

4. DISCUSSION

4.1 ANURAN TADPOLE ECOMORPHOLOGICAL CHARACTERIZATION AND PHYLOGENY

Tadpoles from anuran species which differ in terms of adult habitat, can occupy the same water body (aquatic habitat). Despite this, tadpoles of southern Africa still proved to be a rather diverse group of specimens. From this study it was clear that a number of external tadpole characteristics can be linked to the species' position in the water body. Van Buskirk (2009) identified pelagic or surface-feeding tadpoles as those having lateral eyes, anterior mouths, and tails originating high on the head. He identified benthic tadpoles (opposite of pelagic) as having dorsal eyes, ventral mouths, shallow or bulbous heads and long tails that originates low on the head. Stream-dwelling tadpoles, according to Van Buskirk (2009), have short shallow heads with lateral eyes and long shallow originating tails. These characteristics can be seen in tadpoles that share the same foraging position within the water column. The tadpole species' position within the water body was a major determining factor for guild nomenclature. Taking this into account, position is revealed in almost every guild name. A number of the lentic species may, however, also be able to inhabit backwaters or quiet sections of lotic systems. All species were assigned to the guild and water body it most commonly conformed to.

The phylogenetic approach proved to be very effective for the guild delineation, since a number of the ecomorphological guilds corresponded with previously characterized guilds. Here follows a detailed discussion of the ecomorphological guilds (Figure 7 & Table 3) in relation to the phylogenetic/taxonomic affiliation (Figure 8) of each species assigned to a specific guild, in conjunction with the ecological and morphological characteristics instigating the appointment to a specific guild. The origin/meaning of the guild names are explained, along with the comparison or association of guilds characterized in this study, to previous delineations.

Guild 1- Suspension feeder

This guild includes all species from two genera: *Xenopus* (Pipidae), and *Phrynomantis* (Microhylidae) - previously known as *Phrynomerus*. These genera respectively belong to phylogenetic clade 4 and clade 7 (Figure 8). Both genera were assigned to a group termed Pelagic (open-water filter feeders) by Van Dijk (1972), which indicates the position of these species in the aquatic habitat.

Altig and Johnston (1989) assigned these genera to the guild ‘Suspension feeder’, which as the name indicates are species that feed on food particles that are in suspension. These species remained in this guild even after McDiarmid and Altig’s (1999) revision of the guild.

Tadpoles from this guild are filter feeders that inhabit the water column (midwater) of lentic water bodies. The oral discs are wide, anteriorly positioned and keratinized mouth parts (jaw sheath and labial teeth) are absent. Tail origin in terms of the head is low, with a sharp tip (flagellum). The head is usually strongly depressed. Eyes are laterally positioned and nostrils are small and narrowly spaced. Developmental rate of species from this guild is moderate (35 days to 6 months).

Guild 2 - Macrophagous Type 2 (Nektonic)

The genus *Afrivalus* fell into the same group as the Suspension feeders (Figure 7, guild 1 & 2) because the species in these guilds share a number of characteristics, including: wide anterior oral discs, low origin of the tail in terms of the head, sharp-tipped tail and strongly depressed head with lateral eyes. However, keratinized mouth parts (jaw sheath and labial teeth) are present in *Afrivalus* species, while these are completely absent in the species assigned to the Suspension feeder guild. The oral discs of *Afrivalus* species are wide and anterior, with only posterior labial tooth rows (if present) and delicate jaw sheaths.

Van Dijk (1972) assigned all of the *Afrivalus* tadpoles to a group called Pelagic-hydrophytophilic, due to the species’ position in the water column and the association with aquatic vegetation. This guild was also placed in the same phylogenetic clade as the majority of the Lentic-nektonic species. *Afrivalus*, however, do not belong with genera assigned to the Lentic-nektonic group as there are clear differences in relation to the presence of keratinized labial teeth (absent) and foraging behaviour which is characteristic of the Lentic-nektonic group. McDiarmid and Altig (1999) assigned all the *Afrivalus* species to the Lentic-benthic guild. Unlike this guild, *Afrivalus* have laterally positioned eyes (not dorsal), and anterior oral discs. They are also not associated with the bottom of the water body, since they are found in midwater.

In this study, all the species from the genus *Afrivalus* were assigned to the guild Macrophagous-nektonic according to Altig and Johnston’s (1989) delineation. Nektonic organisms are defined as being able move independently of water currents. Similar to the Suspension feeders, the heads of Macrophagous-nektonic tadpoles are depressed in cross-section, with lateral eyes and small and narrowly spaced nostrils. Tail origin in terms of the head is also low with a sharp tip (flagellum). The tail length is more than twice that of the head. Keratinized mouth parts (jaw sheath and labial teeth) are present in *Afrivalus* species, unlike Suspension feeder tadpoles. The oral discs of *Afrivalus* species are wide and anterior, with only posterior

labial tooth rows (if present) and delicate jaw sheaths (except *Afrivalus aureus*, moderate jaw sheath). These species also filter feed and inhabit the water column (midwater) of lentic water bodies. In addition, they are associated with submerged vegetation where they float motionless (horizontally) in the water column. The development period of species from this guild is also moderate (35 days to 6 months).

Guild 3 - Lentic-nektonic

This guild consists of five genera belonging to three families: *Hyperolius*, *Kassina* and *Semnodactylus* from the family Hyperoiliidae; *Hemisus* from the family Hemisotidae; and *Hildebrandtia* from the family Ptychadenidae. All the species in this guild was placed in the same phylogenetic clade (Figure 8, C4), except *Hildebrandtia ornata*. (Figure 8, C1).

The term Pelagic was first applied to tadpole behaviour and position in the habitat by Van Dijk (1972). This term actually refers to marine organisms that are associated with submerged macroscopic vegetation in mid water (not actively swimming). Hydrophytophilic also indicates these species' association with submerged macroscopic vegetation. The term literally means "a love for water plants". The species from the genera *Hyperolius*, *Kassina* (*Hylambates* including *Kassina*) and *Hemisus* were assigned to the Pelagic-Hydrophytophilic group by Van Dijk (1972), while *Hildebrandtia* was assigned to the Hydrophytophilic group. This genus contains only one species and was most likely assigned to this guild because it is known to actively 'hunt', indicating that *Hildebrandtia ornate* may well inhabit the pelagic zone of a water body.

Semnodactylus wealii is monotypic, and is closely related to *Kassina*. *Semnodactylus wealii* was not addressed by Van Dijk (1972) or Altig and Johnston (1989). Of the Lentic-nektonic tadpoles only the genus *Kassina* was previously assigned to a guild (Suspension-rasper) by Altig and Johnston (1989). They assigned this genus based on the presence of jaw sheaths; the labial teeth row formula (usually 2 rows on the upper labium and 3 on the lower); lateral eyes; the sharp tail tip (flagellum); and the suspended position in midwater. Altig and Johnston (1989) noted that members of the large genus *Hyperolius* have similar mouth parts, but could however not be categorized since data was insufficient.

McDiarmid and Altig's (1999) revision assigned *Hyperolius* and *Hildebrandtia* to the Lentic-benthic guild. However, these species are not associated with the bottom of water bodies, they have lateral eyes (not dorsal), strongly pointed tail tip and their bodies are more compressed than globularly depressed. They assigned *Kassina*, *Semnodactylus* and *Hemisus* to the Lentic-nektonic guild, since they occupy the water column (midwater) of lentic water bodies. Characteristically for midwater dwellers, they have lateral eyes (except *Hemisus marmoratus*, eyes slightly more dorsolateral) and a pointed tail (buoyancy,

slow accurate movement among plants). The Lentic-nektonic tadpoles rasp food from submerged surfaces using their keratinized mouth parts (jaw sheath and labial teeth). Their bodies are compressed and tail tips pointed. *Hyperolius* and *Hildebrandtia* comply with all these criteria and were therefore re-assigned to the Lentic-nektonic guild.

This guild differs significantly from the Suspension feeders and Macrophagous-nektonic species, since Lentic-nektonic tadpoles are not mainly filter feeding. The Lentic-nektonic tadpoles also have keratinized mouth parts (jaw sheaths and labial teeth) on both the upper and lower labium, in comparison to the Suspension feeders having no keratinized mouth parts and the Macrophagous-nektonic species possessing only keratinized jaw sheaths (except *Afraxalus fornasinii*, labial teeth on lower labium).

Guild 4 - Benthic Type 2 (Profundal)

Species from four genera were assigned to the Benthic-profundal guild, and belong to three families. *Ptychadena* and *Hylarana* are from the family Ptychadenidae and Ranidae; *Chiromantis* from the family Rhacophoridae; and the species *Strongylopus grayii* from the family Pyxicephalidae. All these species were placed in one phylogenetic clade (Figure 8, C1). This clade however also contains species from other guilds.

Altig and Johnston (1989) assigned these genera to a group termed Benthic type 2, which are the Benthic-profundal tadpoles. These tadpoles are bottom dwelling, hence the term benthic. They are also profundal, indicating that they may move from littoral areas to deeper areas in the water body. These tadpoles occupy lentic water bodies, but can also be found in non-flowing portions of lotic systems (e.g. backwaters, burrows, pools) which are similar to lentic systems in many respects.

Van Dijk (1972) assigned *Chiromantis* to Pelagic-hydrophytophilic even though *Chiromantis* tadpoles have dorsolateral positioned eyes, not laterally positioned as is the case for Pelagic-hydrophytophilic tadpoles. Tadpoles from the genus *Ptychadena* and *Hylarana* were assigned to the Hydrophytophilic behavioural mode by Van Dijk (1972), stating that these genera “are speckled in such a way as to provide good camouflage in vegetation”. Van Dijk’s behavioural modes (Figure 1) also indicated that these species are bottom dwellers, which coincides with Altig and Johnston’s delineation of *Ptychadena*. Benthic-profundal species inhabit both lentic and lotic water bodies; except *Ptychadena mapacha*, which only inhabits lentic water bodies.

McDiarmid and Altig (1999) grouped all the benthic species together under one rather large Benthic guild. For this study, however, we chose to separate this extensive guild into the Benthic-profundal guild (not Bufonidae); and the Lentic-Benthic guild, including all southern Africa's species belonging to the family Bufonidae (toads). These species are not separated based on being Bufonidae or non-Bufonidae alone (see Lentic-benthic).

In contrast to Suspension feeding, Macrophagous-nektonic and Lentic-nektonic species; Benthic-profundal tadpoles have heads which are depressed to globular-depressed, a sharp tail tip, dorsolateral eyes and anteroventrally positioned oral discs. The jaw sheaths are well developed (moderate) and the labial tooth row formula is often 2 (upper labium)/3 (lower labium). These features are typical for benthic tadpoles (Van Buskirk, 2009).

Guild 5 - Lentic-benthic

Lentic-benthic tadpoles comprise six genera which all belong to the family Bufonidae, also known as toads. The genera include *Amietophrynus*, *Poyntonophrynus*, *Vandijkophrynus*, *Capensibufo*, *Schismaderma* and *Mertensophryne*. This guild formed a phylogenetic clade of its own (Figure 8, C6). These species are bottom-dwelling, and the majority are located along the shoreline of water bodies. Both Van Dijk (1972) and Altig and Johnston (1989) assigned these genera (the majority of which were previously known as *Bufo*) to benthic or bottom-dwelling groups.

Van Dijk (1972) assigned *Schismaderma carens* to the behavioural mode Gregarious, due to the peculiar behaviour of the tadpoles. These tadpoles swarm together to form large masses that rise and sink in the water column. Similarities in aggregation behaviour may however occur in species that are not closely related, and thus aggregation is not a determining factor (Caldwell, 1989). Like the other species assigned to this guild, *S. carens* has a globularly-depressed head with a rounded tail tip, dorsolateral eyes, and well-developed jaw sheaths with two rows of labial teeth on the upper labium and three on the lower. For this reason, it was also assigned to the Lentic-benthic guild. Altig and Johnston (1989) assigned *Mertensophryne* to Arboreal Type 5 due to the presence of the raised ring on the head. However, they noted that aside from this characteristic, this genus resembles benthic forms. McDiarmid and Altig (1999) assigned *Mertensophryne* to Arboreal Type 3, based on this fleshy ring and because the tadpoles are not elongated (similar to Lentic-benthic tadpole), but can occupy very small water pockets. Due to the close resemblance to Lentic-benthic forms in all aspects except the presence of the fleshy ring on their heads, *Mertensophryne* was also assigned to this guild.

Species belonging to this guild share a number of characteristics with Benthic-profundal tadpoles. Lentic-benthic species, like Benthic-profundal species have shorter body lengths (< 40 mm), they have dorsolateral eyes and well-developed jaw sheaths with the labial tooth row formula of 2 (upper labium) / 3 (lower labium). Their heads are also depressed to globular-depressed, although unlike all the guilds discussed thus far, Lentic-benthic tadpoles have rounded tail tips, and most have near ventral oral discs. Lentic-benthic tadpoles inhabit lentic water bodies, the majority of species included in this guild do not move deeper to profundal areas. Instead, they are associated with shallow areas or weedy shorelines.

Guild 6 - Rheophilic

This guild includes species from two genera, namely *Amietia* and *Strongylopus* both of which belong to the family Pyxicephalidae. This guild was also placed in the phylogenetic clade C1 (Figure 8), containing a number of other guilds. Van Dijk (1972) assigned these genera to the behavioural mode Rheophilic, because of these organisms' preference for flowing aquatic habitats. He nevertheless identified *S. fasiatus* as pelagic and *S. grayii* as bottom-dwelling-hydrophytophilic. The guild analysis grouped *S. fasiatus* with the rest of the *Strongylopus* species, thus only one *Strongylopus* species (*S. grayii*) was separated from the genus.

Altig and Johnston (1989) relegated the majority of these species, while still known as *Rana*, to the benthic guilds that occupy lentic and lotic systems. McDiarmid and Altig (1999) did not refer to specific species but assigned all *Rana* species to the lentic-, lotic-benthic and clasping guilds. However, these species differ from other benthic tadpoles in terms of morphology and time to metamorphosis. From Altig and Johnston's assessment, it was assumed that the *Rana* species assigned to the clasping guild in 1999 referred to the same species that were assigned to the clasping guild in 1989, which do not include any southern African species.

Rheophilic tadpoles are bottom dwelling and unlike previous guilds that inhabit lentic water bodies or the lentic sections (e.g. backwaters) of lotic systems, can occupy the margins of lotic water bodies. Although they are also found in lentic systems, they are relatively streamlined, have wide oral discs and dorsolateral eyes (more typical of stream-dwelling tadpoles). All of the species' tail fins, except for those of *S. fasiatus*, originate low in relation to the head.

In contrast to all the guilds discussed thus far, the Rheophilic species have extended developmental times. With the exception of *A. inyangae* and *S. fasiatus*, most Rheophilic species require a period of more than six months to develop before metamorphosing, where previous guilds all have rapid to moderate development periods (this statement was made based on known periods of development, since all *Strongylopus* species time to metamorphosis is still unknown).

Guild 7 - Suctorial

This guild includes species from two genera, namely *Hadromophryne* and *Heleophryne*, both of which belong to the family Heleophrynidae. The phylogenetic delineation caused all the species in this guild to form a clade of their own (Figure 8, C5). The torrent dwelling tadpoles assigned to the Suctorial guild were included in the Rheophilic behavioural mode by Van Dijk (1972). The Suctorial species, based on their phylogeny as well their ecological preferences, strongly relate to other Suctorial species only and were therefore assigned to a guild of their own based on McDiarmid and Altig's (1999) revised terminology, which corresponds with Altig and Johnson's (1989) delineation.

Suctorial tadpoles comply with the following criteria (not present at any of the guilds discussed thus far). They inhabit the benthic zone in fast-flowing waters (in stream flow), and continuously maintain their position by clasping with their oral discs. Suctorial tadpoles are strong swimmers, and the majority of these species occupy water bodies situated at high altitudes. The tadpoles are streamlined, with long tails in relation to head length. The eyes are dorsal, while the oral discs are ventral and expanded into a very broad sucker. Like Suspension feeders the jaw sheaths are absent (except *Hadromophryne natalensis* - delicate posterior jaw sheath). Suctorial species do however have numerous labial tooth rows (more than any other southern African species), and small closely-spaced submarginal papillae. Papillae are believed to assist in attachment to substrates (Altig & Johnston, 1989; Altig, 1999; Saidapur, 2001; Altig, 2010). Like Lentic-benthic tadpoles, the tail originates low in relation to the head, and the tip of the tail is rounded. Suctorial species' tail tips however are not bluntly rounded, unlike those of Lentic-benthic tadpoles.

Guild 8 – Excitus-parageios

This guild includes species from two genera, namely *Tomopterna* and *Pyxicephalus*, both of which belong to the family Pyxicephalidae. These species were previously assigned to the same genus before being revised. This guild was assigned to the same phylogenetic clade as the Benthic-profundal, Rheophilic, Lentophytophilic and some species from the Bentophytophilic and Lentic-nektonic guilds (Figure8). Neither of these genera were addressed by Altig and Johnston. Van Dijk (1972) assigned

Tomopterna to the behavioural mode Bottom-dwelling, while assigning *Pyxicephalus* to the behavioural mode Gregarious. Schools of *Pyxicephalus* tadpoles can often be observed in shallow muddy water. Like *Schismaderma carens*, similarities in aggregation behaviour may occur in species that are not closely related and is thus not a guild determining factor (Caldwell, 1989).

McDiarmid and Altig (1999) assigned these genera to the Lentic-benthic guild. Although there are some similarities between these species and those belonging to the benthic guilds, these species were assigned to this guild since they were completely separated from all other benthic species in the phylogenetic analyses.

The main characteristics that group these species together were the association with shallow temporary lentic water bodies and rapid development period. The term provided for this guild was derived directly from these main attributes. Excitus originates from the Latin terms for rapid (*citus*) and development (*exitus*), while parageios is a Greek term which means to pertain to shallow water. These species can be found at moderate to low (coastal) altitudes. Typical of benthic tadpoles Excitus-parageios species have globular-depressed (rounded heads). In relation to head length, tails are long. The tail originates low and the tip is rounded. The eyes are dorsolateral and the nostrils are narrowly spaced. These tadpoles have similar labial tooth row formulas and three rows of lateral papillae. Excitus-parageios tadpoles are similar to tadpoles from both benthic guilds in terms of morphology and behaviour. There is however no similarity between these tadpoles and tadpoles that belong to the first three guilds, which is expected seeing as they inhabit completely different aquatic habitats.

Guild 9 - Lentophytophilic

This guild includes all species from three genera, *Cacosternum* and *Microbatrachella* (Pyxicephalidae) and *Phrynobatrachus* (Phrynobatrachidae). All the Lentophytophilic species belong to the same phylogenetic clade (Figure 8, C1). The term Lentophytophilic was created for this guild, literally meaning that these organisms love non-flowing waters with submerged vegetation. These tadpoles all occupy the water column (midwater) of shallow lentic water bodies where they are associated with submerged vegetation. These tadpoles may also move from littoral to profundal areas.

Van Dijk (1972) classified *Cacosternum* and *Microbatrachella* as Pelagic-hydrophytophilic. However, these species differ considerably from Pelagic-hydrophytophilic tadpoles in terms of morphology. Pelagic-hydrophytophilic tadpoles mostly have lateral eyes, and anterior to anteroventrally positioned oral discs. Conversely, these tadpoles have dorsolateral eyes (except *Microbatrachella capensis* (lateral) and *Phrynobatrachus acridoides* (dorsal)), and near ventral oral discs. *Phrynobatrachus* was assigned to

the behavioural mode Hydrophytophilic by Van Dijk (1972), yet he acknowledged that these species may occur in midwater. Because of this phenomenon and the morphological similarities between *Phrynobatrachus* and the other genera assigned to the Lentophytophilic guild, these species are also assigned to this guild. None of the Lentophytophilic species were addressed by Altig and Johnston (1989). McDiarmid and Altig (1999) however assigned them all to the Lentic-benthic guild. Unlike Lentic-benthic species, Lentophytophilic tadpoles are associated with submerged vegetation and most commonly occupy the water column, except for some *Cacosternum* species, which inhabit both the benthic and pelagic zones. Lentophytophilic tadpoles have long tails in relation to head length, near ventral positioned oral discs and all species within this guild share similar labial tooth row formulas.

Guild 10 – Bentophytophilic

The tadpoles from the genera *Leptopelis* (Arthroleptidae), *Natalobatrachus* and *Poyntonia* (Pyxicephalidae) were assigned to the guild Bentophytophilic. *Natalobatrachus* and *Poyntonia* are both monotypic species (only one species per genus). The term Bentophytophilic was created using the main characteristics common for all species. The term literally refers to organisms that are bottom-dwelling and associated with submerged vegetation.

Altig and Johnston (1989) assigned *Leptopelis* to the Lotic Benthic guild and all the Bentophytophilic species were assigned to McDiarmid and Altig's (1999) benthic guilds. These tadpoles differ from typical benthic forms in the sense that they do not have globular or depressed body shapes with dorsal eyes; they are streamlined with longer tails than those of typical benthic tadpoles and are always associated with vegetation. *Leptopelis* was assigned to the Pelagic-hydrophytophilic mode of behaviour by Van Dijk (1972), he stated that they differ from the other species in this group because they rest on or hang from hydrophytes. *Leptopelis* species are not known for being pelagic, they are more commonly known for being associated with the benthic zone. Van Dijk (1972) assigned *Natalobatrachus* to the behavioural group Rheophilic because they have been found in forest streams. *Natalobatrachus* was however assigned to Altig and Johnston's (1989) Claspig guild because they inhabit slow water and maintain their position by oral disc. *Natalobatrachus* was re-assigned to the Bentophytophilic guild, since this guild addresses those characteristics as well as the morphological characters which cause this species to be grouped with the other Bentophytophilic species.

The following characteristics caused these species to be allocated to the Bentophytophilic guild. Tadpoles occupy the benthic zone of water bodies where they are associated with vegetation (e.g. grass pools, leaf litter etc.). The tadpoles are streamlined with dorsolateral eyes and small, widely spaced nostrils. The

labial tooth row formulas of these species are also all similar. These tadpoles have considerably long tails (more than twice head length) which originates low in relation to the head.

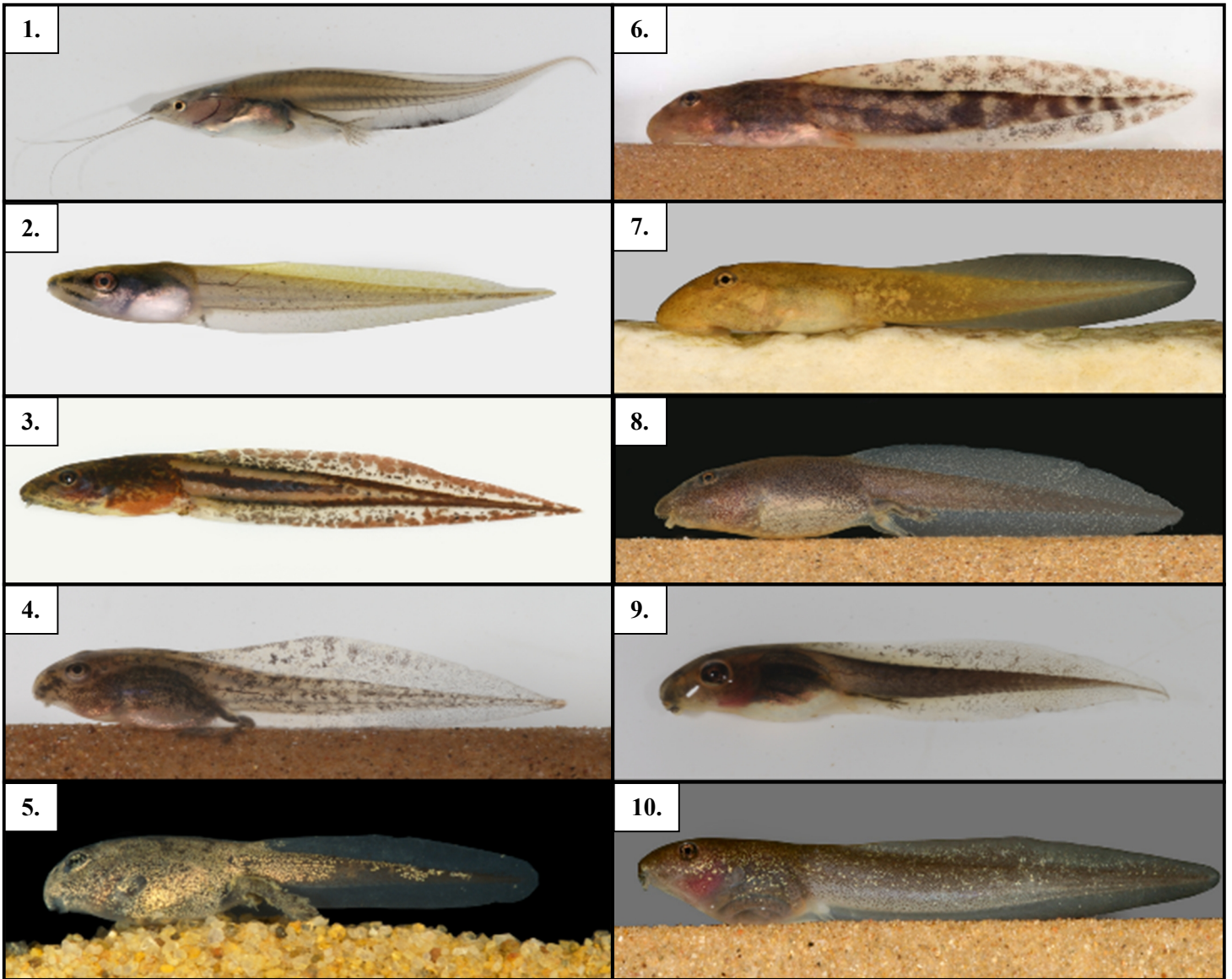


Figure 14: Representative body forms of tadpoles from each ecomorphological guilds. (1.) Suspension feeder, *Xenopus muelleri*; (2.) Macrophagous-nektonic, *Afrixalus aureus*; (3.) Lentic-nektonic, *Hyperolius horstockii*; (4.) Benthic-profundal, *Chiromantis xerampelina*; (5.) Lentic-benthic, *Poyntonophrynus vertebralis*; (6.) Rheophilic, *Amietia quecketti*; (7.) Suctorial, *Heleophryne hewitti*; (8.) Excitator-parageios, *Pyxicephalus edulis*; (9.) Lentophytophilic, *Microbatrachella capensis*; (10.) Benthophytophilic, *Natalobatrachus bonebergi*.

4.2 RELATION BETWEEN ANURAN CONSERVATION STATUS AND ECOMORPHOLOGICAL GUILDS

This study attempted to determine whether certain ecomorphological guilds have suffered more population decline than others. Tadpoles from different genera proved to share ecological characteristics. Figure 9 however indicates that these shared ecological characteristics are not necessarily absent from species that have not suffered population declines. This is why no International Union for Conservation of Nature (IUCN) category was particularly reserved to any specific ecomorphological tadpole guild. The majority of the more critical IUCN categories (CE, EN, and V) appeared to be distributed among the ecomorphological guilds. The following information regarding the threatened status and main threats were all obtained from the IUCN red list (2013).

The majority of genera from all guilds belong to the LC category, since a greater part of the southern African anuran taxa are widespread and abundant. All the species from the guilds Suspension feeder, Benthic-profundal and Excites-parageios are of least concern (LC). This is most likely because the majority of the species belonging to these guilds are widespread (inhabit more than one site over a large geographical area), resilient (tolerant of changes to habitat, can travel long distances to suitable habitats and/or remain dormant for long periods until conditions become suitable), and/or generalists (can inhabit a range of habitats).

The Macrophagous-nektonic, Lentic-benthic, Suctorial and Lentophytophilic guilds all contain species that are critically endangered and therefore face an extremely high risk of becoming extinct in the wild. *Vandijkophrynus amatolicus* from the Lentic-benthic guild belongs to this category. A reduction in population size has been observed, mostly due to loss of grassland (afforestation, overgrazing, and fires). *Heleophryne rosei* individuals from the Suctorial guild occur in and occupy a relatively small area. The decline in the area of occupancy and the quality of habitat (alien vegetation, frequent fires, reduced stream-flow) is still continuing. Water abstraction from streams has also caused the loss of habitat and could limit the vertical movement of these CE tadpoles. *Microbatrachella capensis* (Lentophytophilic) and *Hyperolius pickersgilli* (Lentic-nektonic) both suffer from a continuous decline in area of occupancy (< 10 km²) and habitat size and quality. Both these species are affected by urbanization, as well as drainage for agricultural development and the spread of alien vegetation, all of which cause breeding pools to dry out. Three of the four locations that *Microbatrachella capensis* inhabits are under constant pressure from human development. Some *Hyperolius pickersgilli* breeding sites are being polluted by DDT (controls malarial mosquitoes).

Endangered (EN) taxa are considered to be facing a very high risk of extinction in the wild (though not as high as CE). Five guilds contain species that are EN, these are the Rheophilic, Lentic-benthic, Suctorial, Bentophytophilic and the Macrophagous-nektonic guilds. *Amietia inyangae* (Rheophilic) and *Amietophrynus pantherinus* (Lentic-benthic) are both limited in terms of extent of occurrence (<100 km²) and area of occupancy (< 10 km²). They suffer a continuing decline in quality and extent of habitat. *Amietophrynus pantherinus* suffers decline in quality and extent of habitat at more than one location. *Amietia inyangae* are at risk from human settlement, wood plantations, and overgrazing by livestock. Although tolerant of habitat alteration, *Amietophrynus pantherinus* is threatened by increased urbanization, agricultural expansion, road kills, alien vegetation and introduced fish. The recent introduction of *Amietophrynus gutturalis* (rapidly expanding) poses threats of competition. *Amietophrynus pantherinus*, *Vandijkophrynus inyangae* (Lentic-benthic) and *Heleophryne hewitti*'s (Suctorial) extent of occurrence (<100 km²) is also rather limited, and the extent and quality of their habitats are steadily declining. *Vandijkophrynus inyangae*'s risk from human settlement, wood plantations and overgrazing by livestock is increasing. *Heleophryne hewitti* are suffering loss of suitable non-breeding and breeding habitat due to afforestation. The only area it occupies is within exotic pine plantations which results in a reduction in stream flow and potential fires. The removal of these pine trees further leads to erosion and siltation of streams. *Natalobatrachus bonebergi* and *Leptopelis xenodactylus* (both Bentophytophilic) and *Afrivalus knysnae* (Macrophagous-nektonic) have a limited area of occupancy (< 10 km²) and suffer from a continuous decline in and habitat size and quality. *Natalobatrachus bonebergi* lost much of their forest habitat to agriculture (e.g. sugar cane cultivation), afforestation and urbanization. Pollution and siltation of streams also pose a threat. *Leptopelis xenodactylus* are endangered due to afforestation, unsuitable fire regimes, overgrazing, trampling and eutrophication (cattle). Alien vegetation also leads to breeding sites drying out, due the water table being lowered. *Afrivalus knysnae* also suffers from a continuous decline of a number of mature individuals. Afforestation, agricultural expansion, urban and recreational development, invasive plants, and chemical pollution cause a loss of habitat.

The guilds Lentic-benthic, Lentic-nektonic, and Rheophilic each contain one vulnerable species. These are considered to be facing a high risk (not as high as EC and EN) of extinction in the wild. *Capensibufo rosei* (Lentic-benthic), *Hemisus guttatus* (Lentic-nektonic) and *Strongylopus rhodesianus* (Rheophilic) have a limited area of occupancy (< 10 km²) and suffer from a continuous decline in habitat size and quality. *Capensibufo rosei* can only be found in a range less than 100 km², and is suffering constant decline at a number of locations. *Capensibufo rosei* is losing its fynbos habitat, due to the frequent burning and the spread of alien vegetation. The historical populations from the Cape Peninsula are now

missing, with no justification or identified threat. *Hemisus guttatus* is undergoing constant decline at a number of locations; sugar cane cultivation, afforestation, and urbanization are the main causes of habitat loss. Invasive alien vegetation however also lowers the water table. *Strongylopus rhodesianus* can only be found within a range of less than 100 km² at high-altitudes. This habitat is at risk from human settlement, wood plantations and overgrazing by livestock.

Even though some similarity can be seen between species, based on the criteria for the endangered status, there was still no true correlation between IUCN status and ecomorphological guild. All of the guilds contained a maximum of two anuran species per IUCN category and only the Lentic-benthic guild contained all three categories of greatest concern. This could be due to the large number of species assigned to this guild and the fact that this guild contains the whole Bufonidae family. Even though a diverse group of anuran adults' tadpoles might share the same aquatic habitat, it does not cause those taxa to be grouped together under one IUCN status. The main threats anuran taxa face, seem to be related to urbanisation, afforestation and agricultural activities. The fact that the CE, EN and VU species are distributed over so many guilds further emphasises the extent of amphibian decline. There is no guarantee that any non-generalist species can be exempted from possible future decline based on guild delineation. Based on guild delineation, the Suspension feeder, Benthic-profundal and Excitus-parageios tadpoles are least likely to suffer decline, since none of these species belong to the more critical conservation statuses and are widespread, resilient, and/or generalists (can inhabit a range of habitats).

4.3 TADPOLE TROPHIC ANALYSIS IN RELATION TO ECOMORPHOLOGICAL GUILDS

Tadpole feeding behaviour is frequently associated with functional roles (Saidapur, 2001; Hall *et al.*, 2002; Altig *et al.*, 2007). Information on the feeding behaviour of four representative anuran tadpole species was obtained by studying the gut content of these species, in order to understand the tadpoles' ecological roles as well as to understand the morphological variations and the ecological importance of these features (Saidapur, 2001).

There is no clear relationship between the shape of the labial teeth and the food the majority food tadpoles favour, even though labial tooth shape will affect the way and the extent to which the tadpole can come into contact with the substrate (Altig & Johnston, 1989; Vera Candioti *et al.*, 2010). A rather extensive list of materials ingested by tadpoles has been reported, which include: higher plant material (e.g. Macrophyta) and products (e.g. pollen); algae (benthic, phytoplankton, filamentous) as well as their extracellular products; zooplankton; organic debris or detritus (unidentified organic particles) and associated microorganisms (bacteria and meiofauna); and inorganic material (e.g. minerals, sediments)

(Gosner, 1959; Altig & Kelly, 1974; Altig & Johnston, 1989; Petranka & Kennedy, 1999; Ranvestel *et al.*, 2004; Ghioca-Robrecht & Smith, 2011).

The predominant materials found during this trophic analysis included the following: green algae (Chlorophyta), diatoms (Bacillariophyceae), Macrophyta (fragments of higher aquatic plants), detritus (particulate organic material) and sediment. Tadpole jaws are very flexible, allowing tadpoles to taper their jaws as well as push them forward (Wassersug & Yamashita, 2001). This should aid the rest of the oral structures to better perform their functions. Labial teeth are used to scrape material from substrate surfaces and anchor the tadpole to a substrate (Altig, 2006; Channing *et al.*, 2012). Even though it has not been officially established, it is believed that the jaw sheaths serve as scraping, gouging and biting structures (Altig & Johnston, 1989; Saidapur, 2001).

4.3.1 TROPIC CATEGORIES OF MATERIALS INGESTED BY TADPOLES

The categories based on the position of the ingested materials in the aquatic habitat include: Planktonic/free-swimming; Benthic/Epilithic/Epiphyte; Planktonic-associated with submerged surfaces; and Higher plant material. The organisms allotted to the Planktonic group are found in the photic zone (top layer of water body where light can penetrate). The benthic organisms are attached to a submerged surface, whether attached to stone surfaces (epilithic) or attached to submerged plants (epiphyte) organisms. Some of the benthic organisms are however motile (e.g. *Nitzschia* species move via mucilage secretion), and can therefore easily be dislodged by strong currents, water birds or animals disturbing the submerged surfaces. Due to these species' extremely small surface areas, they are able to stay in suspension for a long time before settling out of suspension. The planktonic species that are associated with submerged surfaces are those organisms that are planktonic, yet easily settle out of suspension (e.g. *Cyclotellameneghiniana*), form long filaments that are only attached to a surface by one end, and are easily dislodged (e.g. *Ocellularia*). Invertebrate eggs and higher plant products (e.g. pollen) which can either be in suspension or settle out to become part of the benthos, are also included among planktonic organisms. The higher plant material include parts of vegetation, for example fine plant roots, moss leaves or just 'epidermal shavings'. Detritus (particulate organic matter) forms an important part of in-stream ecosystems, and is present in suspension as well in the benthos (Cummin & Klug, 1979). Cummin and Klug stated that invertebrates belonging to the functional group designated scrapers may ingest fine particulate organic matter by scraping the surface.

The significance of small particles present in large quantities (e.g. sediment or mineral remains), is overemphasized when using numerical frequencies to point out the contribution of each item (Vera Candioti, 2005). The sediment or mineral remains were thus not quantified, but it was noted that a large amount of sediment particles were present in all the tadpole guts investigated for all the guilds. The turbidity of all the sites where tadpoles were collected, and all the water bodies observed in the Ndumo Game Reserve for that matter, was relatively high. Sediment could be observed in suspension and the majority of the benthos was also covered in a layer of sediment. The mineral remains (e.g. sediment) present in guts of all the tadpoles were therefore relatively high.

4.3.2 ECOMORPHOLOGICAL GUILDS DIETARY PREFERENCES

Suspension feeders

These tadpoles filter feed and inhabit the water column (midwater) of lentic water bodies. The oral discs are wide and anteriorly positioned. All keratinized mouth parts (jaw sheath and labial teeth) are absent. As expected, the Suspension feeders had no higher plant material in their guts. This is most likely due to the position they occupy in the water body, and the lack of keratinized mouthparts. The majority of the materials in the gut either inhabit the photic zone where they stay in suspension or can easily be dislodged to become part of suspension. Purely planktonic materials comprised just more than 75% of the gut content. The percentage of benthic organisms comprised 18% of the ingested material. The benthic materials included a considerable number of motile *Nitzschia* species. These are easily dislodged and set into suspension when the surface it was attached to is disturbed. The sites where tadpoles were sampled had a rather high turbidity; which led to the ingestion of fine suspended particulate organic matter (16% of gut content) by the Suspension feeders.

Lentic-nektonic

Similar to the Suspension feeders, the Lentic-nektonic tadpoles also had a high percentage of ingested planktonic organisms (71%) and virtually no benthic materials. This can also be attributed to the position they occupy within the water body, since these tadpoles are pelagic (suspended position in midwater) and thus spend much of their time in the photic zone where the planktonic organisms are found. Upon observation, it was clear that these tadpoles ingest a rather large amount of fine plant roots. The ingestion of higher plant material was most likely made possible by the presence of a keratinized jaw sheath and labial teeth. The Lentic-nektonic tadpoles had the highest percentage of higher plant material, in comparison to the other guilds. This is likely because these tadpoles are associated with submerged

vegetation, and have keratinized mouth parts (jaw sheaths and labial teeth). The benthic organisms (epiphytes) are also most likely ingested while the tadpoles are feeding on the higher plant material. Similar to the Suspension feeders, the sites where tadpoles were sampled had a rather high turbidity. Thus fine particulate organic matter in suspension was ingested (11% of gut content), often most likely in association with ingested higher plant material.

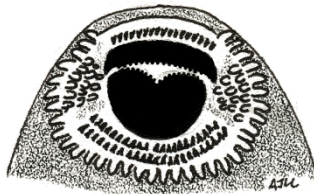


Figure 15: Representation of Lentic-nektonic tadpoles' oral discs (*Kassina senegalensis*). Adopted from Lambiris (1988).

Benthic-profundal

Benthic-profundal species inhabit both lentic and lotic water bodies where they move from the littoral zone of the water body to the profundal zones (deeper) where tadpoles dwell and feed along the bottom. It was not surprising to find that tadpoles from the Benthic-profundal guild consumed 57% benthic organisms. These tadpoles' anteroventrally positioned oral discs and well developed (moderate) jaw sheaths are ideal for removing benthically attached organism from the substrate that they are attached to. The keratinized labial tooth rows present on both upper and lower labium most probably also aid in this removal. The keratinized mouth parts (jaw sheath and labial teeth), however, do seem effective to some extent, in removing more than the benthic layer, considering the presence of 'higher plant material-shavings'. The planktonic materials that are associated with submerged surfaces (30%) were most likely ingested while still attached to the submerged surface, seeing as the planktonic materials ingested comprised only a small percentage of the total material ingested. Particulate organic matter is present in the benthos and can be ingested by scraping the surface, which is how Benthic-profundal tadpoles feed. A total of 14% (non-dimensional number) particulate organic matter was present in the intestines of Benthic-profundal tadpoles, most likely because they scrape the along submerged surfaces with their keratinized mouth parts as they feed.

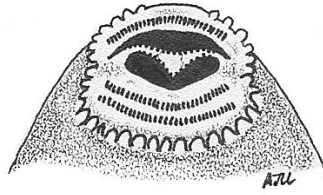


Figure 16: Representation of Benthic-profundal tadpoles' oral discs (*Ptychadena anchietae*). Adopted from Lambiris (1988).

Lentophytophilic

The composition of different materials ingested by the Lentophytophilic tadpoles was not as expected. The materials ingested were distributed evenly among the different categories; except for the higher plant material, which only comprised 4%. Because these tadpoles have near ventrally positioned oral discs it was expected that the percentage of benthic materials ingested would be slightly higher than the other materials. It was also expected that the percentage of higher plant material would be higher since these species are associated with submerged vegetation. The results however indicated that these tadpoles most likely feed as they move between the submerged vegetation. It seems that they do not select one particular position or substrate from which to feed. The non-dimensional number of organic matter present in each sample indicated that Lentophytophilic tadpoles consumed the highest amount of organic matter (18%). This might be attributed to these tadpoles feeding from all substrates as well from the photic zone, considering that particulate organic matter forms an important part of all stream ecosystems and is present in suspension as well in the benthos (Cummin& Klug, 1979).



Figure 17: Representation of Lentophytophilic tadpoles' oral discs (*Phrynomantis natalensis*). Adopted from Lambiris (1988).

Correlations between the phenotypes of organisms and the habitats they occupy have been noted for some time now, this is also true for tadpoles concerning their body forms, oral morphology and the habitats they reside in (Orton, 1953; Orton, 1957; Altig & Johnston, 1989; Altig, 1999; Van Buskirk, 2009). This trophic analysis confirms this statement. The trophic analysis also indicates that the tadpole species from the different guilds choose a diversity of microhabitats within similar water bodies. This selection correlates with their morphological characteristics, and is a vital strategy in ensuring their survival and growth (Saidapur, 2001). Though occupying the same or similar habitats, different tadpole species make use of different feeding methods, and to an extent choose various materials to feed on (Gosner, 1959).

4.4 DIATOM BIOASSESSMENT FOR THE BIOLOGICAL MONITORING OF WATER QUALITY IN THE PHONGOLO RIVER AND FLOODPLAIN

The Water Research Commission (WRC) is currently conducting a regional scale risk assessment to determine the ecological integrity, health and conservation of the very important riverine, wetland and floodplain system in Ndumo. Diatom bioassessment was applied as a tool for inferring water quality. The diatom component of this study will supplement the WRC's assessment.

Diatoms are widely distributed, primary producers, and comprise about 40% of any given algal community. Thus, a change in this fundamental part of the aquatic ecosystem could disrupt the balance of the whole ecosystem (Round, 1990; Stoermer & Smol, 2004). Diatom communities react rapidly and specifically to changes in environmental conditions such as eutrophication, organic enrichment, salinization and changes in pH (Van Dam *et al.*, 1994). Due to their high dispersal rates, rapid growth rate and their direct response to environmental changes, diatoms provide the first indication of environmental changes and are thus one of the most widely used indicators of biological integrity and physico-chemical conditions in aquatic ecosystems. Diatom-based pollution indices are an efficient and cost-effective technique for routine river monitoring, and benthic diatoms provide a time-integrated indication of certain water quality constituents (Hohls, 1996; Bate *et al.*, 2002; Talyor, *et al.*, 2007a; Belton *et al.*, 2005).

4.4.1 WATER QUALITY AS INFERRED BY THE DIATOM INDICES AND DOMINANT DIATOM SPECIES

It has to be taken into account that index scores might have been influenced by the presence of a large number of species from the *Nitzschia* genus. These taxa are assigned somewhat higher ‘indicator values’ due to their association with higher pollution gradients (Van Dam, 1994; Sudhakar, 1994). *Nitzschia* are motile species, and the abundance of these species is often increased by high degrees of sedimentation, which was evident at all the sites due to the artificial floods. The relative abundance of the dominant diatom species is listed in Table 6, followed by the ecological preferences of these species.

Nyamiti Pan:

This site can be classified as poor quality (meso-eutrophic), based on the biological water quality as measured by the diatom indices (Table 8). There was also a high likelihood of organic pollution as the percentage pollution tolerant valves present were above 20%. The percentage of pollution tolerant valves however decreased from 34.6% in 2012 to 23% in 2013, while the index values stayed within the same parameters. This is most probably due to the artificial floods being initiated towards the beginning of 2013, probably washing away or diluting the organic pollutants present. The constantly occurring and dominant diatom species are associated with brackish water which is electrolyte-rich.

Outflow at Nyamiti Pan:

Sampling in 2012 indicated that the biological water quality at the outflow at Nyamiti Pan could be classified as moderate quality, with the trophic state being mesotrophic. Since sampling in 2012 the percentage of pollution tolerant valves increased 21.1%, this site is now classified as poor quality (meso-eutrophic). This is most likely attributable to an increase in organic pollution. These organic pollutants could have been carried down the Nyamiti Pan and accumulated at the outflow of the Nyamiti Pan. The dominant species at the outflow of the Nyamiti Pan are an indication of moderate to high electrolyte content, these species are also frequently found in brackish waters.

Phongolo River:

This site’s biological water quality is moderate to poor, with the trophic state being mesotrophic to meso-eutrophic. There is also a high likelihood of organic pollution, and the percentage of pollution tolerant valves increased only slightly from 26.1% in 2012 to 29.8% in 2013. The dominant species present in the Phongolo River are associated with eutrophic and/or brackish water bodies. These species are also an indication of moderate to high electrolyte content.

4.4.2 ENVIRONMENTAL VARIABLES IN RELATION TO DIATOM INDICES AND DOMINANT DIATOM SPECIES

The majority of the species found in the following sites favour brackish water, with a moderate to high electrolyte content. This indicates that the Phongolo River, and the section of the floodplain considered for this study, is brackish. This means that these water bodies have a higher salinity than fresh water, yet not as high as sea water. This might be due to the water bodies' close proximity to the coast. Salt water is known to be very electronically conductive, hence the associated elevated electrical conductivity (EC) in these water bodies (Dallas & Day, 2004).

The measured physico-chemical water quality parameters also indicated that the EC at all the sites was rather high. The EC was especially high at the Nyamiti Pan outflow (2012) and in the Phongolo River (2012), which is most likely because the artificial floods did not yet lower (dilute) the salt concentration. Typical of brackish water, the pH was also rather high ($> \text{pH } 7$) and all the water bodies were thus alkaline (Table 10) (Dallas & Day, 2004). The DO% at the Nyamiti Pan in 2012 was also higher than any of the other sites. The decrease of DO% in all the sites from 2012 to 2013 can be attributed to the fact that an increase in salinity of water decreases oxygen solubility (Dallas & Day, 2004). All the indices positively correlated to EC, and there was a significant correlation between the %PTV index and EC ($p=0.048$). This further confirms the state of these water bodies as being brackish and slightly eutrophic. The pH had a highly significant correlation to the BDI ($p=0.008$), indicating that a rise in pH (more alkaline conditions) will cause the BDI score to increase.