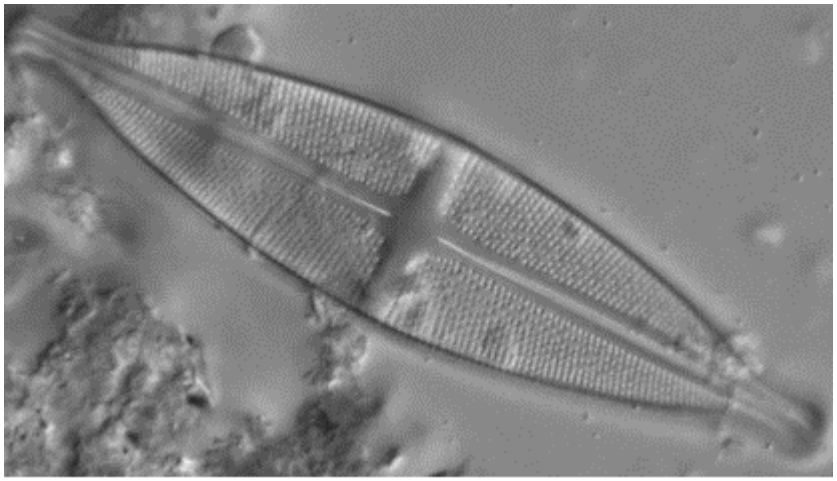


3.18.128

3.18.125: *Pinnularia* sp. 4 3.18.126: *Pinnularia* sp. 5 3.18.127: *Pinnularia* sp. 6 3.18.128: *Pinnularia* sp. 7 3.18.129: *Pinnularia* sp. 9 3.18.130: *Pinnularia* sp. 17 3.18.131: *Pinnularia* sp. 19 3.18.132: *Pinnularia* sp. 21 3.18.133: *Pinnularia viridiformis* 3.18.134: *Pinnularia subcapitata* 3.18.135: *Planothidium frequentissimum* 3.18.136: *Planothidium* sp. 2 3.18.137: *Rhoicosphenia abbreviate*

3.18.138

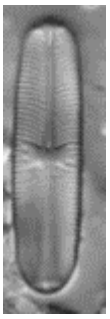
3.18.140



3.18.150

3.18.141

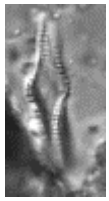
3.18.142



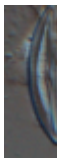
3.18.143

3.18.145

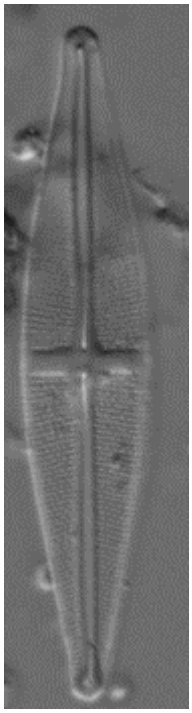
3.18.146



3.18.149



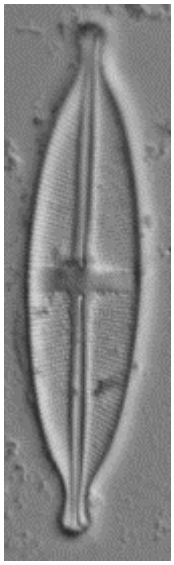
3.18.144



3.18.148

3.18.151

3.18.152



3.18.153

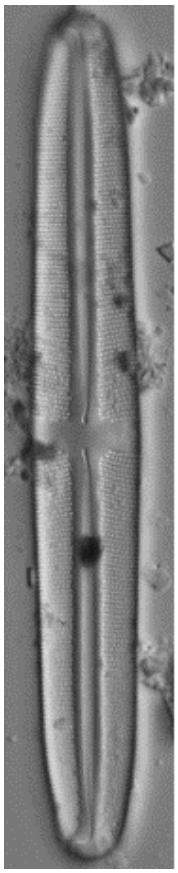
3.18.154

3.18.139

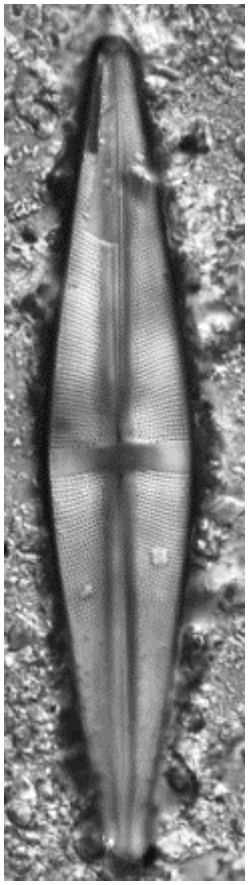
3.18.147

3.18.138: *Pinnularia subbrevistriata* 3.18.139-140: *Rhopalodia gibba* 3.18.141: Sellaphora pupula 3.18.142: *Sellaphora* sp. 1 3.18.143: *Sellaphora stroemii* 3.18.144: *Seminavis* sp. 1 3.18.145: *Stauroneis anceps* 3.18.146: *Stauroneis borrichii* 3.18.147: *Stauroneis gracilis* 3.18.148: *Stauroneis sofia* 3.18.149: Staurosira construens 3.18.150: *Stauroneis kootenai* 3.18.151: *Stauroneis nana* 3.18.152: *Stauroneis* sp. 1 3.18.153: *Stauroneis* sp. 2 3.18.154: *Stauroneis* sp. 3

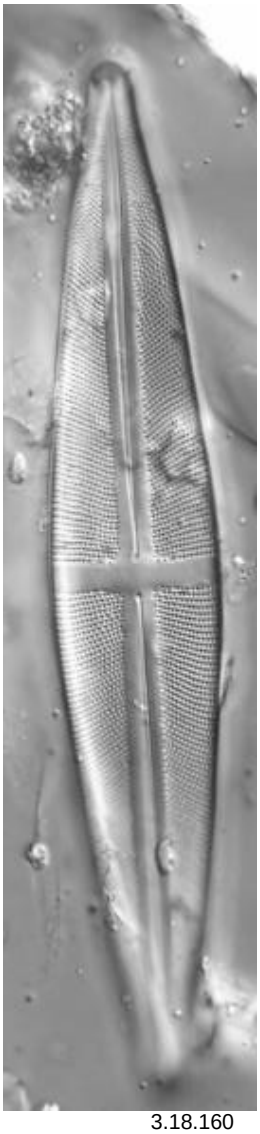
3.18.155



3.18.156



3.18.157



3.18.160

3.18.163

3.18.165

3.18.158

3.18.161

3.18.162

3.18.164

3.18.159

3.18.155: *Stauroneis* sp. 4 3.18.156: *Stauroneis dracomontana* 3.18.157: *Stauroneis* sp. 6 3.18.158: *Stauroneis* sp. 8 3.18.159: *Stauroneis* sp. 9 3.18.160: *Stauroneis* sp. 12 3.18.161: : *Stauroneis* sp. 13 3.18.162: *Stauroneis superkuelbsii* 3.18.163: *Surirella ovalis* 3.18.164: *Tryblionella coarctata* 3.18.165: *Tryblionella littoralis*

4.2. Diatom diversity from environmental samples

The following section will discuss diatom diversity in the context of environmental samples. The information in section 4.1.1. is useful for this context. Diversity was examined by means of calculating Shannon's diversity index (H'). Species richness (S) and evenness (E) are also good indicators of community dynamics (Stevenson, 1984).

Ukutula

Ukutula's third site had the highest diversity (H' = 2.84) and species richness (S = 41) in terms of environmental diatom samples (Table 3.4). It also had a relatively high evenness value (E =0.81), similarly to the environmental samples collected from site 3 (E = 0.81). Considering that E values closer to one indicates a higher similarity of species in terms of distribution, it was observed that environmental diatom species from sites 1 and 3 had a more similar species distribution than the samples taken from site 2 (E = 0.74). This could be attributable to the similarity in ecology between sites 1 and 3, as both were isolated ponds. In fact, site three was the least diverse in terms of environmental diatom samples (H' = 2.84 and S = 20). This could be due to the differences in stream morphology encountered at site 2. It was a deep river with considerably less aquatic vegetation in comparison to sites 1 and 3.

Diatoms from the *Stauroneis* genus were typically the most abundant in the environmental samples from these three sites (Figures 3.1 to 3.3). This is a diverse group of generally large diatoms, typically found in the benthos or sediment of smaller water bodies (Diatoms Of North America, 2020). This, however, is a peculiar discovery, considering that *Stauroneis* diatoms are of the rarer genera found in Southern Africa (Diatoms of North America, 2020; Archibald, 1966; Riato *et al.,* 2014). In fact, it is considered a common European diatom (Diatoms Of North America, 2020; Van de Vijver *et al.*, 2004). Their presence is unforeseen and inexplicable. Therefore, Ukutula provides ample opportunities for research pertaining to *Stauroneis* diatoms in South Africa.

Site 2 is also dominated by *G. gracile* (Figure 3.2). This is a benthic organism often found in sand (Caljon & Cocquyt, 1992; Taylor *et al.*, 2007). Site 2's sandy riverbeds selected for *G. gracile*, justifying its dominance relative to other diatom species.

Aliwal North

Aliwal North's third site was the most diverse in terms of environmental diatom species (H' =2.85 and S = 36) (Table 3.6). In contrast, site 6 had the lowest species richness (S = 9), and site 7 had the lowest diversity (H' = 1.33). Both these sites had relatively low evenness values, indicative of a low population density (0.557 and 0.577 respectively).

Diatom community structure is often attributed to environmental factors acting as filters on community composition, as discussed earlier (Heino *et al.*, 2012; Heino *et al.*, 2014; Jyrkänkallio-Mikkola *et al.*, 2018; Pan *et al.*, 2000; Poff, 1997; Potapova & Charles, 2002; Soininen *et al.*, 2016; Southwood, 1977; Verleyen *et al.*, 2009). Sites 6 and 7 were both small, isolated ponds. Site 7, particularly, was the smallest water body among the sampling localities. These factors could be selective of smaller diatom communities.

Diatoms from the *Fragilaria, Nitzschia*, and *Gomphonema* genera were most prominent in the environmental samples of sites 1 – 7 (Figures 3.4 to 3.11). Diatoms from these genera typically attach to available surfaces (Cox & Cox, 1996; Hill *et al.*, 2001; Stevenson *et al.*, 1996; Villac *et al.*, 2016). These sites had a generous amount of submerged aquatic vegetation, which would have provided sufficient surface for algae attachment. *Nitzschia* diatoms are also motile, and *Gomphonema* diatoms are considered bottom dwellers (Villac *et al.*, 2016).

P. subcapitata and *Denticula* sp., however, dominated site 8 (Figure 3.12). Diatoms from the *Pinnularia* genus is mostly found in ponds and moist soil. They also inhabit the sediment of the ocean and are mostly epipelic (Benke & Cushing, 2011; Diatoms Of North America, 2020). Site 8 is additionally separated from sites 5-7 by a road (R58) (Figure 2.4). It is, therefore, reasonable to assume that anthropogenic and environmental factorsfactors would be driving site 8's diatom community structures. Consequently, resulting in slightly different dominant diatom species.

4.3. Reviewing guild feeding habits in terms of floral gut content

Benthic algae (including diatoms) are a primary source of nutrition for organisms at higher trophic levels (Stevenson *et al.*, 1996; Taylor *et al.*, 2007). Diatom valves consist of opaline silica and can withstand chemical processes occurring in the gastrointestinal tract of tadpoles.

Therefore, there are an abundance of diatoms found when examining the intestinal content of tadpoles. In total, 139 species were found in the gut content of tadpoles sampled from Ukutula and 178 species were counted from the gut content of tadpoles sampled from Aliwal North.

Here follows a comprehensive discussion of the intestinal diatom content and how it pertains to the feeding habits and ecomorphology of the ecomorphological tadpole guilds involved in this study. This section incorporates the results obtained from Table 3.7. It additionally includes an overview of the diversity of diatom species sampled from environmental and intestinal content.

4.3.1. Ukutula

Nine tadpole species and seven ecomorphological guilds were recorded at Ukutula, all of which were sampled at site one (Table 3.1). The least amount of tadpole species and guilds were sampled by site 3 followed by site 2.

Rheophilic tadpoles

Amietia delalandii is classified as a Rheophilic tadpole and was sampled from all three sites at Ukutula. Tadpoles from this guild are usually bottom dwellers (Botha, 2013; Van Dijk, 1972). This theoretically implies that diatoms from the benthos would be ingested by Rheophilic tadpoles. It was therefore fitting to observe diatoms typically inhabiting the benthos in the intestine of this species. These included *C. placentula*, *Craticula* sp., *P. borealis*, and *Gomphonema* sp. (Caljon & Cocquyt, 1992; Diatoms Of North America, 2020; Round *et al.*, 2007; Taylor *et al.*, 2007; Villac *et al.*, 2016).

Amietia delalandii was able to ingest diatoms growing on periphyton in/near the benthos due to re-circulation This included *Eunotia* sp., and *E. formica, G. acuminatum*, *Nitzschia* sp., and *Pinnularia* sp. (Cox & Cox, 1996).

Amietia delalandii was also able to consume known soil diatoms as they are occasionally washed into existing waterbodies (*Hantzschia* sp., *H. elongate, H. amphioxys, Pinnularia* sp., and *Microcostatus* sp.) (Taylor *et al.*, 2007; Taylor *et al.*, 2010). Diatoms from the *Stauroneis* genus is habitually found in moss and soil (Diatoms Of North America, 2020), but because they were consumed by *A. delalandii*, suggests that they could be recirculated from the soil into the water body. This serves as an example of the fact that sampling aquatic tadpoles for diatoms can yield taxonomic information on more than just predominantly aquatic diatoms.

Assessing the diversity of diatom species sampled gives a good indication of diatom community structures across different sites and the related ecomorphological tadpole guilds. The diatom content retrieved from this guild was most diverse for site 2 ($H' = 2.59$) and the second-most diverse for site 3 ($H' = 1.98$) (Table 3.4; Figures 3.12 and 3.13). In contrast, it was the least diverse guild for site 1 ($H' = 2.12$). The H' values correlated well with species richness, as the least amount of diatom species were recovered from the Rheophilic guild at site 1 (S = 19), the most diatom species were sampled from site 2 (S = 23), and the second-most diatom species were sampled from site 3 (22). **Suspension feeder**

Tadpoles from the suspension feeder guild (such as *P. bifasciatus*) were sampled at sites 1 and 3. They typically filter feed in the midwater of lentic water bodies (Altig & Johnston, 1989; Berger *et al.*, 1999). This theoretically implies that motile, unattached, and planktonic diatoms would be ingested by Suspension feeder tadpoles. It was therefore fitting to observe unattached, motile diatoms in the intestine of *P bifasciatus.* These include diatoms like *C. ambigua*, *C. buderi*, *S. kootenai*, and *S. superkuelbsii Phrynomantis bifasciatus* also ingested *C. subleptoceros*, which is generally found in the benthos, attached to various substrates *(Li et al., 2007)*. It's motility, however, made it accessible to Suspension feeder tadpoles.

P. bifasciatus was also able to ingest diatoms residing in the epipelic region at the interface between water and sediment (*Achnanthidium* sp., *Navicula* sp., *P. divergens*). These diatoms are prone to resuspension, making them accessible to organisms filter feeding on suspended algae (Diatoms Of North America, 2020; Steele *et al.*, 2009).

Diatoms usually attached to substrates and aquatic vegetation were also found in the intestines of tadpoles from the Suspension Feeder guild. These include *F. biceps, Fragilaria* sp., *H. amphioxys*, *E. minor, H. amphioxys, P. borealis,* and *G. parvulum*. This suggest the resuspension of attached diatoms due to external disturbances, making them available for consumption by Suspension Feeder tadpoles. This can range from disturbances caused by aquatic organisms, terrestrial animals, and even water flow. The same applies to benthic diatoms also found in the intestinal tract of *P. bifasciatus* (*Craticula sp., Eunotia sp.,* and *Gomphonema parvulum*).Soil diatoms (*Pinnularia* sp., *S. borrichii, Stauroneis* sp., *H. amphioxys,* and *P. borealis*) were also ingested by *P. bifasciatus*, suggesting that they were recirculated in the water column.

Most diatoms from this guild were collected from site 1 (S = 26) and substantially fewer diatoms were collected from the samples collected at site 3 (S = 17) (Table 3.4; Figures 3.12 and 3.13). Therefore, the diatoms collected from the sample taken from site 1 had a considerably higher diversity (H′ = 2.30) than the sample taken from site 3 (H′ = 1.32). Both these samples had low evenness values, indicating lower population densities (E = 0.706 for site 1 and E = 0.4644 for site 3).

Benthic type 2 (Profundal)

Tadpoles (*P. mossambica* and *S. grayii*) from the Benthic type 2 (Profundal) guild were only collected from site 1. They typically inhabit the benthic zone, and often move from littoral to deeper, profundal zones (Altig & Johnston, 1989; Botha, 2013). This theoretically implies that they feed on a range of algae, including benthic, epipelic, planktonic and motile diatoms.

It was therefore reasonable to find many benthic diatoms in the intestinal tract of tadpoles from the Benthic type 2 (Profundal) guild. These included *Achnanthidium* sp., *A. crassum, Craticula* sp., *G. gracile*, *N. recens, Navicula* sp.*, P. borealis, P. divergens, S. biceps, Stauroneis* sp.*, C. buderi, G. angustatum*, *Stauroneis* sp. Benthic type 2 tadpoles were also able to ingest *S. gracilis* due to its motility (Van de Vijver *et al.*, 2004). Other motile diatoms found in the intestinal tracts of Benthic type 2 diatoms included *E. minima*, *E. formica, N veneta,* and *C. ambigua* (Levkov et al., 2016). As expected, epipelic diatoms were also identified from examining gut content Benthic type 2 tadpoles. These included *Pinnularia* sp. and *Tryblionella* sp. (Benke & Cushing, 2011).

Diatoms usually attached to substrates and aquatic vegetation, however, were also found in the intestines of tadpoles the Benthic type 2 guild. These included *Eunotia* sp., *F. biceps,*

Gomphonema sp., *H. amphioxys, Hantzschia* sp., and *Luticola* sp. This suggest the resuspension of attached diatoms due to external disturbances

Diatoms sampled from this guild were the most diverse ($H' = 2.34$ for *P. mossambica* and 2.48 for *S. grayii*) amongst all the guilds examined from Ukutula (Table 3.4; Figures 3.12 and 3.13). A significant number of diatoms were also recorded in both species (S = 25 for *P. mossambica* and 24 for *S. grayii*). Diatoms from both species in this guild had a moderate evenness values, implying moderate population density (E = 0.706 for *P. mossambica* and 0.781 for *S. grayii*).

Lentic-nektonic

Kassina senegalensis is classified as a Lentic-nektonic tadpole and was sampled from sites 2 and 3. Tadpoles from this guild inhabits the midwater and generally do not filter feed (Altig & Johnston, 1989). *Kassina senegalensis* tadpoles often use their keratinized mouthparts to scrape food from surfaces (Wager, 1986). This theoretically implies that they feed on a range of epiphytic and epipelic diatoms growing on available substrates and aquatic vegetation. It was therefore fitting to observe epilithic and/or epipelic diatoms in the intestine of this species (*A. crassum, C. ambigua, C. aspera, N. affine, Nitzschia* sp., *Pinnularia* sp., and *P. subcapitata*).

Diatoms typically found in soil or benthos were also ingested by *K. senegalensis*, feeding on diatoms growing on substrates in close contact with soil/benthos. These included *S. borrichii, Luticola* sp.*, H. amphioxys, Hantzschia* sp., *E. minima*, *Achnanthidium* sp., *Craticula* sp., E. *bilunaris*, *G. gracile, G. angustatum, P. borealis, P. divergens, R. abbreviata, S. biceps, Stauroneis* sp., and *S. ovalis*.

Diatoms examined from this guild had the lowest diversity at site 3 ($H' = 2.46$) and the highest at site 2 ($H' = 2.31$) (Table 3.4; Figures 3.12 and 3.13). However, more diatoms were sampled from site 3 (S = 30) and less from site 2 (S = 22). Both samples had a relatively low species evenness (E = 0.795 for site 2 and E = 0.681 for site 3).

It is evident from the literature that tadpoles sampled from ecomorphological guilds at Ukutula ingested a variety of diatoms. Some of the diatom species ingested were in agreement with the feeding habits characteristic of the involved tadpole guild. Nevertheless, many other unrelated diatom species were consumed. As discussed in the preceding sections, multiple factors could cause the ingestion of diatoms from various substrata.

In fact, there were no statistical or practical significant differences in diatom diversity between samples taken from environmental samples, tadpole species, or ecomorphological tadpole guilds (Annexure D). This was also the case for the sample taken from the terrapin's carapace. Additionally, no clusters were distinguishable between sites and samples obtained from Ukutula (Figure 3.14). This is indicative of no apparent statistical relationship between sample localities and samples.

4.3.2. Aliwal North

Five tadpole species from four guilds were sampled at Aliwal North (Table 3.2). Site 1 was the most diverse, as all species and guilds were sampled there. In contrast, site 3 was the least diverse, with only *K. senegalensis* tadpoles sampled there.

Lentic-nektonic

Kassina senegalensis is classified as a Lentic-nektonic tadpole and was sampled from sites 2-7 and was also sampled at Ukutula. Tadpoles from this guild inhabits the midwater and generally do not filter feed (Altig & Johnston, 1989). *K. senegalensis* tadpoles often use their keratinized mouthparts to scrape food from surfaces (Wager, 1986). This theoretically implies that they feed on a range of epiphytic and epipelic diatoms growing on surfaces and aquatic vegetation. It was therefore fitting to observe epilithic and/or epipelic diatoms in the intestine of this species (*Eunotia* sp., *Fragilaria* sp., *Gomphonema* sp., *C. ambigua, E. pectinalis, F. biceps, Nitzschia* sp., *Pinnularia* sp*., P. viridiformis*, *S. construens,* and *Tryblionella* sp).

Some soil diatoms were also consumed by *K. senegalensis* (*H. amphioxys* and *Hantzschia* sp). This implies that soil diatoms were ingested by Lentic-nektonic tadpoles, while feeding on diatoms growing on substrates in close contact with soil/benthos. The same is applicable to benthic diatoms also ingested by *K. senegalensis* (*Achnanthidium* sp., *Craticula* sp., *E. bilunaris, G. parvulum, Navicula* sp*., N. palea, P. borealis, P. divergens, P. gibba, S. biceps, Stauroneis* sp).

It was, however, unexpected to find unattached diatoms (*E. formica* and *N. symmetrica)* inhabiting the littoral zone (*C. tumida*) in the intestinal tracts of *K. senegelensis.* It is likely

that these diatoms were ingested by Lentic-nektonic tadpoles after settling on surfaces the tadpoles used for grazing.

Diatoms samples obtained from the intestinal tracts of Lentic-nektonic tadpoles ranges from higher to lower diversity (H' = 2.20 for site 7 and 0.94 for site 4). There is additionally variety in terms of evenness and richness values (Table 3.6; Figures 3.15 and 3.16).

Rheophilic tadpoles

Amietia delalandii is classified as a Rheophilic tadpole and was sampled from site 6 at Aliwal North. It was also sampled at Ukutula. Tadpoles from this guild are usually bottom dwellers (Botha, 2013; Van Dijk, 1972). This theoretically implies that diatoms from the benthos would be ingested by Rheophilic tadpoles.

It was fitting to observe diatoms typically inhabiting the benthos in the intestine of this species. These included A*chnanthidium* sp., *Cocconeis* sp., *C. silesiaca, E. sorex, Navicula* sp., *N. amphibia, P. borealis, R. gibba*, and *Stauroneis* sp. *A. delalandii* was able to ingest diatoms found on substrates like plants, rocks and wood (*Sellaphora* sp.). Known soil diatoms (*H. amphioxys*) can occasionally be washed into existing water bodies, and eventually become ingested by tadpoles. *Hantzschia amphioxys*, particularly, was observed in the intestine of *A. delalandii*.

Diatoms from this sample returned a considerably lower evenness in relation to E values from other samples (E = 0.671). This was indicative of lower population densities amongst diatom species from this sample (Table 3.6; Figures 3.15 and 3.16).

Lentophytophilic

Cacosternum boettgeri tadpoles from the Lentophytophilic guild were sampled at various sites from Aliwal North (Sites 1, 4-7). They typically inhabit the midwater of shallow water, demonstrating a preference for aquatic vegetation (Van Dijk, 1972). This theoretically implies that epipelic and epiphytic diatoms would be ingested by Lentophytophilic tadpoles.

It was fitting to observe epipelic and epiphytic diatoms in the intestine of *C. boettgeri* tadpoles. These included *Caloneis* sp., *C. ambigua, E. pectinalis, Navicula* sp., and *P. viridiformis*, *Cymbella* sp., *F. biceps, Pinnularia* sp., *Stauroneis* sp., *N. affine,* and *S. construens*. Unnattached, motile diatoms were also observed in the intestines of

Lentophytophilic tadpoles (*E. Formica*). These diatoms were easily ingested due to their motility (Van de Vijver *et al.*, 2004).

Cacosternum boettergeri ingested various soil and benthic diatoms (*Achnanthidium* sp.*, Craticula* sp., E*. bilunaris, G. affine, G. gracile, G. parvulum, Gomphonema* sp., *H amphioxys, Hantzschia* sp., *N symmetrica, N. palea, P. borealis*, *P. divergens, R. gibba, S. biceps,* and *S. gracilis*). This is due to the tadpole being in close contact with soil and benthos while grazing in shallow water.

There was noticeable variation in the data obtained from analyzing diatoms found in the intestinal tracts of Lentophytophilic tadpoles. Diversity, for instance, ranged considerably ($H' = 2.71$ for site 5 to $H' = 0.82$ for site 6). The same applied to values returned for population density and species richness (Table 3.6; Figures 3.15 and 3.16).

Lentic-benthic

Amietophrynus rangeri tadpoles from the Lentic-benthic guild were sampled from site 1 at Aliwal North. These tadpoles are classified as bottom dwellers in shallow water, and rarely move into deeper water (Altig & McDiarmid, 1999). This theoretically implies that benthic, epipelic and soil diatoms would be ingested by Lentic-benthic tadpoles.

It was fitting to observe abundant benthic diatoms in the intestine of *A. rangeri* tadpoles. These included *Achnanthidium* sp., *Caloneis* sp.*, Craticula* sp., *G. gracile, Navicula* sp.*, P. gibba*, and *Stauroneis*. Similarly, diatoms inhabiting soil and substrates in close contact with shallow water were observed in the intestinal tracts of Lentic-benthic tadpoles. These included *H. amphioxys, Hantzschia* sp.*, Nitzschia* sp.*, S construens,* and *Gomphonema* sp.

Diatoms examined from the intestinal tract of Lentic-benthic tadpoles had a relatively high diversity ($H' =$ for 2.163). It additionally had a moderate species evenness ($E = 0.700$) and species richness ($S = 21$) (Table 3.6; Figures 3.15 and 3.16).

It is evident from the literature that tadpoles sampled from ecomorphological guilds at Aliwal North ingested a variety of diatoms. Some of the diatom species ingested were in agreement with the feeding habits characteristic of the involved tadpole guild. Nevertheless, many other diatom species were consumed due to a combination of factors, as discussed in the preceding chapters.In fact, there were no statistical or practical significant differences in diatom diversity between samples taken from environmental samples, tadpole species, or

ecomorphological tadpole guilds (Annexure D). This is indicative of no apparent statistical relationship between sample localities and samples.

4.4. Absence of amphibian chytrid fungus

A study from 2013 used all available South African records of *Bd* infections and environmental data to construct a prediction model for *Bd* distribution (Tarrant *et al.*, 2013). This model is used to predict the probability of *Bd* occurrence across South Africa.

Ukutula is situated in North-West, which has a low probability of chytrid occurrence (Tarrant *et al*., 2013). As expected, No *Bd* was detected at any of the sites at Ukutula. In fact, the closest confirmed *Bd* positive site relative to Ukutula is located in Central Limpopo. This implies the influence of external factors compromising *Bd*'s survival and infection rate in Ukutula's environment.

5. CONCLUSION

The aim of this study was to determine if tadpole ecomorphological guilds can be used as a proxy for environmental diatom sampling. Tadpole species can also be used as a proxy for environmental diatom sampling. There was no statistical or practical significant difference within or between samples collected from the environment, tadpole species, or ecomorphological guilds. This was also supported by the lack of clusters in the DCA's. Clusters typically form in the case of significant differences in relative abundance values between samples and sites. This suggests that either method of sampling would be sufficient. This was further supported by the lack of clusters in the DCA's. Clusters typically form in the case of significant differences in relative abundance values between samples and sites.

Using tadpoles as a proxy for environmental diatom sampling has many advantages. It can be used to obtain environmental diatom samples in the absence of sufficient substrata. Especially in the case of deep water bodies, where substrata and benthos may be inaccessible. It is additionally a possible alternative to cultivating diatom colonies on introduced artificial materials in an effort to obtain diatoms in cases of minimal substrata. The latter is often time-consuming, as the introduced material should be submerged in the water for several days prior to sampling. The introduced material could also be lost during fieldwork and frequently selects opportunistic species (Taylor *et al.*, 2007).

The usage of ecomorphological tadpole guilds to study environmental diatoms would, however, constitute considerable research. Especially pertaining to endangered anuran species and the ecological impact of tadpole sampling. The advantages and disadvantages of such a survey should thus be carefully considered.

With regards to diatom diversity, an extensive range of diatoms were observed whilst examining environmental samples taken at sites from Ukutula and Aliwal North. In fact, the diatom diversity obtained from environmental samples exhibited variation. Some sites returned high H' values, whilst other sites returned relatively lower H' values. This is attributable to various environmental factors and substrate availability as discussed during the course of this study. It is necessary to consider the possibility of cross-contamination between sites, mediated by migrating mammals, aquatic birds, amphibians, and terrapins. Nutrient availability also significantly influenced the reproductive rate of opportunistic diatom species (Stevenson, 1984).

A variety of diatoms were also observed whilst examining the intestinal content of tadpoles from both Ukutula and Aliwal North. This included diatom species that tadpoles from the involved guilds are expected to ingest according to existing information on guild-associated feeding habits. In fact, the H', E, and S values of diatom samples taken from tadpoles seemed to fluctuate throughout the dataset. Some tadpoles may have eaten more recently than others, causing this variation. However, an abundance of apparent unrelated diatoms were additionally ingested. As outlined in the discussion, some diatom species can be resuspended in the water column. Various factors could attribute to resuspension and re-circulation; including motility, disturbances in the water column, substrate type and so on. Once resuspension occurred, diatoms can be ingested by various tadpoles regardless of their ecomorphological guild feeding habits.

In fact, the H', E, and S values of diatom samples taken from tadpoles seemed to fluctuate throughout the dataset. There was furthermore no statistical or practical significant difference within or between samples collected from the environment, tadpole species, or ecomorphological guilds. This was also supported by the lack of clusters in the DCA's. Clusters typically form in the case of significant differences in relative abundance values between samples and sites.

A variety of tadpole species were also observed. With only one or two species collected per tadpole ecomorphological guild, it would be beneficial to repeat this study including more species per tadpole ecomorphological guild. The results of this study implied that tadpoles could be used as a proxy for environmental diatom diversity. In contrast the results suggested that tadpole intestinal diatom content cannot be used as a reference to ecomorphological guilds. Since there is no statistical or practical significant difference between environmental diatoms and diatoms found when inspecting tadpole gut content, it would not be possible to determine the ecomorphological guild of a tadpole after examining the diatom intestinal content.

An abundant amount of *Stauroneis* diatoms were identified from Ukutula. This is peculiarly significant since these are considered rare and are typically found in Europe (Diatoms of North America, 2020). This discovery can amplify research regarding this *Stauroneis* diatoms in Southern Africa. These sites are of particular value pertaining to this diatoms' diversity and should be conserved as such for future research.

With regards to *Bd*, none of the cultures taken from Ukutula demonstrated growth that resembled chytrid. Hence, Ukutula is considered Bd-negative. Studying tadpole's feeding behaviour in relation to their guilds garners a lot of insight in spatial and temporal behaviour. This can additionally be used to study the influence of tadpole ecomorphological guilds on the susceptibility of chytridiomycosis.

There was also no statistical or practical significant difference between diatoms sampled from the terrapin's carapace in comparison to tadpole or environmental samples. Although this component was supplementary to the study, it is an interesting addition to pursue in this context. In fact, there exists a gap in the literature regarding terrapin diatoms in relation to environmental diatoms. While diatoms on marine turtles have been studied extensively (Azari *et al.*, 2020; Majewska *et al.*, 2015a; Majewska *et al.*, 2015b; Robinson *et al.*, 2016), there remains ample opportunities for research regarding diatoms in relation to terrapins.

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ANNEXURE A: ECOMORPHOLOGIGAL GUILD DELINEATION

TABLE A1: Main criteria in common and previous recognition in guild delineation of the 106 Southern African anuran tadpoles (Botha, 2013).

Guild	Species	Main Characters	Guild Reference
1. Suspension feeder	*Phrynomantis affinis* *Phrynomantis annectens* *Phrynomantis bifasciatus* *Xenopus gilli* *Xenopus laevis* *Xenopus muelleri* *Xenopus petersii*	- Filter feeders - Jaw sheath and labial teeth absent - Eyes lateral	- Van Dijk - Altig & Johnson - Altig &.McDiarmid
2. Macrophagous-nektonic	*Afrixalus aureus* *Afrixalus delicatus* *Afrixalus fornasinii* *Afrixalus knysnae* *Afrixalus spinifrons*	- Drift/rest mid water - Labial teeth absent /posterior - Oral disc anterior	- Altig & Johnston
3. Lentic-nektonic	*Hyperolius acuticeps* *Hyperolius argus* *Hyperolius horstockii* *Hyperolius marmoratus* *Hyperolius mitchelli* *Hyperolius nasutus* *Hyperolius parallelus* *Hyperolius pickersgilli* *Hyperolius pusillus* *Hyperolius semidiscus* *Hyperolius tuberilinguis* *Hemisus guineensis* *Hemisus guttatus* *Hemisus marmoratus* *Kassina maculata* *Kassina senegalensis* *Semnodactylus wealii* *Hildebrandtia ornata*	- Lentic - Mid water - Eyes lateral	-partly as Van Dijk - partly as Altig & McDiarmid
4. Benthic type 2 (Profundal)	*Ptychadena anchietae* *Ptychadena guibei* *Ptychadena mapacha* *Ptychadena mascareniensis* *Ptychadena mossambica* *Ptychadena oxyrhynchus* *Ptychadena porosissima* *Ptychadena schillukorum* *Ptychadena subpunctata* *Ptychadena taenioscelis* *Strongylopus grayii* *Hylarana darlingi* *Hylarana galamensis* *Chiromanthis xerampelina*	- Lentic & Lotic - Eyes dorsolateral - Oral disc anteroventral - Head rounded - Jaw sheath moderate	- partly as Altig & Johnston - partly as Altig & McDiarmid
5. Lentic-benthic	*Amietophrynus garmani* *Amietophrynus gutturalis* *Amietophrynus maculatus* *Amietophrynus pantherinus* *Amietophrynus pardalis* *Amietophrynus poweri* *Amietophrynus rangeri* *Poyntonophrynus dombensis* *Poyntonophrynus fenoulheti* *Poyntonophrynus hoeschi* *Poyntonophrynus vertebralis* *Vandijkophrynus amatolicus* *Vandijkophrynus angusticeps* *Vandijkophrynus gariepensis* *Vandijkophrynus inyangae* *Vandijkophrynus robinsoni* *Capensibufo rosei* *Capensibufo tradouwi* *Schismaderma carens* *Mertensophryne anotis*	- Lentic - Bottom dwellers - Eyes dorsolateral - Oral disc ventral to anteroventral	- partly as Van Dijk - Altig & Johnston - Altig & McDiarmid

TABLE A1: (Continued)

Guild	Species	Main Characters	Guild Reference
6. Rheophilic	*Amietia dracomontana* *Amietia fuscigula* *Amietia inyangae* *Amietia umbraculata* *Amietia vandijki* *Amietia vertebralis* *Amietia queketii* *Strongylopus bonaespei* *Strongylopus fasciatus* *Strongylopus rhodesianus* *Strongylopus springbokensis* *Strongylopus wageri*	- Lotic - Eyes dorsolateral - Long development time	- Van Dijk
7. Suctorial	*Hadromophryne natalensis* *Heleophryne depressa* *Heleophryne hewitti* *Heleophryne orientalis* *Heleophryne purcelli* *Heleophryne regis* *Heleophryne rosei*	- Torrent dwelling - Multiple labial tooth rows - Oral disc -broad sucker	- partly as Van Dijk - Altig & Johnston - Altig & McDiarmid
8. Excitus-parageios	*Pyxicephalus adspersus* *Pyxicephalus edulis* *Tomopterna cryptotis* *Tomopterna delalandii* *Tomopterna natalensis* *Tomopterna tandyi*	- Lentic - Temporary pool - Rapid development	- New delineation - partly as Van Dijk - partly as Altig & McDiarmid
9. Lentophytophilic	*Cacosternum boettgeri* *Cacosternum capense* *Cacosternum karooicum* *Cacosternum namaquense* *Cacosternum nanum* *Cacosternum platys* *Microbatrachella capensis* *Phrynobatrachus acridoides* *Phrynobatrachus mababiensis* *Phrynobatrachus natalensis* *Phrynobatrachus parvulus*	- Lentic - Mid water - Oral disc near ventral	- New delineation - partly as Van Dijk - partly as Altig & McDiarmid
10. Bentophytophilic	*Leptopelis flavomaculatus* *Leptopelis mossambicus* *Leptopelis natalensis* *Leptopelis xenodactylus* *Natalobatrachus bonebergi* *Poyntonia paludicola*	- Lotic (slow flow) - Associated with vegetation - Head streamlined	- New delineation - partly as Van Dijk - partly as Altig & Johnston - partly as Altig & McDiarmid

ANNEXURE B: DIATOM CODES AND SPECIES USED IN THIS STUDY

TABLE B1: Species names and codes used in this study. Species names are adapted from Taylor *et al.,* (2007) while acronyms are adapted from OMIDA version 3 (Lecointe *et al*., 1993).

Diatom species	Code	Diatom species	Code
Achanthidium sp. 1	ACHD1	*Diadesmis* sp. 1	DIAM1
Achanthidium sp. 2	ACHD2	*Diploneis oblongella*	DOOV
Achanthidium sp. 3	ACHD3	*Epithemia adnata*	EADN
Achanthidium sp. 4	ACHD4	*Eunotia bilunaris*	EBIL
Achanthidium sp. 5	ACHD5	*Eunotia formica*	EFOR
Achanthidium sp. 6	ACHD6	*Eunotia minor*	EMIN
Achanthidium sp. 7	ACHD7	*Encyonema* sp. 1	ENCY1
Achanthidium sp. 8	ACHD8	*Encyonema* sp. 2	ENCY2
Achanthidium sp. 9	ACHD9	*Encyonema* sp. 3	ENCY3
Achanthidium sp. 10	ACHD10	*Encyonema* sp. 4	ENCY4
Achnanthes sp. 1	ACHN1	*Encyonopsis leei*	ENLE
Achnanthidium crassum	ADCR	*Encyonema ventricosum*	ENVE
Achnanthes swazi	ASWA	*Eolimna minima*	EOMI
Brachysira neoexilis	BNEO	*Eunotia pectinalis*	EPEH
Cymbella asp. era	CAGI	*Epithemia sorex*	ESOR
Caloneis sp. 1	CALO1	*Eunotia* sp. 1	EUNO1
Caloneis sp. 2	CALO2	*Eunotia* sp. 2	EUNO2
Caloneis sp. 3	CALO3	*Eunotia* sp. 3	EUNO3
Caloneis sp. 4	CALO4	*Eunotia* sp. 4	EUNO4
Craticula ambigua	CAMB	*Eunotia* sp. 5	EUNO5
Cymbella cistula	CCIS	*Eunotia* sp. 6	EUNO6
Capartogramma crucicula	CCRU	*Eunotia* sp. 7	EUNO7
Cymbella neocistula	CNIS	*Eunotia* sp. 8	EUNO8
Cocconeis sp.	COCO1	*Eunotia* sp. 9	EUNO9
Cocconeis placentula	CPTG	*Eunotia* sp. 10	EUNO10
Craticula sp. 1	CRAT1	*Eunotia* sp. 11	EUNO11
Craticula sp. 2	CRAT2	*Eunotia* sp. 12	EUNO12
Craticula sp. 3	CRAT3	*Eunotia* sp. 13	EUNO13
Craticula sp. 4	CRAT4	*Eunotia* sp. 14	EUNO14
Craticula sp. 5	CRAT5	*Eunotia* sp. 15	EUNO15
Craticula sp. 6	CRAT6	*Eunotia* sp. 16	EUNO16
Craticula sp. 7	CRAT7	*Fragilaria anceps*	FANC
Craticula sp. 8	CRAT8	*Fragilaria biceps*	FBCP
Craticula sp. 9	CRAT9	*Fragilaria* sp. 1	FRAG1
Craticula sp. 10	CRAT10	*Fragilaria* sp. 2	FRAG2
Craticula buderi	CRBU	*Fragilaria* sp. 3	FRAG3
Craticula perrotettii	CRPE	*Fragilaria* sp. 4	FRAG6
Cymbella silesiaca	CSLE	*Fragilaria* sp. 5	FRAG7
Cymbella subleptoceros	CSLP	*Fragilaria* sp. 6	FRAG8
Cymbella tumida	CTUM	*Fragilaria* sp. 7	FRAG9
Craticula vixnegligenda	CVIX	*Fragilaria* sp. 8	FRAG10
Cyclotella sp. 1	CYCL1	*Fragilaria* sp. 9	FRAG11
Cyclotella sp. 2	CYCL2	*Fragilaria* sp. 10	FRAG10
Cyclotella sp. 3	CYCL3	*Frustulia sp. 1*	FRUS1
Cyclotella sp. 4	CYMB1	*Gomphonema acuminatum*	GACU
Cyclotella sp. 5	CYMB2	*Gomphonema angustatum*	GADI
Cyclotella sp. 6	CYMB3	*Gomphonema affine*	GAFF
Denticula sp. 1	DENT1	*Geissleria sp. 1*	GEIS1

TABLE B1: (Continued)

Diatom species	Code	Diatom species	Code
Gomphonema gracile	GGRA	*Navicula cryptocephala*	NCRY
Gomphonema lagenula	GLGN	*Neidium affine*	NEAF
Gomphonema laticollum	GLTC	*Neidium* sp. 1	NEID1
Gomphonema sp. 1	GOMP1	*Neidium* sp. 2	NEID2
Gomphonema sp. 2	GOMP2	*Neidium* sp. 3	NEID3
Gomphonema sp. 3	GOMP3	*Neidium* sp. 4	NEID4
Gomphonema sp. 4	GOMP4	*Nitzschia* sp. 1	NITZ1
Gomphonema sp. 5	GOMP5	*Nitzschia* sp. 2	NITZ2
Gomphonema sp. 6	GOMP6	*Nitzschia* sp. 3	NITZ3
Gomphonema sp. 7	GOMP7	*Nitzschia* sp. 4	NITZ4
Gomphonema sp. 8	GOMP8	*Nitzschia* sp. 5	NITZ5
Gomphonema sp. 9	GOMP9	*Nitzschia* sp. 6	NITZ6
Gomphonema sp. 10	GOMP10	*Nitzschia* sp. 7	NITZ7
Gomphonema sp. 11	GOMP11	*Nitzschia* sp. 8	NITZ8
Gomphonema sp. 12	GOMP12	*Nitzschia* sp. 9	NITZ9
Gomphonema sp. 13	GOMP13	*Nitzschia* sp. 10	NITZ10
Gomphonema sp. 14	GOMP14	*Nitzschia* sp. 11	NITZ11
Gomphonema parvulum	GPAR	*Nitzschia* sp. 12	NITZ12
Gomphonema pumilum	GPUM	*Nitzschia* sp. 13	NITZ13
Gomphonema venusta	GVNU	*Nitzschia* sp. 14	NITZ14
Gyrosigma sp. 1	GYRO1	*Nitzschia* sp. 15	NITZ15
Hantzschia amphioxys	HAFC	*Nitzschia microcephala*	NMIC
Hantzschia sp. 1	HANT1	*Navicula nivalis*	NNIV
Hantzschia sp. 2	HANT2	*Nitzschia palea*	NPAL
Hantzschia sp. 3	HANT3	*Navicula recens*	NRCS
Hantzschia sp. 4	HANT4	*Navicula symmetrica*	NSYM
Hantzschia sp. 5	HANT5	*Nitzschia valdecostata*	NVLC
Hantzschia sp. 6	HANT6	*Navicula vetita*	NVTA
Hantzschia elongata	HELO	*Pinnularia acrosp. haeria*	PAUN
Lemnicola sp. 1	LEMN1	*Pinnularia borealis*	PBRT
Luticola kotschyi	LKOT	*Pinnularia divergens*	PDML
Luticola mutica	LMUT	*Pinnularia divergens* var. *sublinearis*	PDSL
Luticola sp. 1	LUTI1	*Pinnularia gibba*	PGIB
Luticola sp. 2	LUTI2	*Pinnularia* sp. 1	PINU1
Luticola sp. 3	LUTI3	*Pinnularia* sp. 2	PINU2
Luticola sp. 4	LUTI4	*Pinnularia* sp. 3	PINU3
Luticola sp. 5	LUTI5	*Pinnularia* sp. 4	PINU4
Mayamaea atomus	MAAT	*Pinnularia* sp. 5	PINU5
Mayamaea sp. 1	MAYA1	*Pinnularia* sp. 6	PINU6
Microcostatus sp. 1	MCCT1	*Pinnularia* sp. 7	PINU7
Nitzschia amphibia	NATG	*Pinnularia* sp. 8	PINU8
Navicula sp. 1	NAVI1	*Pinnularia* sp. 9	PINU9
Navicula sp. 2	NAVI2	*Pinnularia* sp. 10	PINU10
Navicula sp. 3	NAVI3	*Pinnularia* sp. 11	PINU11
Navicula sp. 4	NAVI4	*Pinnularia* sp. 12	PINU12
Navicula sp. 5	NAVI5	*Pinnularia* sp. 13	PINU13
Navicula sp. 6	NAVI6	*Pinnularia* sp. 14	PINU14
Navicula sp. 7	NAVI7	*Pinnularia* sp. 15	PINU15
Navicula sp. 8	NAVI8	*Pinnularia* sp. 16	PINU16
Navicula sp. 9	NAVI9	*Pinnularia* sp. 17	PINU17
Navicula sp. 10	NAVI10	*Pinnularia* sp. 18	PINU18
Navicula sp. 11	NAVI11	*Pinnularia* sp. 19	PINU19
Navicula sp. 12	NAVI12	*Pinnularia* sp. 20	PINU20
Nitzschia clausii	NCLA	*Pinnularia* sp. 21	PINU21

TABLE B1: (Continued)

Diatom species	Code	Diatom species	Code
Planothidium frequentissimum	PLFR	*Stauroneis anceps*	STAN
Planothidium sp. 1	PLTD1	*Stauroneis* sp. 1	STAU1
Planothidium sp. 2	PLTD2	*Stauroneis* sp. 2	STAU2
Pinnularia microstauron var. *rostrata*	PMRO	*Stauroneis* sp. 3	STAU3
Pinnularia subbrevistriata	PSBV	*Stauroneis* sp. 4	STAU4
Pinnularia subcapitata	PSCA	*Stauroneis* sp. 5	STAU5
Pinnularia viridiformis	PVIF	*Stauroneis* sp. 6	STAU6
Rhoicosp. henia abbreviata	RABB	*Stauroneis* sp. 7	STAU7
Rhopalodia gibba	RGIB	*Stauroneis* sp. 8	STAU8
Stauroneis borrichii	SBOR	*Stauroneis* sp. 9	STAU9
Staurosira construens	SCBI	*Stauroneis* sp. 10	STAU10
Sellaphora sp. 1	SELL1	*Stauroneis* sp. 11	STAU11
Sellaphora sp. 2	SELL2	*Stauroneis* sp. 12	STAU12
Stauroneis gracilis	SGRC	*Stauroneis* sp. 13	STAU13
Stauroneis kootenai	SKOO	*Stauroneis* sp. 14	STAU14
Seminavis sp. 1	SMNA1	*Stauroneis* sp. 15	STAU15
Surirella ovalis	SOVI	*Stauroneis dracomontana*	STDR
Sellaphora pupula	sp. UP	*Stauroneis nana*	STNN
Stauroneis sofia	SSOF	*Tryblionella coarctata*	TCOA
Stauroneis superkuelbsii	SSRU	*Tryblionella littoralis*	TLIT
Sellaphora stroemii	SSTM	*Tryblionella* sp. 1	TRYB1

ANNEXURE C: RAW DIATOM DATA

TABLE C1: Raw dataset obtained from Ukutula's samples. Env: Environmental sample taken at site 1; Env2: Environmental sample taken at site 2; Env3: Environmental sample taken at site 3. Tadpole guild samples are indicated by blue spots (Ter: Terrapin (*Pelomedusa galiata*) sample taken at site 1; G1-1s1: *Phrynomantis bifasciatus* (Guild 1, Suspension feeder) sampled at site 1; G4-1s1: *Ptychadena mossambica* (Guild 4, Benthic type 2) sampled at site 1; G4-2s1: *Strongylopus grayii* (Guild 4, Benthic type 2), and G6-1s1: *Amietia delalandii* (Guild 6, Rheophilic) sampled at site 1; G3-1s2: *Kassina senegalensis* (Guild 3, Lentic-nektonic) sampled at site 2; G6-2s2: *Amietia delalandii* (Guild 6, Rheophilic) sampled at site 2; G3-1s3: *Kassina senegalensis* (Guild 3, Lentic-nektonic) sampled at site 3; U3-1s3: *Phrynomantis bifasciatus* (Guild 1, suspension feeder) sampled at site 3; G6-2s3: *Amietia delalandii* (Guild 6, Rheophilic) sampled at site 3. Rows highlighted in green indicates samples removed when statistical noise was removed. See Annexure B for diatom species codes.

Sites and samples →	Ukutula site 1						Ukutula site 2			Ukutula site 3				Totals
Code	Env	Ter	G6-1s1	G1-1s1	G4-1s1	G4-2s1	Env2	G3-1s2	G4-2s2	Env3	G3-1s3	G1-1s2	G4-2s3	
ACHD1	0	0	0	0	0	8	0	0	0	0	1	0	0	9
ACHD2	1	0	0	35	1	3	0	0	0	3	28	0	0	71
ACHD3	89	0	0	14	0	0	0	0	0	5	0	0	0	108
ACHD4	0	0	0	1	0	0	0	0	0	0	0	0	0	1
ACHD5	0	0	0	0	0	0	0	0	0	45	0	0	0	45
ACHD6	0	0	0	0	0	3	0	0	0	0	0	0	0	3
ACHD7	0	0	0	1	0	0	0	0	0	0	0	0	0	1
ADCR	0	0	0	0	4	10	0	0	0	2	2	0	0	18
ASWA	0	0	0	0	0	0	0	0	0	1	0	0	0	1
BNEO	0	0	0	0	0	0	0	0	0	1	0	0	0	1
CAGI	0	0	0	0	0	0	0	0	0	0	1	0	0	1
CAMB	0	0	0	11	30	0	0	56	0	0	0	0	0	97
CCRU	0	1	0	0	0	0	0	0	0	0	0	0	0	1
CNIS	0	0	0	0	0	0	0	0	0	6	0	0	0	6
CPTG	0	0	1	1	0	0	0	0	0	0	0	0	0	2
CRAT1	0	12	0	0	0	0	9	0	0	0	0	0	0	21
CRAT2	0	0	0	0	0	0	0	0	4	0	14	10	13	41
CRAT3	0	0	16	0	0	0	0	0	0	8	0	0	0	24
CRAT4	0	6	0	0	0	0	0	0	0	0	0	0	0	6
CRAT5	0	0	0	0	0	0	0	5	0	0	0	0	0	5
CRAT6	19	0	0	0	0	0	0	0	0	0	0	0	0	19
CRBU	0	0	0	35	6	0	0	0	0	0	0	0	0	41
CRPE	0	0	0	0	0	31	0	0	70	0	0	0	0	101
CSLP	0	0	0	0	0	0	0	0	0	0	0	1	0	1
CTUM	0	0	0	0	0	0	0	0	0	7	0	0	0	7
CVIX	0	0	0	0	0	0	0	0	0	0	31	1	13	45
CYMB1	0	0	0	0	0	0	0	0	0	3	0	0	0	3
DOOV	0	0	0	0	0	0	0	0	0	5	0	0	0	5
EBIL	0	0	0	0	0	0	0	0	0	0	4	0	0	4
EFOR	14	5	53	0	34	1	0	0	1	3	0	11	1	123
EMIN	0	0	0	7	2	0	0	0	0	0	0	0	0	9
ENCY1	6	0	0	0	0	0	0	0	0	0	0	0	0	6
ENLE	0	1	0	0	0	0	0	6	0	1	0	0	0	8
ENVE	0	0	0	0	0	0	0	1	0	0	0	0	0	1
EOMI	0	9	0	0	2	0	0	0	0	0	1	0	1	13
EUNO1	0	0	0	1	0	0	0	0	0	0	0	0	0	1
EUNO2	0	0	0	0	0	1	3	7	0	1	0	0	0	12
EUNO3	0	0	0	0	2	0	0	0	0	0	1	0	1	4
EUNO4	15	0	0	0	0	4	0	0	0	0	4	0	1	24
EUNO5	16	9	0	0	1	0	0	30	22	0	0	2	0	80
EUNO6	47	0	32	0	0	0	0	0	0	0	0	0	0	79
FANC	0	0	0	1	0	0	0	0	0	0	0	0	0	1
FBCP	0	0	0	0	0	0	0	0	0	0	0	0	1	1
FRAG1	0	0	11	0	0	0	0	0	0	2	0	0	0	13
FRAG2	0	0	0	0	0	0	0	0	0	0	0	7	0	7

TABLE C1: (Continued)

Sites and samples → Code	Ukutula site 1						Ukutula site 2			Ukutula site 3				Totals
	Env	Ter	G6-1s1	G1-1s1	G4-1s1	G4-2s1	Env2	G3-1s2	G4-2s2	Env3	G3-1s3	G1-1s2	G4-2s3	
FRUS1	1	0	30	0	0	0	0	0	0	0	0	0	0	31
GACU	0	0	4	0	0	0	0	0	0	0	0	0	0	4
GADI	0	0	0	0	4	0	0	0	0	0	1	0	0	5
GAFF	11	4	0	0	0	0	0	0	0	0	0	0	0	15
GGRA	0	50	0	7	0	86	182	22	12	48	8	0	8	423
GLTC	0	2	0	0	0	0	0	0	0	0	0	0	0	2
GOMP1	14	0	0	10	63	57	134	29	0	6	9	0	0	322
GOMP2	0	22	0	0	0	1	0	0	9	17	0	0	0	49
GOMP3	0	0	0	0	0	0	0	0	0	0	0	0	1	1
GOMP4	0	36	0	0	0	0	0	0	0	0	0	0	0	36
GOMP5	4	6	1	1	4	0	0	6	0	0	0	0	0	22
GOMP6	0	0	0	0	33	0	0	0	0	0	0	0	0	33
GPAR	0	5	0	20	33	0	0	4	3	15	0	0	0	80
GPUM	0	0	0	0	0	0	0	0	0	20	0	0	0	20
GVNU	0	4	0	0	0	11	0	0	0	10	0	0	0	25
HAFC	0	11	9	7	10	21	29	18	31	24	11	8	35	214
HANT1	142	11	0	0	0	44	26	23	23	0	0	0	0	269
HANT2	0	55	6	0	8	0	0	0	0	0	0	0	0	69
HANT3	18	0	0	10	0	46	0	4	16	0	0	0	0	94
HANT4	12	0	0	0	0	0	0	0	0	0	0	0	0	12
HELO	0	0	6	0	25	3	1	0	3	4	0	0	0	42
LEMN1	0	0	0	0	0	0	0	8	0	0	0	0	0	8
LKOT	0	0	0	0	0	0	0	0	0	0	2	0	0	2
LMUT	20	11	10	9	11	6	27	13	10	27	11	2	15	172
LUTI1	1	0	0	0	0	0	0	0	2	0	4	0	0	7
LUTI2	8	0	0	1	0	0	0	0	0	0	20	0	0	29
LUTI3	10	0	0	1	0	0	0	0	0	0	0	0	0	11
MAAT	0	0	0	0	0	0	0	2	0	0	0	0	0	2
MCCT1	0	0	1	0	0	0	0	0	0	0	0	0	0	1
NATG	0	0	0	1	0	0	0	0	0	0	0	0	0	1
NAVI1	0	0	0	0	0	0	0	0	0	0	0	3	0	3
NAVI2	0	0	0	0	0	0	0	0	0	15	0	0	0	15
NAVI3	32	0	0	0	5	3	0	0	0	0	0	0	0	40
NCLA	0	2	0	0	0	0	0	0	0	0	0	0	0	2
NCRY	0	0	0	0	0	0	0	0	0	1	0	0	0	1
NEAF	9	8	0	0	2	8	0	0	0	0	1	0	0	28
NEID1	0	0	0	0	0	0	0	0	0	0	3	0	0	3
NEID2	0	0	0	0	0	3	0	0	0	0	0	0	0	3
NEID3	0	0	0	0	0	0	0	0	0	0	3	0	39	42
NEID4	0	0	0	0	0	5	0	0	0	0	0	0	0	5
NITZ1	99	0	0	44	0	0	0	0	0	35	70	35	49	332
NITZ2	10	1	0	0	0	0	0	0	0	0	58	0	0	69
NITZ3	0	0	0	0	0	0	0	13	38	0	0	1	0	52
NITZ4	16	1	0	0	5	0	0	0	0	0	0	0	0	22

TABLE C1: (Continued)

Sites and samples → Code	Ukutula site 1						Ukutula site 2			Ukutula site 3				Totals
	Env	Ter	G6-1s1	G1-1s1	G4-1s1	G4-2s1	Env2	G3-1s2	G4-2s2	Env3	G3-1s3	G1-1s2	G4-2s3	
NITZ5	0	0	0	0	0	0	3	14	0	0	0	0	0	17
NITZ6	18	18	0	0	0	15	9	0	0	0	4	6	0	70
NITZ7	0	0	0	0	0	0	0	0	0	2	0	0	0	2
NITZ8	0	0	35	0	0	0	10	56	3	0	0	0	0	104
NITZ9	0	0	0	0	0	0	0	0	1	0	0	0	0	1
NNIV	0	0	3	0	3	0	0	1	4	7	1	1	3	23
NPAL	0	14	0	211	84	0	0	0	0	0	0	0	0	309
NRCS	0	0	0	0	0	0	0	0	0	0	0	0	3	3
NSYM	0	0	0	1	0	0	0	0	0	1	0	0	0	2
NVLC	0	0	0	0	0	0	0	0	0	40	0	0	0	40
NVTA	0	1	0	0	0	3	0	0	0	0	0	0	0	4
PBRT	36	43	15	1	25	33	31	44	72	24	18	11	27	380
PDML	140	70	0	14	0	17	5	52	35	17	0	71	64	485
PDSL	0	54	0	0	0	0	0	0	0	0	0	0	0	54
PGIB	0	0	0	0	0	0	40	0	0	0	0	0	24	64
PINU1	0	12	0	0	0	0	0	6	0	0	0	0	0	18
PINU2	77	4	72	0	0	0	0	0	0	0	0	0	0	153
PINU3	20	20	0	0	0	0	35	5	3	8	12	12	67	182
PINU4	0	0	0	0	0	0	0	0	0	8	0	0	0	8
PINU5	0	0	0	0	0	0	0	0	0	0	35	0	0	35
PINU6	0	0	0	7	0	0	0	34	3	0	0	2	17	63
PINU7	19	0	0	7	0	1	0	0	0	4	0	0	0	31
PINU8	0	0	0	0	0	0	0	0	0	0	0	0	1	1
PLFR	0	0	0	0	0	0	0	0	0	0	1	0	0	1
PLTD1	0	0	0	0	0	0	0	1	0	0	0	0	0	1
PLTD2	0	0	0	0	0	0	0	0	0	3	0	0	0	3
PMRO	0	0	0	0	2	24	0	0	28	0	71	0	0	125
PSBV	0	0	0	77	0	0	0	0	0	0	0	0	0	77
PSCA	0	0	0	0	0	0	0	0	0	0	1	0	0	1
PVIF	0	0	0	0	0	0	1	0	0	0	0	0	0	1
RABB	0	0	0	0	0	0	0	5	0	0	0	0	0	5
SBOR	0	0	3	2	0	0	1	0	0	35	2	10	16	69
SELL1	12	0	0	0	0	1	0	0	0	0	0	0	0	13
SGRC	100	94	0	0	0	166	0	0	0	0	0	0	0	360
SKOO	251	0	0	51	187	0	0	0	0	0	0	0	0	489
SOVI	0	0	0	0	0	0	0	0	0	0	1	0	0	1
SSOF	0	0	0	0	0	0	2	0	2	5	5	0	10	24
SSRU	0	0	0	7	0	0	0	0	0	0	0	0	0	7
SSTM	0	0	0	0	0	0	0	0	2	0	0	0	0	2
STAN	0	91	0	0	0	0	56	159	15	0	210	0	0	531
STAU1	0	0	0	0	1	0	3	2	2	37	11	9	0	65
STAU2	83	0	188	0	0	32	0	0	13	106	0	429	377	1228
STAU3	0	4	0	0	0	0	0	0	0	0	0	0	0	4
STAU4	0	0	0	1	37	0	0	0	0	0	0	0	0	38
STAU5	0	0	0	0	2	0	0	0	0	0	0	0	0	2
STNN	0	0	0	0	0	0	10	0	0	0	4	0	2	16
TCOA	0	0	0	0	0	0	2	0	0	0	0	0	0	2
TLIT	0	0	0	0	0	0	0	0	1	0	0	0	0	1
TRYB1	10	0	0	0	1	0	0	0	0	0	0	0	0	11

TABLE C2: Raw dataset obtained from Aliwal North's samples. Environmental samples includes EnvFE1, EnvFE2, EnvFE3, EnvFE7, EnvTN1B, EnvTN2, EnvTN4, and EnvX taken at sites 1, 2, 3, 4, 5, 6, 7, and site 8 respectively. Samples includes G9-1sFE1; *Cacosternum boettgeri* from guild 9 (Lentophytophilic) taken at site 1, G5-1sFE1; *Amietophrynus rangeri* from guild 5 (Lentic-benthic) taken at site 1, G3-1sFE2; *Kassina senegalensis* from guild 3 (Lentic-nektonic) taken at site 2, G3-1sFE3 ; *Kassina senegalensis* from guild 3 (Lentic-nektonic) taken at site 3, G3-1sFE7; *Kassina senegalensis* from guild 3 (Lentic-nektonic) taken at site 4, G9-1sFE7; *Cacosternum boettgeri* from guild 9 (Lentophytophilic) taken at site 4, G3-1sTN1B; *Kassina senegalensis* from guild 3 (Lentic-nektonic) taken at site 5, G9-1sTN1B; *Cacosternum boettgeri* from guild 9 (Lentophytophilic) taken at site 5, G3-1sTN2; *Kassina senegalensis* from guild 3 (Lentic-nektonic) taken at site 6, G9-1sTN2; *Cacosternum boettgeri* from guild 9 (Lentophytophilic) taken at site 6, G3-1sTN4; *Kassina senegalensis* from guild 3 (Lentic-nektonic) taken at site 6, G9-1sTN4; *Cacosternum boettgeri* from guild 9 (Lentophytophilic) taken at site 7, and G6-1sX; *Amietia delalandii* from guild 6 (Rheophilic) taken at Site 8. Rows highlighted in green indicates samples removed when statistical noise was removed. See Annexure B for diatom species codes.

Sites and samples →	**Site 1**			**Site 2**		**Site 3**		**Site 4**			**Site 5**			**Site 6**			**Site 7**			**Site 8**		
Code	**EnvFE1**	**G9-1sFE1**	**G5-1sFE1**	**EnvFE2**	**G3-1sFE2**	**EnvFE3**	**G3-1sFE3**	**EnvFE7**	**G3-1sFE7**	**G9-1sFE7**	**EnvTN1B**	**G3-1sTN1B**	**G9-1sTN1B**	**EnvTN2**	**G3-1sTN2**	**G9-1sTN2**	**EnvTN4**	**G3-1sTN4**	**G9-1sTN4**	**EnvX**	**G6-1sX**	**Totals**
ACHD1	9	0	0	4	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	15
ACHD2	9	0	0	0	0	0	0	0	2	5	0	3	1	0	0	0	0	0	0	0	5	25
ACHD3	0	4	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	13
ACHD7	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
ACHD8	0	0	0	0	0	0	2	4	0	1	0	0	0	0	0	0	0	0	0	0	0	7
ACHD9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
ACHD10	0	0	0	0	0	0	0	0	0	1	0	0	3	0	0	0	0	0	0	0	0	4
ACHN1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
CALO1	3	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	7
CALO2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
CALO3	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	2
CALO4	0	0	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18

TABLE C2: (Continued)

Sites and samples →	Site 1			Site 2		Site 3		Site 4			Site 5			Site 6			Site 7			Site 8		
Code	EnvFE1	G9-1sFE1	G5-1sFE1	EnvFE2	G3-1sFE2	EnvFE3	G3-1sFE3	EnvFE7	G3-1sFE7	G9-1sFE7	EnvTN1B	G3-1sTN1B	G9-1sTN1B	EnvTN2	G3-1sTN2	G9-1sTN2	EnvTN4	G3-1sTN4	G9-1sTN4	EnvX	G6-1sX	Totals
CPTG	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
COCO1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
CAMB	0	9	0	0	0	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	22
CRAT1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
CRAT4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
CRAT5	1	0	0	0	25	0	6	0	1	1	0	0	0	0	0	0	0	0	0	0	0	34
CRAT6	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7
CRAT7	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	6
CRAT8	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
CRAT9	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
CRAT10	0	7	10	4	0	0	0	16	0	8	0	0	0	0	0	0	0	0	0	1	0	46
CYCL1	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	6
CYCL2	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	8
CYCL3	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	10
CAGI	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CCIS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CSLE	6	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	4	12
CYMB2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	4
CYMB3	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	5
CTUM	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
DENT1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	69	0	69
DIAM1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
ENLE	0	0	0	0	0	1	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	5
ENCY2	0	0	5	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	7
ENCY3	0	0	0	0	0	0	0	0	0	2	0	0	1	0	0	0	0	0	0	0	0	3
ENCY4	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
ENVE	0	0	0	0	0	0	0	0	0	0	38	0	0	0	0	0	0	0	0	0	0	38
EADN	19	0	0	0	0	8	1	0	0	0	0	0	3	0	0	0	0	1	1	10	69	112
ESOR	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3
EBIL	0	0	0	0	10	4	5	0	0	0	0	0	0	0	0	2	0	3	8	0	0	32
EFOR	0	0	0	136	19	3	5	31	1	2	0	54	27	52	9	9	223	59	66	0	0	696
EMIN	1	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	10	0	0	0	0	14
EPEH	0	14	0	23	9	18	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	66

TABLE C2: (Continued)

Sites and samples →	Site 1			Site 2		Site 3		Site 4			Site 5			Site 6			Site 7			Site 8		
Code	EnvFE1	G9-1sFE1	G5-1sFE1	EnvFE2	G3-1sFE2	EnvFE3	G3-1sFE3	EnvFE7	G3-1sFE7	G9-1sFE7	EnvTN1B	G3-1sTN1B	G9-1sTN1B	EnvTN2	G3-1sTN2	G9-1sTN2	EnvTN4	G3-1sTN4	G9-1sTN4	EnvX	G6-1sX	Totals
EUNO2	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
EUNO3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	21	0	0	0	23
EUNO5	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	3
EUNO6	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
EUNO7	0	0	0	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13
EUNO8	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
EUNO9	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	3
EUNO10	0	0	0	0	0	0	0	0	0	1	0	0	0	0	11	0	0	0	0	0	0	12
EUNO11	0	0	0	0	3	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	7
EUNO12	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
EUNO13	0	0	0	0	0	0	0	0	0	1	0	33	0	0	0	0	0	23	0	0	0	57
EUNO14	0	0	0	0	0	0	1	0	0	0	0	0	5	0	0	0	0	0	16	0	0	22
EUNO15	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	50	0	0	52
EUNO16	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
FANC	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	1	0	0	0	0	0	3
FBCP	6	0	16	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	20	0	0	47
FRAG1	0	0	0	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13
FRAG2	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
FRAG3	80	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	80
FRAG6	0	0	0	0	0	0	0	0	0	0	0	0	0	40	0	0	0	0	0	0	0	40
FRAG7	0	0	0	0	0	0	0	22	0	0	0	0	0	0	0	0	14	0	0	0	0	36
FRAG8	0	0	0	0	0	2	0	0	0	0	6	0	0	0	0	0	0	0	0	4	0	12
FRAG9	0	0	0	0	0	4	7	0	0	0	20	0	0	0	0	0	0	0	0	4	0	35
FRAG10	0	0	0	0	0	24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	24
GSDC	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
GAFF	0	0	0	0	0	7	0	5	0	0	0	0	0	29	0	4	14	3	0	0	0	62
GGRA	0	0	2	27	1	7	19	22	0	2	1	0	0	0	5	0	71	6	12	10	0	185
GLGN	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
GLTC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	7	8
GPAR	28	13	0	7	2	25	50	74	18	19	199	1	2	190	155	16	0	56	0	6	0	861
GPUM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2
GOMP1	0	18	0	0	4	0	0	6	1	41	0	0	8	0	73	248	13	0	9	0	6	427
GOMP2	0	0	0	0	0	0	30	0	0	0	0	0	0	0	0	0	0	23	0	0	5	58
GOMP3	23	0	0	0	0	0	21	0	0	0	0	0	0	0	0	1	0	0	1	0	0	46
GOMP4	0	0	0	0	1	0	56	0	0	0	0	0	0	0	0	0	0	0	0	0	0	57
GOMP7	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
GOMP8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	0	0	0	0	11
GOMP9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	13	0	0	0	17
GOMP10	0	0	1	0	0	94	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	95

TABLE C2: (Continued)

Sites and samples →	Site 1			Site 2		Site 3		Site 4			Site 5			Site 6			Site 7			Site 8		
Code	EnvFE1	G9-1sFE1	G5-1sFE1	EnvFE2	G3-1sFE2	EnvFE3	G3-1sFE3	EnvFE7	G3-1sFE7	G9-1sFE7	EnvTN1B	G3-1sTN1B	G9-1sTN1B	EnvTN2	G3-1sTN2	G9-1sTN2	EnvTN4	G3-1sTN4	G9-1sTN4	EnvX	G6-1sX	Totals
GOMP11	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	3
GOMP12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	22	0	0	22
GOMP13	0	0	0	0	0	0	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18
GOMP14	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7
GVNU	0	0	0	0	0	0	0	0	0	0	0	0	0	0	48	0	0	35	0	0	0	83
GYRO1	4	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
HAFC	7	22	4	2	1	6	8	0	0	0	0	2	7	0	0	9	0	1	14	0	2	85
HELO	0	8	24	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	36
HANT1	0	0	1	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7
HANT3	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	3
HANT5	0	0	0	0	3	0	3	0	0	0	0	1	5	0	0	0	0	1	0	0	0	13
HANT6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
LEMN1	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	2
LMUT	0	0	1	0	1	1	0	0	0	0	1	0	3	0	0	0	0	2	9	2	1	21
LUTI4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LUTI5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MAAT	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	5
MAYA1	0	0	50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	50
NNIV	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	3
NRCS	0	0	0	0	0	0	0	0	0	0	28	0	0	0	0	0	0	0	0	11	0	39
NAVI3	0	0	0	0	0	4	0	2	0	0	0	0	0	0	0	0	0	0	0	1	0	7
NAVI4	18	0	28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	62
NAVI5	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
NAVI6	2	28	0	2	0	0	0	3	0	0	1	1	0	0	0	0	0	0	1	0	0	38
NAVI7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
NAVI8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NAVI9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NAVI10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	3
NAVI11	0	0	51	0	0	0	0	0	1	154	0	0	0	0	0	0	0	0	0	0	0	206
NAVI12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	44	0	44
NSYM	2	0	0	0	0	0	2	35	0	0	0	0	3	0	0	0	0	0	0	0	0	42
NEAF	0	1	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	9
NATG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	14
NMIC	0	2	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
NPAL	0	0	0	71	0	0	17	0	249	7	0	0	0	0	0	0	0	0	0	0	0	344

TABLE C2: (Continued)

Sites and samples →	Site 1			Site 2		Site 3		Site 4			Site 5			Site 6			Site 7			Site 8		
Code	EnvFE1	G9-1sFE1	G5-1sFE1	EnvFE2	G3-1sFE2	EnvFE3	G3-1sFE3	EnvFE7	G3-1sFE7	G9-1sFE7	EnvTN1B	G3-1sTN1B	G9-1sTN1B	EnvTN2	G3-1sTN2	G9-1sTN2	EnvTN4	G3-1sTN4	G9-1sTN4	EnvX	G6-1sX	Totals
NITZ1	43	0	0	0	17	0	0	0	0	0	0	0	4	0	0	5	0	0	3	0	3	75
NITZ2	0	81	56	0	94	0	0	70	0	25	19	3	49	0	4	0	0	6	2	30	0	439
NITZ3	0	0	0	5	0	0	4	0	0	0	0	0	5	0	0	0	0	0	0	0	0	14
NITZ6	0	0	6	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	8
NITZ7	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
NITZ8	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	67	73
NITZ10	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
NITZ11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	2	0	9
NITZ12	0	0	0	0	0	0	0	0	12	16	0	0	0	0	1	0	0	0	0	0	0	29
NITZ13	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	5
NITZ14	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	1	0	0	0	0	0	8
NITZ15	0	0	21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	21
PAUN	0	0	0	0	0	0	0	0	3	0	0	135	10	0	0	0	0	0	0	0	0	148
PBRT	0	6	0	0	3	0	4	0	0	1	1	63	7	0	0	0	0	6	12	1	1	105
PDML	0	25	3	20	52	11	14	2	9	0	1	3	17	0	0	0	6	1	30	0	3	197
PGIB	0	0	0	3	6	0	0	0	0	3	0	0	20	1	0	0	0	0	0	0	0	33
PMRO	0	0	0	3	0	3	8	0	0	0	0	0	7	0	0	0	0	9	17	0	0	47
PINU1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	2
PINU3	0	0	0	0	0	0	1	0	2	0	0	4	0	0	0	0	0	0	0	0	0	7
PINU5	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
PINU7	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
PINU9	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	2
PINU10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	6
PINU11	0	0	0	0	0	16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16
PINU12	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
PINU13	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
PINU14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	9
PINU15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3
PINU16	0	0	0	0	0	0	0	0	0	0	0	0	33	0	0	0	0	0	0	0	0	33
PINU17	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	6
PINU18	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	3
PINU19	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11
PINU20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	2
PSBV	0	0	0	0	0	2	0	0	0	0	0	0	0	0	1	0	0	0	13	0	0	16
PSCA	0	0	0	0	0	4	0	2	0	0	0	7	0	0	0	3	2	22	0	133	0	173
PVIF	2	2	0	0	0	2	2	0	0	0	0	0	0	0	0	0	0	1	3	0	0	12
RGIB	30	0	1	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	93	126

TABLE C2: (Continued)

Sites and samples →	Site 1			Site 2		Site 3		Site 4			Site 5			Site 6			Site 7			Site 8		
Code	EnvFE1	G9-1sFE1	G5-1sFE1	EnvFE2	G3-1sFE2	EnvFE3	G3-1sFE3	EnvFE7	G3-1sFE7	G9-1sFE7	EnvTN1B	G3-1sTN1B	G9-1sTN1B	EnvTN2	G3-1sTN2	G9-1sTN2	EnvTN4	G3-1sTN4	G9-1sTN4	EnvX	G6-1sX	Totals
SPUP	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	6
SELL2	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2
SMNA1	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	5
STAN	0	1	0	0	0	0	0	0	0	0	0	0	25	0	0	1	0	4	0	0	0	31
SGRC	0	0	0	0	0	0	1	0	0	0	0	0	25	0	0	0	0	0	0	0	0	26
SSOF	0	0	0	0	8	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	14
STAU1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	2
STAU2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
STAU3	1	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7
STAU4	0	0	1	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	6
STAU6	0	24	0	7	0	14	13	0	0	0	2	0	0	0	0	0	0	0	0	0	1	61
STAU7	0	7	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	24
STAU8	0	0	0	0	6	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	7
STAU9	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
STAU10	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	2
STAU11	0	0	0	0	0	0	0	0	0	0	0	21	0	0	0	0	0	0	0	0	0	21
STAU12	0	0	0	0	0	0	0	0	0	0	0	3	3	0	0	0	0	0	0	0	0	6
STAU13	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	5
STAU14	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
SCBI	0	3	6	11	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	22
TRYB2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
TRYB3	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	3
TRYB4	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
Sum	**309**	**304**	**313**	**375**	**301**	**300**	**305**	**314**	**318**	**312**	**319**	**349**	**304**	**320**	**313**	**303**	**372**	**310**	**328**	**354**	**310**	**6733**

ANNEXURE D: RESULTS OF STATISTICAL ANALYSES

TABLE D1: Sig. (P) values calculated from relative abundance data cleared from statistical noise for Ukutula

Variable	Sig.
Environmental samples	0,434
Species	0,982
Guilds	0,954

TABLE D2: Matrix indicating effect sizes calculated from relative abundace data cleared from statistical noise for sample types of Ukutula

	Mean	Environment	Terrapin
Environment	5,001	-	-
Terrapin	4,663	0,042652932	-
Tadpole	6,374	0,173152995	0,215805926

TABLE D3: Matrix indicating effect sizes calculated from relative abundace data cleared from statistical noise for tadpole species of Ukutula

	Mean	*Phrynomantis bifasciatus*	*Kassina senegalensis*	*Ptychadena mossambica*	*Amietia delalandii*
Phrynomantis bifasciatus	6,281	-	-	-	-
Kassina senegalensis	5,790	0,053092305	-	-	-
Ptychadena mossambica	7,115	0,090054996	0,143147301	-	-
Strongylopus grayii	6,318	0,003919077	0,057011382	0,086135919	-
Amietia delalandii	7,492	0,130787968	0,183880272	0,040732972	0,126869

TABLE D3: Matrix indicating effect sizes calculated from relative abundace data cleared from statistical noise for ecomorphological tadpole guilds of Ukutula

	Mean	Suspension feeder	Lentic-nektonic	Benthic type 2 (Profundal)
Suspension feeder	6,281			
Lentic-nektonic	5,790	0,053282239		
Benthic type 2 (Profundal)	6,496	0,023308491	0,076590731	
Rheophilic	7,492	0,131255854	0,184538093	0,107947362

TABLE D4: Sig. (P) values calculated from relative abundance data cleared from statistical noise

for Aliwal North

Variable	Sig.
Environmental samples	0,376
Species/Guilds	0,755

TABLE D5: Effect sizes calculated from relative abundace data cleared from statistical noise for sample types of Ukutula

	Mean	Effect size
Environment	8,076	0,110287
Species/Guilds	6,828	

TABLE D6: Matrix indicating effect sizes calculated from relative abundace data cleared from statistical noise for tadpole species of Aliwal North

Species	Guild	Mean	*Kassina senegalensis*	*Amietophrynus rangeri*	*Ametia delalandii*
Kassina. senegalensis	Lentic-nectonic	7,883			
Amietophrynus rangeri	Lentic-benthic	6,669	0,110208		
Ametia delalandii	Rehophilic	6,936	0,085909	0,0243	
Cacosternum boettgeri	Lentophytophilic	5,824	0,186852	0,076644	0,100943