INFLUENCE OF WATERFALLS ON PATTERNS OF ASSOCIATION BETWEEN TROUT AND NATAL CASCADE FROG *HADROMOPHRYNE NATALENSIS* TADPOLES IN TWO HEADWATERSTREAMS OF THE UKHAHLAMBA DRAKENSBERG PARK WORLD HERITAGE SITE

by

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SUMMARY

Current literature suggests that little, if any, research has been conducted in South Africa to determine the impact of alien invasive trout on indigenous amphibian biodiversity. The primary aim of the research project was to establish whether waterfalls are seasonally important in protecting the indigenous Natal Cascade Frog *Hadromophryne natalensis* tadpole populations from predation by alien rainbow trout *Oncorhynchus mykiss* and brown trout *Salmo trutta* at two sampling areas located at Injesuthi and Monk's Cowl Nature Reserve within the uKhahlamba Drakensberg Park World Heritage Site (UDPWHS). The dissertation argues in favour of a biotic rationale, namely trout predation, as being the primary cause for the decline of *H. natalensis* tadpoles below the waterfalls and systematically negates the influence of geo-physical (abiotic) environmental factors on tadpole abundance. Habitat isolation and fragmentation is identified as a latent threat to the continued persistence of *H. natalensis* populations in the UDPWHS.

***Key words* –** amphibian, Brown Trout *Salmo trutta*, Rainbow Trout *Oncorhynchus mykiss*, alien, invasive, predation, waterfall, isolation, fragmentation.

Dissertation submitted in honour of my friends and colleagues Mark Coetzee and James Wakelin who died in a light aircraft crash in Mozambique, 2007.

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DISCLAIMER

These studies represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any University or other academic institution. Where use has been made of the work of others, it is duly acknowledged in the text. All the experimental work described in this dissertation was carried out in the School of Agriculture and Environmental Sciences, University of South Africa (UNISA) under direct supervision of my UNISA supervisor Dr. Kerry Slater in close collaboration with my co-supervisor Dr. Nicholas Rivers-Moore.

PREAMBLE

I am currently employed as a Chief Aquatic Research Technician with the provincial conservation agency Ezemvelo KwaZulu-Natal Wildlife, South Africa. I was tasked in 2004 to carry out field surveys in the uKhahlamba Drakensberg Park World Heritage Site (UDPWHS), South Africa, to assess what impact, if any, invasive alien trout may have had on indigenous biodiversity. A number of field surveys were conducted above (trout absent) and below (trout present) selected waterfalls within the UDPWHS. These preliminary field surveys suggested that the populations of the Natal Cascade Frog *Hadromophryne natalensis* tadpoles are substantially reduced below certain waterfalls where trout occur. This study is primarily aimed at understanding the pivotal role that waterfalls play in conserving *H. natalensis* populations in the UDPWHS by empirically investigating the link that exists between trout and tadpole abundance. The project argues in favour of a biotic reason (trout predation) being primarily responsible for the decline in tadpole abundance below waterfalls in the UDPWHS, and conversely argues against the role that abiotic environmental factors may have played, relative to the similarities in geo-physical conditions occurring both above and below two selected waterfalls in the UDPWHS.

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CHAPTER 1

1 INTRODUCTION

1.1 General

Conservation efforts to protect the planet's vertebrate biodiversity have tended to favour mammals and birds. The so-called 'lower vertebrates', i.e. fish, amphibians and reptiles, generally have a lower public appeal and are typically neglected in conservation programmes, although these groups are fundamentally important at an ecosystem level (Anon., 2000). In terms of species richness, amphibians outnumber mammals with more than 4 700 living species currently recognized and with an expected global total exceeding 5 000 (Glaw and Kohler, 1998). Ironically, at a time when taxonomists are unraveling and describing amphibian species richness at an unprecedented rate, alarming reports of amphibian population declines and species extinctions are being recorded around the world. Amphibians are proportionally the most threatened group of vertebrates globally (Branch, 1994).

With the human population more than doubling during the second half of the 20th century and reaching six billion in October 1999 (Brown *et al*., 1999), a concurrent increase in the rate of habitat loss and species extinction has become the greatest conservation concern to maintaining biodiversity. At the first World Congress of Herpetology in 1989, many of the participants expressed concern regarding the marked declines in amphibian populations observed in many parts of the world over several decades. It is evident that the declines cannot be attributed to a single cause, but are the result of various factors acting in isolation or in combination. The principal and most widespread local cause appears to be habitat loss, degradation and fragmentation, while other local factors include pollution by agricultural and industrial chemicals, the introduction of exotic predators and road kills. Amphibians are particularly vulnerable to habitat degradation because they may require a variety of discrete habitats to support different life stages or to survive seasonally stressful conditions (Knapp *et al*., 2001a; Matthews *et al*., 2001;

Pilloid *et al*., 2002). Examples of more widespread or global causes of decline are an increase in ultra-violet radiation due to ozone depletion in the upper atmosphere, acid precipitation and global warming. A novel frog pathogen, a chytrid fungus, has been responsible for mass mortalities and extinctions of numerous frog species in Australia and Central America (Berger *et al.,* 1998; Longcore *et al*., 1999). Chytrid is now reported in 43 countries and 36 U.S. states. It survives at elevations from sea level to 6000 m.a.s.l and has affected 200 amphibian species globally. The fungus has been the catalyst for the newly formed Amphibian Ark in which 500 species have now been quarantined globally in zoos, research institutions, and even hotels until this crisis has been resolved (National Geographic Magazine, April 2009). Irido-viral infections are similarly implicated in mass amphibian mortalities (Daszak *et al*., 1999).

1.2 The importance of researching amphibians

Amphibians are integral components of many ecosystems, and they may constitute the highest fraction of vertebrate biomass in some ecosystems (Burton & Likens, 1975). Through their contribution to trophic dynamics in a variety of communities, a worldwide decline in amphibians could have an important impact on other organisms. Larval amphibians can be important herbivores (Dickman, 1968; Seale, 1980; Morin *et al*., 1990) as well as prey (Duellman & Trueb, 1986) in aquatic habitats, whilst adults can play the role of both predator and prey in both aquatic and terrestrial habitats (Porter, 1972). Due to many amphibians requiring a variety of discrete habitats to support different life stages or to survive seasonally stressful conditions, they are particularly vulnerable to habitat degradation and can therefore be used as ecological indicators. (Knapp *et al*., 2001b; Matthews *et al*., 2001; Pilloid *et al*., 2002).

1.3 Threat of alien invasive species to freshwater biodiversity

The practice of introducing wild animals to new countries and new localities is a long established practice worldwide. The introduction of exotic animals seems to have been especially prevalent in the 19th century when there was a great deal of human migration. At this time most introductions were made with little thought for consequences, and it is largely the species involved in such early introductions that have become serious exotic pests (Duff, 1995; Fraley, 1996).

Alien species have many vectors, including biogenic means, but most species considered invasive are associated with human activity. Natural range extensions are common in many species, but the rate and magnitude of human-mediated extensions in these species tend to be much larger than natural extensions, and the distances that species can travel to colonise new areas are often much greater with human assistance (Cassey *et al.*, 2005). Cottam (1950) remarked: “The tremendous impact of man upon nature as a result of introductions can be seen in every country.” Elton (1958) noted that introductions, whether deliberate, accidental or the result of natural dispersal, often lead to ecological explosions, due to the breakdown of ecological balance of biotic communities. Bump (1951) stated, “It should be realised that no species can succeed in a new habitat without causing some changes to the plant and animal associations already established there”. The critical difference between natural dispersals and man’s introductions is that man is able to greatly increase the invasion pressure for long periods of time. In some instances it is possible for man to weaken the ecosystem by removing some of the resisting agencies and modifying the environment to favour the alien form. Wilson (1965) noted that a crucial factor affecting the success of the colonising species is the degree of saturation of the fauna into which the species is entering – the more unsaturated the fauna, the greater the number of vacant niches. Thus, in many cases there is little or no competition for the available niche the invader is attempting to colonise. Elton (1958) and Pearsall (1959) similarly drew attention to the importance of species diversity of the ecosystem in resisting invasions.

Because they have the potential to affect a wide range of organisms, both directly and indirectly, exotic fish have often caused changes to natural communities (Li & Moyle, 1981, Balon & Bruton, 1986).Their introduction may alter communities via competition (Minckley, 1973; Meffe *et al*., 1983), predation (Hutchinson, 1971; Zaret & Paine, 1973; Meffe, 1985), the introduction of parasites (Petrushevski & Shulman, 1961) and habitat change (Mitchell, 1986).

In many cases it is difficult to assess, quantify or predict the impact of introducing an alien fish species (Fausch, 1988; Moyle & Light, 1996: McDowall, 2003). Early studies on the impact of alien introductions on indigenous fish were simplistic, noting mainly competition and/or predation scenarios (McDowall, 2003). It is now apparent that there are more subtle interactions with altered animal behaviours that impact the availability of resources shared with other species and also feedback effects that influence interspecific interactions (Power *et al*., 1985; Wootton, 1994). The impact of introduced species is one of the leading causes of biodiversity loss (Meffe & Carroll, 1997), and introduced species are probably the most important anthropogenic impact on freshwater ecosystems (Olsen *et al*., 1991; Kolar & Lodge, 2000).

Protected areas are increasingly important in the global preservation of biodiversity (Soulė & Sanjayan, 1998), and although it is widely recognised that species diversity within reserves can be reduced by external factors (Janzen, 1986), the importance of internal anthropogenic effects is often overlooked (Cole & Landres, 1996). With the potential to provide the best standards of relatively unmodified landscapes, protected areas in North America (such as wilderness areas and national parks) have tremendous ecological and scientific value (Cole & Landres, 1996). Although the montane ecosystems of western North America are particularly well presented in this complex of protected lands, aquatic habitats within these protected areas are often subject to management practices that are inconsistent with the goal of maintaining natural processes. The most prevalent of these practices is the introduction of salmonid fishes (such as trout) into historically fishless ecosystems to create recreational fisheries (Knapp *et al*., 2001a). The management of non-native trout populations in protected areas is highly controversial due in large part to increased awareness of the ecological effects of introduced fishes on naturally fishless ecosystems.

Liss & Larson (1991) noted that salamander abundance was relatively high in the North Cascades National Park when predatory fish were absent. Salamanders were also noted to move freely within the high mountain lakes during the daylight hours, suspending themselves in the water column and basking openly on submerged rocks and logs. When fish were present, salamander abundance was reduced and the animals became much more secretive, hiding in crevices during the day and presumably feeding mostly under the cover of darkness. Evidence of salamander larvae were also found in the fishes stomachs (Liss & Larson, 1991).

Recent studies have addressed more complicated interactions that involve more than one alien predator and their effects on amphibians. In a study of the impact of bullfrogs (*Bufo.* spp) and mosquito fish (*Gambusia.*spp) on Red-legged Frog (*Rana aurora*) tadpoles (Lawler *et al*., 1999), the effects of bullfrogs were so great that they dominated the smaller effects of the mosquito fish. Nyström *et al*., (2001) examined the effects of multiple-introduced predators on a littoral pond community and found that alien crayfish and Rainbow trout (*O. mykiss)* had negatively impacted on the native common frog (*Rana temporaria*) tadpoles, as well as having both direct and indirect impacts on multiple-trophic levels in the community. Both snail biomass and macrophyte coverage decreased with alien predators. Leavitt *et al*., (1994) used a fish bioenergetics model to evaluate the effects of fish introductions on nutrient cycles in naturally fishless oligotrophic lakes. The model suggested that trout introductions routinely increased phosphorous (P) regeneration from previously inaccessible benthic and terrestrial sources. Because P derived from benthic and terrestrial sources represents a new source of nutrients for plankton, even small increases in nutrient availability can result in increased algal biomass and production. To support the importance of this increased nutrient subsidy to pelagic algae, they presented paleo-limnological evidence that algal production increased 10-fold following trout introductions and showed that this increased production was maintained for the duration of fish presence.

Clearly the best solution would be to increase and preserve the number of aquatic habitats that are free from alien predators (Kats & Ferrer, 2003). International agreements will be necessary to prevent the further spread of species from one continent to another (McNeely, 2000). The effectiveness of these laws and policies will be dependant on public education programmes that inform people about the negative impacts of alien species as well as authority's actions against those that do not comply (McNeely, 2000).

1.4 Threat of introduced alien fish on amphibian diversity

Debates continue as to the role of disease and climate change as contributors to amphibian population declines, while other potential causes of population declines such as habitat loss and the spread of alien species have generally become accepted as detrimental to amphibians (Kiesecker, 2003). Many studies have implicated alien species in amphibian declines by competing with native species (Kiesecker, 2003), carrying disease (Kiesecker *et al*., 2001; Blaustein & Kiesecker, 2002), hybridizing (Riley *et al*., 2003) or preying on amphibians.

Over the last two decades, both experimental studies and correlative field studies have implicated alien species of fish, bullfrogs and crayfish as major contributors to amphibian population decline, and in some instances to local extinction (Kats & Ferrer, 2003). Additional studies (Townsend, 2003, Matthews *et al*, 2002, Simon & Townsend, 2003) have shown that alien predators also caused long-term changes in aquatic communities. The negative impacts of introduced predacious fish on native amphibian populations have been documented in Russia (Reshetnikov, 2003), Australia (Gillespie, 2001), Europe (Martinez-Solano *et al*., 2003), and North America (Bull and Marx, 2002). One third of all endangered and threatened species in the USA are listed (at least in part) due to the action of alien species (Bright, 1995).

Alien predators have almost exclusively affected amphibians with complex life cycles (adult and larval stages) (Stebbins & Cohen, 1995). Amphibian eggs and aquatic larvae are particularly vulnerable to alien aquatic predators, with fish being the most widespread alien predator on amphibians (Stebbins & Cohen, 1995). In many cases, these fish have been placed into habitats to provide game for sport fisherman (Cory, 1963; Knapp, 1996; Stein *et al*., 2000). Amphibian populations are now frequently absent in habitats where alien predators have been introduced (Kats & Ferrer, 2003). Of the numerous organisms that prey upon amphibian larvae, fish are probably the most destructive. Because many of the damaging aliens (e.g. fish, crayfish, bullfrogs) are dependant on permanent or near permanent water for their survival, amphibians that typically inhabit permanent water are frequently documented as those most impacted by aliens (Kats & Ferrer, 2003).

Trout are considered highly effective predators and their impacts on prey species is well-documented (Northcote, 1998). One common perception is that high mountain lakes in protected areas, such as wilderness or national parks are pristine, and can be viewed as reserves for native biota (Knapp & Matthews, 1998). Scientific studies (Duff 1995; Carter, 1997) have discussed how stocking non-native fish may have profound impacts on native biota and that the introduction of non-native fish disrupts “naturalness” that should be an integral part of wilderness (Carter, 1997).

In many river basins, remaining populations of native fishes are concentrated in headwater refugia where they are protected by natural barriers from introduced predatory fishes that are already established at lower elevations. The introduction of non-native fishes into headwater lakes provide point sources capable of invading all downstream habitats, as the fish surmount barriers that normally hinder upstream-directed invasions (Knapp *et al.*, 2001b).

1.5 Trout as alien invaders

A growing body of evidence suggests that non-native trout can substantially change aquatic ecosystems wherever they are present (Simon & Townsend, 2003). At the individual level, grazing invertebrates showed changes in behavior as a result of the introduction of Brown trout (*Salmo trutta)* (Simon & Townsend, 2003). At the population level, trout have replaced non-migratory indigenous galaxiid fish in some New Zealand streams but not others, and have affected the distribution of crayfish and other large invertebrates (Simon &Townsend, 2003). At the community level, trout have suppressed grazing pressure from invertebrates and are thus responsible for enhancing algal biomass and changing algal species composition. Finally at an ecosystem level, essentially all annual production of invertebrates has been found to be consumed by trout and the algal primary production six times higher in a trout stream (Simon & Townsend, 2003). This leads to an increased flux of nutrients from the water to the benthic community. The trout invasion led to a strong top-down control of community structure and ecosystem functioning via its effect on individual behaviour and population distribution and abundance (Townsend, 2003). Benthic invertebrate data from John Muir Wilderness in the U.S.A. showed that species diversity, mean abundance, and mean size were all lower in lakes containing trout compared to fishless lakes, and that guild composition was greatly altered in trout-containing lakes (Matthew *et al*., 2002).The effects on zooplankton appear to be similar (Rowan, 1996). Alien trout have also had a negative impact on the Garter Snake population of the Sierra Nevada by decreasing the availability of amphibian prey (Matthews *et al*., 2002). The effects of alien invasive trout range across several biological domains, from genetic and ecological influences on individual species, to ecosystem processes. Despite concerns over these effects, the popularity of many fisheries, and the difficulty of eradicating established populations, will culminate in non-native trout remaining ubiquitous in many aquatic ecosystems into the foreseeable future (Simon and Townsend, 2003).

Most alien species fail to arrive in distant locations. Thus, one characteristic that may readily be linked with the probability of invasion is long distance dispersal ability. Of those species that do arrive in new locations, it is likely that a large proportion, perhaps the vast majority, fail to become established (Williamson, 1989; Ross, 1991; Williamson & Fitter, 1996). The probability of establishment increases both with the size of the founder population (Crowell, 1973; Ebenhard; 1989) and with the number of invasion attempts (Crowley, 1978). However, when invasions are assisted, as with *S. trutta* in New Zealand, the need for good dispersal ability is by-passed and multiple invasions are created by human agency. Large numbers of trout ova were imported into New Zealand and a large-scale and widespread rearing programme was instigated (Townsend, 1996). Sizable propagules, usually in the order of thousands of fry, fingerlings and yearlings, were introduced to many locations, and successive repeat stockings were routinely made for a number of years. It is estimated that more than 60 million *S. trutta* had been raised and liberated in New Zealand by 1921 (Mc Dowall, 1990b). It is generally accepted that *S. trutta* is an aggressive competitor, particularly for good foraging sites, and it is unlikely that any native species has the ability to out-compete the invader. The practice of importing ova has ensured the exclusion of many parasites of *S .trutta* that occur in its native range: in New Zealand there are only 17 parasites associated with the species compared to 63 in the United Kingdom (Boustead, 1982).

Large body size may also enhance the likelihood of successful establishment. (Lawton & Brown, 1986) because bigger animals tend to have fewer predators (Crawley, 1989) and may possess enhanced competitive ability (Rummell & Roughgarden, 1985; Roughgarden *et al*., 1984; Crawley, 1986). Bigger animals also tend to exhibit less variation in population size (Pimm, 1989), a feature that may reduce the chance of a small population going extinct. These arguments can be applied to *S. trutta*, which as an adult reaches 20-50 cm in length (Maitland, 1972) and is more or less invulnerable to predators and is an aggressive predator for territorial space (Newman, 1956; Kalleberg, 1958; Chapman, 1966a). Species whose adults are long-lived may be more likely to establish than species with short-living adults (Crawley, 1986). The long-lived *S. trutta* (up to eight years) is able to produce offspring over a long period, increasing the chance of encountering good conditions and establishing successfully (Townsend & Crowl, 1991). Invading species with wide tolerance limits and a broad habitat range (Swincer, 1986; Crawley, 1987; Ehrlich, 1989) are more likely to survive in the receiving habitat. Species with a wide geographic range tend to be more successful as invaders (Bruton, 1986; Moulton & Pimm, 1986; Crawley, 1987). Given the large natural range of *S. trutta* and its ability to exploit a range of habitats ranging from small streams to large rivers and lakes and a generalist diet of terrestrial and aquatic invertebrates and fish (Frost & Brown, 1967), the successful establishment of this adaptable species in New Zealand was predictable. Studies of growth and reproduction show that trout do just as well in New Zealand as their native region (Allen, 1951; Burnet, 1970; Hayes, 1988).

In general, it appears that species-rich communities are more resistant to invasion than species-poor communities (Elton, 1958; Ross, 1991), possibly because a larger number of interacting species more fully utilise the available resources (Fox & Fox, 1986). Hobbs (1955) listed one of the environmental conditions essential for invasion of a new species as either a biotic vacancy or a place weakly-held by a displaceable species. The fish fauna of New Zealand is relatively impoverished, with only 27 native species so the success of

S .trutta could be due to the presence of empty niches. Because *S. trutta* is such an aggressive competitor it would seem that they are likely to be effective as an invader of occupied niches too.

S .trutta may profoundly affect the functioning of stream communities, reducing the abundance of grazing invertebrates and altering their grazing behaviour so that algal biomass increases. A trophic cascade was predictable on the basis of the attributes of the invader and of the stream community. *S. trutta* seems to have been responsible for the evolution of invertebrates of novel anti-predator behaviours with far reaching community consequences (Townsend & Crowl, 1991). When stream invertebrates are reduced in abundance by an alien predator, their behaviour changes as they become more cryptic, leading to less algal grazing, that in turn depresses the production of benthic insects, resulting in the benthos becoming less accessible to indigenous fish predators (McDowall, 2003). Introduced trout profoundly affected the structure and composition of faunal assemblages in Californian High Sierra lakes. Large and/or conspicuous taxa, including tadpoles, large bodied micro-crustacean zooplankton and many epi-benthic or limnetic macro-invertebrates were rare or absent in lakes containing trout (Bradford *et al*., 1998).

An intensive study of the Tairei River in New Zealand has revealed that several native galaxiid fishes are now restricted to the headwaters above large waterfalls that prevent the upstream migration of *S. trutta* (Townsend, 1991). In a study on indigenous galaxiids Townsend (1991) found that in over 198 sites examined the best predictor of the presence of galaxiids was the absence of trout. Galaxiids only existed in ‘fringe’ upstream habitats to which trout were excluded by natural barriers. In Lesotho the same applies for the endangered Maloti Minnow (*Pseudobarbus quathlambae*) (Skelton, 2001).

1.6 The importance of recreational angling as a medium for introduction of invasive species

Non-native trout have been successfully introduced into a variety of freshwaters and represent one of the most widespread invasions of non-native species on the planet (Lever 1996; Lowe *et al*., 2000). Most introductions were intended to provide recreational fisheries, with only a minority conducted for conservation of threatened species (Young & Harig, 2001). *S. trutta* was first introduced to the South Island of New Zealand in 1867 and into North Island in 1870. Self-sustaining populations are now found in many streams, rivers, and lakes in the region (Mc Dowall 1990a; Townsend & Crowl, 1991).

Common carp *Cyprinus carpio* and *S. trutta* were intentionally introduced from Europe into South Africa during the late 1800s. The reasons for both introductions were to provide more fishing opportunities. Both species have been extremely successful in colonising across the continent, and the cost to native species has been high. *C. carpio* and *S. trutta* were introduced at a time when people had little concept of the impact these species would have on ecosystems. Their introductions were part of a long tradition in western culture in tinkering with nature in order to “improve” it. With today’s knowledge of ecological systems, fisherman rarely recommend fish introduction because of the subsequent social, economic, and ecological impacts. However, uninformed anglers do move carp, *S. trutta* and other fish around, creating problems for native fish populations and often for other anglers (Moyle & Mount, 2007)

The first consignment of imported British (*S. trutta*) eggs into South Africa in 1875 perished, and was followed by an additional 10 000 *S. trutta* ova in 1882 (Endangered Wildlife Trust News, 2004). Survivors from the third shipment in 1890 were more successful and batches of 500 fry were distributed by John Clarke-Parker into the Mooi, Bushmans and uMngeni Rivers respectively. By 1891 *S. trutta* had been released into many other rivers in the former Natal province. *O. mykiss* (Rainbow trout) was first successfully reared at Jonkershoek in the Cape in 1898. By 1923, trout had become firmly established in the headwaters of virtually every major drainage system rising in the high berg, from the Eastern Cape through to Natal and Lesotho. Trout introductions were often championed by provincial conservation departments, such as the former Natal Parks Board, who actively produced trout in a number of hatcheries. The last remaining state-owned trout hatchery, at Kamberg Nature Reserve, was closed in 2004. The production of trout now only takes place within the private sector.

Many people have grown up fishing for alien species, which they now assume are indigenous. In some cases, such as, rainbow trout (*O. mykiss*) a certain cult status has been created for angling for this species (Cowx, 2002), with some anglers in South Africa wanting alien trout to be declared an ‘honorary indigenous species’ because it has been in the country for over 100 years (Hamman, 2002). Cambray (1997) tried to inform South African readers to the plight of freshwater fish globally, but trout anglers immediately saw this as an attack on their favourite alien fish hobby and wrote emotive, ill-informed letters (Herbst, 1997; Kirby, 1997). Angling for most people is a pastime for pleasure. The main motivation is to be able to relax in pleasant surroundings with like-minded angling friends; the number of fish caught in many cases is only of secondary importance (Wedekind *et al.* 2001). In many cases indigenous fishes are considered to be of marginal importance (Cowx, 2002) as alien species are better known due to their established economic value and vast amount of global literature compared to little known and often poorly studied indigenous species. Many members of the general public, such as farmers and many freshwater anglers, still believe that we can improve on the initial biotic ‘hand’ of nature (Mooney, 1998). Recreational angling in fresh water is now big business, and has thus become a socio-economic problem (Cambray, 2003). In South Africa, certain indigenous species have only recently appeared in the fly-fishing spotlight due to the dedication of conservation officials (Impson, 2001). For example, Yellowfish (*Labeobarbus* spp.) are proving to be a popular and excellent angling species and now adorn the covers of many local angling magazines. However, this has created a new demand for *Labeobarbus* spp. and there are now more requests for private hatcheries to rear these species. In retrospect, there was no real need to introduce trout and bass (*Micropterus* spp.) into South Africa where they now compete and prey on the juveniles of indigenous angling species, some of which are now endangered (Skelton, 1987).

Trout have created a huge industry in terms of aquaculture, property development, manufacturing, retailing and ecotourism with at least 300,000 South Africans fly-fishing (Wildlife Trust Newsletter, May, 2004). Currently there isn’t a single South African province where you cannot fly-fish, ranging from trout fishing in Mpumalanga, KwaZulu Natal, eastern and western Cape, eastern Free State, North West Province and Gauteng, to catching *Labeobarbus* spp. countrywide. Trout have contributed greatly to the local economies of South African towns such as Waterval Boven, Machadodorp, Belfast and Dullstroom. A statement by Dr. Tom Sutcliffe, president of the Federation of Southern African Flyfishers (FOSAF) suggested that trout introductions were an environmental mistake in this country but that it would now become difficult to get rid of them (Endangered Wildlife Trust Newsletter, May, 2004). He further stated that trout were restricted to cooler climates and that these fish were unlikely to spread elsewhere. A further argument by FOSAF was that trout provided an income on land that could have been set aside for forestation or some other agricultural practice which may have had a greater impact on biodiversity (Endangered Wildlife Trust Newsletter, May, 2004). Compared with many other invaders, few would make the case that *S. trutta* has negative economic effects. The recreational salmonid fishery in New Zealand, of which *S. trutta* is the prime component, is conservatively valued at more than U.S. $300 million per year (Townsend, 2003).

In defense of trout stocking, Crass (1986) suggests that provided the environment in which trout have been introduced remains stable, predation and competition between individuals is not undesirable. Even with the introduction of a new predator such as trout no more than minor adjustments will occur as they are a part of the natural community and will be decimated by otters and cormorants in any case. According to Cambray (2003), in the 19th and early 20th centuries, ’good intentions’ fueled the spread of alien recreational fish. Introductions were thought to ‘improve’ biodiversity in local water bodies for anglers (Hey, 1926). These ‘well-intentioned introductions’ have resulted in serious detrimental effects to natural ecosystems, and in some cases the complete loss of indigenous species, resulting in trophic cascades (Hazzard, 1946; Mc Dowell, 2003). In some national Parks in South Africa, such as the Bontebok National Park, the number of alien freshwater fish species now exceeds the number of indigenous species in the Breede River (Russel, 1999; 2001).

One of the most significant threats to fish conservation around the world is the deliberate or accidental introduction of alien fish species (Cambray, 2003). The impact of alien invasive sport fish is typically unpredictable in time and space, with the introductions of relatively few species having resulted in the loss of indigenous fish species worldwide. More nations need to quantify aquatic biodiversity losses caused by alien sport fishes. The spread of alien invasive fishes does not respect political boundaries, therefore total global costs to aquatic biodiversity and ecosystem functioning also needs to be assessed. The global invasive species database of the Global Invasive Species Program (GISP) highlights eight fish species among the world's "One Hundred Worst Invasive Alien Species". Three of these fish species (*O. mykiss*, *S. trutta* and one bass species (*Micropterus* sp.) were introduced purely for sport. Historically, the social value of recreational fishing was usually far more important than conserving biodiversity. Globalisation of alien fish species for sport is best illustrated by *O. mykiss* now in 82 countries, and still spreading, along with associated expensive angling gear, magazines and accommodation infrastructure. Such sport species have become an integral part of a global consumer society.

1.7 The impact of trout on indigenous amphibians.

The introduction of exotic fishes such as trout and bass into African streams and other wetlands deserves special mention. This practice may have had a serious impact on frogs, probably restricting some species, such as the large-mouthed frog (*Amietia vertebralis)*, Johnstone's river frog (*Afrana johnstoni*), and ghost frogs *Heleophryne*, to only part of their former ranges (Channing, 2001). Tadpoles are generally slower-moving than small fish which probably accounts for the fact that tadpoles are more frequently found in trout stomachs than are adults, e.g.: the adult Common River Frog (*Afrana angolensis*) (Crass, 1986).

There are generally too few data that demonstrate how introduced species affect native species (Simberloff, 1981). Furthermore, we have no idea under what circumstances such effects ripple (Simberloff, 1990) or cascade through the food web of the receiving community. Theory predicts that species declines will have ramifications throughout the food web (MacAuthur, 1955; De Angelis, 1975; Pimm, 1980). These effects are particularly marked on the predators that rely on the species removed, especially when there is limited prey available (Paine, 1966; Lynch, 1979; Pimm, 1980). Matthews *et al*., (2002) found that whenever frog populations were reduced or absent so were the aquatic garter snake (*Thamnophis elgans*). When habitats did not contain introduced trout, amphibians still existed in lakes and garter snakes were also present. This suggests that garter snakes might survive the disappearance of some amphibians, but would probably not survive if all amphibians were impacted by alien predators (Jennings *et al*., 1992).

1.8 Case studies involving the decline of amphibians due to trout predation

1.8.1 Mountain Yellow-legged Frogs *Rana muscosa*

Amphibians were the most common vertebrate above 1 800m within the historically fishless aquatic habitats of the High Sierra Nevada (Grinnell and Storer, 1924). Starting more than a century ago, anglers began stocking western streams, rivers, and lakes with, *S. fontinalis, O. mykiss* and golden trout *Oncorhynchus aguabonita*, and other non-native fish. Trout stocking is now conducted by the California Department of Fish and Game, and the current programme is intended to supplement and maintain existing populations of trout. As a result of such stocking, the proportion of trout containing lakes has increased to 63 % of the larger lakes in excess of 4 000 ha (Knapp, 1996). This is in spite of experimental surveys in the Sierra Nevada mountain range finding a direct link between the widespread introduction of non-native trout and the decline of the native *R. muscosa* (Knapp, 1996). Many high mountain lakes in the Sierra Nevada, formerly brimming with *R. muscosa*, are now almost completely depleted of this species (Knapp, 1996). As early as 1924, Grinnell and Storer (1924) reported that *R. muscosa* tadpoles and introduced trout rarely co-exist in lakes and ponds in the Sierra Nevada, and biologists observed and reported trout preying on *R. muscosa* as early as in 1938 (Bradford, 1989). This observation has subsequently been quantified repeatedly in different parts of the Sierra Nevada (Bradford 1989; Bradford *et al*., 1993; Drost & Fellers 1996). Despite this evidence, trout were stocked by the tens of thousands in mid-1960 via aircraft into nearly every conceivable lake, including dozens of previously inaccessible lakes at the top of watersheds. By the mid-1990's, some researchers suspected that the introduced trout were the most significant cause of the decline in *R. muscosa* in the Sierra Nevada (Avery, 2007).

Predation by trout has also caused dramatic changes in zooplankton and benthic invertebrate species composition in lakes, shifting the dominant species in these communities from large bodied to small bodied forms. The majority of lakes stocked by the California Department of Fish and Game lie within designated wilderness areas, areas managed for their natural values (Knapp, 1996). Because amphibian populations fluctuate widely under natural conditions (Pechmann *et al*., 1991; Gulve 1994), and small populations are more likely to go under stochastic population fluctuations than are large populations (Wilcox 1980; Hanski 1989; Hanski & Gilpin, 1991), the reduction in *R. muscosa* size caused by trout introductions is likely to have increased the rate at which individual populations are destroyed. In addition, they suggested that the increased isolation of *R. muscosa* populations would reduce the probability of re-colonisation of formerly occupied sites. This might lead to populations becoming less resilient to extinction and environmental change. *R. muscosa* are not considered good candidates for translocation since they have a high site fidelity and move short distances to relocate to previously used breeding and feeding sites (Pope & Matthews, 2001; Matthews, unpubl.data). Unfortunately, little is known about the extent to which ranids home or what mechanisms (e.g., olfaction, site recognition, etc) may be involved (Sinsch, 1990).

1.8.2 Pacific Treefrogs *Hyla regilla*

This species is commonly found in sympatry with *R. muscosa* in the high elevation lakes, ponds and streams of the Sierra Nevada (Storer & Usinger, 1963). *H. regilla* is the most abundant amphibian in western North America (Brattström &Warren, 1955), and occurs from sea level to 3 540 m (Stebbins, 1985). *H.regilla* were most abundant in portions of the Kings Canyon National Parks (KCNP) where the possibility of finding lakes with trout is lowest, and least abundant in the northern part of the John Muir Wilderness where the probability of finding lakes with trout is highest (Matthews *et al.* 2001). At the water body scale, after accounting for the effects of all significant habitat and isolation variables, the odds of finding *H. regilla* in water bodies with no trout was 2.4 times greater than in water bodies with trout (Matthews *et al.* 2001). Compared to *R. muscosa*, *H. regilla* may not be as vulnerable to introduced fish, because *H. regilla* can breed successfully in shallow ephemeral ponds, adults are more terrestrial, and larvae metamorphose within one season (Schaub & Larsen, 1978). Even so, in aquatic systems of the High Sierra Nevada, predation by introduced trout on the egg and larval stages of *H. regilla* may be strong enough to influence the current distribution and abundance of *H. regilla* both at a local and landscape scale (Matthews *et al*., 2001). The consequence of the decline of both the *H. regilla* and *R. muscosa* is likely to extend beyond the boundaries of water bodies and impact native predators of amphibians and other species in the High Sierra food web (e.g. garter snakes, Jennings *et al*., 1992).

1.8.3 Spotted Tree Frog *Litoria spenceri*

S. trutta and *O. mykiss* were first introduced into south-eastern Australia in the late 1800's and stocking by State Fisheries agencies was widespread by the 1940's (Jackson, 1981, Clements, 1988). Two observations support the hypothesis that introduced trout have played a role in the decline of *L. spenceri*. Firstly, *L. spenceri* was last seen along Buffalo Creek, a near pristine stream in north-eastern Victoria (Watson *et al*., 1991), coinciding with the first reports of trout in this stream (G.Johnson[1], pers.comm, Gillespie, 2001). Secondly, *L. spenceri* was found to occur in high densities along a short reach of the Bogong Creek, in Kosciuszko National Park, New South Wales (Gillespie & Hollis, 1996; Hunter & Gillespie, 1999). This population was restricted to a discrete section of stream only 1, 6 km long, which is inaccessible to trout due to the presence of high waterfalls and an impoundment (Hunter & Gillespie, 1999). All other extant populations of *L. spenceri* persist at densities from one to two orders of magnitude lower than at

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[1] Victorian Department of Natural Resources and Environment, Melbourne, Victoria, Australia.

Bogong Creek (Gillespie & Hollis, 1996). Trout occur in all of these streams and all streams where *L. spenceri* has disappeared (Gillespie & Hollis, 1996). Collectively these observations suggest that introduced trout may have had a major adverse impact on populations of *L. spenceri.* At least five other riverine frog species in south-eastern Australia have declined in upland streams (Anstis & Littlejohn, 1996; Tyler, 1997; Gillespie & Hines. 1999). These are the Booroolong Frog (*Litoria booroolongensis*), Pearson's Tree Frog (*Litoria pearsoniana*), Peppered Tree Frog *(Litoria piperata*), New England Tree Frog (*Litoria subglandlosa*) and stuttering frog (*Mixophyes balbus*). Trout occur in many of the streams in which these species historically occurred. These species are likely to be similarly vulnerable to trout predation, which may be a major causative factor in the observed declines (Gillespie, 2001).

1.8.4 Tailed Frog *Ascaphus truei*

Tadpoles of the Tailed Frog (*Ascaphus truei*) are common within many small, high gradient streams of the Pacific Northwest (United States and southern Canada), where they typically graze periphyton from exposed cobble (Metter, 1964). Ventral flattening and the presence of a suctorial disc allow tadpoles to preferentially inhabit surfaces of cobbles in swift water (e.g., ≥ 1 m/s, Hawkins *et al*., 1988). The tadpoles usually forage at night and hide under crevices during daylight hours (Altig & Brodie, 1972). Tadpoles take two to three years to complete development (Metter, 1967) and attain individual size of about 1g wet mass prior to metamorphosis. Because of their large individual size, tadpoles often represent >90% of the total herbivore biomass in the streams they inhabit (Hawkins, *et al*., 1988).The near absence of *A. truei* from larger streams has been attributed to the predation of trout (Metter, 1964). Feminella & Hawkins (2007) observed significant reductions in the activity of tadpoles when exposed to upstream Cutthroat Trout (*Oncorhynchus clarkia*) (three-fold reduction) and *S. fontinalis* (six-fold reduction) compared with unexposed tadpoles.

1.8.5 Other amphibian case studies involving trout predation

Amphibian species richness, including larval and adult stages sampled in water, was significantly lower in Mountain lakes of the Cantabrian range ((Asturias and León, northern Spain, inhabited by alien predatory fish, versus those than lakes without (Braňa *et al*., 1996)). Despite the extreme scarcity of amphibians in Calabazosa Lake, two specimens of *S. trutta* sampled contained identifiable remains of one larval salamander and three larval anurans (Braňa *et al*., 1996). Further evidence of fish predation on newts (*Triturus* spp.) and the sudden decline of amphibians during the few years following the earliest salmonid introductions (by 1880) was reported in Lake Enol (Braňa *et al*., 1996). Burger (1950) reported the wide scale elimination of the tiger salamander (*Ambystoma tigrinun*) larvae from ponds in Colorado following the stocking of trout. Introduced salmonids are thought to be responsible for the extinction of several amphibian species (*Atelopus* spp.) in Costa Rica (Pough *et al*., 1998). Macan (1966) noted a dramatic decrease in the numbers of toad, *Bufo* spp. and frog, *Rana* spp. tadpoles following the introduction of *S. trutta* into a British tarn.

1.9 Anti-predator mechanisms of amphibians

Studies have found that amphibian larvae grow less or metamorphose at smaller size when they are raised with alien predators than when they are raised without them (Kats & Ferrer, 2003). Mechanisms for mediating these changes in growth and metamorphosis are probably the result of standard responses to predators e.g. reduced movement and reduced feeding on the part of amphibians in the presence of predators (Kats & Ferrer, 2003).

1.9.1 The important role of cover in protecting amphibians

Sections of streams along which *L. spenceri* has persisted in South-East Australia are characterised by extensive cobble or loose rock habitats (Gillespie & Hollis, 1996). The small spaces between loose rocks and cobbles provide suitable oviposition sites and also provide tadpoles with gaps and crevices in the shallows. These microhabitats may provide enough protection from trout predation to allow adequate survival of populations, albeit at a reduced density. Many of the streams from which *L. spenceri* has disappeared, such as Buffalo Creek and lower Bogong creek, lack cobble habitats and are characterised by larger boulders or bedrock (Gillespie & Hollis, 1996) and therefore may offer little protection from trout predation (Gillespie, 2001). However, trout may not eliminate species such as the *L. spenceri* from streams directly, rather reducing recruitment levels or restricting populations to optimal sections of streams, thus decreasing population sizes and increasing population fragmentation. These small, isolated populations then become increasingly vulnerable to local extinction. Thus, the persistence of a species along streams containing trout does not necessarily mean that trout are not having a significant impact on the population (Gillespie, 2001). Shifts in behaviour and habitat use to avoid intra- and interspecific predation, while increasing the probability of larval survival (Frigiel & Semlitsch, 1990) may reduce food consumption and growth by decreasing foraging efficiency (Semlitsch, 1987; Frigiel & Semlitsch, 1990). Eventually larvae may need to leave a refuge to forage and become vulnerable to predation (Petranka *et al*., 1987). Thus, refuge use within a lake may not necessarily ensure long-term survival (Tyler *et al*., 1998).

1.9.2 Defence mechanisms of amphibian larvae

Amphibian larvae have evolved a variety of defences against fish, including reduced mobility (Woodward, 1983), cryptic coloration (Wassersug, 1971), chemical repellents (Voris & Bacon, 1966; Kruse & Francis, 1977; Brodie *et al*., 1978), shifts in diet activity patterns (Taylor, 1983) and the use of chemical cues to detect predatory fish (Petranka *et al*., 1987). Choice of oviposition sites by breeding adults, development of chemical defences, rapid growth rates, and behavioural modifications by larval stages to reduce detection is common responses of amphibian populations evolved under intense predation pressure (Petranka *et al.* 1987; Lawler, 1989; Resetaris & Wilbur, 1989; Holomuzki, 1995; Manteifel, 1995).

Although subjected to modification by experience, such anti-predator behaviours have been shown to be inherited in some amphibian species (Semlitsch & Reyer, 1992). Other defences of tadpoles include rapid growth rate coupled with large body size (Heyer *et al*., 1975). Behaviours that are thought to reduce predation risk include schooling (Waldman, 1982; Kruse & Stone, 1984) and protean flight (Taylor, 1983). A growing body of data suggests that larval behaviour is a more important defense strategy against predators than previously suspected (Petranka *et al*., 1987). Taylor (1983, 1984) reported that the larvae of the North-Western Salamander (*Ambystoma gracile*) are active during the day and at night in lakes lacking predatory fish, but are strictly nocturnal in those with trout. Prey activity level or microhabitat use could affect prey vulnerability. Active prey may be more conspicuous to predators and predator-prey encounter rates may differ among micro-habitats.

Predators attack tadpole species, even when tadpoles are similar in size and colouration (Morin, 1983). Some competitively inferior tadpoles can survive in communities with predators, while competitively superior tadpoles fail to persist (Morin, 1983). A behavioural mechanism may possible be responsible for persistence with predators. Spring Peepers (*Hyla* sp.) a competitively inferior but persistent species, shifts its microhabitat use to avoid predators (Morin, 1986). Such shifts indicate that behavioural responses to predators might explain different vulnerabilities to predators. In research carried out by Lawler (1989), involving four species of larval anurans, tadpoles decreased activity in the presence of predators although they were not exposed to predators until the trials. An innate response to predators in an inexperienced prey would be advantageous when any experience with a predator is potentially lethal. A response to predators in naïve animals implies that predation has historically been a strong selective force (Seghers, 1974; Giles, 1984). The Pine Barrens treefrog *Hyla andersonii* became more benthic in the presence of predators. A benthic habitat could be defense against visually foraging predators for several reasons: light decreases with depth, tadpoles are more cryptic against an irregular, similarly coloured background than in a water column, and motion near the bottom can stir up silt, further hiding the prey (Lawler, 1989). Use of microhabitat refugia has also been proposed as an important adaptation of tadpoles to avoid predators (Heyer *et al*., 1975; Sredl & Collins, 1992). Inadequate anti-predator defences can explain why many species are excluded from permanent habitats. Petranka *et al*., (1987) discovered that certain amphibian larvae showed strong anti-predator responses when placed into a tank that previously held fish. Some species dramatically increased protean flight responses, actively swimming about the aquaria, while others noticeably reduced time spent outside of refuges. Experiments using the larvae of two-lined salamanders (*Eurycea bislineata*) and Grey Tree Frogs (*Hyla chrysocelis*) in Y flow-through systems showed that the avoidance response was specific for fish (Petranka *et al*., 1987).

Chemical defences have been documented in many larval amphibians (Liem, 1961; Voris & Bacon, 1966; Wassersug, 1971: Kruse & Francis, 1977; Brodie *et al*., 1978) and are most prevalent in species that live in permanent habitats with fish. Nonetheless, many amphibian species that co-exist with fish are palatable, and presumably rely on behavioural defences to reduce predation risk. Recent work has revealed a surprising number of behavioural defences in amphibian larvae that reduce the risk of predation from aquatic predators (Taylor, 1983: Woodward, 1983; Holomuzki, 1986; Petranka *et al*., 1987). Kats *et al*., (1988) found that the genus *Rana* includes species that are palatable, and others that are unpalatable, to sunfish. Tadpoles of the family Bufonidae are well known for their toxicity to predators (Licht, 1968; Lawler & Hero, 1997); yet in a study conducted by Hero (2001) several species of Hylidae (*H. boans and H. buckley*) were more unpalatable to the fish tested than bufonids.

Chemical detection of predators has been documented in several lower vertebrates, including snakes (Weldon & Burghardt, 1979) and lizards (Thoen *et al*., 1986). Chemical cues used to detect predators are fairly widespread amongst amphibians (Petranka *et al*, 1987). Species that significantly increased refuge use when exposed to fish chemicals included members of the Ranidae, Hylidae, Ambystomatidae and Plethodontidae. Trends among species suggest that chemically mediated predator avoidance is an adaptive feature that has involved independently in several families in the response to fish predation. The origin and evolution of this behaviour may have been influenced to some extent by factors other than direct natural selection, such as pre-adaption (Gould & Vrba, 1982) or phylogenetic constraints. However, these are insufficient to explain patterns of variation among congenerics and conspecifics. Only species at great risk from predatory fish responded to water conditioned by fish (Kats *et al*., 1988). Simple evolutionary patterns are evident regarding palatability. Three *Rana* species *(R. catesbeiana, R. clamitans, and R. chalconota*) used in feeding trials were unpalatable to fishes (Kats *et al*., 1988; Kruse & Francis, 1977 and Liem, 1961). All of these species breed in permanent habitats with predatory fish. In contrast, other congenenrics (e.g., *R. sylvatica, R. blairi* and *R. pipiens*) that typically breed in fishless, temporary ponds were palatable to fishes. Kats *et al*., (1988) found that larvae of virtually all amphibian species surveyed from permanent habitats had at least one defence against predatory fish, while temporary pond species consistently lacked defences. The ability to detect fish chemically appears to be a major behavioural defence of palatable species that coexist with fish. Work carried out by Kats *et al*., (1988) and other authors suggest that fish avoid all the developmental stages of noxious species. A case in which the defense mechanisms are effective against native predators, but apparently unsuccessful against an alien predator occurs with the newt *Taricha* sp. A powerful neurotoxin makes them unpalatable to almost all predators (Petranka, 1988); yet alien crayfish will attack even adult newts, undeterred by the toxin (Gamradt *et al*., 1997).

The effect of tadpole body size, abundance and fish body size on predation of Grey Tree Frog tadpoles (*H.chrysocelis*), were studied in laboratory and artificial pond experiments (Semlitsch & Gibbons, 1988). Tadpole body size had a significantly positive effect on the survival of tadpoles in all experiments. The relationship between tadpole biomass eaten and biomass available suggested that fish were not satiated when consuming the largest tadpoles. Large tadpoles were probably better able to evade predators. Genetic differences in predator avoidance behaviour or palatability were probably secondary in importance to body size. Fish body size had a significantly negative effect on the survival of tadpoles. Larger fish consumed a larger number and proportion of tadpoles as well as greater biomass. These factors indicate that environmental factors affecting the growth rate of tadpoles can dramatically alter the vulnerability to gape-limited predators (Semlitsch & Gibbons, 1988)

Kats *et al*., (1988) demonstrated that some temperate zone tadpoles, which were found in habitats with fish, were either unpalatable to fish or demonstrated behavioural avoidance of fish. Cory (1963) reported that larval and post-metamorphic *R. muscosa* showed distinctive escape behaviour in waters containing fish but lack such behaviour in waters devoid of fish. The impact of salmonid introductions was less significant for populations of *H. regilla* and two other aquatic breeding anurans, *Bufo boreas* and *B. canorus* that occur at high elevation in some parts of the Sierra Nevada. These species metamorphose within one season (Karlstrom, 1962) and are not restricted to permanent or deep water (Bradford, 1989). These authors suggested that tadpole size, use of microhabitat refugia, and unpalatability were possible mechanisms allowing tadpoles to survive with fish.

1.10 Recovery of amphibian populations after removal of alien predators

Amphibians generally move smaller distances than other small bodied tetrapods (Sinsch, 1990). Small mammals, birds, and reptiles may move great distances during migrations and generally seem to move longer distances than amphibians on a daily basis (Cockrum, 1962; Orr, 1970; Southern, 1979, Welty & Baptista, 1988). Jameson (1956) estimated that *H. regilla* disperse less than 200 m while the home range of terrestrial small mammals, including small bodied rodents, are often several hectares in size (Cockrum, 1962; Southern, 1979; Vaughn, 1986). Due to the relatively short distances travelled, site fidelity, and physiological constraints, amphibians may not readily re-colonise locally extirpated populations. Blaustein *et al.* (1994) reported that it took 12 years for *R.cascadae* to re-colonize a site in the central Cascade Mountains of Oregon despite the presence of a population of *R. cascadae* frogs only 2km away.

Amphibian populations can be reduced to such low numbers by alien predators that they will probably become isolated from other populations and may ultimately disappear (Bradford *et al.*, 1998). Predation by introduced fish and perhaps by other aquatic vertebrates (e.g. *Rana catesbeiana*, Hayes & Jennings, 1986) has been reported as a certain cause in the reduction of local amphibian populations (Macan, 1966: Honegger, 1978; Bradford, 1989; Aronsson & Stenson, 1995), even leading to virtual extinction (Bradford, 1991). This can be particularly risky in the case of meta-populations comprising a number of local populations, each associated with isolated breeding ponds, as this situation makes the balance between local extinction and re-colonisation critical for amphibian persistence (Bradford, 1991; Pechmann & Wilbur, 1994).

Knapp *et al*., (2001b) studied alpine lakes that fell into three categories: lakes with introduced trout, lakes where trout were unable to persist and lakes that never contained trout. Native *R. muscosa,* crustaceans and macro-invertebrates were greatly reduced in lakes containing fish. In lakes where fish had disappeared, the frogs and invertebrates began to increase 5-10 years after fish disappeared and converged on fishless lakes 11-20 years after fish were removed. The study points out that the recovery of lakes after the removal of aliens could be a slow process and might depend on the length of time the aliens had persisted in the habitat before they disappeared (Kats & Ferrer, 2003).

Pope (2007) conducted a four year, replicated whole-lake experiment in the Klamath Mountains of northern California (U.S.A.) to quantify changes in population density, survival, population growth rate and recruitment of Cascades Frog *R. cascadae* in response to the removal of trout species. She compared the demographic responses in lakes where trout were eradicated, lakes which were naturally fish-free and lakes that remained stocked with trout. The frog density increased by a factor of 13,6 following the eradication of trout from three lakes. The survival of young adult frogs increased from 59 to 94 %, and realised population growth and recruitment rates at the fish-removal lakes were more than twice as high as the rates for fish-free reference lakes and lakes that contained fish. Although recruitment rates were extremely high in the first two years following fish removals, they decreased in the third year and were comparable to the fish-free reference sites. The suggestions for these results were: a) the recovery of aquatic insect predators at the fish removal sites increased the predation rates of young frogs; b) intraspecific competition for space and resources increased as the population increased so that young frogs had more difficulty surviving or remaining on site (Pope, 2007). *R. cascadae* has a high reproductive output with about 300-500 eggs/egg mass (Pearl & Adams, 2005), but a similar response may not be expected from species with low reproductive outputs (Pope, 2007). Funk & Dunlap (1999) studied a similar situation where stocked trout disappeared from certain high elevation lakes. Long-toed salamanders (*Ambystoma macrodactylum*) had been eliminated from lakes with fish, but, salamanders re-colonised lakes where trout had gone extinct within 20 years of fish disappearance despite the fact that dispersal in this amphibian was thought to be minimal

(Funk & Dunlap, 1999). Diatom assemblages in lakes (Mt Rainier National Park, Washington, USA.) where trout were removed did not return to pre-disturbance assemblages during the 20-30 years after fish removal (Drake & Naiman, 2000). Diatoms are sensitive indicators of ecological conditions and the study suggested that a more thorough recovery in these aquatic communities is complex and that recovery times are often unpredictable (Drake & Naiman, 2000). Similarly, McNaught *et al*. (1999) found that the invertebrate community in alpine lakes recovered very slowly (>15 years) after the disappearance of stocked salmonids. Following the removal of *S. fontinalis* by gill netting in Bighorn Lake, (Canadian Rocky Mountains), one of the two large zooplankton species believed to have been present in the lake prior to fish introductions, reappeared while another failed to do so, apparently because the egg bank of the latter species had been depleted during the 30 years of fish presence (Parker *et al*., 2001). Waiting for natural events to remove alien predators may prove to be late for many local amphibian populations that are currently decreasing as a result of recently introduced predators and some scientists have proposed removing alien predators aggressively as a way of restoring aquatic habitats for amphibians (Knapp & Matthews, 1998).

1.11 Why the Natal Cascade Frog *Hadromophryne natalensis* is an important research subject

In the southern African region there are 237 species of amphibians, 32 of which are now threatened with extinction (IUCN, 2004). Amphibians are the most threatened group of vertebrates in southern Africa followed by the freshwater fishes (Bruton, 1995). Southern Africa is the only place in the world where entire families of frogs, Heleophrynidae, are found; and two members of this family are currently listed as being critically endangered (IUCN, 2005). There are only seven species in the family (du Preez & Carruthers 2009), of which six are endemic to South Africa. The Natal Cascade Frog which is investigated in this study, was previously known as the Natal Ghost Frog *Heleophyrne natalensis,* but has recently been reclassified as the Natal Cascade Frog *Hadromophryne natalensis* and now occurs within its own genus (du Preez & Carruthers 2009), and is found only in South Africa, Lesotho and Swaziland. The World Conservation Union (IUCN) does not list this species being at risk but does note that their numbers are declining. Major threats to Ghost Frogs are habitat loss due to agriculture and large scale wood plantations, groundwater extraction, dam building, invasive alien species, sedimentation and pollution (du Preez & Carruthers, 2007). In some areas, such as the Cascades in Pietermaritzburg, human impact on previously suitable streams has resulted in the loss of physiognomically suitable vegetation cover, altered hydrodynamic patterns, siltation and accumulated litter unacceptable to the species (Lambiris, 1990).

1.12 Trout Ecology

1.12.1 Distribution of Brown Trout *S. trutta* and Rainbow trout *O. mykiss*

The natural range of *S. trutta* is Europe and North-East Africa where populations occur in streams of the Atlas Mountains of Morocco. *S. trutta* was first introduced into the United States in 1883 and is now found in all states where trout fishing occurs (MacCrimmon & Marshall, 1968; Needham, 1969). Within southern Africa *S. trutta* inhabits mountain streams and upland reservoirs in the western and eastern Cape, KwaZulu-Natal, Mpumalanga, Lesotho and Zimbabwe. They are regarded as the smartest and most difficult trout species to catch. The average size attained is usually 0,1 to 1,8 kg in inland streams.

The native range of *O. mykiss* is the eastern Pacific Ocean and freshwater, mainly West of the Rocky Mountains, from northwest Mexico, to the Kuskokwim River, Alaska. Following its widespread introduction outside of its normal range, in now occurs throughout the United States in all suitable localities (Scott & Crossman, 1973) and has been widely introduced in temperate and high-altitude regions throughout the world.

1.12.2 General habitat requirements of trout

Besides correct physical conditions and chemistry, certain environmental parameters of streams and rivers are required for the growth and successful survival of trout. Trout have a preference for perennial streams with a late summer stream-flow. Cover (defined as sheltered areas in a stream where trout can rest or hide from predators, i.e. snags, logs, undercut banks, large rocks etc.) is positively correlated with trout abundance (Molony, 2001). The best trout areas have in excess of 55% of the available area of stream containing some form of cover (Molony, 2001).

Stream width does not exhibit a linear relationship with trout abundance (Molony, 2001) but unfavoured streams seem to be those that are either very narrow (less than 0,6m) or very wide (greater than 46m). The most preferred trout streams vary between 5,4 and 6,6m wide and are probably a function of the ratio of the stream width to the cover available (i.e. the relative area of overhanging banks) (Molony, 2001). The highest abundance of trout is also found where there is little or no erosion of the river banks and surrounding landscape (0-9%)(Molony, 2001). Trout prefer water depths less than 2m, with an uneven bed that offers a suitable habitat for insects, crabs and tadpoles (Molony, 2001).

Moderate maximum summer water temperatures are optimal for trout density (12.6 -18,6 C) with few trout recorded in areas with maximum summer water temperatures less than 6^0C or greater than $26,4^0$C in North American streams (Molony, 2001). They can however, withstand higher temperatures (20 – 24^0C) if exposure is temporary (usually < 1 or 2 hrs) and infrequent (usually no more than 2 or 3 consecutive days). *O. mykiss* are however, sometimes found in temperatures as high as 24^0C sustained over longer periods (Molony, 2001).The growth rate of trout increases to a maximum near 19^0C and declines at temperatures above and below 19^0C (Myrick & Cech, 2004). The greater heat tolerance of *O. mykiss*, compared to *S. trutta*, is perhaps correlated with their existence at altitudes somewhat lower than those at which *S. trutta* survive in KwaZulu-Natal (Crass, 1964).

One of the most critical factors in determining trout survival is the level of dissolved oxygen (DO) in the water and for many species of salmonids exposure to low levels of DO (less than approximately 5.0 – 6.0 mg/l) can result in mortality (Weithman & Haas, 1984). The most productive trout streams have a relatively low gradient, from 0.5 to 2 percent which converts to a drop of 4.5 to 19 m per kilometre (Hunter *et al.*, 1990). If the gradient falls below 0.5% a stream is likely to have a silty bottom and water temperatures that become too warm for trout (Hunter *et al*., 1990). Trout prefer streams with deep undercut banks, therefore a moderately sinuous stream is ideal (Hunter *et al*., 1990). To conserve energy, salmonids actively seek areas where the water current is slower (Hunter *et al*., 1990). This can be anything from large rocks to submerged logs in the current that create an eddy behind them with slower moving water (Hunter *et al*., 1990). Another favourite haunt is in pools at the bottom of riffles where the current is slower.

Optimal *S. trutta* lotic habitat is characterised by clear, cool to cold water; a relatively silt-free rocky substrate in riffle-run areas; a 50 - 70% pool to 30 - 50% riffle-run habitat combination with areas of slow, deep water; well vegetated, stable stream banks; abundant in-stream cover and relatively stable annual water flow and temperature regimes (US Fish and Wildlife Service Report, 1986). *S. trutta* tend to occupy the lower reaches of low to moderate gradient rivers (<1%) in suitable, high gradient river systems (US Fish and Wildlife Service Report, 1986). High gradient, headwater trout streams are relatively unproductive. Most energy inputs to the stream are generated outside of the water body in the form of terrestrial vegetation and terrestrial insects (Idyll, 1942; Chapman, 1966b; Hunt, 1971). The gradient, water velocity, and substrate size tend to decrease downstream, whereas the pool to riffle ratio, temperature, productivity and species biodiversity tend to increase (US Fish and Wildlife Service Report, 1986). *S. trutta* lives and breeds in many types of streams and they colonise waters of small size provided there is adequate cover (US Fish and Wildlife Service Report, 1986). A water depth of 15cm or more and a focal velocity of less than 15cm/s are recommended for optimal adult *S. trutta* resting and feeding habitat (Wesche, 1980). A large, deep pool is seldom as well populated by *S. trutta* as a shallower one with an average depth of 1 or 1,2m. This is due to the better food supplies which are carried by the shallow water (Crass, 1964). Cover is recognised as one of the essential components of trout streams as, *S. trutta* along with other salmonids, show a strong hiding or cover response during winter (Hartman, 1963). Winter hiding behaviour in salmonids is triggered by low (4-8 C) temperatures (Everest, 1969). Adult *S. trutta*, except during the spawning season, occupy the same stations with very little movement to other stream sections from day to day or year to year (Schuck, 1943; Allen, 1951; Solomon & Templeton, 1976). The species is very territorial and aggressively defends feeding areas from conspecifics and other trout species (Jörgen *et al.* 2000).

S. trutta occur within a pH range of 5,0 to 9,5 (Marshall & MacCrimmon, 1970; Mills, 1971; Heacox, 1974), with optimal growth occurring at a pH of 6,8 to 7,8 (Heacox, 1974). Wingfield (1940) reported faster growth and greater longevity for *S. trutta* in alkaline water than in acidic water. In general, it appears that *S. trutta* are better at surviving in acidic waters than *O. mykiss* (Runn & Milbrink, 1977; Edwards, 1978) Grande *et al*., 1978) with Ikuta *et al*., (1992) recording 24h LC_{50} of pH 3,83 for *O. mykiss* and pH 3,63 for *S. trutta*. Water temperatures ranging between 12,6-18,6^{0}C are optimal for high trout density with few trout occurring at temperatures <6^{0}C or>26,4^{0}C (Molony, 2001). Stream temperatures which exceed the normal range, a lack of spawning habitat, high sedimentation and a lack of preferred food items will limit the population and range of *O. mykiss* (Scott & Crossman, 1973; Wydoski and Whitney, 1979). It would appear that *S. trutta* is slightly more tolerant to wider environmental conditions than *O. mykiss,* although *O. mykiss* has the tendency to grow much faster (Molony, 2001).

1.12.3 Diet and feeding habits of trout.

Both *S .trutta* and *O. mykiss* are opportunistic feeders and generally prey upon aquatic and terrestrial insects, crabs, frogs and small fish if available. *S. trutta* will even feed on zooplankton such as daphnia (Skelton, 2001). Trout in streams feed from the bottom or from drift on the water surface (Skelton, 2001). Overeating is never likely to occur under natural conditions where food shortage is often the cause of a short life expectancy (Crass, 1986). Few fish find enough food to grow as large as their genetic potential will allow (Crass, 1986). The need to conserve energy plays a large part in the feeding behaviour of trout (Crass, 1986). In general a trout takes whatever food is readily available (Crass, 1986). Crass (1986) noticed that trout only responded to eating tadpoles in a small pond in Underberg when the prey attempted to flee suggesting that the movement of the prey is pivotal to the predatory response. Trout select regular foraging sites in a stream which are defended territorialy (Crass, 1986). Bachman's (1984) research indicates that *S. trutta* have about six feeding stations. Any particular station may be used by more than one fish but never simultaneously (Crass, 1986). The selection of foraging sites is based on the physical nature of the stream bed and the flow of water. All sites occurred out of the main current and minimal energy was required to obtain a regular supply of drifting insects (Crass, 1986). A well ordered social system existed in which there little conflicted occurred. Bigger fish dominated with juvenile fish occurring a respectable position behind the owner of the foraging site. Trout prefer water depths less than 2m, with an uneven bed that offers a suitable habitat for insects, crabs and tadpoles (Crass, 1986). A mixture of rock, gravel, sand and loam provides for a diversity of bottom organisms upon which trout feed. Shallow water is more productive than deep pools and feeding trout move into the shallows particularly after sunset (Crass, 1986). Frog tadpoles are relished by trout (Crass, 1986). Tadpoles are slower moving than minnows which probably accounts for the fact that tadpoles, in general, are more often found in trout stomachs than fish (Crass, 1986). Adult Common River Frog *Afrana angolensis* frequently fall victim to large trout.

1.12.4 Life history

There is a large variety in the growth and developmental stages within a species, with factors such as water temperature, food quality and food availability playing a major role. Trout in a nutrient-rich system grow and develop faster than trout in a nutrient-poor system (Crass, 1986). Under favourable conditions in a river *S. trutta* may grow to 150-180mm within their first year and up to 260mm by the end of their second year (Skelton, 2001). Their lifespan is three or four years; rarely five years in Southern Africa (Skelton, 2001). *O. mykiss* tends to move downstream as they grow. They are more active and voracious than *S. trutta* and are less inclined to remain in one area in the river. The growth rate of young *O. mykiss* in KwaZulu-Natal is generally somewhat faster than that of young *S. trutta* (Crass, 1986). This may be due partly to the greater activity and voraciousness which is evident in *O. mykiss* compared to *S. trutta*. Associated with a faster growth rate is a slightly higher condition factor in the majority of *O. mykiss* from rivers compared with *S. trutta* from similar waters (Crass, 1986).

1.13 Biology of Natal Cascade Frog *Hadromophryne natalensis*

H. natalensis is endemic to South Africa, Lesotho and Swaziland. It occurs throughout the Drakensberg and Maluti mountains and along the escarpment of South Africa, Swaziland and Lesotho. The recorded altitudinal range is 580-2 675m (Boycott 2004; Boycott, in prep). The adults are very secretive, and during the day they are usually found under stones, other debris, or in caves (Gow, 1963). *H. natalensis* inhabits clear, swift-flowing streams in mountainous terrain and these waters flow through wooded and forested habitats and have headwaters in montane grasslands. Annual rainfall in these habitats is 800-2 700mm (Boycott, 2004). The fast running water environment they metamorphose in is unique to *H .natalensis* (Carruthers, 2001). Adults more often frequent waterfalls and cascades, where they may be found beneath submerged rocks, in rock cracks, in caves, or sometimes in exposed positions on wet rock faces (Boycott, 2004). Tadpoles live on rocky substrates in swift flowing streams. When disturbed they take cover beneath rocks or in cracks (Boycott 2004; Boycott, in prep).This species occupies both forest and grassland biomes; vegetation types include Afromontane Forest, Wet Cold Highveld Grassland, Afro Mountain Grassland and Short Mistbelt Grassland (Boycott, 2004). Breeding usually takes place in late summer (March-May) when stream flow is reduced, and before winter temperatures become severe (Boycott, 2004). The eggs and oviposition sites of *H. natalensis* have not been described, but it is unlikely that its breeding biology differs greatly from that of other *Heleophyrne* species (Boycott, 2004).The tadpoles are extremely specialised and adapted to live in fast flowing streams (Wager, 1965). The body is flattened, and the mouth is enlarged to become a huge sucker with which it can cling to smooth rocks in running water (Wager, 1965). It has a 'walking' mechanism, and by alternatively thrusting forward the upper lip, and bringing forward the lower lip it can climb against the stream, or even a wet rock face out of the water, and can travel backwards in the same way (Wager, 1965). The tadpoles are usually found in the gloomiest of tree-sheltered streams, but occasionally in the high mountains they may inhabit sunlit stretches of streams (Wager, 1965). Their food consists of the thin layer of algae attached to rocks which they scrape off with their sucker-mouths, and their tracks are clearly visible as wide, clean paths on the rocks showing where they had 'walked' (Wager, 1965). The tadpoles reach 85mm in length, the body is broad and flat, light brown in colour with darker mottling (Wager, 1965). Metamorphosis is slow and may take up to two years (Wager, 1965). As soon as the front legs appear, the sucker-like mouth shrinks and disappears and the frog, 30mm long and still with a long tail, hides in partially submerged vegetation or in pockets or cracks in the rocks while the tail is very slowly absorbed (Wager, 1965). The forested ravines and high altitude montane grasslands are mostly protected with remote wilderness areas. Threats include afforestation with exotic trees, damming of rivers, water extraction and the introduction of alien fishes (Boycott, 2004). Owing to the wide distribution of this species, it is not considered to be a conservation priority (Boycott, 2004).

1.14 Relevance of the study

Much controversy exists in the perception of both the angling and scientific fraternity in South Africa regarding the impact of trout on indigenous biodiversity. Many of the views expressed have been emotionally based or speculative, and have in most instances lacked empirical information. Although introduced invasive alien organisms are regarded as being the second biggest threat to indigenous biodiversity, following habitat change (IUCN Invasive Species Specialist Group (ISSG), 2010), much of the research work to date has centered on larger, more charismatic animal species with a greater public appeal. It appears that little, if any, research has been conducted in South Africa to determine the impact of trout on indigenous amphibian biodiversity. This study is not only aimed at filling an important local information gap but is also directed at the greater global scientific community where there is currently a lack in data highlighting the impact of alien invasive trout on indigenous amphibian biodiversity. From a conservation perspective it becomes imperative that this problem becomes more fully researched to guide future management practices and policy formulation.

Previous field studies conducted by myself within the uKhahlamba Drakensberg Park World Heritage Site (UDPWHS) revealed that the abundance of Natal Cascade Frog *H. natalensis* tadpole populations appeared to be greatly reduced below certain waterfalls where alien trout occurred. The primary objective of this study was aimed at determining whether variability in abiotic environmental conditions above and below selected waterfalls was primarily responsible for the decline or whether certain waterfalls in the UDPWHS function as natural barriers to the upstream migration of predatory fish forming refugia for indigenous species like *H. natalensis* to flourish.

1.15 Aim of the study

The aim of this study was to gather empirical data to determine whether the decline in tadpoles below waterfalls is due to abiotic factors or if alien invasive trout have a significant predatory impact on *H. natalensis* populations in the UDPWHS. A further aim of this study was to reduce some of the controversy that currently exists in South Africa between trout fishing and scientific fraternities regarding the impact of alien invasive trout on indigenous biodiversity.

From the above the following hypothesis was tested:

H_0 Abiotic environmental conditions above and below waterfalls in the UDPWHS are different and therefore are responsible for the decline of tadpoles below waterfalls.

or

H_1 Abiotic environmental conditions above and below waterfalls in the UDPWHS are similar and therefore predatory trout are responsible for the decline of tadpoles below waterfalls and the waterfalls prevent upward movement of trout and therefore act as refugia for the tadpoles.

1.16 Specific research questions

1. Is there a difference in the basic habitat requirements of *H. natalensis* tadpoles and trout above and below two selected waterfalls in the UDPWHS

2. Is there a difference in seasonal distribution and relative abundance of *H. natalensis* tadpoles and trout above and below two selected waterfalls in the UDPWHS

3. Is there an association of species between *H. natalensis* tadpoles and trout below two selected waterfalls in the UDPWHS

1.17 Constraints to this dissertation

Only four of the 12 sampling sites used during this study were chosen to be representative of the geo-physical environmental conditions existing both above and below the two selected waterfalls. The four sites chosen were from the upstream and downstream extremities of each sampling area where the variability between the geo-physical environmental conditions is expected to be the greatest. The bulk of the geo-physical data related to the specific sampling sites was collected in spring when water levels were lowest. Although only limited sampling was carried out in the summer due to incidences of local flooding, data collected in summer was incorporated into the study wherever possible. Hiking into mountainous terrain and carrying heavy sampling equipment placed limitations on time and site access. Two water temperature loggers were lost during the study resulting in the loss of some seasonal data.

1.18 Dissertation outline

Chapter 1 (Introduction) is a review of past and present information related to the research work being conducted on trout as invasive species. The chapter gives the reader a greater insight into the environmental threats faced by amphibians both nationally and globally. It outlines the importance of protected areas for biodiversity conservation, discusses the socio-economic importance of the sport fishing industry and investigates the threat of alien trout as a potential invader of pristine aquatic ecosystems. It describes pertinent aspects of the biology of the Natal Cascade Frog, (*H. natalensis)*, Brown trout (*S. trutta)* and rainbow trout (*O. mykiss)*. This chapter also reviews the defense mechanisms of tadpoles to predatory fish, and investigates some of the threats posed through the isolation and fragmentation of habitat.

Chapter 2 (Methods) explains how the sampling sites were chosen and delineated. The methodology used in the collection and processing of field data is described and an overview of the field equipment used is provided. It also gives the reader some insight into the overall research design and deployment of statistical methods.

Chapter 3 (Results) collates the information collected in the field and evaluates the preferences of *H. natalensis* tadpoles and trout for specific habitat types based on a series of descriptive tables and graphs. Use is also made of a series of Principal Component Analyses to establish which geo-physical features have the greatest influence on the variability between sampling sites. PCA's also establish which sites are more closely related in terms of their geomorphology, hydrology and water quality. Emphasis is given to the establishment of a Catch per Unit of Effort (CPUE) for both *H. natalensis* tadpoles and trout, sampled above and below the two selected waterfalls, as a tool for evaluating the population abundances. A mean CPUE of *H. natalensis* tadpole's electrofished at sampling sites located above the selected waterfalls vs. sampling sites below was subsequently the main criteria used in estimating the relative abundance of populations. Length and mass data was used to identify seasonal cohorts of tadpole populations and to establish a Condition Factor (CF) of tadpoles. The extent of habitat availability for *H. natalensis* tadpole and trout populations based upon their species' specific habitat preferences were estimated using a grid based modeling technique. Finally, the proportion of habitat overlap between *H. natalensis* tadpoles and trout was calculated using the same method.

Chapter 4 (Discussion) compares the findings of this study to that of others conducting similar studies involving alien aquatic predators. The result suggests strongly in favour of a biological reason being the cause for the drastic decline in tadpole abundance below the waterfalls and systematically negates the influence of geo-physical variables as being the most probable cause. The assumption is made, in solidarity with other researchers, that trout predation is the most logical cause for decline of *H. natalensis* tadpoles occurring below waterfalls in the UDPWHS.

In Chapter 5 (Conclusion) the aim and the objectives of the research study are reviewed relative to past and new information presented in the preceding chapters.

Recommendations are made regarding the long term management of *H. natalensis* populations in the UDPWHS relative to the presence of alien trout occurring below certain waterfalls.

CHAPTER 2

2. METHODS

2.1 Description of study area.

A desktop study taking into account the findings of earlier field surveys (Karssing & Craigie, 2004a, 2004b; Karssing & Mickleburgh, 2005) indicated the presence of Natal Cascade Frog *Hadromophryne natalensis* tadpoles and trout in the UDPWHS relative to waterfalls. The Ezemvelo KZN Wildlife Species Database was queried for the recorded distribution of both *H. natalensis* and trout within the UDPWHS. Information from both the field reports and the database was used to identify suitable sampling areas for this study within the UDPWHS. Two study areas were chosen based on their relative ease of accessibility, the Injesuthi and Monk's Cowl Nature Reserves. Injesuthi Nature Reserve forms the northern border of the greater 34 638 ha Giant's Castle Game Reserve which is located within the central Drakensberg region of the UDPWHS while Monk's Cowl Nature Reserve, which borders on the northern boundary of Injesuthi, forms part of the northern UDPWHS management area (Figure 2.1).

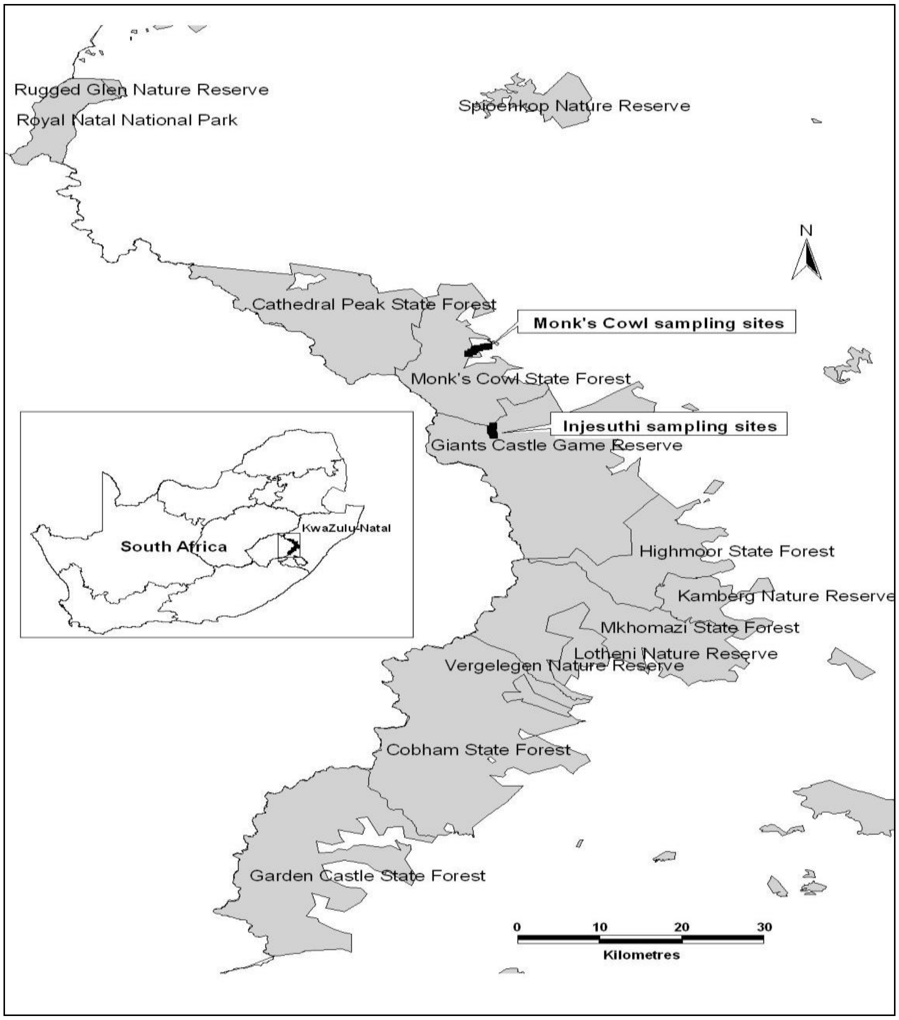

Figure 2.1 Location of sampling sites within the uKhahlamba Drakensberg Park World Heritage Site (UDPWHS), KwaZulu-Natal, South Africa.

Two headwater rivers, the Mobovaneni River at Injesuthi and the Sterkspruit River at Monk's Cowl, were chosen as the study sites. Both rivers have waterfalls that function as natural barriers to the upstream migration of fish, and both rivers form part of the upper reaches of the greater Tugela River drainage system that drains eastward into the Indian Ocean.

2.2 GIS interpretation of sampling areas.

Geographical Information System (GIS) data layers were queried to establish the degree of uniformity between the sampling sites in terms of land-cover, geology, mean annual precipitation, mean annual air temperature, water yield, aspect, slope and vegetation cover types (Appendix A). Sterkspruit Falls (ca. 20m) on the upper Sterkspruit River was chosen at Monk's Cowl Nature Reserve, while an approximately five metre waterfall on the Mbovaneni River, a major tributary of the Injesuthi River, was chosen at Injesuthi Nature Reserve.

2.3 GIS delineation of sampling sites.

A system of twelve potential sampling sites was identified and delineated on a 1:50 000 topographical map (Champagne Castle, 2929 AB, Chief Directorate: Surveys and Mapping, Private Bag X10, Mowbray, 2003). Three sampling sites were identified above, and three sampling sites below, each of the prospective waterfalls. The six sampling sites above the two waterfalls, known to be populated only by *H. natalensis* tadpoles, were treated as control sites. The six sampling sites below the two waterfalls, also known to be populated by trout (Karssing & Craigie, 2004b; Karssing *et al.*, 2007), were treated as experimental sites based on the assumption of amphibian mortality occurring due to of fish predation. The six sampling sites occurring at each river waterfall i.e. three above, and three below, were confined to within 1.5km of each waterfall, the midpoint axis of each sampling area. The maximum distance between the uppermost and lowermost sampling site at each sampling venue was consequently limited to a distance of three kilometres. Each independent sampling site was then limited to a maximum distance of 150m.

2.4 Delineation of sampling sites in the field.

Each sampling site was delineated by laying out a 30m length of rope along the upstream river bank until the total sampling length of 150m was reached. Site codes were given to each of the sampling sites based on their locality and position in relation to the waterfall midpoint (Figure 2.2). Sites at Injesuthi and Monk's Cowl were given the locality code IN and M respectively and subsequently numbered sequentially upstream. GPS waypoints relating to the starting and finishing point of each sampling site is shown in Appendix C. The locality of actual sampling sites in the field is shown in Figures 2.3 and 2.4.

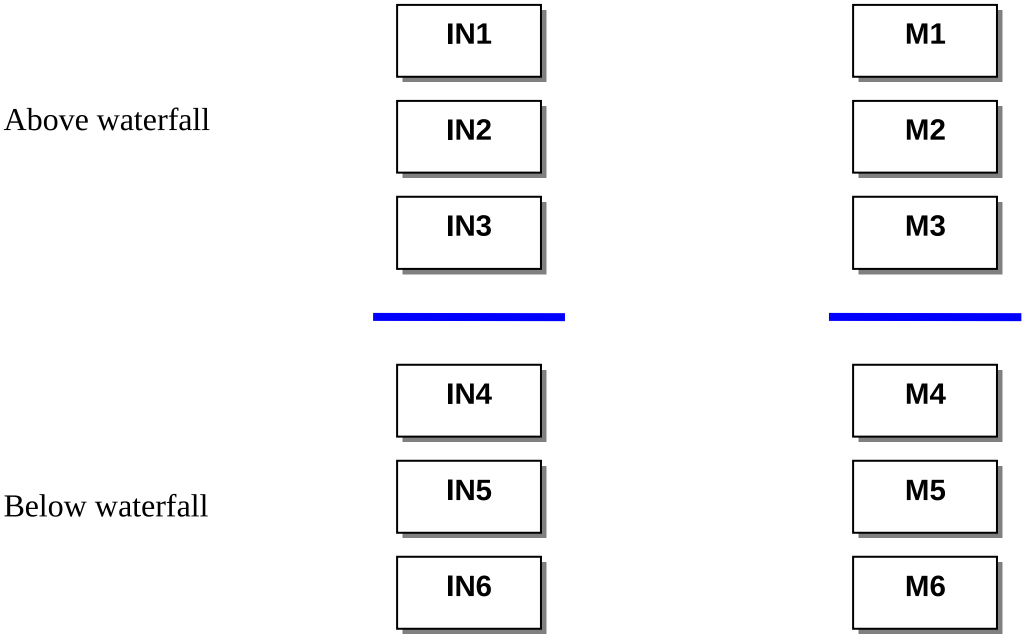

Figure 2.2 Sampling sites located above and below the selected waterfalls at Injesuthi (IN) and Monk's Cowl (M) respectively.

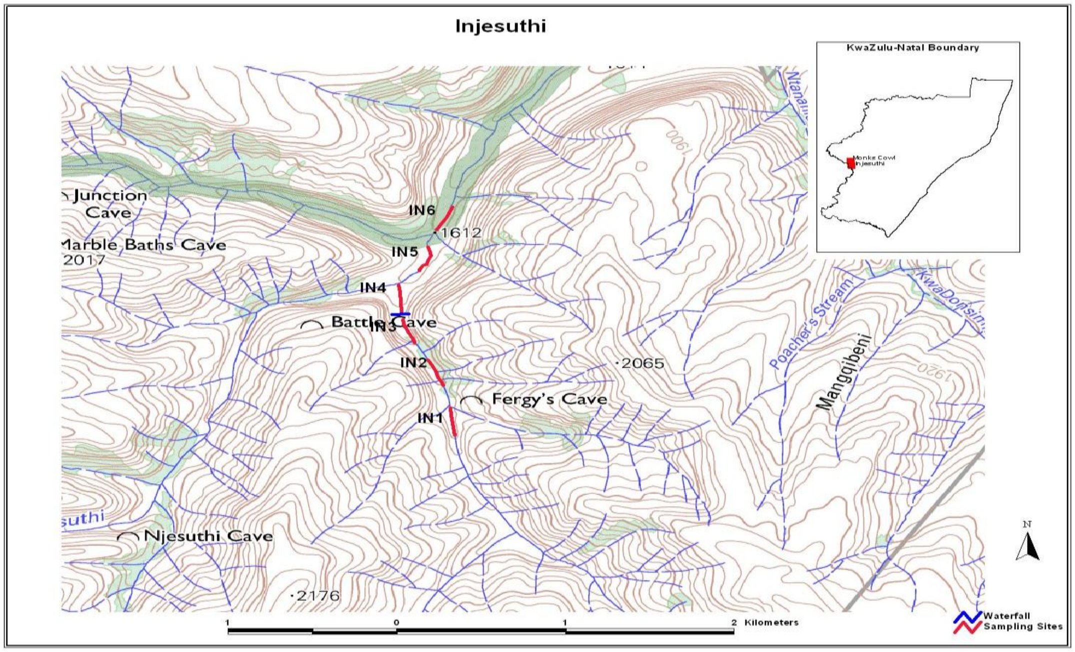

Figure 2.3 Locality of sampling sites at Injesuthi Nature Reserve IN1- Injesuthi 1, IN2 – Injesuthi 2, IN3 - Injesuthi 3, IN4 – Injesuthi 4, IN5 – Injesuthi 5, IN6 - Injesuthi 6.

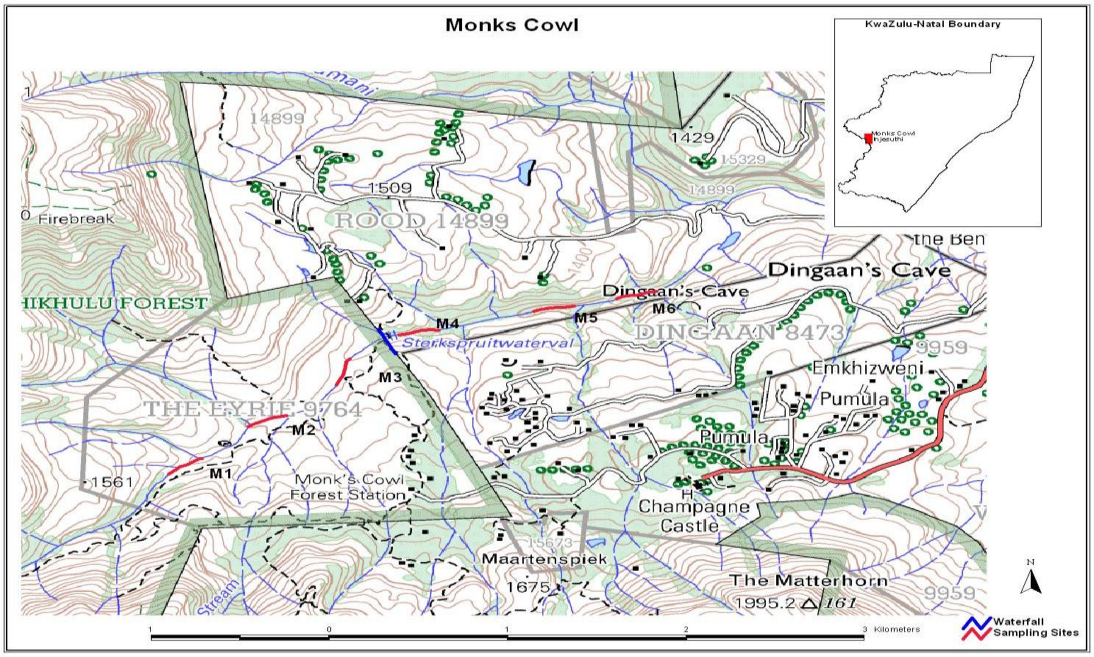

Figure 2.4 Locality of sampling sites at Monk's Cowl Nature Reserve .M1- Monk's Cowl 1, M2 – Monk's Cowl 2, M3 – Monk's Cowl 3, M4 – Monk's Cowl 4, M5- Monk's Cowl, M6 - Monk's Cowl 6.

2.5 Sampling frequency.

Sampling periods were timed to take into account the effect that seasonal influences may have had on both the physical and chemical characteristics of the sampling sites and their associated biota, particularly in terms of *H. natalensis* tadpoles and trout abundance. The data source, frequency of collection, variables, sampling sites, datasets collected and equipment used in data collection is shown in Appendix B. The first field survey was conducted in September 2007, representing spring, the second in February 2008, representing summer, the third in May 2008, representing autumn and the last in July 2008, representing winter. Gridded geomorphological site details in terms of physical attributes were only collected once at four sites (IN1, IN6, M1 and M6) in spring due to labour, access and budget constraints. Water quality parameters relative to pH, Dissolved

Oxygen (DO), Electrical Conductivity (EC) and Total Dissolved Solids (TDS) were collected seasonally at all sampling sites. These sites, located at the extremities of each sampling venue above and below the two selected waterfalls represent the main focus of physical site comparisons. To achieve a more integrated measure of water quality, the SASS 5 Rapid Bio-monitoring method (Dickens & Graham, 2002) was used at sampling sites IN2, IN5, M2 and M5 to assess the general health of the river section, both upstream and downstream of the two selected waterfalls, during each of the four seasonal sampling periods. Sampling sites IN2, IN5, M2 and M5 broadly represent the mid-point of each river section, above and below each of the two selected waterfalls.

2.6 Gridded geomorphological data collection.

Sampling sites IN1, IN6, M1 and M6, representing the upstream and downstream extremities of each main sampling area, were further divided into five metre upstream transects along the full length of the site by marking out points with a 30m rope that had been pre-knotted at five metre intervals. Starting at the main starting point (point 0m), each site was consecutively divided up into a system of 30 x 5m longitudinal sampling points until the upstream finishing point of 150m was reached. The 30m rope was fixed upstream to the river bank which was most clear of obstructions, in five 30m stages, starting at 0-30m and ending at 120-150m. During each 30m stage of marking out five metre upstream sampling points, a second procedure of marking out 0.5m cross-sectional (lateral) sampling points across the breadth of the river was repeated at each of the five metre upstream sampling points. Steel pegs were driven into each bank on opposite sides of river (coinciding with each five metre upstream sampling point) at a height that approximated the full channel width of the river. A second rope knotted at 0.5m intervals, was then draped laterally across between the two pegs. Starting off from the starting peg (a point representing zero), depth, biotope and benthic structure type data was recorded across the breadth of the river in 0,5m stages until the second peg, on the opposite side of the river, was reached. This procedure was repeated for each five metre upstream longitudinal sampling point until the finishing point at 150m was reached. A system of creating a series of five metre upstream longitudinal sampling points, in conjunction with a series of cross-sectional 0,5m sampling points across the breadth of the river, effectively created a matrix of sampling points at their point of intersection. River biotope, benthic structure, riverbank structure, and riparian vegetation categories recorded at each sampling point is shown in Tables 2.1, 2.2 and 2.3 respectively.

Table 2.1 Categories of river biotopes.

Category	River biotope type	Description
1	Run	A run has tranquil flow, no broken water on the surface and has greater depth than riffles (Wadeson,1994; Rowntree & Wadeson, 1999)
2	Riffle	Riffles are defined as shallow, fast-flowing reaches of a river where the water flows over cobbles and gravel, causing turbulent flow, and broken water is observed on the surface (Gerber & Gabriel, 2002)
3	Glide	A glide is a section of river that moves more smoothly and gently than a run (Gerber & Gabriel, 2002)
4	Pool	A pool is an area of a stream that is deep and where the water flows more slowly than in other parts of the river. It can also be a collection of water that is not in the main stream of the water flow e.g. in hollows formed in the bedrock (Gerber & Gabriel, 2002)
5	Eddy-current	A contrary turbulence that creates circular upstream currents behind rocks and other obstructions and along the edges of a stream or river channel. (Glossary of river ecology terms. New Hampshire Volunteer Assessment Programme (2007).
6	Backwater	A small, generally shallow body of water attached to the main current with little or no current of its own pushed back by a dam or current (Glossary of river ecology terms. New Hampshire Volunteer Assessment Programme 2007).
7	Exposed	Aerially exposed benthic structure.

Table 2.2 Categories of benthic structure (Dickens & Graham, 2002).

Category	Benthic Structure type	Description
1	Silt	< 0.06 mm
2	Silt/Sand	A mix of category 1 & 3
3	Sand	0.06 – 2 mm
4	Sand/Gravel	A mix of category 3 & 5
5	Gravel	2 – 20 mm
6	Gravel/Stone	A mix of category 5 & 7
7	Stone	2 – 30 cm
8	Boulder	> 30 cm
9	Bedrock	Slabs of rock

Table 2.3 Categories of riverbank structure and riparian vegetation types.

Category	Riverbank type	Riverbank vegetation type
1	Undercut	Forest
2	Vertical	Shrub
3	Sloped	Grassland
4	Boulder	Mixed

2.7 Water flow velocity and depth.

Cross-sectional river profiles, with the aim of comparing seasonal differences in water flow velocity, were developed at sampling sites IN1, IN4, IN6, M1, M4 and M6. The Head-Rod Measuring System (Carufel, 1980) was then deployed using the equation:

$$V = \sqrt{(2gh)}$$

where V = mean velocity (m/s), g is the gravitational constant of 9.81 m/s and h is the mean head height (m). The procedure of measuring water velocity is illustrated in Figure 2.5 (A). A level monofilament line is stretched horizontally across the river attached to a steel peg embedded on opposites of the river (B) and set at a height that approximates the active channel width of the river (C). A 0.5m knotted rope (D) is then draped between the two pegs to designate each 0.5m cross-sectional sampling point. Starting from the left (upstream) bank, depth measurements are taken at every 0.5m interval using a 1m stainless steel ruler marked in centimeters, across the full wetted breadth of the river until the second peg is reached. Depth measurements were taken by, firstly holding the flat edge of the ruler against the full force of the current (E) and secondly using the sharp edge (F). The mean difference in depth between these two measurements was then used to calculate the mean head height (m).

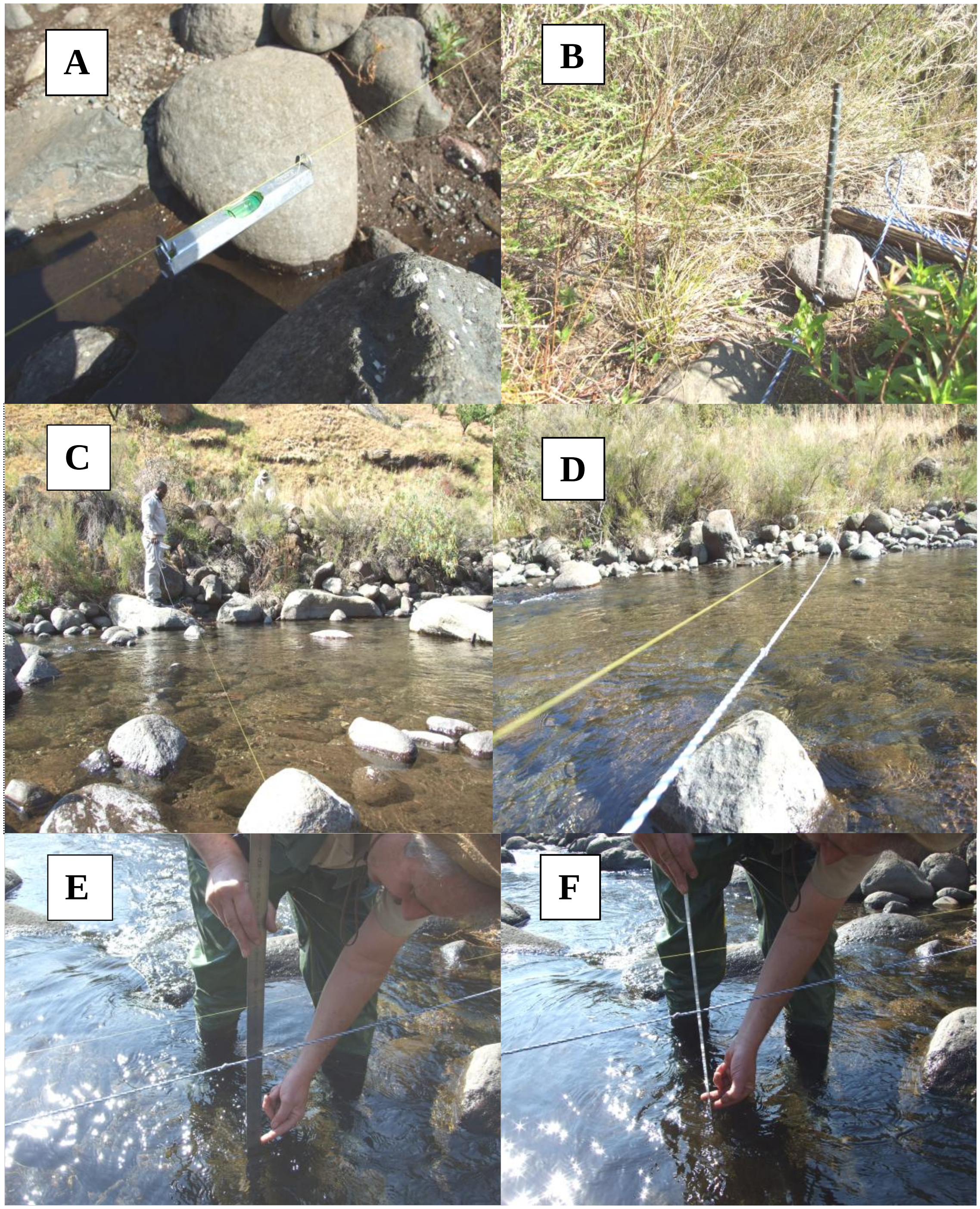

Figure 2.5 Measurement of stream velocity using the Head-Rod Measuring System.

2.8 Water quality variables.

Point data water quality measurements (DO, EC, TDS,^{0}C) were taken at each sampling site during each of the four seasonal sampling periods using a Hanna (HI 9143) DO meter and Hanna (HI 991300) pH/EC/TDS/Temperature multi-meter. Water temperature data was collected by placing temperature data loggers at sampling sites IN1, IN4, IN6, M1, M4 and M6, at locations broadly corresponding with the upstream extremity, midpoint axis (waterfall) and downstream extremity at the Injesuthi and Monk's Cowl sampling venues. The temperature recording system utilized six Dallas® Thermochron i-Buttons (Figure 2.6) pre-programmed to measure water temperatures at 90 minute intervals.

Figure 2.6 Dallas® Thermochron i-button programmed to measure daily water temperature in 90 minute intervals

The activated i-Buttons were sealed into Ziplock® plastic bags before being placed into 60 x 35mm plastic medicinal bottles. i-Buttons were further protected from rocks by placing the sampling bottle in a 20cm section of steel water pipe (50mm). Each of the six sections of pipe was predrilled diagonally at both ends with 4 x 6.5mm holes. Two stainless steel bolts were then inserted at each end of the water pipe and secured effectively encapsulating the sample bottle inside. An additional hole was drilled into one end of the pipe through which a 1.5m x 3mm stainless steel cable was inserted and secured to the device using 3mm steel Crosby clamps (Figure 2.7).

Figure 2.7 Dallas® Thermochron i-buttons protected by a 20cm section of water piping

The protective device was secured in position by attaching the loose end of the cable through a fence standard that had been firmly knocked into the stream substrata. Water temperature data was seasonally downloaded into a spreadsheet for further processing. Daily maximum, minimum and mean water temperatures were recorded**.** The data was processed into monthly datasets that display the maximum monthly water temperature, minimum monthly water temperature and mean monthly water temperature. The mean monthly water temperature dataset for the full sampling cycle (one year) was then tested for normality using a Shapiro-Wilks statistical test (p = 0.05). Mean monthly water temperature datasets collected at sites located above the selected waterfalls at Injesuthi and Monk’s Cowl were then compared to respective sampling sites occurring below, using a Student’s t-test (p = 0.05).

2.9 Electrofishing.

Electrofishing was carried out at all 12 sampling sites during the four seasonal periods using a standard 220 volt electrofisher powered by a portable petrol-driven generator. Electrofishing uses electricity to stun fish before they are caught. Electrofishing is a common scientific survey method used to sample fish populations to determine abundance, density, and species composition. Electrofishing should be conducted in a manner that minimises harm to the fish (National Marine Fisheries Service, 2000). Stream segments should be sampled systematically, moving the anode continuously in a herringbone pattern (where feasible) (National Marine Fisheries Service, 2000). Do not electrofish in one location for an extended period and note that the zone of potential injury for fish is 0.5m from the anode (National Marine Fisheries Service, 2000). Electrofishing crews should be observant of the condition of the fish and change or terminate sampling when experiencing problems with recovery time, banding, injury, mortality or other indications of stress (National Marine Fisheries Service, 2000). Netters should not allow the fish to remain in the electrical field any longer than necessary by removing stunned fish from the water immediately after netting (National Marine Fisheries Service, 2000). Electrofishing was conducted by moving upstream from the starting point of each sampling site until the finishing point of 150m was reached. The chosen method was to use yellow cork floats (wine corks) that were sequentially numbered (1-30) and attached to lead sinkers with 0.5m sections of monofilament. These markers were dropped in the immediate vicinity where specimens of tadpoles or fish had been stunned.

Time keeping was kept by an assistant with a stopwatch. The stopwatch was temporarily paused for the time it took to secure the specimen(s) of tadpole / fish into a bucket and note the number of the floating marker. Once these tasks had been completed the electrofishing continued until the full length of the sampling site (150m) had been completed, pausing only to secure specimens. The time taken to complete the full site (less pauses) was also noted on a field sampling sheet. The sampling team systematically returned back downstream to the numbered markers. Measurements relating to river biotope type, benthic structure type, depth and flow velocity preferences (Head-Rod Measurement Method) were noted on a field data sheet. This procedure was repeated downstream until the original starting point was reached. The time taken to sample each site, versus the number of specimens sampled, formed the basis of the Catch per Unit of Effort (CPUE) i.e. number of tadpoles / fish electrofished per minute. The electrofishing technique was adapted at sampling sites located below the two selected waterfalls to accommodate for a potential bias that exists between tadpoles and trout, relative to their inherent motility. Electrofishing was repeated at these sites, first quickly, with the aim of catching trout, then more slowly, with the aim of securing tadpoles. Time keeping was subsequently combined for both these electrofishing sessions. Fish and tadpoles that had been captured were weighed and measured (Figure 2.8) in a special device (A) consisting of a clear plastic bottle equipped with an internal ruler. A smaller unit was reserved for the weighing and measuring of tadpoles and small fish (100 x 0.5g laboratory pencil scale) while a larger unit, using a 200 x 20g spring balance, was used for fish > 10cm total length (TL). Both tadpoles and fish would be inserted head-first into their appropriate holding devices and individual total length (TL) (mm) established by measuring between the tip of the snout and the tail (B). Tadpoles and fish were then weighed individually (C).

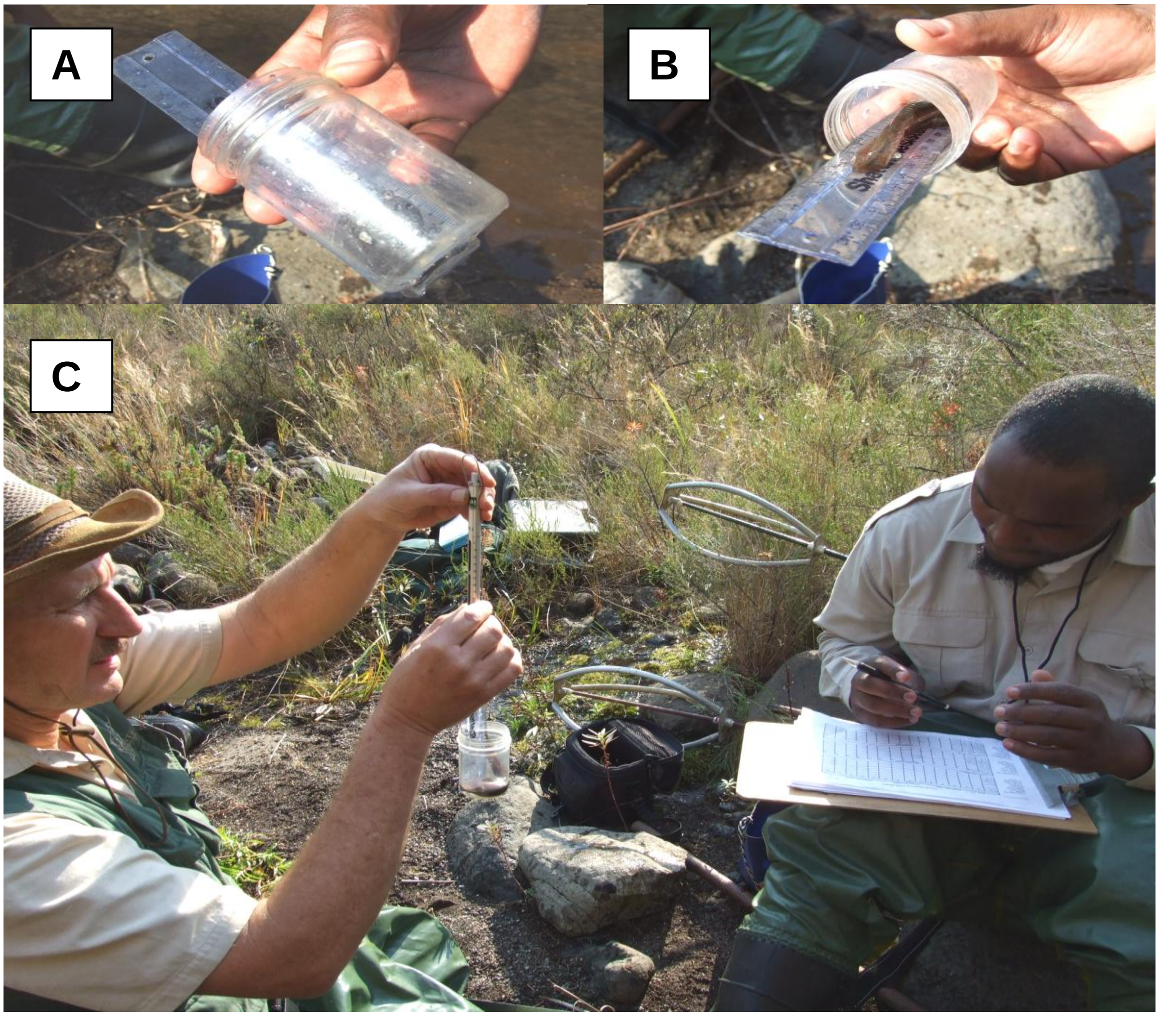

Figure 2.8 Tadpoles being measured and weighed in the field.

2.10 Data Analysis.

The source of data, frequency of collection, data variables, site of collection, complete data sets and equipment used during field sampling, is shown in Appendix B. Where applicable all data was subjected to a Shapiro-Wilk Normality test and then subjected to appropriate statistical tests.

2.10.1 Geo-physical parameters.

Broad landscape comparisons were made between the Injesuthi and Monk's Cowl sampling sites using GIS layers (Appendix A) relative to geology, soil, vegetation, forest type, water yield, mean annual air temperature, mean annual precipitation, site gradient and aspect. Geophysical comparisons at a sampling site level were made between sampling sites IN1 – Injesuthi 1, IN6 – Injesuthi 6, M1 – Monk's Cowl 1 and M6 - Monk's Cowl 6 relative to the percentage broad riparian vegetation type; altitude, mean wetted and active channel width, mean wetted and active channel surface area, percentage bank structure type, river biotope type, benthic structure, depth and current velocity.

2.10.2 Water quality.

Graphs were developed for all sampling sites showing the seasonal differences between pH, Electrical Conductivity (EC) and Dissolved Oxygen (DO). A graph was similarly developed showing the mean river health scores recorded seasonally at sampling sites IN2, IN5, M2 and M6 using the South African Scoring System (SASS) Version 5 (Dickens & Graham, 2002) monitoring technique. Mean monthly water temperature datasets derived from sampling sites located above the selected waterfalls at Injesuthi and Monk's Cowl, was compared to sampling sites located below using a Student's t-test ($p = 0.05$).

2.10.3 Principal Component Analysis (PCA).

Geo-physical environmental templates were developed and compared for sampling sites IN1, IN6, M1 and M6 with a series of PCA's using PC-Ord Version 4.17 (MjM Software Design). The variables were divided into four broad groups (1) Landscape (2) Geomorphology (3) Hydrology and (4) Water quality.

2.10.4 Electrofishing.

Graphs were developed depicting the CPUE's of *H. natalensis* tadpoles and trout electrofished seasonally at sampling sites located above and below the two selected waterfalls at Injesuthi and Monk's Cowl. The methodology used in applying the Student's t-test for two independent variables, based on the mean CPUE of *H. natalensis* tadpoles electrofished from combined sampling sites occurring above the selected waterfalls at Injesuthi and Monk's Cowl, versus combined sampling sites located below, and is shown in Figure 2.9. The test was carried out to determine whether there was a significant difference between selected *H. natalensis* tadpoles populations located above waterfalls, versus populations located below (p = 0.05).

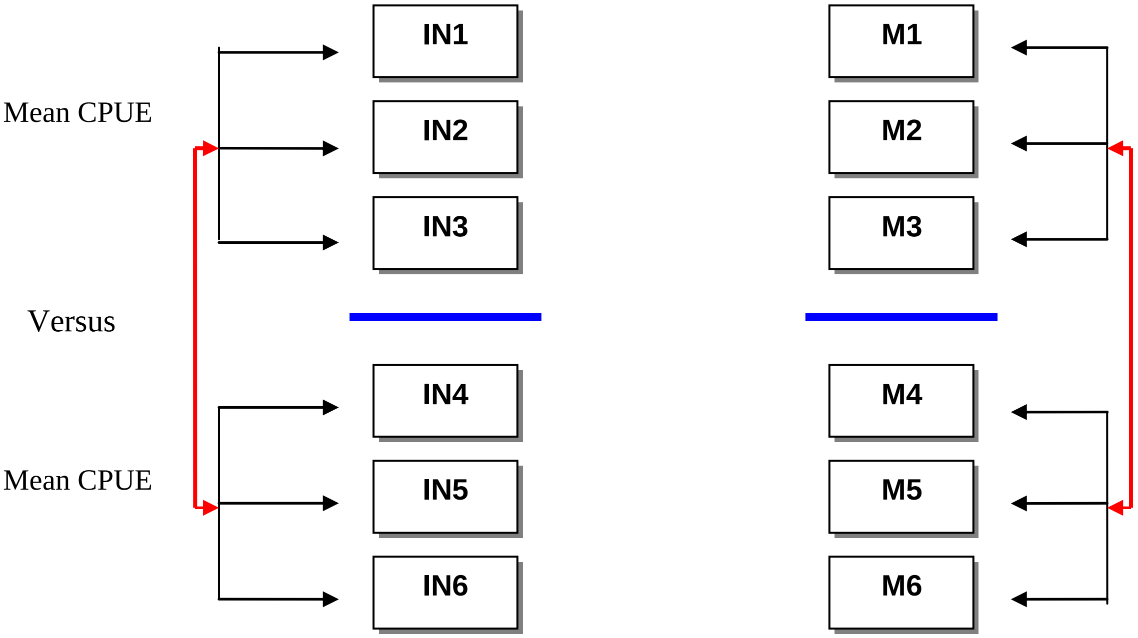

Figure 2.9 Mean CPUE of *H. natalensis* tadpole's electrofished at combined sampling sites located above the selected waterfalls at Injesuthi and Monk's Cowl, compared seasonally to respective sampling sites located below, using Student's t-test (p = 0.05).

The CPUE of *H. natalensis* tadpoles' electrofished seasonally at sampling sites located below each selected waterfall was paired off with the corresponding CPUE of trout and interpolated with a scatter plot. The association between *H. natalensis* tadpoles versus trout was analysed with a straight line regression graph. Tadpole length data was used to identify the percentage of tadpoles occurring seasonally within 2.5mm size classes (high resolution based on small body size) <120mm TL, both above and below the two selected waterfalls. Tadpole mass to length ratios were then used to approximate the mean condition factor (CF) of *H. natalensis* tadpoles occurring at sampling sites located above the two selected waterfalls at Injesuthi and Monk's Cowl, versus sampling sites located below**.** The proportion of tadpoles congregating seasonally into specific group sizes, ranging between two and eight individuals, at sampling sites located above and below the selected waterfalls was quantified. The association of *H. natalensis* tadpoles and trout for specific river biotope types, ranked in order of associated flow velocity was tested using Spearman's Rank Correlation tests. The association of *H. natalensis* tadpoles and trout for benthic structure ranked in descending order of particle size was similarly tested using Spearman's Rank Correlation tests. Habitat overlap between *H. natalensis* tadpoles and trout relative to river biotope and benthic structure type, depth, and current velocity was also investigated.

2.10.5 Habitat overlap modeling between *H. natalensis* tadpoles and trout.

Habitat overlaps between trout and *H. natalensis* tadpoles were estimated in 2-D & 3-D space. A 2-D model was developed based on their preferred range of flow velocity and depth i.e. mean ± one standard deviation. A 3-D habitat model was developed for both trout and tadpoles using grid based techniques in Idrisi Kilamanjaro (Version 14.0 (1987-2003), J. Ronald Eastman., Clark University)**.** Habitat data relative to river biotope type, benthic structure and river depth occurring at sampling site IN6 (surrogate for sampling sites IN1, M1 and M6) (Figure 2.10) was incorporated into spreadsheets and then converted into Idrisi raster images. Three independent raster images, each specific to each site's longitudinal (5m) and lateral transect (0.5m) dimensions, were then created for each of the three habitat variables captured – biotope, depth and benthic structure. Each raster image was then reclassified according to the range of preferences of *H. natalensis* tadpoles for specific river biotope types, depth and benthic structure derived from the spring electrofishing results. These images were then overlaid with each other and queried for commonality. The results of the computation highlighted the approximated suitable habitat locations within each sampling site. Grid based modeling was then used to overlay the approximated available habitat for trout with that of *H. natalensis* at sampling sites situated below the selected waterfalls for the spring sampling period. The resultant image was an approximation of the extent of shared habitat between trout and *H. natalensis* tadpoles based on species specific preferences for river biotope type, benthic structure and depth.

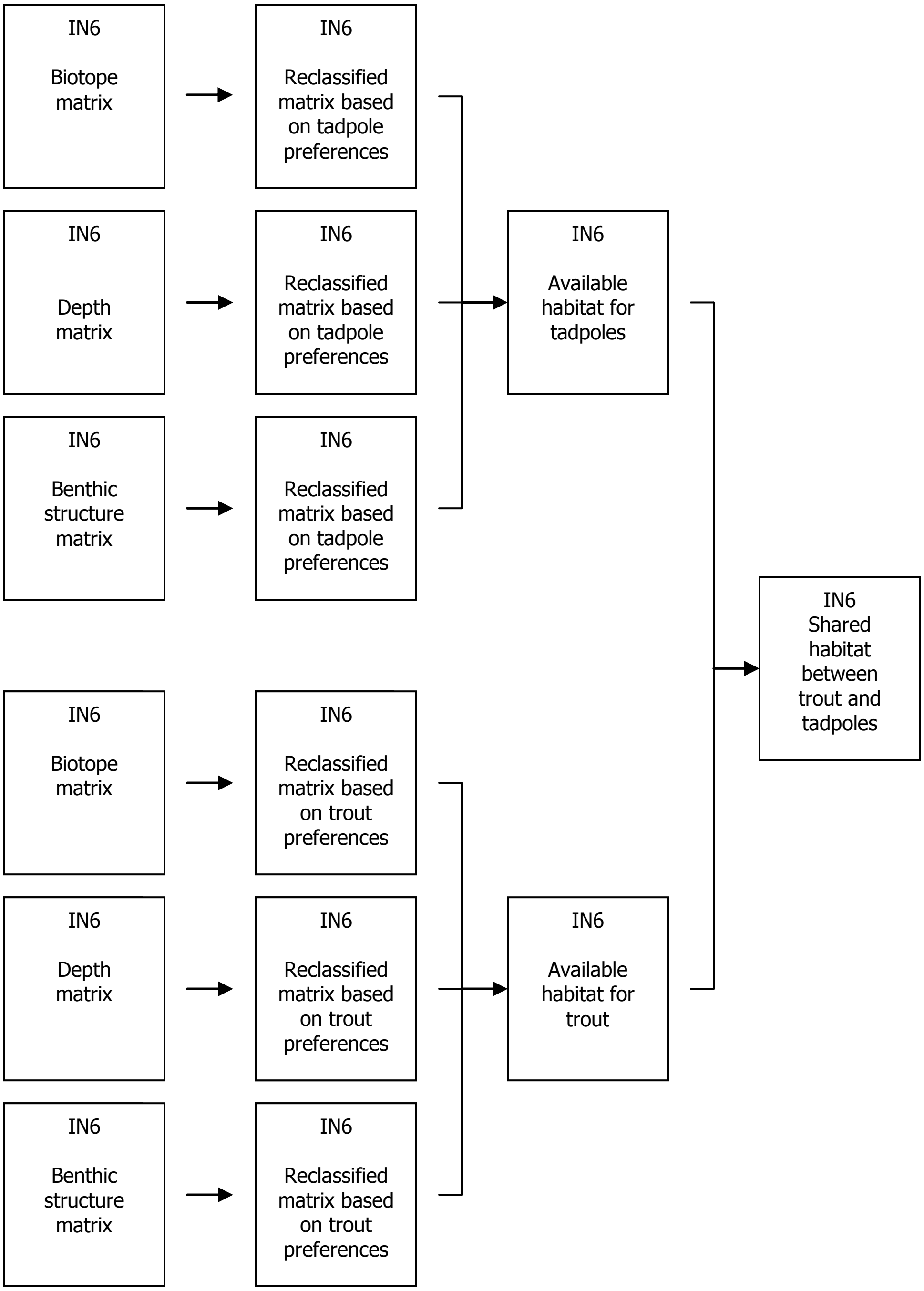

Figure 2.10 Modeling procedure (Idrisi Kilamanjaro) at sampling site IN6 – Injesuthi 6 used to approximate percentage habitat overlap between *H. natalensis* tadpoles and trout based on specific preferences for categorized river biotope and benthic structure type and depth.

CHAPTER 3

3. RESULTS

3.1 Landscape-scale description of sampling sites.

Sampling sites located above and below the selected waterfalls at Injesuthi and Monk's Cowl Nature Reserve are similar in soil type, forest type and water yield. Differences between the Monk's Cowl and Injesuthi sampling areas are only evident in the geology and vegetation type (Table 3.1).

Table 3.1: Landscape-scale attributes of Injesuthi and Monk's Cowl.

GIS Layer	**Injesuthi**	**Monks Cowl**
Geology	Stormgroup Basalt	Mudstone
Soil Type	Red-Yellow Apedal, freely drained soils, red and yellow, dystrophic and/ or mesotrophic soils	Red-Yellow Apedal, freely drained soils, red and yellow, dystrophic and/ or mesotrophic soils
KZN Vegetation Type	Drakensberg Foothill Moist Grassland	Northern KwaZulu-Natal Moist Grassland
KZN Forest type	Northern Afro-temperate Forest	Northern Afro-temperate Forest
KZN Water Yield	High	High

Sampling sites located above the selected waterfalls (IN1, IN2, IN3, M1, M2, M3) typically had a slightly higher mean rainfall than sampling sites occurring further downstream (IN4, IN5, IN6, M4, M5, M6), although this observation is less evident at the Injesuthi sampling sites. The mean annual rainfall of the Monk's Cowl sampling sites (1 375mm) is approximately 36% higher than the Injesuthi sampling sites (878mm) (Table 3.2).

Table 3.2 Large-scale climate and topographical attributes of sampling sites derived from rasterised GIS topographical layers.

Sampling sites	**Mean annual air temperature ^{0}C**	**Mean annual precipitation mm**	**Site Gradient $^{0}/_{00}$**	**Site aspect ^{0}**
IN1	15	896	10.00	34.00
IN2	15	874	21.50	22.00
IN3	15	874	22.00	43.00
IN4	15	874	14.00	82.00
IN5	15	874	13.00	96.00
IN6	15	874	20.50	119.00
M1	15	1428	11.50	121.00
M2	15	1428	6.00	134.00
M3	15	1385	20.00	116.00
M4	15	1385	5.00	42.00
M5	15	1385	4.50	155.50
M6	15	1240	14.50	131.00

The mean gradient of the river at sampling sites located above the selected waterfall at Injesuthi is slightly higher than those sampling sites occurring below (17.83 vs.15.83$^{0}/_{00}$). The mean gradient of the sampling sites located above the selected waterfall at Monks Cowl is similarly steeper than those sampling sites located below (12.50 vs. 8.00$^{0}/_{00}$). The gradient at Injesuthi is comparatively steeper than Monk's Cowl. The mean aspect of sampling sites located above the waterfall (33) at Injesuthi, varied from sampling sites occurring below the waterfall (99). The mean aspect of sampling sites occurring above the selected waterfall (124) at Monk's Cowl varied slightly with sampling sites located below (110). The mean aspect of sampling sites at Injesuthi (66) and Monk's Cowl (117) generally face in a north easterly and south easterly direction respectively.

3.2 Physical attributes of sampling sites.

3.2.1 Altitude.

The altitude of the Injesuthi sampling sites consistently exceeded those of Monks Cowl by approximately 300m (Figure 3.1). The altitude at the Injesuthi sampling sites ranged between 1 600m.a.s.l and 1 700 m.a.s.l over a distance of approximately 1 500m, while the sampling sites at Monk's Cowl ranged between 1 280m.a.s.l and 1 440m.a.s.l over a distance of approximately 2 850m.

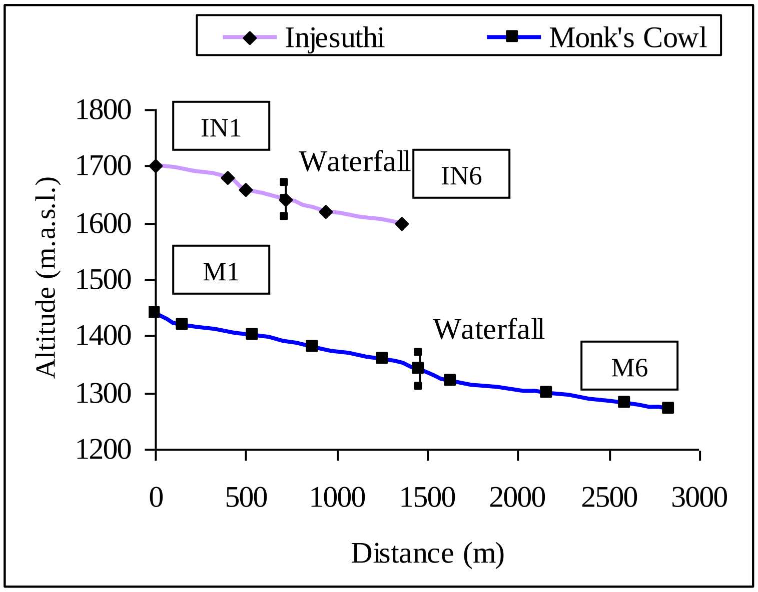

Figure 3.1 Altitude and distance between sampling sites located above and below the selected waterfalls at Injesuthi and Monk's Cowl.

3.2.2 River biotope type.

The percentage of categorised river biotope types occurring at all sampling sites in spring is shown in Figure 3.2. Exposed river bed, mostly as a result of low water conditions in spring (dry season) accounted for the greatest proportion of river biotope type followed by runs. Sampling site M1 had the greatest proportion of exposed river bed (38%) followed by sampling site IN6 (34%), M6 (25%) and IN1 (24%). The greatest proportion of run biotope type occurred at sampling site IN6 (27%) followed by M1 (26%), M6 (19%) and IN1 (19%) respectively. IN1 had the greatest proportion of riffles (11%), IN6 the greatest proportion of glides (17%), IN6 the greatest proportion of back-eddies (10%) and IN1 the highest proportion of backwater habitat (13%).

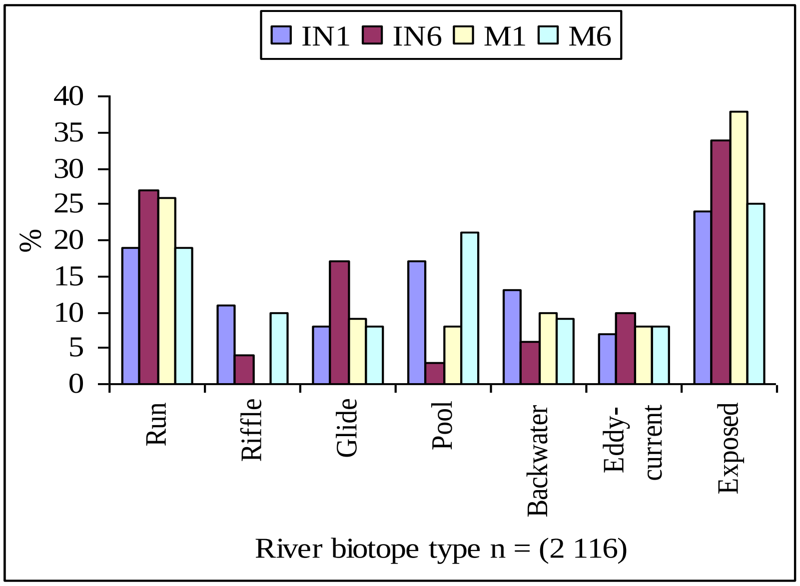

Figure 3.2 Percentage of river biotope types occurring at sampling sites IN1, IN6, M1 and M6 during the spring sampling period.

3.2.3 Benthic structure type.

The percentage of categorised benthic structure types occurring at sampling sites IN1, IN6, M1 and M6 in spring is shown in Figure 3.3. Stones accounted for 40% of the benthic structure type at sampling site M6, 39% at IN1 and IN6, and 33% at M1. Boulders accounted for 35% of the benthic structure of IN6, 27% at M6, 25% at M1 and 18% at IN1. The greatest percentage of gravel occurred at sampling sites M1 (30%) and M6 (24%). Gravel accounted for 19% of the benthic structure at IN1 and 10% at IN6. Collectively sand accounted for 10%, bedrock 2% and silt 1% of all benthic structure. The high percentage of boulder, stone and gravel, and limited sand and silt deposits, is characteristic of a typical mountain headwater zone (Gerber & Gabriel, 2002).

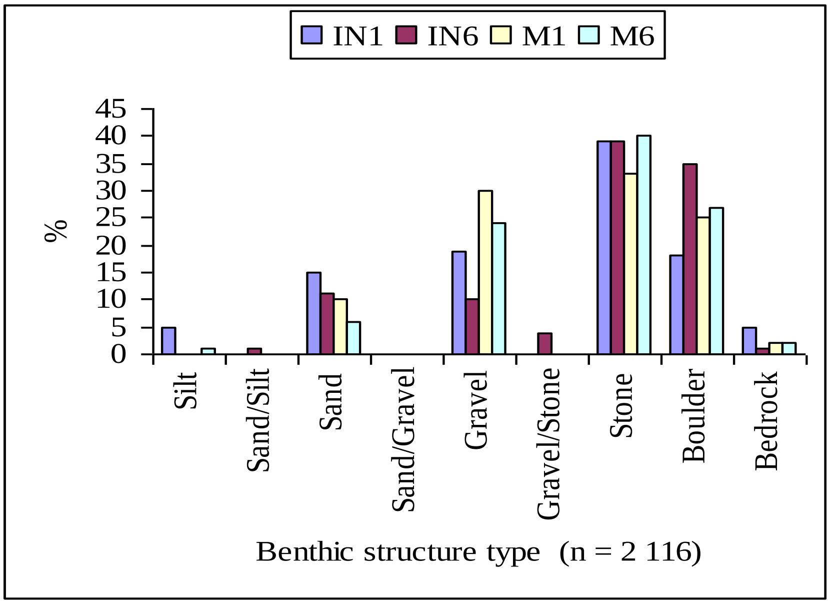

Figure 3.3 Percentage of benthic structure occurring at sampling sites IN1, IN6, M1 and M6 during the spring sampling period.

3.2.4 Riverbank type.

Sloped banks (≥ 41.90%) were the most prominent bank type occurring at all sampling sites (Table 3.3). Sampling site M6 had the greatest percentage of undercut bank (24.20%), IN6 the greatest percentage of vertical bank (30.60%) and IN1 the greatest percentage of large boulders (27.40%) in the riparian zone.

Table 3.3 Percentage riverbank structure type occurring at sampling plots IN1, IN6, M1 and M6 during the spring sampling period.

Bank type	**Sampling sites**			
	IN1 %	IN6 %	M1 %	M6 %
Undercut	14.50	3.20	6.50	24.20
Vertical	4.80	30.60	27.40	11.30
Sloped	53.20	66.10	48.40	41.90
Boulder	27.40	0.00	17.70	22.60
n	62	62	62	62

3.2.5 Riparian vegetation type.

Percentage of broad riparian vegetation type occurring along the riverbanks at sampling sites IN1, IN6, M1 and M6 during the spring sampling period is shown in Table 3.4. Sampling sites IN6 and M1 consisted of 100% mixed vegetation (grasses and shrubs), M6 largely riparian forest, and IN1 predominantly grassland interspersed with some riparian forest and shrubs.

Table 3.4 Percentage broad riparian vegetation type occurring at sampling sites IN1, IN6, M1 and M6 during the spring sampling period.

Vegetation type	**Sampling sites**			
	IN1 %	IN6 %	M1 %	M6 %
Grass	65.00	0.00	0.00	0.00
Mixed	14.00	100.00	100.00	18.00
Forest	21.00	0.00	0.00	82.00
n	62	62	62	62

3.2.6 River width.

Wetted-width measurements taken at sampling sites IN1, IN6, M1 and M6 during spring is shown in Table 3.5. Comparisons were made at Injesuthi and Monk’s Cowl between sampling sites occurring furthest upstream from the two selected waterfalls, i.e. IN1 and M1, versus sampling sites occurring furthest downstream below, i.e. IN6 and M6 respectively. The mean wetted width of sampling site IN6 (7.65m) was 58 % greater than IN1 (4.83m), while the mean wetted width of sampling site M6 (9.20m) was 99% greater than sampling site M1 (4.62m). The greatest degree of variation in river width occurred at sampling site M1 (38.96%) and M6 (28.72%). Both streams became wider in a downstream direction subject to a normal river continuum.

Table 3.5 Wetted width measurements at sampling sites IN1, IN6, M1 and M^ during the spring sampling period.

Parameters	Sampling sites			
	IN1	IN6	M1	M6
Maximum (m)	8.00	13.60	9.10	14.30
Minimum (m)	2.20	4.90	2.00	4.10
Range (m)	5.80	8.70	7.10	10.20
Mean (m)	4.83	7.65	4.62	9.20
SD (m)	1.19	1.93	1.80	2.93
CV (%)	24.63	25.23	38.96	28.72
n	31	31	31	31

A comparison was made between the active channel width measurements taken at sampling sites IN1, IN6, M1 and M6 during the spring sampling period (Table 3.6). The mean active channel width at IN6 (9.59m) was 57% greater than IN1 (6.10m) while the mean active channel width at M6 (10.78) was 77% greater than M1 (6.10m). All sampling sites indicated a similar degree of variability in active channel width. The greatest variability in active channel width occurred at sampling site M1 (29.84%).

Table 3.6 Active channel width measurements at sampling sites IN1, IN6, M1 and M6 during the spring sampling period.

Parameters	Sampling sites			
	IN1	IN6	M1	M6
Maximum (m)	9.80	16.00	10.50	15.40
Minimum (m)	2.90	5.10	3.40	5.70
Range (m)	6.90	10.90	7.10	9.70
Mean (m)	6.15	9.59	6.10	10.78
SD (m)	1.50	2.36	1.82	2.90
CV %	24.39	24.61	29.84	26.90
n	31	31	31	31

3.2.7 Surface area.

The total surface area of sampling sites IN1, IN6, M1 and M6 was approximated by multiplying the mean width by the fixed length (150m) of each sampling site (Figure 3.4). Both the wetted surface area and active channel surface areas situated below the selected waterfalls i.e. IN6 and M6 are noticeably greater than the sites above i.e. IN1 and M1. The two river systems are similar in terms of their total surface area.

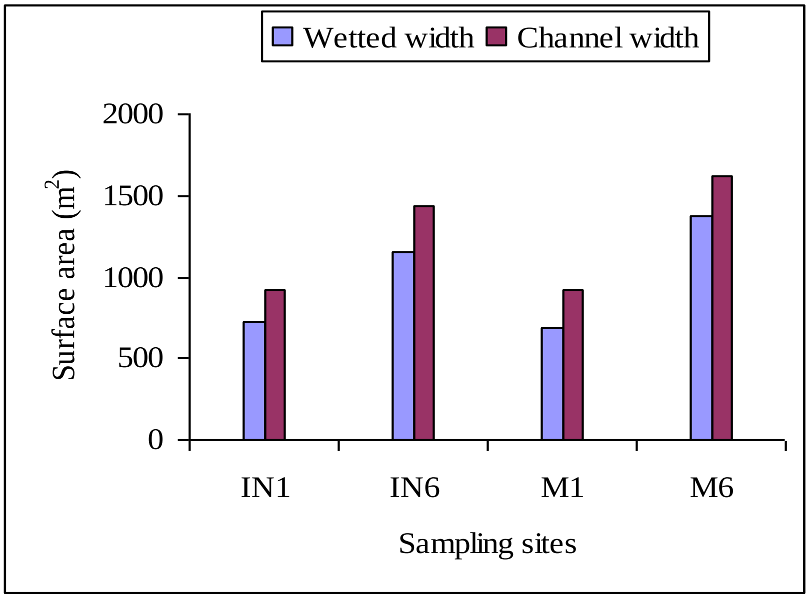

Figure 3.4 Approximated wet and dry surface areas (m^2) occurring at sampling plots IN1, IN6, M1 and M6 during the spring sampling period.

3.2.8 River depth.

Sampling site M6 had the greatest mean depth (21.72cm) followed by IN6 (19.89 cm), IN1 (18.67cm) and M1 (12.54cm) (Table 3.7). Sampling site M6 had the greatest maximum depth (78.00cm) followed by IN1 (75.00cm), IN6 (64.00cm) and M1 (58.00cm). Sampling site M1 has the greatest percentage of shallow water (< 20cm) while sampling site M6 has the greatest percentage of deep water ranging between 40 and 70cm (Figure 3.5). All sampling sites were highly variable in terms of depth (≥ 65.26%) with the highest degree of variability occurring at the Monk's Cowl sites.

Table 3.7 Depth measurements collected at sampling sites IN1, IN6, M1 and M6 in spring.

Parameter	**Sampling sites**			
	IN1	IN6	M1	M6
Maximum (cm)	75.00	64.00	58.00	78.00
Mean (cm)	18.67	19.89	12.54	21.72
SD (cm)	12.32	12.98	9.26	15.97
CV %	65.99	65.26	73.84	73.53
n	402	640	377	697

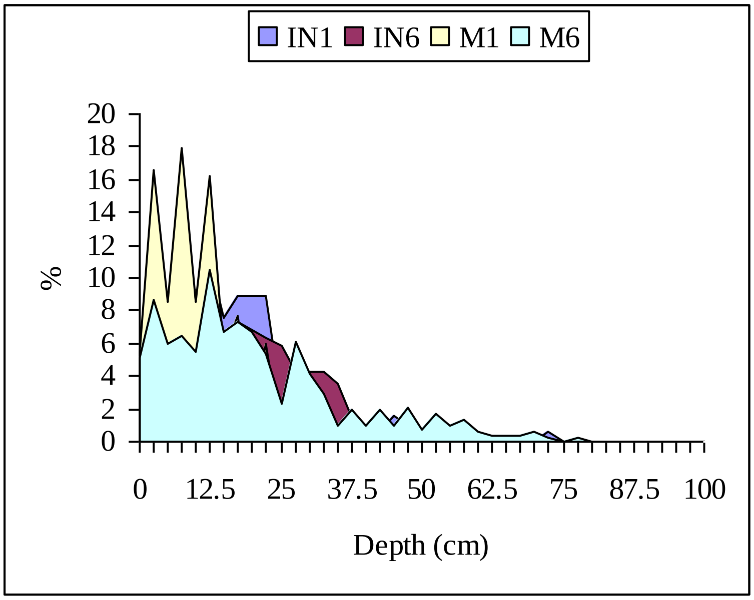

Figure 3.5 Percentage depths occurring at sampling sites IN1, IN6, M1 and M6 during the spring sampling period.

3.2.9 Current velocity.

The highest current velocities occurred in summer and the lowest in spring (Figure 3.6). Sampling sites occurring immediately below the selected waterfalls (IN4, M4) recorded the highest flow velocities. Sampling sites IN6 and M6, located furthest downstream from the selected waterfalls at Injesuthi and Monk's Cowl respectively recorded the lowest flow velocities.

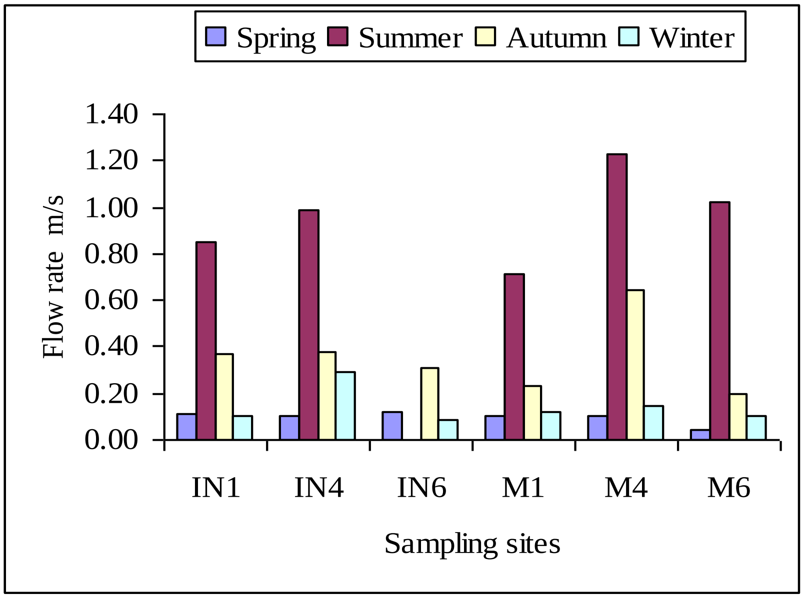

Figure 3.6 Flow velocities (m/s) recorded seasonally at sampling sites IN1, IN6, M1 and M6 during the spring sampling period.

3.3 Water Quality.

3.3.1 pH.

pH readings taken seasonally at all sites ranged between a minimum of 6.90 at sampling site M4 in spring and a maximum of 9.02 at sampling site M3 in autumn (Figure 3.7).

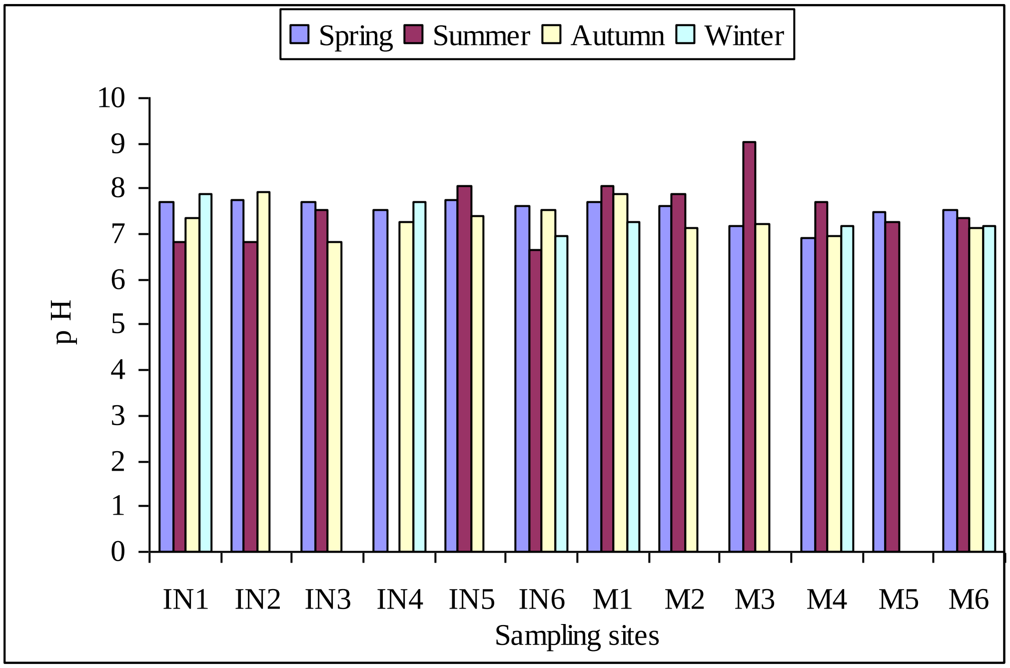

Figure 3.7 pH measurements recorded seasonally at all sampling sites during the spring sampling period.

3.3.2 Electrical conductivity (EC).

The highest EC readings were recorded in spring and the lowest in summer (Figure 3.8). All sampling sites displayed low conductivity values concurrent with oligotrophic ecosystems. Results suggest a trend for the mean EC to decrease below waterfalls (Table 3.8).

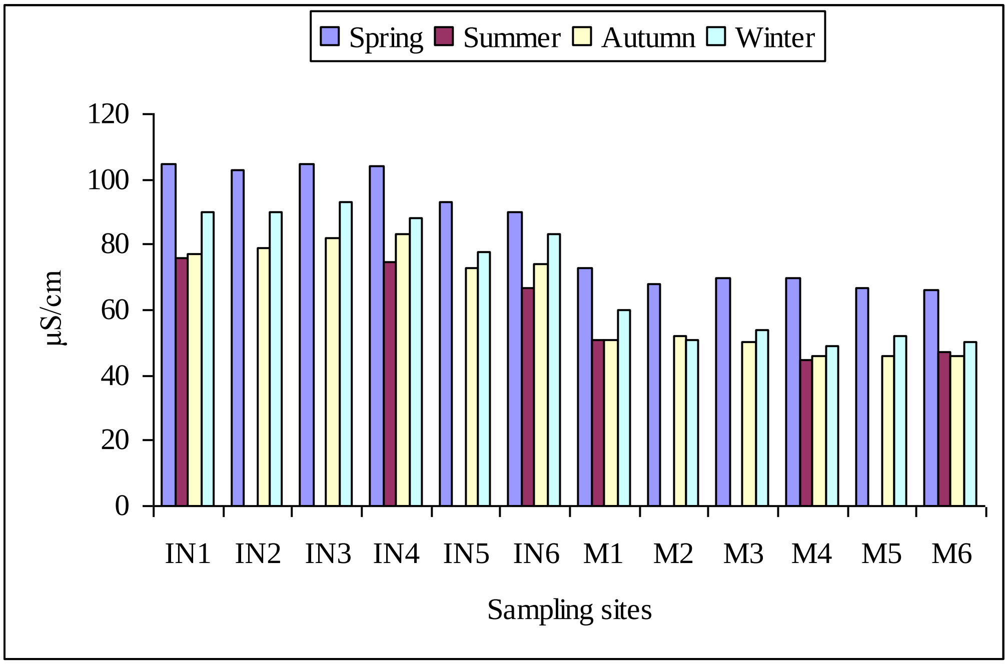

Figure 3.8 Electrical conductivity measurements (µS/cm) recorded seasonally at all sampling sites during the spring sampling period.

Table 3.8 Mean seasonal electrical conductivity (µS/cm) of combined sampling sites occurring above the two selected waterfalls at Injesuthi and Monk’s Cowl vs. sampling sites located below (n = 41).

Sampling seasons	**Injesuthi**		**Monk’s Cowl**	
	Sites above waterfall µS/cm	Sites below waterfall µS/cm	Sites above waterfall µS/cm	Sites below waterfall µS/cm
Spring	104.30	95.70	70..30	67.70
Summer	76.00	71.00	51.00	46.00
Autumn	79.30	76.70	51.00	46.00
Winter	91.00	83.00	55.00	50.30

3.3.3 Dissolved oxygen (DO).

Dissolved oxygen levels (DO) fluctuated between a minimum of 6.40mg/l at sampling site IN4 in summer and a maximum of 12.32mg/l at sampling site M1 in winter (Figure 3.9). Oxygen concentrations were highest in winter when water temperatures were lowest and lowest in summer when water temperatures were highest. DO was only taken at sampling sites IN1, IN4, IN6 and M1 during the summer sampling period due to site access problems.

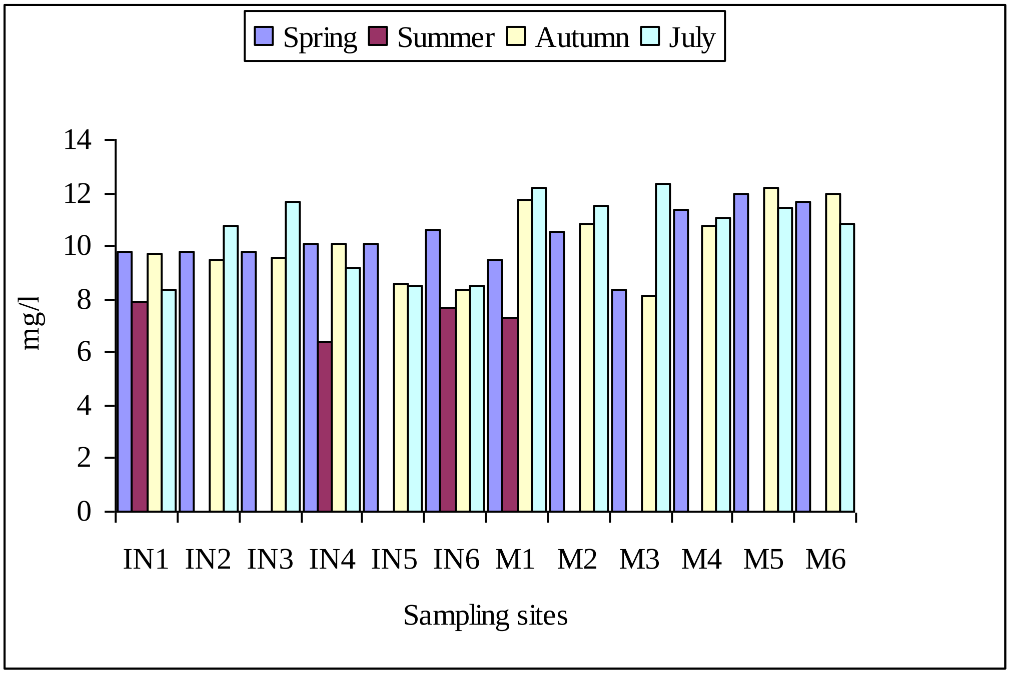

Figure 3.9 Dissolved oxygen (DO) concentrations (mg/l) recorded seasonally at all sampling sites.

3.3.4 River health.

River health bio-monitoring was conducted using the South African Scoring System (SASS) Version 5 (Dickens & Graham, 2002). The Average Score per Taxon (ASPT) determined seasonally at sampling sites IN2, IN5, M2 and M5 is indicated in Figure 3.10. The combined seasonal ASPT mean for sampling site IN2, situated above the selected waterfall at Injesuthi is 7.41, compared to 7.27 at sampling site IN5 located below the waterfall. Similarly, the combined seasonal ASPT mean for sampling site M2, located above the selected waterfall at Monk’s Cowl is 7.33, compared to 7.48 at sampling site

M5 located below the waterfall. All sites showed comparable results and indicated good river health i.e. ASPT ≥ 6.

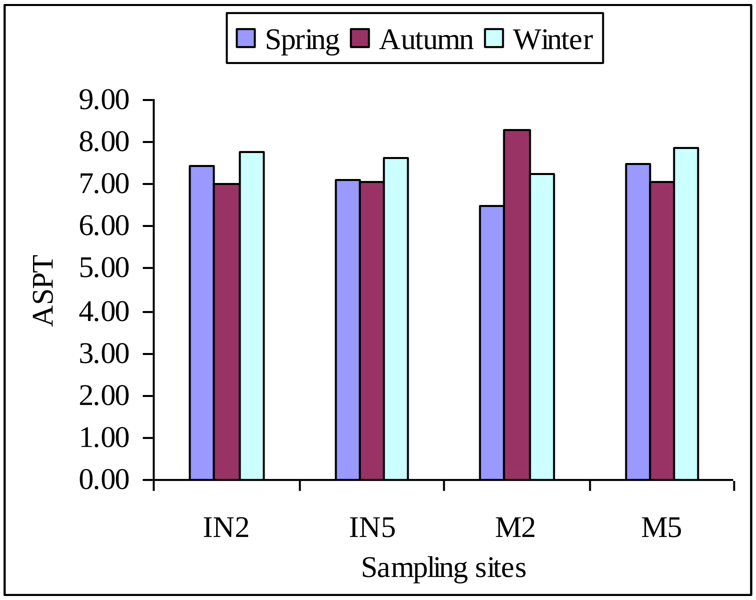

Figure 3.10 Average Score per Taxon (ASPT) recorded seasonally at sampling sites IN2, IN5, M2 and M5 using the SASS version 5 River Health Bio-monitoring System.

3.3.5 Water temperature.

The mean monthly water temperatures measured seasonally at sampling sites IN1, IN4, IN6, M1, M4 and M6 is shown in Figure 3.11. Mean monthly temperatures were highest in January-February (summer) and lowest in June, July and August (winter). The highest degree of temperature variation between sampling sites occurred in summer, with comparatively little variation occurring in winter. No temperature records were collected from sampling sites M6 and M4 during the period February-May 2008 and August-September 2008 respectively due to the loss of temperature loggers in the field.

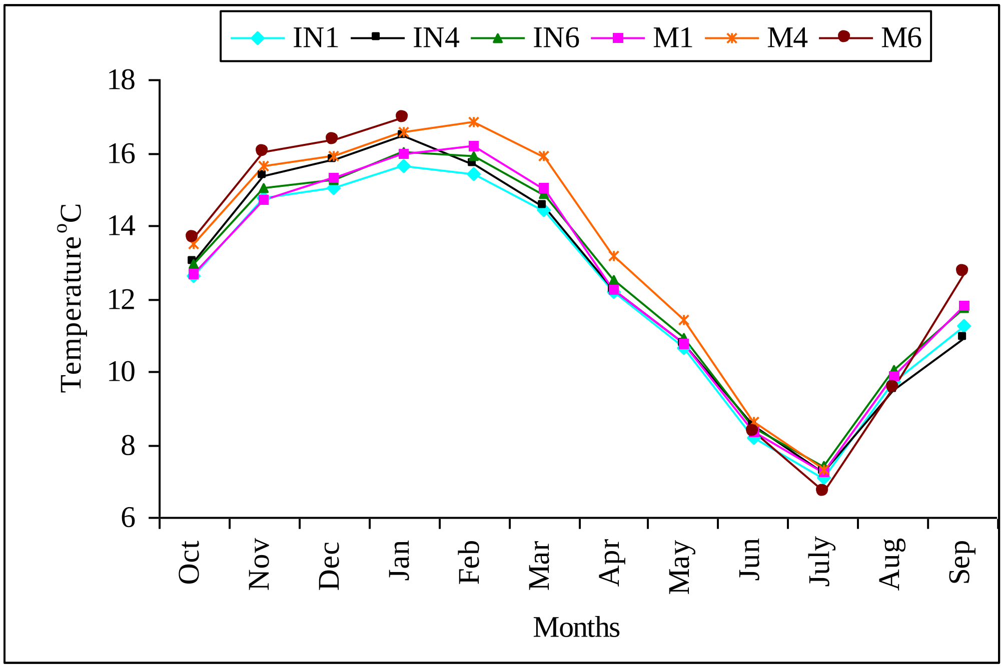

Figure 3.11 Mean monthly water temperatures at sampling sites IN1, IN4, IN6, M1, M4 and M6 for the period 1 October 2007 to 30 September 2008.

The mean monthly water temperatures datasets from sampling sites IN6, IN6, M1 and M6 were tested for normality using the Shapiro-Wilk Test with p-values of 0.300, 0.317, 0.177 and 0.096 respectively, indicating normally distributed datasets. Results from a Student's t-test for two independent variables indicate there are no significant differences in mean monthly water temperature occurring between sampling site IN1, located above the selected waterfall at Injesuthi, versus sampling site IN6 located below ($t = 0.299$; df =22; $p = 0.767$) at $p \leq 0.05$. Similarly, no significant differences existed between the mean monthly water temperature occurring at sampling site M1, located above the selected waterfall at Monk's Cowl, versus sampling site M6, located below ($t = -0.434$; df = 18;
$p = 0.669$) at $p \leq 0.05$.

3.4 Principal Component Analyses (PCA).

3.4.1 Landscape.

Variables incorporated into a PCA for assessing landscape variability between sampling sites IN1, IN6, M1 and M6 is displayed in Table 3.9. Eigenvalues, eigonvector and a correlation matrix between the selected landscape variables is provided in Appendix D. 97.38 % of the variability between sampling sites is accounted for in Axis 1 with 2.47 % placed in Axis 2. The correlation matrix suggests a strong positive association between geology, soil and vegetation types with mean annual precipitation and altitude having the greatest influence on sampling site variability. Similarly, a strong negative association exists between the percentage forest cover, aspect, vegetation and soil type. Figure 3.12 indicates that sampling sites M1 and M6 are more closely associated with each other than either IN1 or IN6, relative to the same selected landscape variables. The influence of selected variables on the variability of the PCA is seen in Figure 3.13. The graph suggests altitude and precipitation have the greatest influence on sampling site variability. All remaining landscape variables are closely correlated with each other.

Table 3.9. Variables utilized in landscape PCA.

Variables	**Sampling sites**			
	IN1	IN6	M1	M6
Precipitation (mm)	896.00	874.00	1428.00	1240.00
Gradient $^{0}/_{00}$	10.00	20.00	11.50	14.50
Aspect ^{0}	34.00	119.00	121.00	131.00
Altitude (m)	1727.00	1615.00	1483.00	1294.00
Geological category	1.00	1.00	2.00	2.00
Soil category	1.00	1.00	2.00	2.00
Vegetation category	1.00	1.00	2.00	2.00
% Forest cover	65.00	0.00	0.00	0.00
% Mixed forest/grass cover	14.00	100.00	100.00	18.00
% Grass cover	21.00	0.00	0.00	82.00

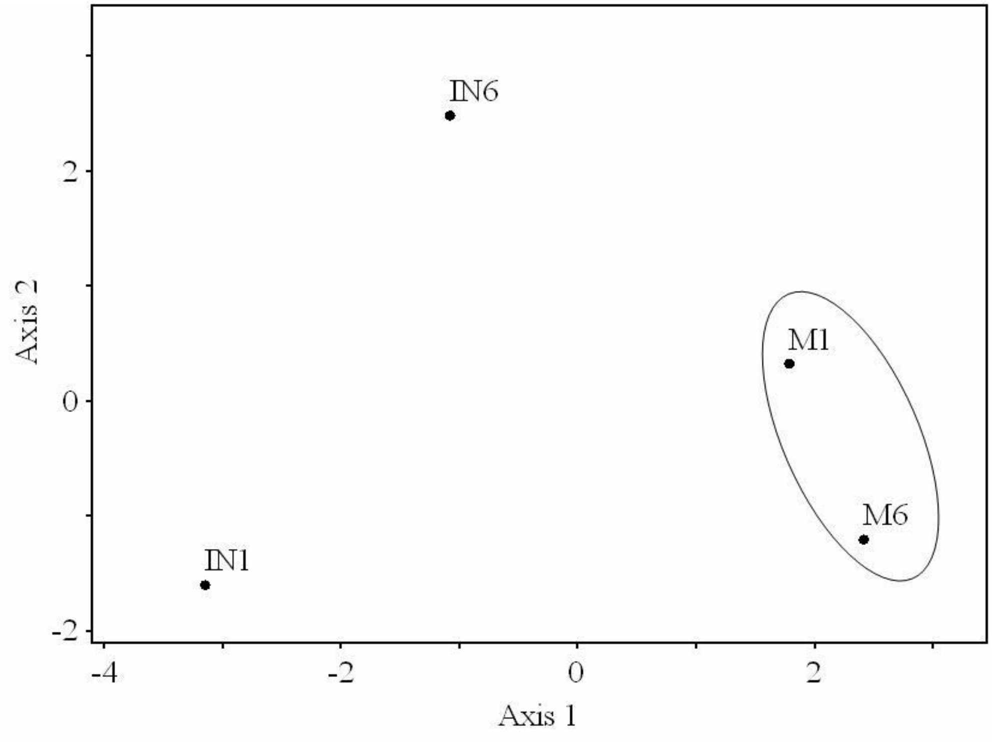

Figure 3.12 Landscape PCA conducted between sampling sites IN1, IN6, M1 and M6 using mean annual precipitation, site gradient, site aspect, altitude, geology; soil vegetation type, % forest cover, mixed, grassland cover; as selected variables.

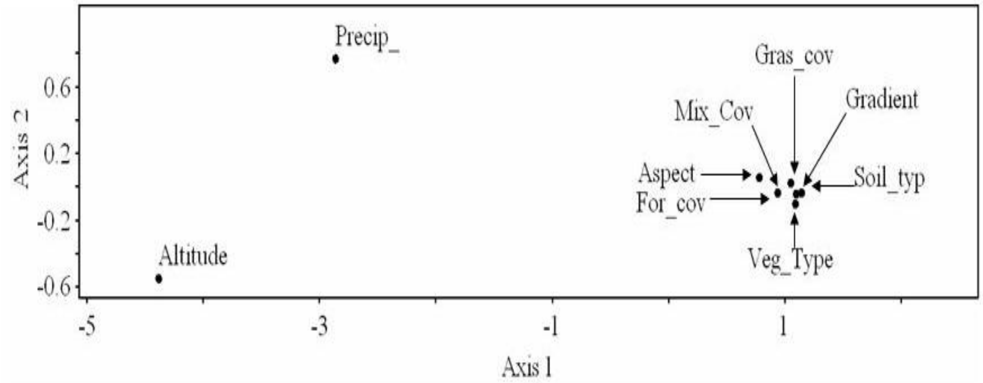

Figure 3.13 Influence of selected landscape attributes on variability between sampling sites IN1, IN6, M1 and M6.

3.4.2 River geomorphology.

Variables incorporated into a PCA for assessing geomorphologic variability between sampling sites is shown in Table 3.10. Eigenvalues, eigonvector and a correlation matrix associated with the PCA is shown in Appendix E. 87.62 % of the variability is represented in Axis 1 of the PCA followed by 7.75%, 3.63% and 0.99 % in Axes 2-4 respectively suggesting a greater degree of variability amongst the selected geomorphologic variables compared to the broader landscape features mentioned in section 3.4.1 The correlation matrix shown in Appendix E reveals a strong positive correlation between exposed substrata and stones, as well as in the occurrence of runs and riffles. A strong positive correlation also exists between boulders and the presence of glides. A strong negative relationship exists between the occurrence of riffles versus runs and pools. Figure 3.14 indicates sampling sites IN1 and M6 are more closely associated with one another relative to the selected geomorphologic variables than IN6 and M1. The influence of specific variables upon on the overall variability of the PCA is seen in Figure 3.15.

Table 3.10. Categories of variables utilized in geomorphologic PCA.

Variables	**Sampling sites**			
	IN1	IN6	M1	M6
% Run	19.00	27.00	26.00	19.00
% Riffle	11.00	4.00	0.00	10.00
% Glide	8.00	17.00	9.00	8.00
% Pool	17.00	3.00	8.00	21.00
% Back eddy	7.00	10.00	8.00	8.00
% Backwater	13.00	6.00	10.00	9.00
% Exposed substrata	24.00	3.00	38.00	25.00
% Bedrock	5.00	1.00	2.00	2.00
% Boulder	18.00	35.00	25.00	27.00
% Stone	39.00	39.00	33.00	40.00
% Stone/Gravel	0.00	4.00	0.00	0.00
% Gravel	19.00	10.00	3.00	24.00

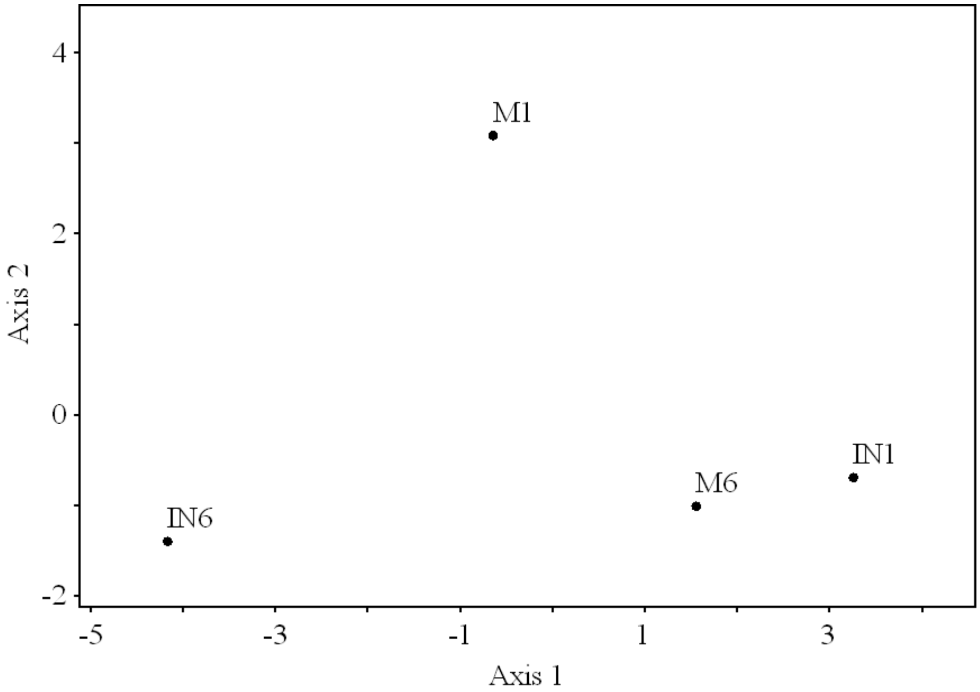

Figure 3.14 Geomorphologic PCA conducted between sampling sites IN1, IN6, M1 and M6 using percentage run, riffle, glide, pool, back-eddy, backwater, exposed substrata river biotope types; bedrock, boulder, stone, stone/gravel and gravel benthic structure types; as selected variables.

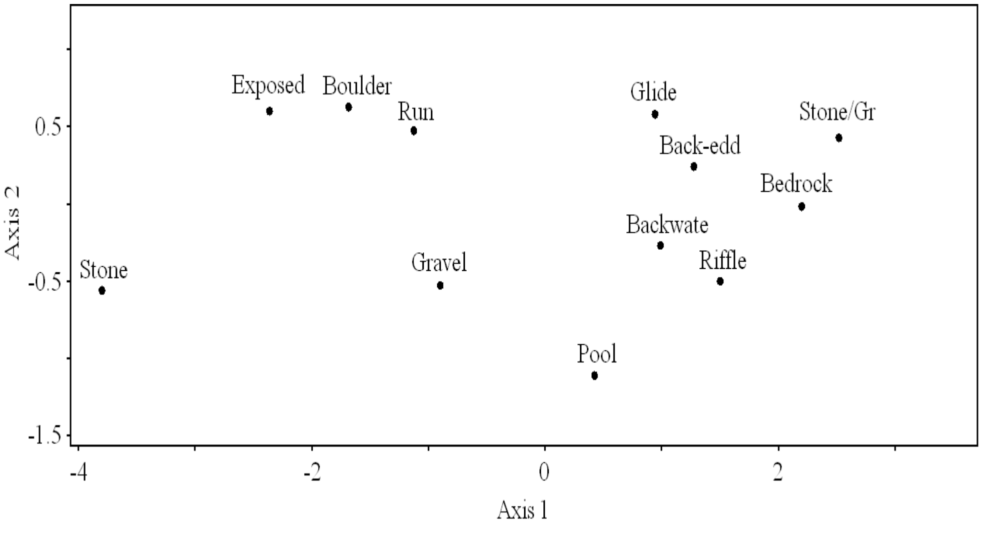

Figure 3.15 Influence of selected geomorphologic attributes on the variability occurring between sampling sites IN1, IN6, M1 and M6.

3.4.3 Hydrology.

Selected variables incorporated into a PCA for assessing the hydrological variability between sampling sites IN1, IN6, M1 and M6 is shown in Table 3.11. Eigenvalues, eigonvectors and a correlation matrix associated with the selected PCA variables is provided in Appendix F. 51.60 % of the variability occurred in Axis 1, followed by 28.89 % and 19.51 % in Axes 2-3 respectively, suggesting a fairly high degree of variability between the selected hydrological variables. The correlation matrix indicates a strong positive association between undercut banks and maximum depth. Similarly, a strong negative association exists between exposed substrata and maximum depth. Figure 3.16 indicates sampling sites IN1 and M1 are more closely associated with each other than either IN6 or M6 for the same set of hydrological variables. The influence of specific variables upon the overall variability of the PCA is shown in Figure 3.17. The graph indicates a high degree of variability between the variables in terms of hydrological functioning. Stone, boulders and exposed substrata had the greatest influence on variability between sampling sites.

Table 3.11 Variables utilized in hydrological related PCA.

Variables	**Sampling sites**			
	IN1	IN6	M1	M6
Mean depth (cm)	18.67	19.94	12.54	21.72
Maximum depth (cm)	75.00	64.00	58.00	78.00
% Exposed substrata	24.10	33.60	37.70	25.00
Mean wetted width (m)	4.80	7.70	4.60	9.20
Active channel width (m)	6.10	9.60	6.10	10.80
Spring flow velocity (m/s)	0.11	0.12	0.10	0.04
Autumn flow velocity (m/s)	0.37	0.31	0.23	0.04
Winter flow velocity (m/s)	0.10	0.09	0.12	0.10
% Undercut bank	14.50	3.20	6.50	24.20
% Vertical bank	4.80	30.60	27.40	11.30
% Sloped bank	53.20	66.10	48.40	41.90
% Boulder bank	27.40	0.00	17.70	22.60

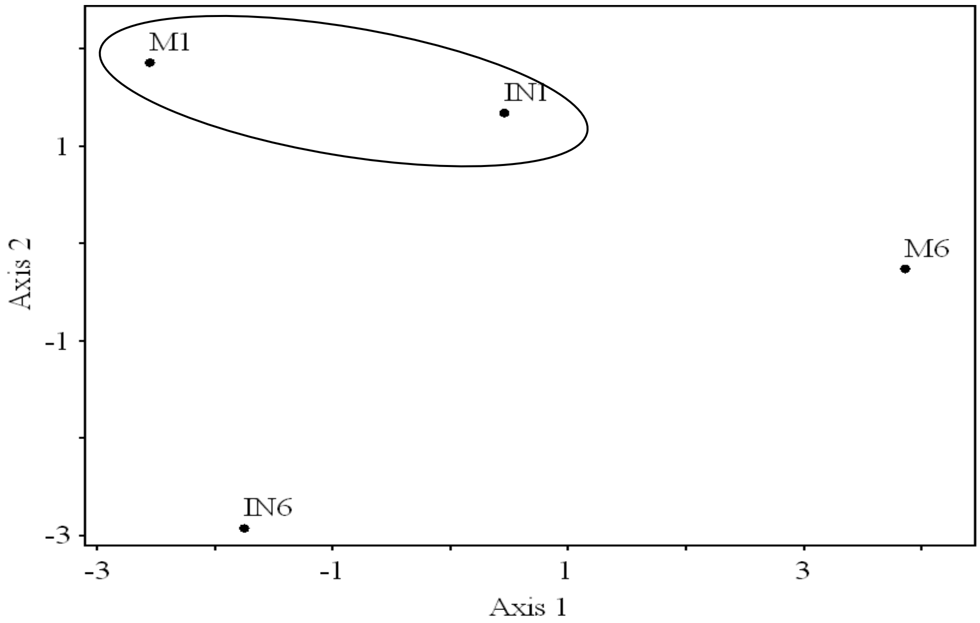

Figure 3.16 Hydrological PCA conducted between sampling sites IN1, IN6, M1 and M6 using mean depth, maximum depth, exposed substrata, mean wetted width, active channel width spring, autumn and winter flow velocity; percentage undercut, vertical, sloped and boulder river bank types; as selected variables.

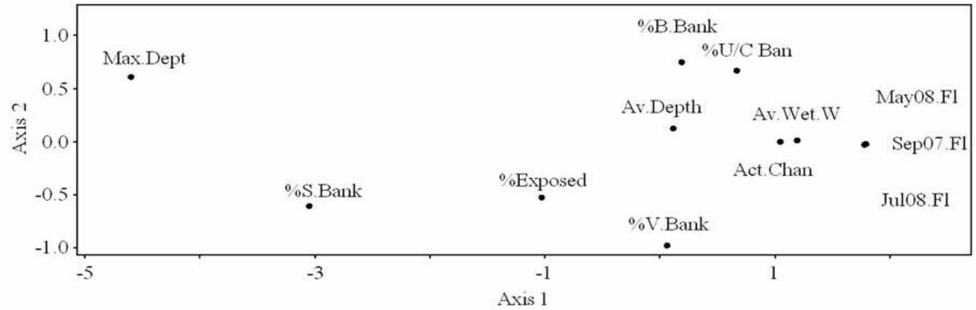

Figure 3.17 Influence of selected hydrological attributes on variability between sampling sites IN1, IN6, M1 and M6.

3.4.4 Water quality.

Selected water quality variables incorporated into a PCA for assessing variability between sampling sites is shown in Table 3.12. Eigenvalues, eigonvectors and a correlation matrix associated with the selected PCA variables is depicted in Appendix G. 62.73 % of the variability occurred in Axis 1 followed by 29.64% and 7.63 % in Axes 2-3 respectively suggesting a moderate degree of variability between the selected water quality variables. The correlation matrix indicates a strong positive association between DO and pH in autumn; and TDS in autumn and winter. A strong negative association occurs between the DO and pH in spring. Figure 3.18 indicates that sampling sites IN1 and IN6 are more closely associated with one another than either M1 or M6 for the same set of selected water quality variables. The influence of selected variables upon on the overall variability of the PCA is shown in Figure 3.19. Seasonal changes in TDS had the greatest influence on the PCA with a high degree of correlation existing between the other variables.

Table 3.12 Variables utilized in water quality and temperature related PCA.

Variables	**Sampling sites**			
	IN1	IN6	M1	M6
pH (Spring)	7.72	7.62	7.70	7.54
pH (Autumn)	6.82	6.65	8.04	7.34
pH (Winter)	7.37	7.54	7.88	7.12
TDS (Summer) mg/l	52.00	45.00	36.00	33.00
TDS (Autumn) mg/l	38.00	37.00	25.00	23.00
TDS (Winter) mg/l	45.00	41.00	30.00	25.00
DO (Spring) mg/l	9.80	10.59	9.51	11.64
DO (Autumn) mg/l	9.69	8.32	11.74	12.00
DO (Winter) mg/l	8.35	8.50	12.23	10.82
Mean monthly summer water temp.^{0}C	15.63	16.03	16.00	16.95
Mean monthly winter water temp.^{0}C	7.09	7.45	7.28	6.71
Mean monthly spring water temp.^{0}C	11.28	11.77	11.81	12.74

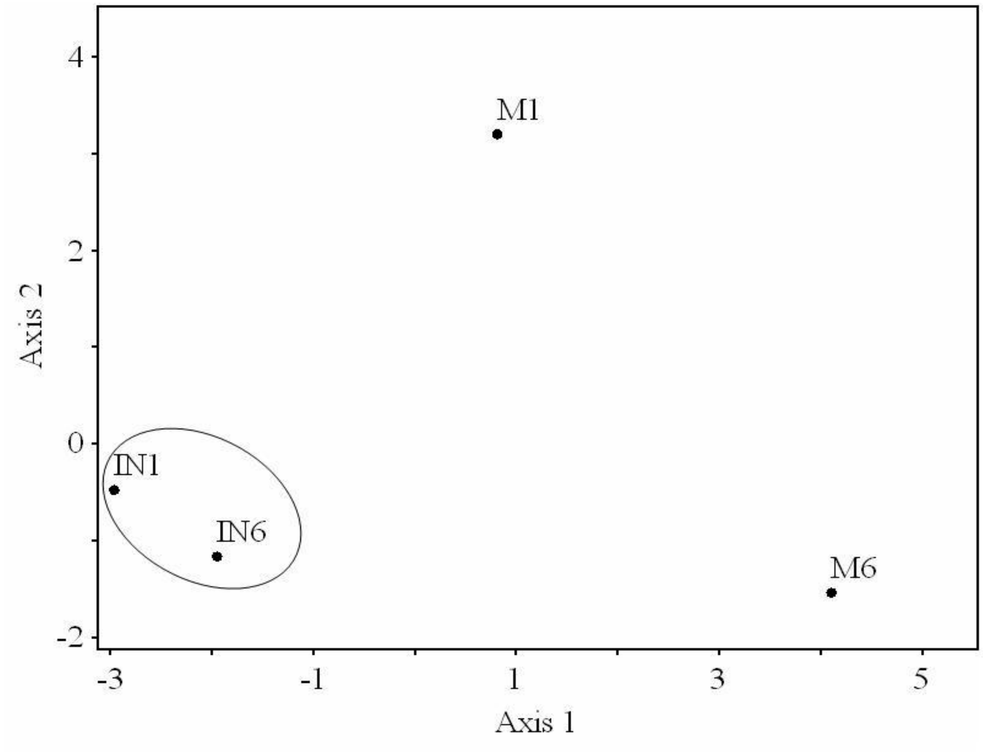

Figure 3.18 Water quality PCA conducted between sampling sites IN1, IN6, M1 and M6 using pH in spring, autumn and winter; TDS in spring, autumn and winter; DO in spring, autumn and winter; mean monthly summer, winter and spring water temperatures; as selected variables.

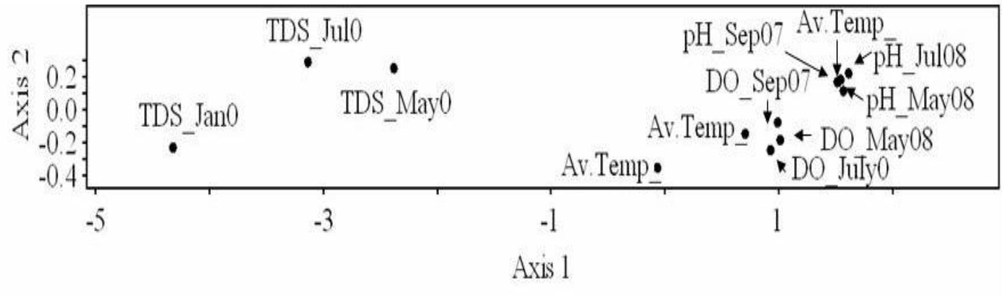

Figure 3.19 Influence of selected water quality variables on variability between sampling sites IN1, IN6, M1 and M6.

3.5 Seasonal electrofishing results.

Rainbow Trout *Oncorhynchus mykiss*, Natal Mountain Catfish *Amphilius natalensis* and Natal Cascade Frog *Hadromophryne natalensis* tadpoles were electrofished at Injesuthi. *S. trutta*, *A. natalensis* fish species and *H. natalensis* tadpoles were electrofished at Monks' Cowl.

3.5.1 Spring.

The CPUE (number specimens electrofished per minute) of *H. natalensis* tadpoles, trout and *A. natalensis* electrofished in spring is shown in Figure. 3.20. *H. natalensis* tadpoles (n = 151) accounted for the bulk of the catch, followed by trout (n = 42) and *A. natalensis* (n = 8). Only one specimen of *H. natalensis* tadpoles was electrofished at each sampling site below the selected waterfalls at Injesuthi (IN4) and Monk's Cowl (M4) respectively. *A. natalensis* was only encountered at Monks Cowl at sampling sites M4 (n = 1), M5 (n = 5) and M6 (n = 2). The abundance of *H. natalensis* tadpoles occurring above the selected waterfall at Monk's Cowl is notably higher than Injesuthi. Similarly the abundance of *S. trutta* at Monk's Cowl is greater than *O. mykiss* at Injesuthi.

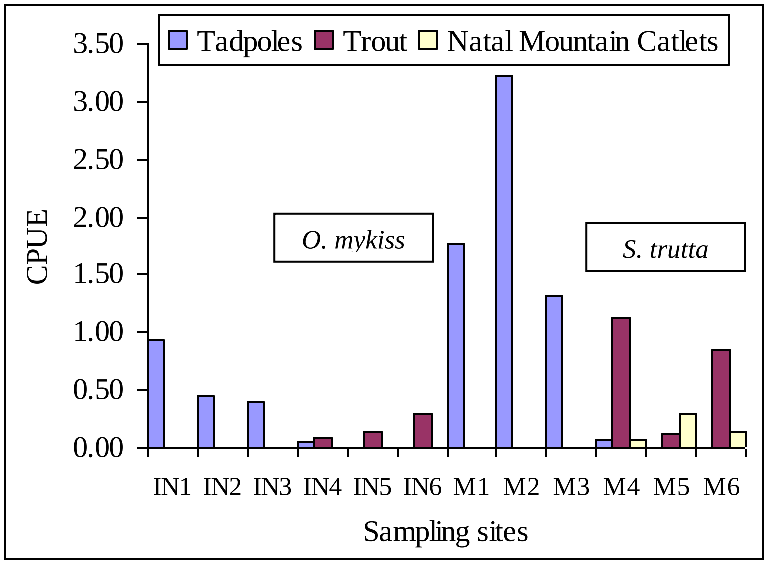

Figure 3.20 CPUE of *H .natalensis* tadpoles, *O. mykiss*, *S. trutta* and *A. natalensis* electrofished at all sampling sites during the spring seasonal sampling period.

3.5.2 Summer.

Electrofishing was only undertaken at sampling sites IN1, M1, M4 and M6 in summer due to site access problems (Figure 3.21). The greatest concentration of *H. natalensis* tadpoles occurred at sampling site IN1, followed by M1, M6 and M4.

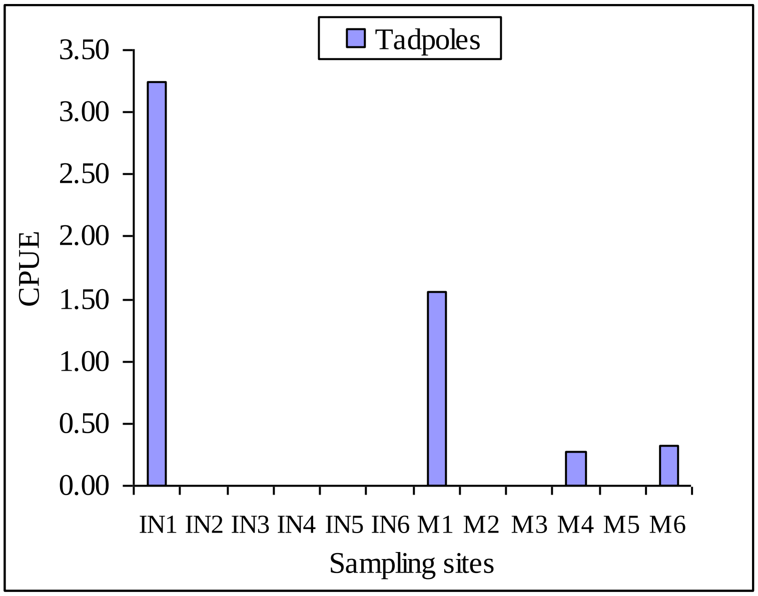

Figure 3.21 CPUE of *H. natalensis* tadpoles' electrofished at sampling sites IN1, M1 and M6 per during the summer sampling period.

3.5.3 Autumn.

The CPUE of *H. natalensis* tadpoles (n = 419) remained high at all sites situated above the two selected waterfalls but was much reduced at sampling sites located below (Figure 3.22). Moderate numbers of *H. natalensis* tadpoles occurred below the waterfall at sampling site IN4. With the exception of sampling site IN6 trout were electrofished at all sampling sites located below the selected waterfalls. *A. natalensis* (n = 15) only occurred below the selected waterfall at Monks Cowl.

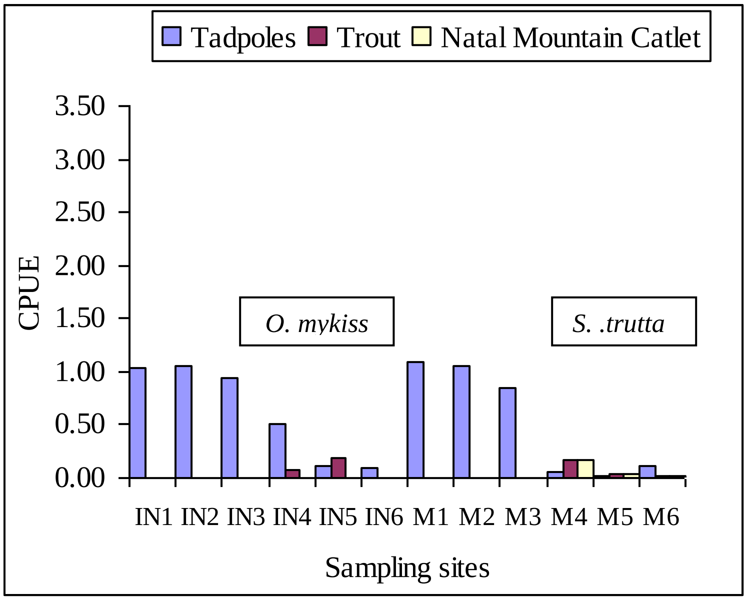

Figure 3.22 CPUE of *H. natalensis* tadpoles, *O. mykiss*, *S. trutta* and *A. natalensis* electrofished at all sites during the autumn sampling period.

3.5.4 Winter.

The greatest abundance of *H. natalensis* tadpoles consistently occurred at the six sampling sites located above the selected waterfalls (Figure 3.23). Moderate numbers of *H. natalensis* tadpoles were still however encountered at sampling sites IN4 and M4 located immediately below the selected waterfalls at Injesuthi and Monk’s Cowl respectively. *A. natalensis* was present in moderate numbers at sampling sites IN6, M5 and M6.

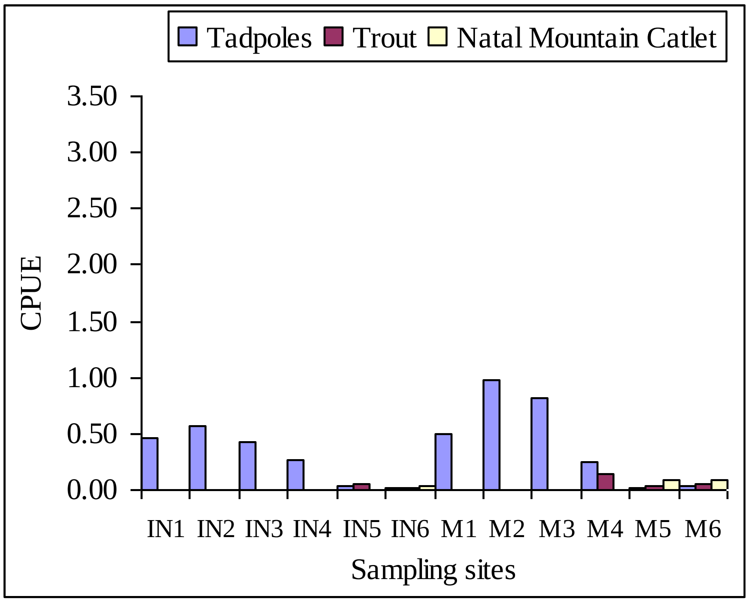

Figure 3.23 CPUE of *H. natalensis* tadpoles, *O. mykiss*, *S .trutta* and *A. natalensis* electrofished at all sampling sites during the winter sampling period.

3.6 Catch per Unit of Effort (CPUE) of *H. natalensis* tadpoles and trout.

3.6.1 Mean CPUE of *H. natalensis* tadpoles' electrofished at sampling sites located above the selected waterfalls at Injesuthi and Monk's Cowl, versus sampling sites located below.

The combined mean CPUE of *H. natalensis* tadpoles electrofished seasonally above the selected waterfall at Injesuthi is 0.75 tadpoles per minute vs. 0.16 tadpoles below the waterfall (Table 3.13). The mean abundance of *H. natalensis* tadpoles occurring at sampling sites located above the selected waterfall at Injesuthi is consequently 4.69 times greater than the sampling sites located below. Similarly, the combined mean CPUE of *H. natalensis* tadpoles electrofished above the selected waterfall at Monk's Cowl is 1.10 tadpoles per minute vs. 0.07 below (Table 3.14). The abundance of *H. natalensis* tadpoles occurring at sampling sites located above the selected waterfall at Monk's Cowl is consequently 15.71 times greater than the sampling sites below.

Table 3.13 Mean CPUE of *H. natalensis* tadpoles' electrofished seasonally at sampling sites located above the selected waterfall at Injesuthi versus mean CPUE at sampling sites located below waterfall (n = 378).

Injesuthi							
Season	Sites above waterfall			Sites below waterfall			Change in CPUE
	Number of tadpoles	Time in minutes	CPUE	Number of tadpoles	Time in minutes	CPUE	
Spring	41.00	75.57	**0.54**	1.00	62.30	**0.02**	**- 27.00 x**
Autumn	216.00	213.24	**1.01**	37.00	124.27	**0.30**	**-3.34** x
Winter	70.00	145.13	**0.48**	13.00	142.25	**0.09**	**-5.33 x**
Total	327.00	433.94	**0.75**	51.00	328.82	**0.16**	**-4.69 x**

Table 3.14 Mean CPUE of *H. natalensis* tadpoles' electrofished seasonally at sampling sites located above the selected waterfall at Monk's Cowl versus mean CPUE at sampling sites located below waterfall (n = 400).

Monks Cowl							
Season	Sites above waterfall			Sites below waterfall			Change In CPUE
	Number of tadpoles	Time in minutes	CPUE	Number of tadpoles	Time In minutes	CPUE	
Spring	108.00	51.45	**2.10**	1.00	46.47	**0.02**	**-105 x**
Autumn	158.00	158.56	**1.00**	12.00	186.72	**0.06**	**-16.67 x**
Winter	106.00	127.36	**0.83**	15.00	168.45	**0.09**	**-9.22 x**
Total	372.00	337.37	**1.10**	28.00	401.64	**0.07**	**-15.71 x**

3.6.2 Student’s t-test conducted on the mean CPUE of *H. natalensis* tadpoles.

The mean CPUE of tadpoles’ electrofished seasonally at sampling sites located above the selected waterfall at Injesuthi versus sampling sites located below waterfall is shown in Table 3.15. Results from the Student’s t-test for two independent variables found that *H. natalensis* tadpole numbers are significantly less below the selected waterfall at Injesuthi than above during the spring (t = 3.455, df = 4, p = 0.026) and autumn (t = 5.509, df = 4, p = 0.005) sampling periods. Although the winter sampling period also revealed more tadpoles above the waterfall, the difference was not significant (t = 4.092, df = 4, p = 0.049).

Table 3.15 Mean seasonal CPUE of tadpoles located at sampling sites above the selected waterfall at Injesuthi versus sampling sites below the waterfall (n = 378).

Injesuthi											
Spring				**Autumn**				**Winter**			
Sites above	CPUE	Sites below	CPUE	Sites above	CPUE	Sites below	CPUE	Sites above	CPUE	Sites below	CPUE
IN1	0.94	IN4	0.05	IN1	1.04	IN4	0.51	IN1	0.47	IN4	0.27
IN2	0.46	IN5	0.00	IN2	1.05	IN5	0.12	IN2	0.57	IN5	0.03
IN3	0.41	IN6	0.00	IN3	0.94	IN6	0.09	IN3	0.42	IN6	0.02
Mean	**0.60**		**0.02**		**1.01**		**0.38**		**0.49**		**0.11**
Median	0.46		0.00		1.04		0.12		0.47		0.03
SD	0.29		0.03		0.06		0.23		0.08		0.14
Variance	0.09		0.00		0.00		0.05		0.01		0.02

The mean CPUE of tadpoles’ electrofished seasonally at sampling sites located above the selected waterfall at Monk’s Cowl versus sampling sites located below is shown in Table 3.16. Results from the Student’s t-Test for two independent variables found that *H. natalensis* tadpole numbers are significantly less below the selected waterfall at Monk’s Cowl than above during the spring (t= 0.455; df = 4; p = 0.026) and autumn (t = 5.509; df = 4; p = 0.005) sampling periods. Although the winter sampling period also revealed more tadpoles above the waterfall, the difference was not significant (t =4.092, df = 4, p = 0.049).

Table 3.16 Mean seasonal CPUE of tadpoles located at sampling sites above the selected waterfall at Monk's Cowl vs. sampling sites below (n = 400).

Monk's Cowl											
Spring				**Autumn**				**Winter**			
Sites above	CPUE	Sites below	CPUE	Sites above	CPUE	Sites below	CPUE	Sites above	CPUE	Sites below	CPUE
M1	1.76	M4	0.07	M1	1.09	M4	0.05	M1	0.49	M4	0.24
M2	3.22	M5	0.00	M2	1.05	M5	0.02	M2	0.98	M5	0.02
M3	1.32	M6	0.00	M3	0.84	M6	0.11	M3	0.82	M6	0.03
Mean	**2.10**		**0.02**		**0.99**		**0.06**		**0.76**		**0.10**
Median	1.76		0.00		1.05		0.05		0.82		0.03
SD	0.99		0.04		0.13		0.05		0.25		0.12
Variance	0.99	0	0.00		0.02		0.00		0.06		0.02

3.6.3 CPUE of *H. natalensis* tadpoles versus trout below selected waterfalls.

Results of a straight line regression between the CPUE of *O. mykiss* (n = 26) versus the corresponding CPUE of *H. natalensis* tadpoles' (n = 51) electrofished seasonally at all sampling sites located below the selected waterfall at Injesuthi is shown in Figure 3.24. The graph suggests a weak ($R^2 = {}^{-}0.0712$) negative species association between *O. mykiss* and *H. natalensis* tadpoles. A weak ($R^2 = -0.0145$) negative species association similarly exists between *S. trutta* (n= 57) and *H. natalensis* tadpoles (n = 28) at all sampling sites located below the selected waterfall at Monk's Cowl (Figure 3.25).

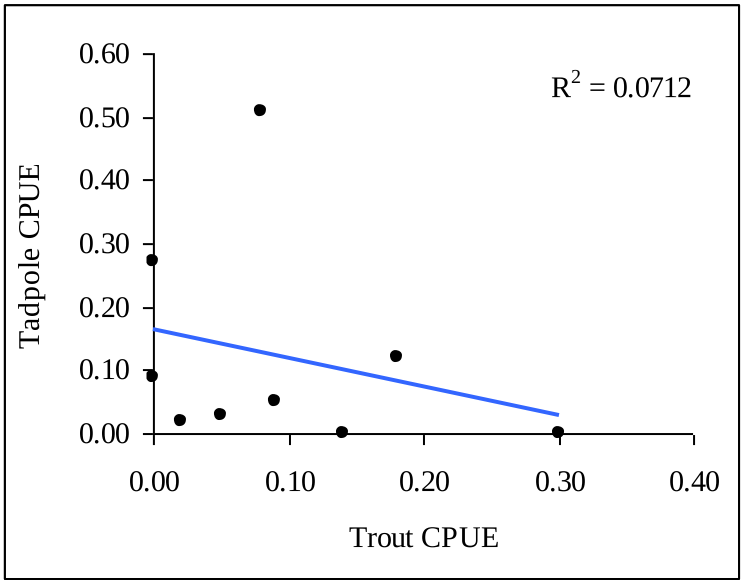

Figure 3.24 CPUE of *O. mykiss* (n = 26) vs. CPUE of *H. natalensis* tadpoles' (n = 51) electrofished seasonally at sampling located below the selected waterfall at Injesuthi

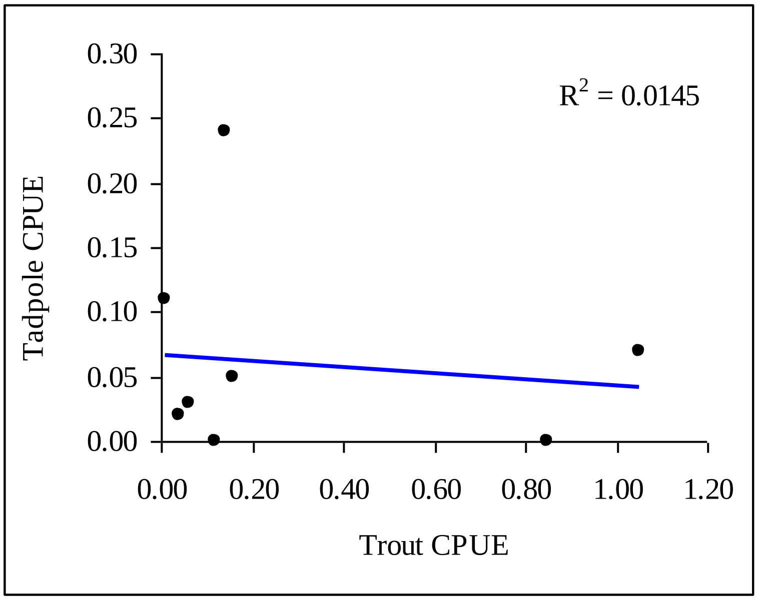

Figure 3.25 CPUE of *S. trutta* (n = 57) vs. CPUE of *H. natalensis* tadpoles' (n = 28) electrofished seasonally at sampling located below the selected waterfall at Monk's Cowl.

3.7 Length frequencies of seasonal *H. natalensis* tadpole cohorts.

Identification of *H. natalensis* cohorts in relation to length occurring seasonally at sampling sites located above and below the selected waterfalls at Injesuthi and Monk's Cowl found the following:

3.7.1 Spring vs. summer (Figure 3.26A).

The population of *H. natalensis* tadpoles electrofished in spring (n = 88), compared with the population electrofished in summer (n = 103), indicates a broadly bi-modal tadpole length distribution. Summer results suggest the emergence of a new tadpole cohort that was not present in spring indicating *H. natalensis* adults had bred. Many of the larger tadpoles sampled in spring (70 -100 mm) had since developed into adults by the summer and a new cohort ranging between 25-50mm had since hatched. The assumption is the 40-60mm tadpole cohort sampled in spring had since matured into the 60 – 80 mm yearlings in summer.

3.7.2 Summer vs. autumn (Figure 3.26B).

The length distribution of *H. natalensis* tadpoles electrofished in summer (n = 103) compared to those sampled during autumn (n = 420), also give a bi-modal distribution of *H. natalensis* tadpoles. The dual cohorts present in autumn are assumed to be the more advanced metamorphic stage of tadpoles that had been previously sampled in summer. A small contingent of large tadpoles (±100 mm) was still present in autumn.

3.7.3 Autumn vs. winter (Figure 4.26C).

The length distribution of *H. natalensis* tadpoles present in autumn (n = 420), compared with those sampled in winter (n = 204), showed that the bimodal distribution of *H. natalensis* tadpoles that existed in autumn had shifted to the right of the graph in winter, indicating tadpole growth. The 30 – 50 mm tadpole cohort sampled in autumn is assumed to be the 50 – 70 mm cohort sampled in winter.

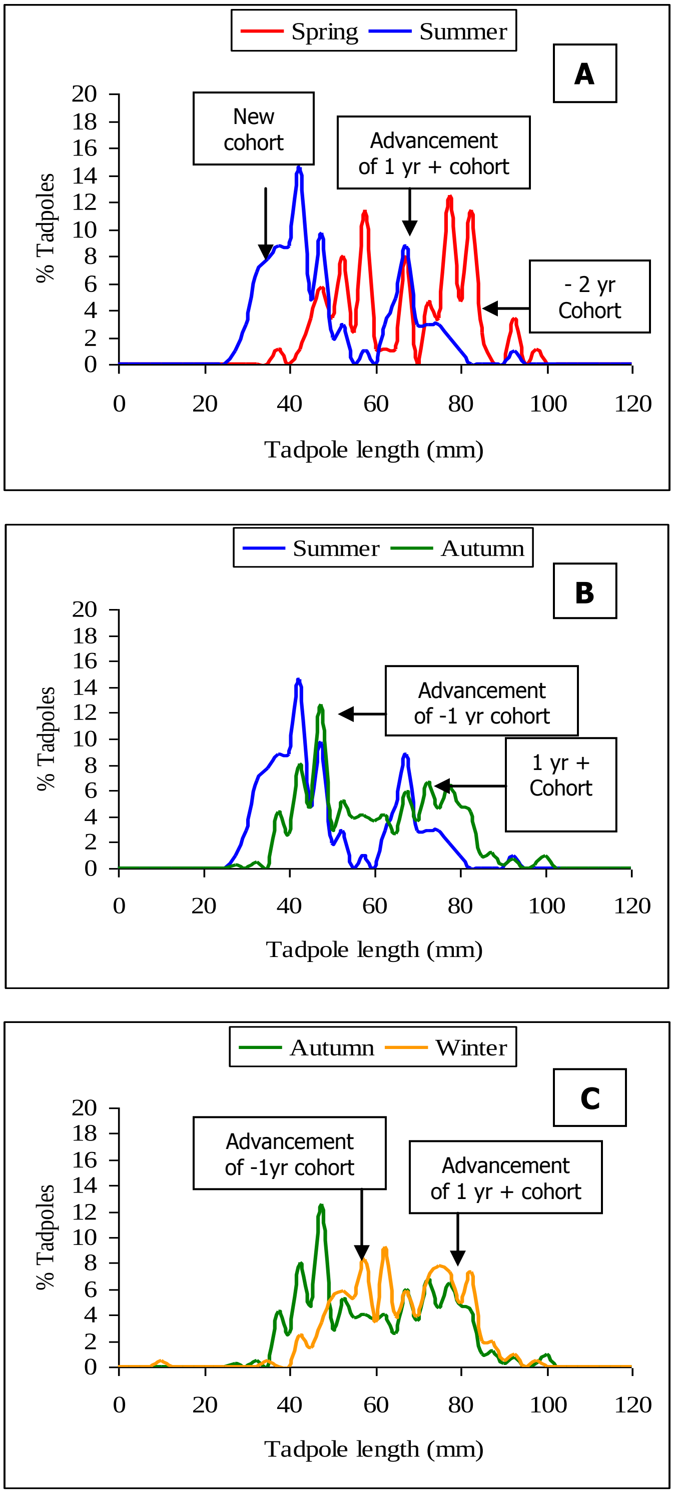

Figure 3.26 Combined length frequencies of *H. natalensis* tadpoles' electrofished seasonally at all sampling sites (n = 743).

3.7.4 Length distribution of *H. natalensis* tadpoles electrofished at sampling sites located above the selected waterfalls at Injesuthi and Monk's Cowl versus sampling sites located below the waterfalls.

The length distribution of *H. natalensis* tadpoles' (n = 743) electrofished seasonally at sampling sites located above the selected waterfalls versus sampling sites located below the waterfalls is shown in Figure 3.27. In spring, only single specimens of *H. natalensis* tadpoles were sampled above and below the selected waterfalls at Injesuthi and Monk's Cowl respectively. The minimum length of tadpoles found below the waterfalls is consistently larger than above, with tadpoles > 100 mm only occurring at sampling sites located below waterfalls. The smallest (30 mm) and largest tadpole (110 mm) were both sampled above and below the selected waterfall in autumn.

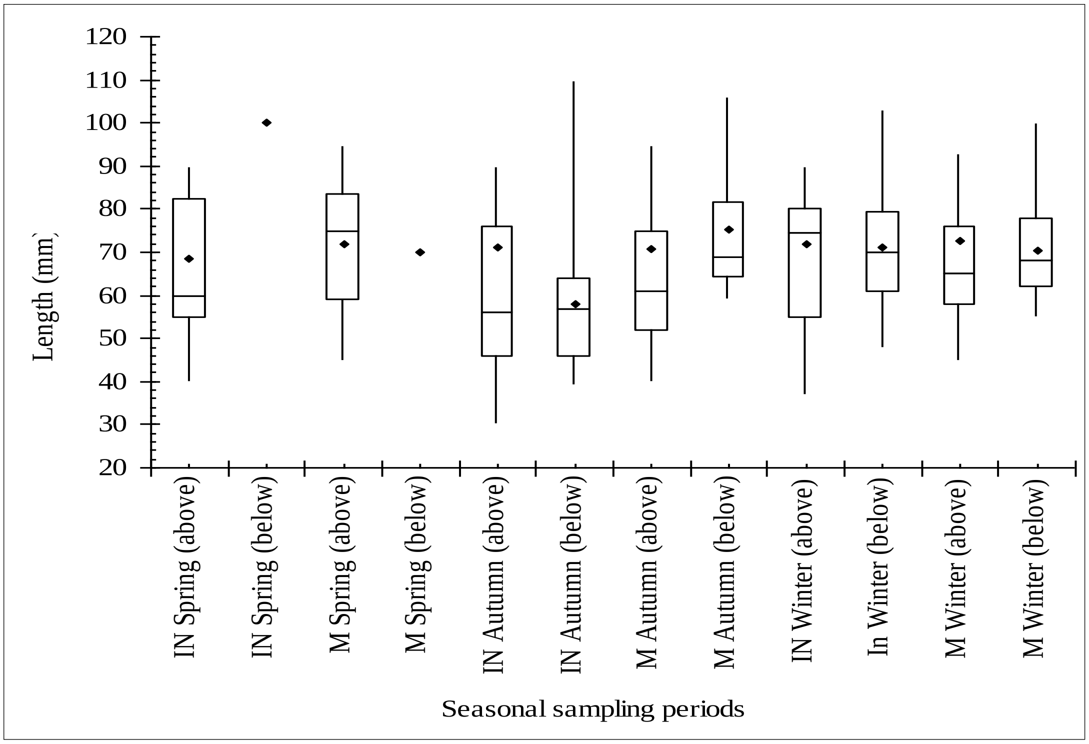

Figure: 3.27 Length intervals of *H. natalensis* tadpoles electrofished seasonally above and below the selected waterfalls at Injesuthi and Monk's Cowl (n = 743).

3.7.5 Length / mass relationships of *H. natalensis* tadpoles' electrofished seasonally above and below the selected waterfalls at Injesuthi and Monk's Cowl.

Length mass regression curves relative to the combined number of tadpoles (n = 743) electrofished during the four seasonal sampling periods (Figure 3.28) suggest that the tadpoles had a higher mass to length ratio at the Monk's Cowl sampling sites versus those at Injesuthi. Results also indicate that the mass to length ratio of tadpoles is lower below the selected waterfalls .The graph also confirms very large tadpoles of ≥100mm only occur below the selected waterfalls.

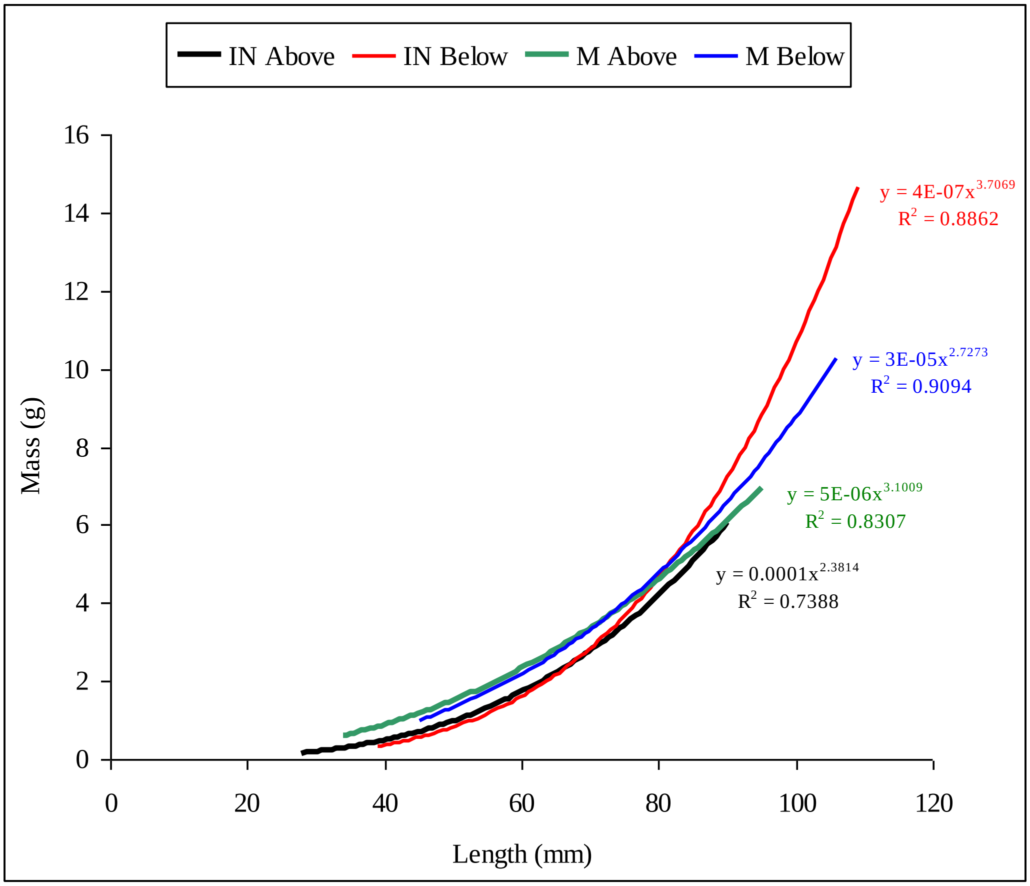

Figure: 3.28 Length / mass relationship of *H. natalensis* tadpoles electrofished seasonally above and below the selected waterfalls at Injesuthi and Monk's Cowl (n = 743).

3.8 Group sizes of *H. natalensis* tadpoles electrofished above and below the selected waterfalls at Injesuthi and Monks Cowl.

The occurrence of tadpoles' forming groups (0 – 8 individuals) is substantially lower at sampling sites occurring below the selected waterfalls, versus those located above (Figure 3.29). 74 % of tadpoles occur as single individuals below the selected waterfall at Injesuthi, versus 51 % above the waterfall.. Similarly, 71% of tadpoles occur as single individuals below the selected waterfall at Monk's Cowl, versus 47% above the waterfall.

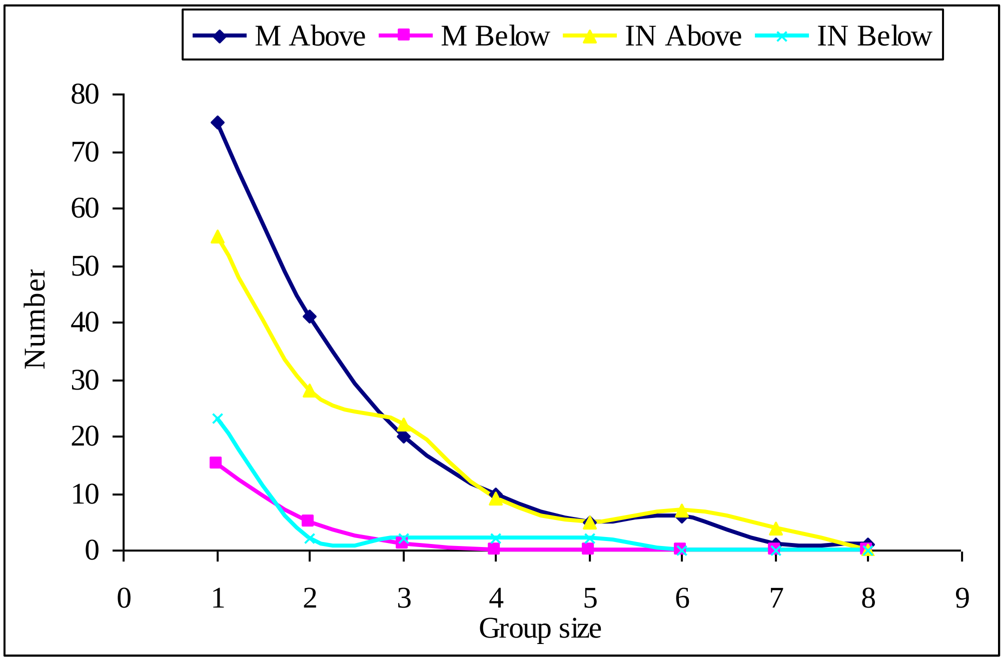

Figure 3.29 Formation of groups of *H. natalensis* tadpoles at sampling sites located above the selected waterfalls at Injesuthi and Monk's Cowl, versus sampling sites located below the waterfalls, derived from combined seasonal electrofishing results (n = 743).

3.9 Habitat preferences of *H. natalensis* tadpoles and trout.

3.9.1 River biotope and benthic structure type preferences of *H. natalensis* tadpoles.

The seasonal preference of *H. natalensis* tadpoles for specific river biotope types is shown in Figure 3.30. Tadpoles in spring alternatively utilize runs (60 %), glides (19 %) and pools (14 %) in the absence of favoured riffle habitat. Tadpoles display a distinct preference for riffle habitat in autumn (76%) and winter (86%) following a summer rainfall season and increased flow velocities. The abundance of *H. natalensis* tadpole abundance displays a significantly positive correlation (r = 0.94) to river biotope types ranked in descending order of current velocity (Table 3.17). The seasonal preference of *H. natalensis* tadpoles to specific benthic structure types is shown in Figure 3.31. Tadpoles display a strong seasonal association to benthic structure characterized by gravel/stone, stone and boulders in descending order of importance. Tadpoles generally avoided benthic structure dominated by gravel, sand/gravel, sand, sand/silt and silt.

H. natalensis tadpole abundance indicates a strong positive correlation (r = 0.84) to benthic structure ranked in descending order of particle size (Table 3.18).

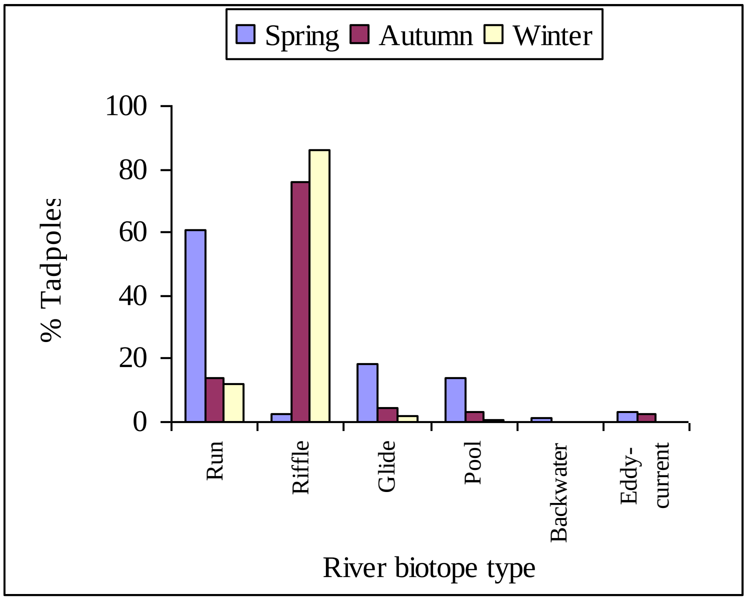

Figure: 3.30 Percentage of *H. natalensis* tadpoles inhabiting categorized river biotope types derived from seasonal electrofishing results (n = 743).

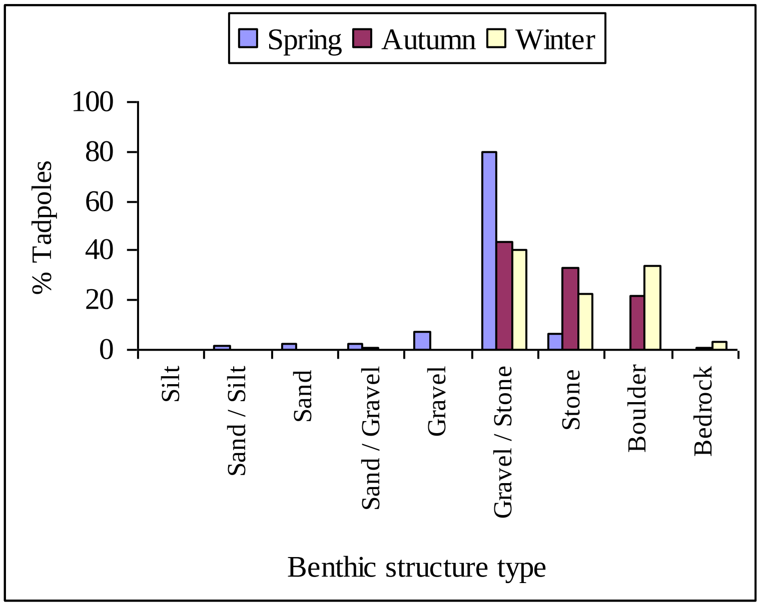

Figure: 3.31 Approximated percentage of *H. natalensis* tadpoles inhabiting categorized benthic structure types derived from seasonal electrofishing results (n = 743).

Table 3.17 Abundance of *H. natalensis* tadpole linked to categorized river biotope types ranked in descending order of associated flow velocity using Spearman's Rank Correlation Test (n = 743).

Category	**River biotope ranked in descending order of associated flow velocity**	**Number of tadpoles**	**Trout ranked in descending order of relative abundance**
Riffle	1	488	1
Run	2	159	2
Glide	3	44	3
Back-eddy	4	16	5
Pool	5	34	4
Backwater	6	2	6
n		743	
r = 0.94			

Table 3.18 Abundance of *H. natalensis* tadpoles linked to categorized benthic structure types ranked in descending order of particle size using Spearman's Rank Correlation Test (n = 743).

Category	**Benthic structure type ranked in descending order of associated flow velocity**	**Number of tadpoles**	**Tadpoles ranked in descending order of relative abundance**
Bedrock	1	11	4
Boulder	2	156	3
Stone	3	188	2
Gravel/Stone	4	367	1
Gravel	5	10	5
Sand/Gravel	6	5	6
Sand	7	4	7
Silt/Sand	8	2	8
Silt	9	0	9
n		743	
r = 0.83			

3.9.2 Depth preferences of *H. natalensis* tadpoles.

The percentage of *H. natalensis* tadpoles utilising specific depth classes, relative to seasonal electrofishing data, is shown in Figure 3.32. The greatest percentage of tadpoles in spring is confined to a mean depth 15.81 ± 9.31 cm (n =134), when water levels are lowest. Following summer rains and increased flow velocities and water levels, *H. natalensis* tadpoles utilised a greater range of depth classes in autumn with a mean depth of 23.57 ± 7.62 cm (n = 404). The distribution of tadpoles in winter subsequently regressed into a narrower mean depth range of 17.43 ± 5.68 cm sd, (n = 205) following reduced flow velocities and water level. Results suggest that *H. natalensis* tadpoles occupy a greater range of depth when flow velocities and associated water levels are seasonally higher.

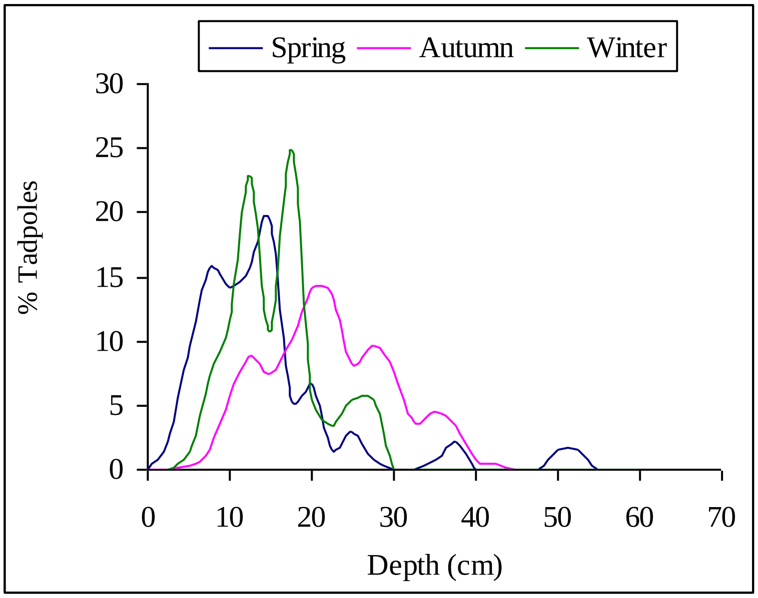

Figure: 3.32 Approximated percentage *H. natalensis* tadpoles seasonally frequenting various depth ranges derived from seasonal electrofishing results (n = 743).

3.9.3 Flow velocity preferences of *H. natalensis* tadpoles.

The seasonal preference of *H. natalensis* for specific flow rates derived from seasonal electrofishing data is displayed in Figure 3.33. *H. natalensis* tadpoles utilize a narrower range of flow velocities (mean 0.71 ± 0.41 m/s n = 134) in spring when water levels are lowest. In contrast *H. natalensis* tadpoles utilize a wider range of flow velocities (mean 3.19 ± 1.74 m/s sd, n = 404) in autumn when flow velocities and water levels are higher. The tadpoles subsequently reverted to utilising a narrower range of water velocity (mean 2.05 ± 0.88 m/s, n = 205) in winter when the flow velocity reduced and the water level subsided. Results indicate that *H. natalensis* tadpoles actively seek high velocity flow when it is seasonally available.

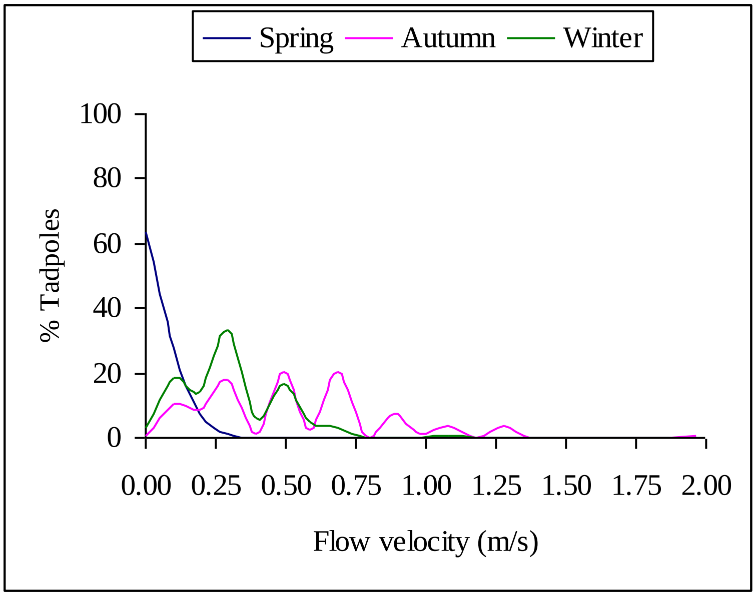

Figure: 3.33 Percentage *H. natalensis* tadpoles seasonally inhabiting specific flow velocity classes relative to seasonal electrofishing results (n = 743).

3.9.4 River biotope type and benthic structure type preferences of trout.

The river biotope preference of trout, relative to seasonal electrofishing results, is shown in Figure 3.34. Trout mostly inhabit pools (45 %), runs (41 %) and glides (14 %) in spring. Trout indicate a preference for pools (52 %), riffles (3 2%) and runs in autumn followed by riffles (31 %), glides (31 %) and pools (25 %) in winter. Pools are the most sought after river biotype type (43%), followed by runs (25%) and riffles (16%). Trout, like *H. natalensis* tadpoles seldom frequent back-eddies and backwaters. The association of trout to specific river biotope types, ranked in descending order of associated flow velocity, is shown in Table 3.19. Trout display a weak positive relationship (r = 0.26) to faster flowing river biotope types. The percentage of trout utilising specific benthic structure types, relative to seasonal electrofishing data, is shown in Figure 3.35. Trout display a high degree of seasonal variation in respect of categorised benthic structure types. Trout indicate a preference for gravel/stone (36 %), stone (28 %) and boulders (16 %) in spring, gravel/stone (36 %), stone (28 %) and boulder (16 %) in autumn, boulder (56 %), gravel/stone (25 %) and stone (19 %) in winter. Trout collectively indicate a preference for a gravel/stone (24 %), stone (23 %) and boulder (22 %). Trout display a fairly strong association (r = 0.66) to benthic structure ranked in descending order of relative particle size (Table 3.20).

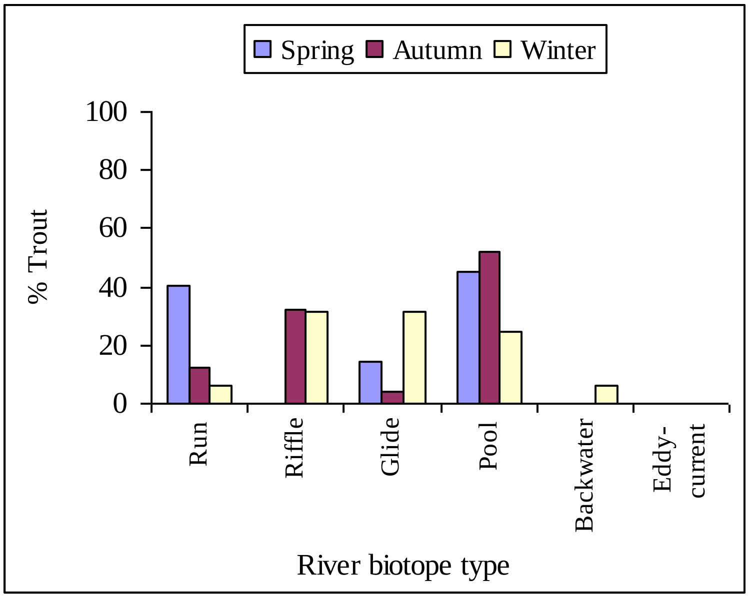

Figure 3.34 Percentage river biotope type seasonally frequented by trout derived from electrofishing results (n = 83).

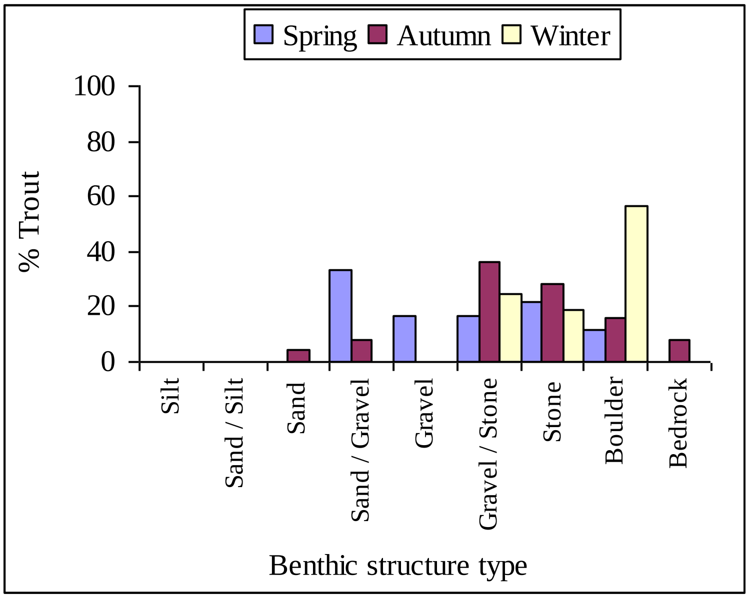

Figure 3.35 Percentage benthic structure type seasonally frequented by trout derived from electrofishing results (n = 83).

Table 3.19 Abundance of trout linked to categorized river biotope types ranked in descending order of associated flow velocity using Spearman's Rank Correlation Test (n = 83).

Category	**River biotope ranked in descending order of associated flow velocity**	**Number of trout**	**Trout ranked in descending order of relative abundance**
Riffle	1	13	3
Run	2	21	2
Glide	3	12	4
Back-eddy	4	0	6
Pool	5	36	1
Backwater	6	1	5
n		83	
r = 0.26			

Table 3.20 Abundance of trout linked with categorized benthic structure types ranked in descending order of associated particle size using Spearman's Rank Correlation Test (n = 83).

Category	**Benthic structure type ranked in descending order of associated flow velocity**	**Number of tadpoles**	**Tadpoles ranked in descending order of relative abundance**
Bedrock	1	2	6
Boulder	2	18	3
Stone	3	19	2
Gravel/Stone	4	20	1
Gravel	5	7	5
Sand/Gravel	6	16	4
Sand	7	1	7
Silt/Sand	8	0	8
Silt	9	0	8
n		83	
r = 0.66			

3.9.5 Depth preferences of trout.

The depth preferences of trout based on combined seasonal electrofishing data is shown in Figure 3.36. Trout indicate a strong preference for depth ranging from 15 – 40 cm with a small proportion of trout seeking deeper water ranging from 50 – 80 cm. The highest percentage of trout occurred at a depth of 32.5 cm (16 %) followed by 17.5 cm (11 %) and 22.5 cm (10 %). Trout were sampled at a mean depth 29.57 ± 13.83 cm (n = 42), in spring; 38.42 ± 14.82 cm (n = 25) in autumn; and 30.63 ± 14.31cm (n = 16), in winter.

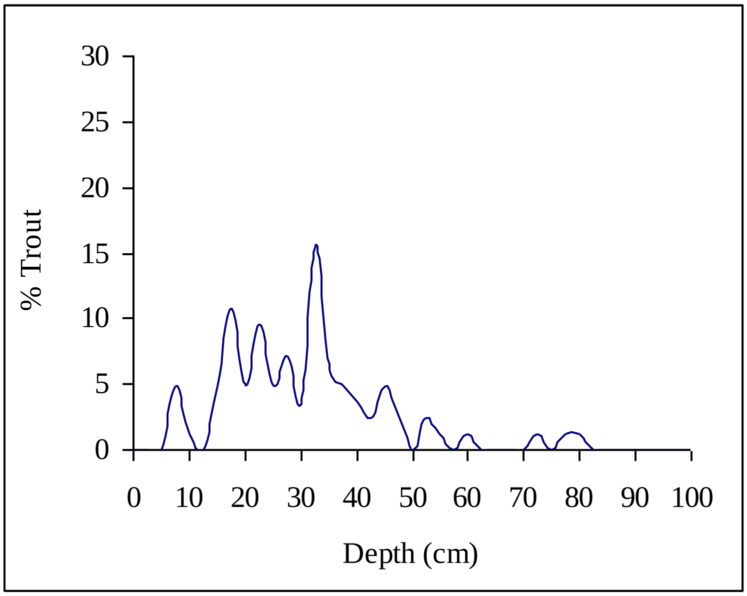

Figure 3.36 Percentage depth frequented by trout derived from combined seasonal electrofishing results (n = 83).

3.9.6 Flow velocity preferences of trout.

The flow velocity preference of trout relative to combined seasonal electrofishing results is displayed in Figure 3.37. The graph indicates that the abundance of trout decreases sharply with a concomitant increase in flow velocity. The greatest percentage of trout occurred at a flow velocity of < 0.25 m/s. A small percentage of trout occur above a flow velocity of 1m/s suggesting that they have a preference for slower moving water. Trout display a weak negative correlation (r = -0.17) to an increase in flow velocity (Table 3.21).

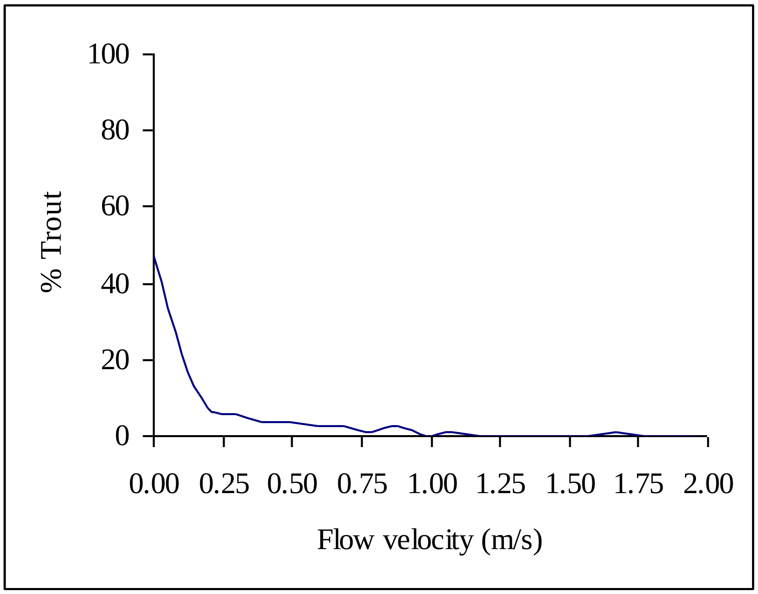

Figure 3.37 Percentage of trout inhabiting specific flow velocities relative to combined seasonal electrofishing results.

Table 3.21 Abundance of trout linked to categorized 0.10 m/s flow velocity classes ranked in ascending order of magnitude using Spearman's Ranked Correlation Test (n = 83).

Flow Rate (m/s)	Ranking of flow rate	Number of trout	Ranking of trout
0.00	21	39	1
0.10	20	18	2
0.20	19	6	3
0.30	18	5	4
0.40	17	3	5
0.50	16	3	5
0.60	15	2	6
0.70	14	2	6
0.80	13	1	7
0.90	12	2	6
1.00	11	0	8
1.10	10	1	7
1.20	9	0	8
1.30	8	0	8
1.40	7	0	8
1.50	6	0	8
1.60	5	0	8
1.70	4	1	7
1.80	3	0	8
1.90	2	0	8
2.00	1	0	8
Total		83	
r = -0.17			

3.9.7 Seasonal patterns in the distribution and abundance of trout at sampling sites located below the selected waterfalls at Injesuthi and Monk's Cowl.

A total of 83 trout were sampled in 705.49 minutes of electrofishing, equating to a mean CPUE of 0.12 trout per minute. 26 *O. mykiss* (31.33 %) and 57 *S. trutta* (68.67 %) were electrofished below the selected waterfalls at Injesuthi and Monk's Cowl respectively. The greatest concentration of trout occurred in spring with 42 fish (50.60 %), followed by 25 fish in autumn (30.12%), and 16 fish in winter (19.28%). The mean CPUE of *S. trutta* electrofished at Monk's Cowl (0.16 fish per minute) is twice the mean CPUE of *O. mykiss* at Injesuthi (0.08). The mean Total Length (TL) of *S. trutta* electrofished at Monk's Cowl (TL 17.93 ± 3.31 cm, n =57) is similar to *O. mykiss* at Injesuthi (TL 18.17 ± 4.62 cm sd, n=26). The largest *O. mykiss* and *S. trutta* electrofished at Injesuthi and Monk's Cowl is TL 27.00 and 28.50 cm respectively. Results indicate that relatively low (n=16) to moderate numbers (n=42) of small to moderate sized trout ≤ 28.50cm occur seasonally below the selected waterfalls at Injesuthi and Monk's Cowl respectively in association with *H. natalensis* tadpoles.

3.10 Habitat overlap between trout and *H. natalensis* tadpoles.

3.10.1 Depth versus flow velocity preferences

Figure 3.40 indicates the modeled range of habitat overlap between *H. natalensis* tadpoles (n = 743) and trout (n = 83) relative to species specific preferences for depth and flow velocity ranges derived from combined seasonal electrofishing data. Results suggest that *H. natalensis* tadpoles prefer depths ranging between 12.00 - 29.00 cm and flow velocities between 0.15 - 0.80m/s. Conversely, trout seek deeper water ranging between 18.00 – 47.00cm and slower flow velocities < 0.60 m/s. The apportionment habitat overlap occurring between trout and *H. natalensis* tadpoles, relative to depth and flow velocity preferences, is modeled at 27.43 % and 42.08 % respectively.

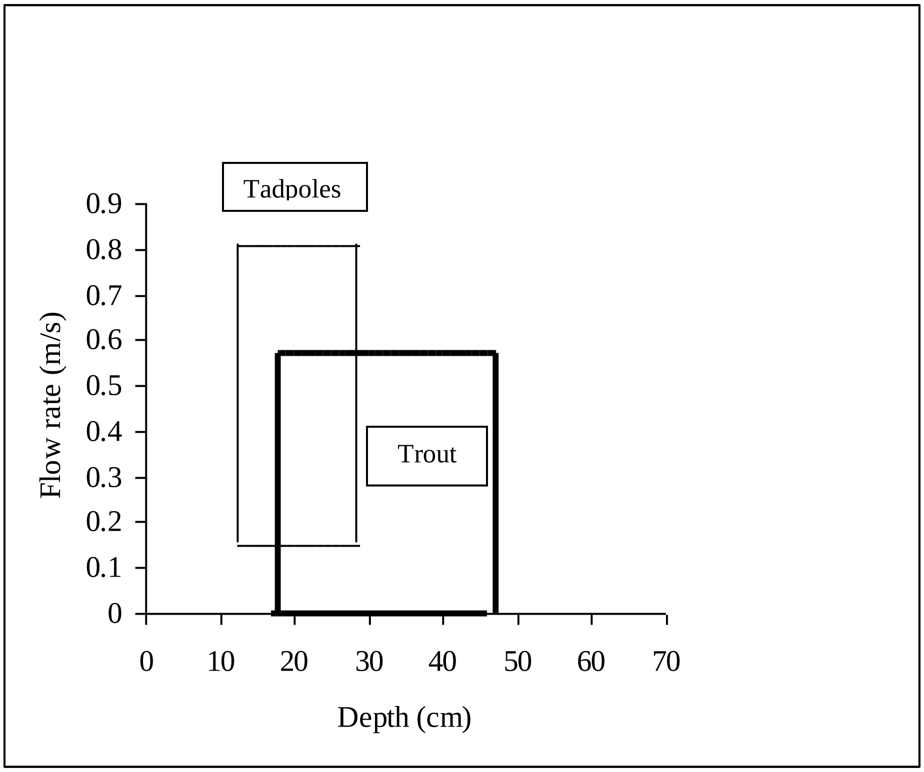

Figure: 3.38 2-D Habitat overlap model between *H. natalensis* tadpoles (n = 743) versus trout (n = 83) relative to frequented flow velocity and depth classes derived from combined seasonal electrofishing results.

3.10.2 River biotope type utilization by trout and *H. natalensis* tadpoles.

Figure 3.39 indicates that the greatest possibility of habitat overlap occurring between *H. natalensis* tadpoles and trout is within run type habitat, followed by riffles and glides. *H. natalensis* has a greater preference for riffles (66 %) versus trout which occur more commonly in pools (43 %). On the other hand, *H. natalensis* tadpoles are seldom found in pools (5 %) while trout are much less frequently encountered in riffle habitat (17%). Both *H. natalensis* tadpoles and trout show signs of avoiding slower flowing back-eddies and backwaters.

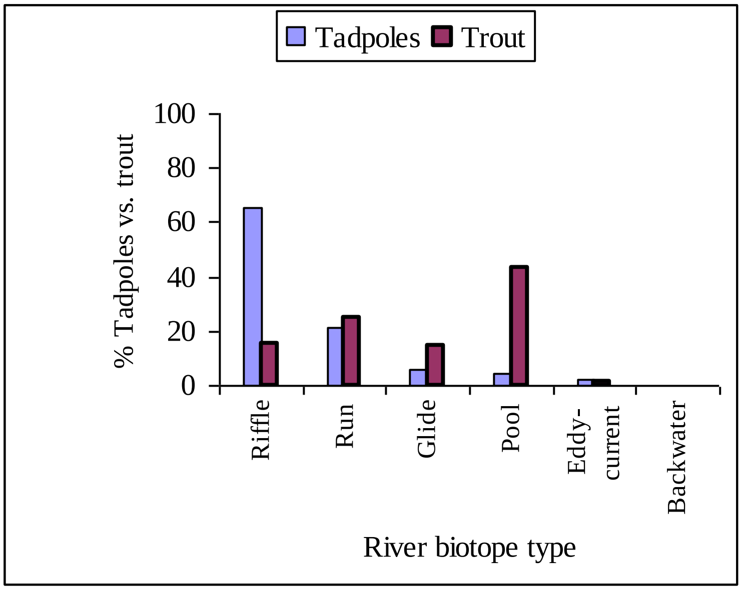

Figure 3.39 Percentage habitat overlap between *H. natalensis* tadpoles (n = 743) and trout (n = 83) relative to river biotope type preferences derived from combined seasonal electrofishing results.

3.10.3 Benthic structure type utilisation by trout and *H. natalensis* tadpoles.

Results indicate that the greatest degree of shared utilisation of habitat between *H. natalensis* tadpoles and trout is more likely to occur in habitats associated with a high proportion of gravel/stone, stone and boulder related benthic structure (Figure 3.40). Tadpoles indicate a significant preference for gravel associated with stone, followed by stone and boulder habitat. Results alternatively indicate that trout utilise a greater range in benthic structure, ranging from sand/gravel (common pool sediment), stones and boulders. Both *H. natalensis* tadpoles and trout indicate signs of avoiding benthic structure dominated by silt, silt/sand and bedrock, suggesting that a lack of favourable cover may be a limiting factor.

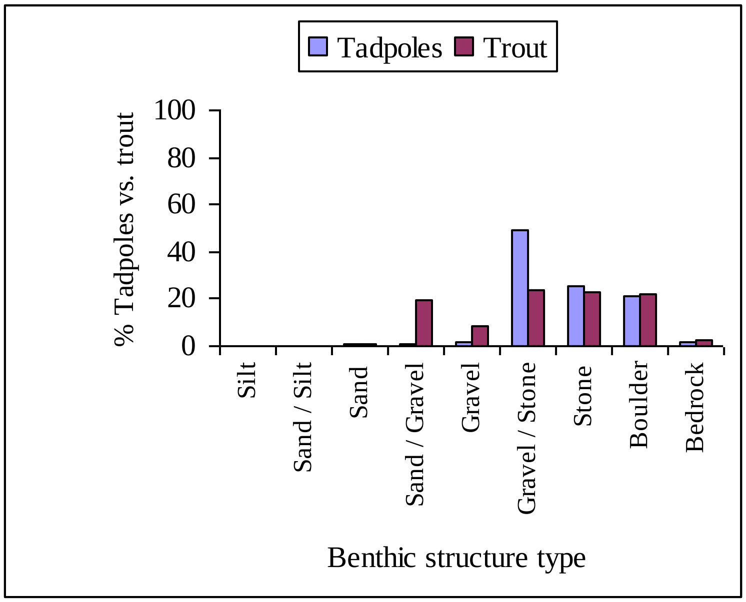

Figure 3.40: Approximated percentage habitat overlap occurring between *H. natalensis* tadpoles (n = 743) and trout (n = 83) subjected to benthic structure type preferences derived from seasonal electrofishing results.

3.10.4 Habitat utilization of *H. natalensis* tadpoles and trout above and below the selected waterfalls at Injesuthi and Monk's Cowl using grid based modeling.

Raster images relating to the main preferences of *H. natalensis* tadpoles (n = 743) for river biotope type (runs), depth (6.50 - 25.12 cm) and benthic structure type (gravel, stone) at sampling site IN 6 in spring are shown in Figure 3.41(A-C) respectively. Figure 3.43(D) gives an approximation of the total available suitable habitat occurring for *H. natalensis* tadpoles at sampling site IN6. Table 3.22 gives an approximation of the modeled percentage of suitable *H. natalensis* tadpole habitat occurring at sampling site IN1, IN6, M1, and M6 using the results of IN1 as a modeling surrogate for all sites. Habitat availability for trout relative to the preference of trout (n = 83) for river biotope types (pools, runs, glides), depth (14.50 ± 9.31cm), and benthic structure (gravel/stone, stone, boulders), at sampling sites located below the selected waterfalls is shown in Table 3.23. The highest percentage of suitable habitat for *H. natalensis* tadpoles occurred at sampling site M1 (30.60 %), followed by IN1 (16.07 %), IN6 (10.70%), and M6 (7.76 %) respectively. Modeled results suggest the environmental habitat conditions for

H. natalensis tadpoles are comparably more suitable at sampling sites located above the selected waterfalls, versus those below the waterfall.

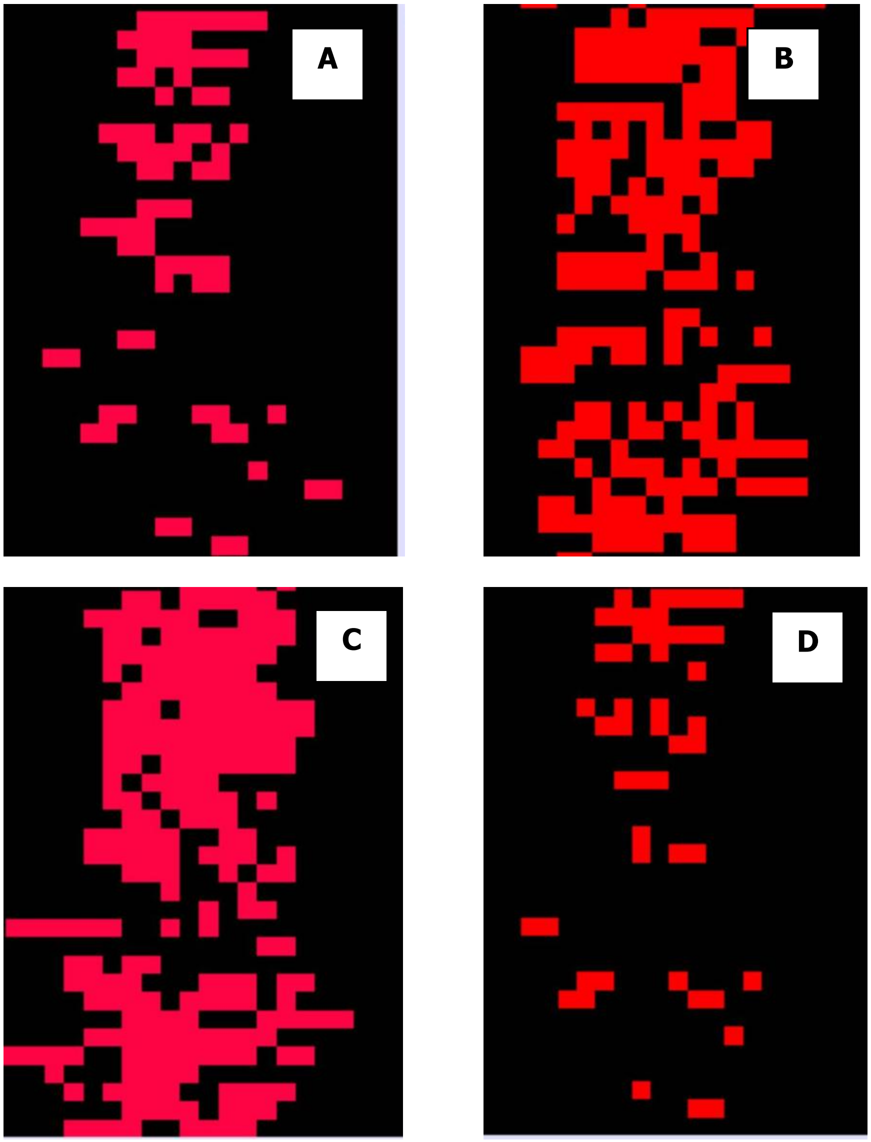

Figure 3.41 Modeled rasterized images relative to the availability of suitable habit at sampling site IN 1 for *H. natalensis* (n = 743) tadpoles subject to preferred biotope type (A), depth (B) and benthic structure type (C) with the modeled amount of suitable available habitat (D) derived by overlaying and querying images A, B and C.

Table 3.22 Modeled percentage of suitable *H. natalensis* tadpole habitat occurring at selected sampling sites relative to species specific river biotope type, depth and benthic structure type preferences (n = 743).

Sampling site	**Sampling points**	**Exposed sampling points**	**Inundated sampling points**	**Suitable Sampling points**	**% Suitable habitat**
IN1	402	97	305	49	16.07
IN6	640	215	425	33	7.76
M1	377	145	232	71	30.60
M6	697	174	523	56	10.70

Table 3.23 Modeled percentage suitable trout habitat occurring at sampling sites located below the selected waterfalls based on combined river biotope type, benthic structure type and depth preferences (n = 83).

Sampling site	**Sampling points**	**Exposed sampling points**	**Inundated sampling points**	**Suitable Sampling points**	**% Suitable habitat**
IN6	640	215	425	83	19.53
M6	697	174	523	83	15.87

3.10.5 Habitat sharing between *H. natalensis* tadpoles and trout below the selected waterfalls relative to grid based modeling.

The result of overlaying and querying the modeled suitable habitat raster image for *H. natalensis* tadpoles versus trout at sampling site IN6, is shown in Figure 3.42 (A). Modeled results suggest limited habitat sharing and utilisation (5.88 %) between

H. natalensis and trout based on species-specific preferences for river categorised river biotope type, benthic structure type and depth. The modeled results indicate no habitat sharing between *H. natalensis* tadpoles and trout at sampling site M6 (Figure 3.42 (B)). Results suggest that minimal competition exists between *H. natalensis* tadpoles and trout for habitat space occurring below the selected waterfalls at Injesuthi and Monk’s Cowl.

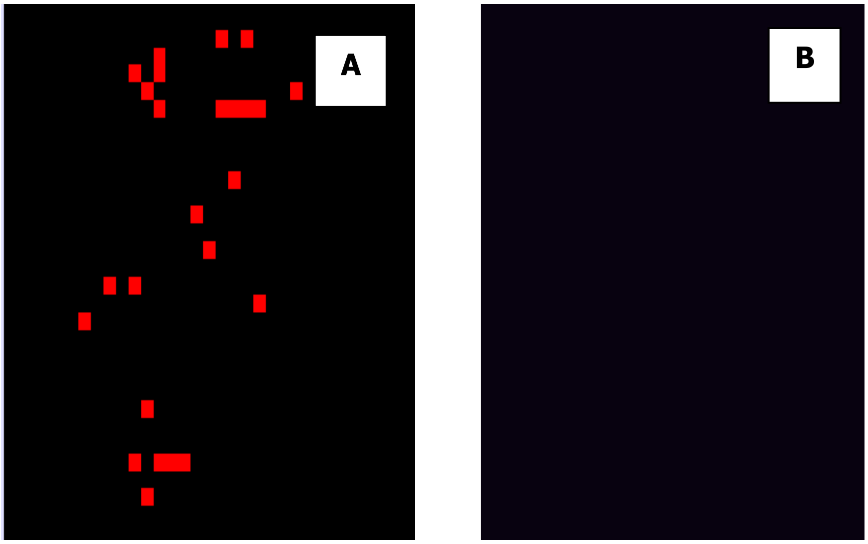

Figure 3.42: Modeled habitat sharing between *H. natalensis* tadpoles and trout below the selected waterfalls at sampling sites IN6 (A) and M6 (B).

CHAPTER 4

4. DISCUSSION

4.1 Review of habitat utilisation of *Hadromophryne natalensis* tadpoles and trout occurring at sampling sites.

4.1.1 Landscape-scale description of sampling area.

GIS results indicate that the geology, soil type, vegetation type, forest type and water yield are categorically the same at sampling sites located above and below the two selected waterfalls at Injesuthi and Monk's Cowl respectively (Table 3.1). Spatial differences in terms of geology and broad vegetation type did occur regionally between Injesuthi and Monks Cowl but soil and forest type remain the same. Results suggest that both *H. natalensis* tadpoles and trout are tolerant of a fairly broad range of geomorphological conditions.

4.1.2 Large-scale climate and topographical features of sampling sites.

A Principal Component Analysis (PCA) conducted on selected large-scale climate and topographical features suggest that precipitation and altitude (Figure 3.13) are the two main variables influencing sampling site differences at a landscape level. Results show that *H. natalensis* tadpoles occurred at all sampling sites ranging between 1 280 and 1 700m.a.s.l. (Figure 3.1) which is consistent with that of Boycott (2004) who claims that *H. natalensis* occurs within an altitudinal range of 580 - 3 675 m.a.s.l. The type locality of *H. natalensis* is the Krantzkloof Nature Reserve, in KwaZulu-Natal, located at 450 m.a.s.l (pers.obser.). The wide range in altitudinal tolerance of *H. natalensis* populations suggests that altitude is not a primary variable regulating their distribution in KwaZulu-Natal.

Altitude is, however, an important limiting factor in the occurrence of trout in KwaZulu-Natal. Crass (1986) suggested that trout mainly occur in the Drakensberg foothills at altitudes of between 1 200-1 800 m.a.s.l. Above 1 800 m, the streams are generally too small and steep, and become too silted or sluggish below 1200m, whilst the dams tend to be too warm for trout. The altitude range of sampling sites located below the selected waterfalls (Appendix C) varied between 1 615 and 1 653m.a.s.l at Injesuthi and between 1 292 and 1 727 m.a.s.l at Monk's Cowl, indicating that all the sampling sites fall within the altitudinal limits as suggested by Crass (1986).

Precipitation is higher at the Monk's Cowl sampling sites with a mean annual precipitation (Table 3.2) ranging between 1 240 and 1 428mm vs. 874 - 896mm at Injesuthi. With such variability in the mean annual precipitation between the two sites, rainfall is not considered to be a primary factor limiting the occurrence of *H. natalensis* in the study. Crass (1986) suggested that flow volume and water temperature are the two main factors that affect the occurrence of river trout – these factors are closely correlated as the effect of hot weather is accentuated by a low river, while a particularly rapid change in temperature can take place with floodwaters associated with a thunderstorm.

A third factor is turbidity, also related to stream flow. The most productive streams are those that have a relatively constant flow, no great fluctuations in temperature and clear water (Molony, 2001). Mountain catchments in South Africa have a relatively small capacity for storing and releasing groundwater, and river flow is dependent on rainfall. The result is that the dry season discharge is a minute fraction of that occurring after heavy precipitation (Crass, 1986). In KwaZulu-Natal, little rain is expected between April and October and once the water exceeds 25 ºC the trout are under stress (Crass, 1986). Flow velocities measured at the sampling sites during this study confirm the high variability in amplitude (Figure 3.6). Stream flow velocities at sampling site IN1 increased from 0.11m/s in spring (low flow period) to 0.85m/s (+7.71 x) in summer (high flow period). Flow rates at sampling sites M1 and M6 similarly increased by a magnitude of 7.10 x and 25.50 x respectively. The high variability in flow indicates that sampling sites located below the selected waterfalls at Injesuthi and Monk's Cowl is not always optimal for trout colonisation and persistence. Results from this study showed that mean water temperatures (Figure 3.11) were highest in January ((summer sampling period/high rainfall season; IN4 (16.45 ºC), IN6 (16.03 ºC), M4 (16.60 ºC), M6 (16.95 ºC)) falling well below the 25 ºC threshold suggested by Crass (1986) for trout under South African conditions.

H. natalensis tadpoles were sampled in a wide range of river gradients, ranging from 8.00 ‰ at sampling sites located below the selected waterfall at Monk's Cowl to 17.83 ‰ above the selected waterfall at Injesuthi (Table 3.2). The greatest concentration of *H. natalensis* tadpoles occurred above the selected waterfall at Monk's Cowl (Table 3.14) at 12.50 ‰. The variability in gradient between all sampling sites is not considered to be a major factor limiting the distribution of *H. natalensis* in the UDPWHS. Hunter *et al.* (1990) states that the most productive trout streams have a relatively low gradient of 0.50 - 2.00 ‰ Crass (1986) states that the Ngwangwana River in KwaZulu-Natal has an ideal gradient of 0.80 ‰ combined with excellent holding water for large (>500 g) trout, confirming Hunter's findings. The average gradient below the selected waterfalls at Monk's Cowl and Injesuthi varied between 5.00 and 6.00 ‰ respectively, suggesting that both river sections are considered precipitous for trout and consequently may lack suitable holding water for large trout. The largest fish sampled at Injesuthi and Monk's Cowl measured 27.00 and 28.50 cm TL respectively, supporting both Crass's (1986) and Hunter's *et al.* (1990) statements that trout productivity may be reduced at sub-optimal gradients.

All sampling sites supported *H. natalensis* tadpoles despite the site aspects varying between 22 º and 155 º. The mean annual air temperature was 15 ºC for all sampling sites. The fact that *H. natalensis* tadpoles tolerate such wide variability in mean annual precipitation, water temperature, flow velocity, site aspect and gradient, suggests that the species should occur in similar abundances both above and below the selected waterfalls at Injesuthi and Monk's Cowl respectively.

4.1.3 Physical features of sampling sites.

Exposed river bed (24-38 %), mostly as result of low flow conditions (Figure 3.2), accounted for the greatest proportion of river biotope type at all sampling sites in spring, followed by runs (19-27 %). Stones accounted for 33-40% and boulders (18-35%) of all benthic structure at all sites (Figure 3.3). 41.9-66.10% of all sampling sites similarly had sloped river banks (Table 3.3). A wide spectrum of riparian vegetation types (Table 3.4), ranging from grassland (65 %), mixed vegetation (100 %), and forest (82 %), each supported large numbers of *H. natalensis* tadpoles, suggesting that riparian vegetation is not a major factor limiting the distribution of the species in the UDPWHS. A wide range in wetted stream width (8.00-14.30 m) and active channel width (9.80 - 16.00 m) similarly supported *H. natalensis* tadpole populations (Table 3.5). The mean depth of all sampling sites (Figure 3.5) varied between 12.54cm at sampling site M1 (highest tadpole concentration) and 21.72cm at M6 (lowest tadpole concentration). Sampling site IN1 with a mean depth of 18.67cm, however, supported the largest concentration of *H. natalensis* tadpoles at Injesuthi, suggesting that depth, independently, is not a primary factor regulating the abundance of tadpoles. The results also indicate that *H. natalensis* tadpoles seek fast flowing riffle water dominated by gravel, stone and boulder for colonization (Figure 3.30). Sampling sites below the selected waterfalls had the highest flow velocities (Figure 3.6) as well as the highest incidence of stone (M6) and boulder (IN6), second highest incidence of gravel (M6) (Figure 3.3), yet supported the lowest abundance of *H. natalensis* tadpoles (Table 3.13 & 3.14). PCA's conducted on the geomorphologic variability (Figure 3.14) and hydrological variability (Figure 3.16) between all sampling sites suggest that both sampling site IN1 (high tadpole density) and M6 (low tadpole density) are the most closely associated. The CPUE of tadpoles electro-fished at IN1 ranged seasonally between 0.47 (Figure 3.23) and 1.04 (Figure 3.22) while M6 ranged between 0.00 (Figure 3.20) and 0.11 (Figure 3.22) tadpoles per minute. The similarity between sampling site IN1 and IN6 in terms of geomorphologic and hydrological variables, suggest that they should support similar abundances of *H. natalensis* tadpoles (Figures 3.20, 3.22 & 3.23).

Results suggest that all sampling sites shared a wide range of river biotope type (Figure 3.2), benthic structure (Figure 3.3), bank structure (Table 3.3), depth (Figure 3.5), wetted and active channel width (Figure 3.4) and riparian vegetation features (Table 3.4) yet the abundance of *H. natalensis* tadpole populations remained notably reduced at all sampling sites located below the selected waterfall at Injesuthi and Monk's Cowl.

Cover is recognised as one of the essential components of trout streams. Boussu (1954) was able to increase the number and weight of *S. trutta* in stream sections by adding artificial bush cover. Lewis (1969) reported that the amount of cover was important in determining the abundance of *S. trutta* in sections of a Montana stream. Cover for adult *S. trutta* consists of areas of obscured stream bottom where the velocity is low and the depths are at least 15cm. Wesche (1980) reported that in larger streams, the abundance of *S. trutta* ≥15cm in length increased with depth; most occurred at depths ≥15cm. Escape cover is provided by overhanging and submerged vegetation, undercut banks, in-stream objects (such as debris piles, logs, and large rocks), pool depth and surface turbulence. These habitat conditions occurred commonly at sampling sites located below the selected waterfall of Monk's Cowl, inhabited by *S. trutta*, but were largely lacking at the lower Injesuthi sites inhabited by *O. mykiss*. A lack of cover associated with a steeper gradient may explain why the trout population occurring below the selected waterfall at Injesuthi is half that of Monk's Cowl. Results also suggest that the incidences of habitat overlap between *H. natalensis* tadpoles and trout is most likely to occur in runs dominated by gravel, stone and boulder substrate (Figure 3.40). Our results also suggest that 60% of *H. natalensis* tadpoles utilised runs in the absence of riffle habitat in spring (Figure 3.30). Coincidentally, this is also the season in which tadpole abundance is at its lowest (Figure 3.20) and trout abundance is at its highest (50.60%) below the selected waterfalls. 40 % of the trout (Figure 3.34) were also sampled in the runs during spring suggesting that predation may have been highest during this period. Results suggest that the greater abundance of *S. trutta* below the waterfall at Monk's Cowl had a greater negative impact on *H. natalensis* tadpoles than *O. mykiss* at Injesuthi.

4.1.4 Water Quality.

All sampling sites located above the selected waterfalls had similar seasonal pH and EC values as sampling sites located below the waterfalls (Figures 3.7 & 3.8) All sites seasonally similarly displayed good indications of river health based on the South African

Scoring System (SASS) Version 5 (Dickens & Graham, 2002) (Figure 3.10). Mean monthly water temperatures occurring at sampling sites located above the selected waterfalls at Injesuthi and Monk's Cowl, vs. sites below the waterfalls were found to be significantly similar in terms of their thermal regimes (Figure 3.11).

The importance of water temperature to aquatic biota has been well documented (Claska & Gilbert, 1998; Eaton & Scheller, 1996; Sullivan *et al.*, 2000). Vannote & Sweeney (1980) consider the most important aspect of a river's thermal regime to be its temporal predictability. Stuckenberg (1969) highlighted the links between temperature, topography and faunal assemblages, notably for snakes and amphibians. Rivers-Moore *et al.*, (2004), also highlighted the major impacts of water temperatures on aquatic organisms, and showed how water temperatures are one of the primary environmental drivers structuring fish communities in the Sabie River, arguably the most species-rich river in South Africa in terms of fish biodiversity. Results indicate that the water quality occurring above the selected waterfalls is quite similar to the sites below, suggesting that the abundance of *H. natalensis* should be similar both above and below the selected waterfalls at Injesuthi and Monk's Cowl.

4.2 Electro-fishing results.

4.2.1 CPUE of *H. natalensis* tadpoles above and below the selected waterfalls.

The mean CPUE of *H. natalensis* tadpoles electro-fished seasonally at combined sampling sites occurring above the selected waterfalls at Injesuthi and Monk's Cowl, in the absence of trout, exceeded the mean CPUE of *H. natalensis* populations sampled below in association with trout by a magnitude of 4.69 x (Table 3.13) and 15.71 x (Table 3.14) respectively. Results from this study suggest a negative species association between trout and *H. natalensis* tadpoles (Figures 3.24 & 3.25).

4.2.2 Habitat suitability for *H. natalensis* tadpoles and trout at sampling sites located above and below the selected waterfalls based on grid based modeling.

Grid based modeling identified suitable cells as optimal *H. natalensis* tadpole habitats based on their combined preferences for specific river biotope type, benthic structure type and depth. The modeling results indicate that the percentage of suitable *H. natalensis* habitat cells was higher at sampling sites located above each selected waterfall than sites below the waterfall. The greatest percentage of suitable tadpole habitat (30.60%) occurred at sampling site M1 (Table 3.22), one of three sampling sites located above the selected waterfall at Monk's Cowl that also recorded the highest mean CPUE of tadpoles (Figure 3.14), therefore reinforcing the grid based modeling technique. Sampling site M6, which occurred below the selected waterfall at Monk's Cowl, only had 10.70% suitable tadpole habitat (Table 3.22). Although the percentage of suitable habitat is clearly lower below the waterfall, sampling site M6 still supported 56 suitable modeled sampling points (Table 3.22) for *H. natalensis* habitation, compared to 71 suitable modeled sampling site at M1 (Table 3.22), and should consequently support a comparable abundance of *H. natalensis* tadpoles. The combined seasonal abundance of *H. natalensis* tadpoles was however a magnitude of 15.71 x lower at sampling sites located below the waterfall (Table 3.14).

4.2.3 Seasonal patterns in the distribution and abundance of *H. natalensis* tadpoles at sampling sites located above and below the selected waterfalls.

The CPUE of *H. natalensis* tadpoles remained seasonally much greater above the selected waterfalls at Injesuthi (Table 3.15) and Monk's Cowl (Table 3.16), in the absence of trout, and significantly lowered below the waterfall in the presence of trout, supporting the hypothesis that waterfalls are important in protecting *H. natalensis* tadpoles from assumed trout predation. The results suggest that the Monk's Cowl sampling sites, in comparison to Injesuthi, had the greatest abundance of *H. natalensis* tadpoles occurring above the selected waterfall, and conversely, the lowest concentration below the selected waterfall in the presence of *S. trutta*. The greatest number of *H.*

natalensis tadpoles occurred in the summer following a seasonal spawning period. High water levels in summer limited the electrofishing effort with sampling only taking place at sampling sites IN1, M1, M4 and M6 with mean CPUE's of 3.25, 1.56, 0.27 and 0.3.2 respectively. With the exception of sampling site M1, these are the highest CPUE's recorded during this study. An analysis of the tadpole length data also revealed that a new tadpole cohort in summer, not present during the earlier spring sampling period, had emerged, indicating that adult *H. natalensis* had bred in the interim. Very large tadpoles, close to metamorphosis into adults, were no longer present in the summer sampling period, but had been replaced by a younger cohort that had matured into yearlings. Results clearly indicate that *H. natalensis* tadpoles take approximately two years to metamorphose into adults and that spawning in the UDPWHS takes place sometime between late spring and early summer. The findings are contrary to Boycott (2004) who suggests that breeding takes place in late summer (March-May) when stream flow is reduced and before winter temperatures are become severe. The variability in tadpole length within a cohort could assumedly be attributable to size differences between adult male and female tadpoles. Females are generally larger than males (Passmore & Carruthers, 1979).

The CPUE of *H. natalensis* tadpoles remained consistently high at all sampling sites located above the selected waterfalls during autumn. The CPUE averaged between 0.94 – 1.05 at sampling sites IN1 – IN3, and 0.84 – 1.09 at sampling sites M1 – M3 during this period. The CPUE during winter is much lower ranging between 0.42 – 0.57 at sampling sites IN1 – IN3 and 0.49 – 0.98 at sampling sites M1 – M3 respectively. These results suggest that decreases in numbers also takes place within *H. natalensis* tadpole populations located above selected waterfall in the absence of alien predators like trout. The progressive decline in tadpole abundance, from summer seasonally onwards, could be linked to a decrease in available riffle habitat as water levels drop, exposure to natural predators, downstream drift losses or even disease factors.

Sampling site IN4 is the only sampling site located below the selected waterfalls that has smaller secondary waterfall located within the main sampling area (Figure 4.1). This is also the only sampling site below the selected waterfalls that maintained a moderate population (CPUE) of *H. natalensis* during autumn and winter measuring 0.51 and 0.27 respectively. In contrast, sampling sites IN5 and IN6, located further downstream, only maintained CPUE's of between 0 and 0.12, during the same seasonal sampling periods, suggesting that tadpole abundance decreases further away from the waterfall. It is proposed that the secondary waterfall occurring at sampling site IN4 provided an additional barrier to the upstream migration of trout, reinforcing the hypothesis that waterfalls are important in protecting *H. natalensis* tadpoles from potential trout predation.

Figure 4.1 Secondary waterfall located within sampling IN4.

4.2.4 Species association between *H. natalensis* tadpoles and trout below the selected waterfalls.

Species-rich communities are more resistant to invasion than species-poor communities (Elton, 1958; Ross, 1991). This is possibly because a greater number of interacting species maximise utilisation of the available resources (Fox & Fox, 1986; Hobbs, 1955) and environmental conditions essential for invasion of a new species as either a biotic vacancy or a place weakly held by a displaceable species are not available. *S. trutta* and

O. mykiss occur in most streams and rivers in the UDPWHS and have formed self-sustaining populations following their historical introduction.

The general attributes of a successful invader species is that it is a habitat generalist, has a broad dietary requirement, and has a high reproductive potential (Meffe & Carroll, 1997). Trout are very aggressive towards other species (Gamradt *et al.*, 1997), a behavioural component that contributes to their success as an invader (Holway & Suarez, 1999). Both *S. trutta* and *O .mykiss* meet these criteria and are currently listed within the top 100 worst alien invasive species in terms of the Global Invasive Species Programme (GISP)

This study showed that *H. natalensis* tadpole populations were of a magnitude of 4.69 x less below the selected waterfall at Injesuthi in association with *O. mykiss* and of a magnitude of 15.71 x less below the selected waterfall at Monk's Cowl in association of *S. trutta.* Results indicate that the greatest decrease in *H. natalensis* abundance occurred below the selected waterfall at Monk's Cowl where the mean CPUE of *S. trutta* (1.60) is twice that of *O. mykiss* (0.08) at Injesuthi. Knapp & Matthews (2000) similar found that the expected number of *R. muscosa* tadpoles in water bodies without trout was 6.8 times greater than in water bodies with trout.

Kats & Ferrer (2003) demonstrated that amphibians either do poorly (slowed growth, smaller size) in the presence of aliens or are eliminated in short term studies due to high mortality rates e.g. *O. mykiss* vs. *Rana temporaria* (Nyström *et al.,* 2001), *O. mykiss* vs. *L spenceri* (Gillespie, 2001), *O. mykiss* vs. *L. phyllochroa* (Gillespie, 2001), *O. mykiss* vs. *A. macrodactylum* (Tyler *et al.*1998), *O. mykiss* vs. *Ambystoma gracile* (Tyler *et al.*1998).

Studies have found that amphibian larvae grow less or metamorphose at a smaller size when they are raised with alien predators compared to when they are raised without them (Kats & Dill, 1998). Mechanisms for mediating these changes in growth and metamorphosis are probably the result of standard responses to predators e.g. reduced movement and reduced feeding on the part of amphibians in the presence of predators.

The CF of tadpoles electrofished at sampling sites located below the selected waterfalls was consistently lower below the selected waterfalls, in the presence of trout vs. sampling sites above the waterfall, where trout were absent. This observation is consistent with the findings of Kats & Dill (1998) suggesting that some form of predation was taking place. Many amphibian species apparently lack prior evolutionary experience of such predators, and even with species functionally similar to most alien predators (Diamond & Case, 1986). Consequently, amphibians are particularly vulnerable to alien predators. *H. natalensis* populations sampled above the selected waterfalls at Injesuthi and Monk's Cowl occur in environments devoid of fish (Figures 3.20, 3.22 & 3.23). *H. natalensis* tadpoles occurring below the selected waterfalls co-exist with small numbers of indigenous Natal Mountain Catlet *Amphilius natalensis* that reputedly only prey on mayfly and midge larvae (Skelton, 2001). *A. natalensis* is itself preyed upon by trout and is now scarce in certain streams (Skelton, 2001). *H. natalensis* has similarly not co-evolved with a fish predator in the UDPWHS as suggested by Diamond & Case (1986), indicating that this species may also be highly vulnerable to predation. Species-poor insular ecosystems made up mainly of few endemic species seem particularly prone to disruption by invasions (Elton, 1958; Orians, 1986; Loope & Mueller-Dombois, 1989). Thorp (1986) also stated that the effect of predation was generally most pronounced in systems where prey had no common evolutionary history with a newly introduced predator. This observation is supported by Townsend & Crowl (1991), McDowall (1968, 1984, & 1987) and Tilzey (1977). Similarly, *H. natalensis* tadpoles have no common evolutionary history with trout and also occur in a species-poor insular ecosystem within the UDPWHS as suggested by the previous authors.

Knapp & Matthews (2000) suggested that amphibians typically utilise shallow water bodies and have larvae that complete metamorphosis within weeks to months, but in the high elevation habitats of the Sierra Nevada, *R. muscosa* require two to four years to complete metamorphosis (Zwiefel, 1955), extending the risk of predation.

H. natalensis also has a complex life cycle since they take approximately two years to metamorphose into adults and are dependent on permanent, fast flowing water dominated by gravel, stones and boulders. Knapp & Matthews (2000) also hypothesised that the strong effect of introduced trout on *R. muscosa* is due to similar habitat requirements between trout and the amphibian. Both trout and *H. natalensis* tadpoles are equally dependent on clean permanent running water for their survival in the UDPWHS.

The minimum size of *H. natalensis* tadpoles occurring at sampling sites below the selected waterfalls was consistently larger than those above the waterfall, suggesting prey selection based on body size. The effects of tadpole body size versus fish body size on predation in a study on Grey Treefrog tadpoles (*Hyla chrysocelis*), was studied in laboratory and artificial pond experiments (Semlitsch & Gibbons, 1988). Tadpole body size had a significantly positive effect on the survival of tadpoles in all experiments. Large tadpoles were probably better able to evade predators (Semlitsch & Gibbons, 1988)

Fish body size had a negative effect on the survival of tadpoles. Larger fish consumed a larger number and proportion of tadpoles as well as greater biomass. These factors indicate that environmental factors affecting the growth rate of tadpoles can dramatically alter the vulnerability to gape-limited predators (Semlitsch & Gibbons, 1988). It has been suggested that some species of tadpole could avoid gape-limited fish predators when they are larger in size (Hecnar & McCloskey, 1997). This appears to be the case amongst the small number of large *H. natalensis* tadpoles surviving below the selected waterfalls in the presence of trout.

H. natalensis has large tadpoles (100 - 120mm TL) that take approximately two years to metamorphose into adult frogs within the UDPWHS. The large “surviving” tadpoles were found exclusively below the waterfalls of Injesuthi and Monk’s Cowl and is assumed to have mostly outgrown the gape capability of resident trout population. Research conducted by L’abée-Lund *et al.* (1992) confirm that *S. trutta* become more piscivorous at ≥13 cm body length and can consume prey up to one third of their body length. The mean length of *O. mykiss* and *S. trutta* electrofished at Injesuthi and Monk’s Cowl measured 18.71 ± 4.62 cm and 17.93 ± 3.31 cm respectively suggesting that most fish would be capable of ingesting tadpoles in the 5.31 - 5.83 cm size class range. This suggests that tadpoles of ≤ 6.0 cm (- 1 year) would fall well within the gape limitations of *O. mykiss* and *S. trutta* populations at Injesuthi and Monk's Cowl respectively.

Large trout of approximately 30cm TL capable of ingesting a tadpole of 10.0 cm (third trout length) were not caught during field sampling. These findings confirm the results of an earlier field survey in which it was found that indigenous Natal Mountain Catfish (*A natalensis*) "co-existed" with rainbow trout in a 1:2 ratio survey (Karssing *et al.*, 2007). In addition to the size effects of predation, Crowley (1978) and Crowder & Cooper (1982) suggest that greater habitat structural complexity may reduce predator efficiency, which may then allow the persistence of both predators and prey. Sampling sites located below the selected waterfalls at Injesuthi and Monk's Cowl both displayed complex benthic structure, potentially affording the tadpoles a certain degree of cover from trout predation. *H. natalensis* tadpoles have been observed being active at night (Passmore & Carruthers, 1995) which may further protect them from predatory fish like trout.

Heyer *et al.* (1975) suggested that predation by aquatic predators (primarily fish) was the most important biotic factor influencing the temporal and spatial composition of tadpole assemblages. He further implied that tadpole size, use of microhabitat refugia, and un-palatability were possible mechanisms allowing tadpoles to survive with predatory fish. *H. natalensis* tadpoles were predominantly electrofished in the crevices found between gravel, stones and boulders in fast flowing riffle areas free of sand and silt deposits (Table 3.18). Results from this study suggest that the complex nature of the benthic structure (Figure 3.3) and availability of cover at sites occurring below the selected waterfalls possibly offers the *H. natalensis* a certain amount of protection against predation by trout. The incidence of tadpoles forming small social groups at sampling sites located below the selected waterfalls was also reduced when compared to sampling sites above the waterfall. This phenomenon may simply be related to a reduced capacity of *H. natalensis* to form social groups based on reduced abundance, or alternatively, a higher order behavioural response to evade predators.

4.2.5 Extinction, isolation and fragmentation of *H. natalensis* tadpole populations within the UDPWHS.

Bradford *et al.*, (1993) proposed that fish could also impact populations by isolating remaining populations. They reported that fish introductions into lakes in the Sequoia and King Canyon National Parks have resulted in a fourfold reduction in effective *R. muscosa* population sizes and a tenfold reduction in connectivity between these populations. Amphibian populations can be reduced to such low numbers by alien predators that they will probably become isolated from other populations and may ultimately disappear. Increased isolation of remaining *R. muscosa* populations could result in increased inbreeding with a decrease in genetic diversity (Reh & Seitz, 1990). This can lead to populations becoming less resilient to extinction and environmental change. In the John Muir Wilderness area, alien trout occupy 90 % of the total water body in study areas with the result that the *R. muscosa* population has become restricted to extremely isolated marginal habitats. They now more than likely represent non-equilibrium meta-populations where the extinction rate exceeds the colonisation rates (Bradford *et al.*, 1993; Hanski & Simberloff, 1997). Results from this study similarly indicate that the *H. natalensis* populations in the UDPWHS exist as isolated populations above waterfalls. Recent studies have demonstrated that multiple stressors, when combined, can impact on amphibians severely (Relyea & Mills, 2001; Blaustein & Kiesecker, 2002). Stochastic events in the UDPWHS such as flooding (exacerbated by too-frequent burning of the river catchment, either due to runaway fires or poor catchment management) could lead to the *H. natalensis* habitat becoming progressively clogged by silt, sand and other debris. Sedimentation of the in-stream habitat of *H. natalensis* populations in the UDPWHS (where they currently occur) in conjunction with trout predation below waterfalls could be a major threat to the continued persistence of *H. natalensis* populations in the UDPWHS. Kats & Ferrer (2003) suggest that surveys where alien predators and amphibians co-exist may reflect a more recent colonisation by the aliens. Surveys in those instances might capture a ‘snapshot’ of a longer process that might lead ultimately to complete elimination of amphibians by alien predators (Kats & Ferrer, 2003). This may also be the case in the UDPWHS where *H. natalensis* populations have observably become isolated to living above waterfalls (Karssing & Craigie, 2004a, 2004b; Karssing

& Mickleburgh, 2005) following the introduction and acclimatisation of trout since 1890 (Crass, 1964).

CHAPTER 5

5. CONCLUSION

5.1 Geo-physical variability between sampling sites.

Resul**ts** indicate that sampling sites located above both the selected water falls at Injesuthi and Monk’s Cowl are similar at the landscape level in respect of geology, soils, broad vegetation type and water yield. Results also suggest that Natal Cascade Frog *H. natalensis* tadpoles are tolerant of a broad range of physical environmental conditions in respect of altitude, mean precipitation, gradient, riparian vegetation and site aspect differences. Commonality existed between all sampling sites in respect of river biotope, benthic structure, river bank and riparian vegetation type, river width, surface area, depth, current velocity and water quality. River health conditions at all sampling sites are good. The thermal regime of water temperatures occurring both above and below the waterfalls at Injesuthi and Monk’s Cowl is similar. A series of Principle Component Analyses (PCA) conducted at a landscape level suggested that all sampling sites were similar and that differences only occurred regionally in respect of mean precipitation and altitude. Results of a geomorphologic and hydrological PCA conducted between selected sampling sites confirmed that sampling site IN1, located above the selected waterfall at Injesuthi, is closely associated with sampling site M6, and located below the selected waterfall at Monk’s Cowl.

5.2 Seasonal electrofishing results.

5.2.1 Abundance of *H. natalensis* populations in association with trout below the selected waterfalls.

Despite the similarity in geo-physical environmental habitat conditions existing between sampling sites electrofishing results confirmed that the abundance of *H. natalensis* tadpoles is seasonally significantly higher above the selected waterfalls in the absence of predatory fish, versus sampling sites located below the selected waterfalls which are inhabited by alien *S. trutta* and/ or rainbow trout *O. mykiss*. The abundance of *H. natalensis* populations above the selected waterfall at Injesuthi and Monk's Cowl is 4.69 x and 15.71 x higher than sampling sites located below the waterfall, in the presence of alien *O. mykiss* and *S. trutta*. These findings concur with the findings of other researchers who found decreases in the abundance of indigenous amphibians in the presence of alien trout.

5.2.2 Spawning, longevity, survival, condition factor and clustering of *H. natalensis* tadpoles.

This study indicated that *H. natalensis* tadpoles take approximately two years to metamorphose into adults and that the latter have a spawning period between spring and summer following the first seasonal rains. Results also suggest that prey selection relative to size is occurring below the waterfalls and that the large surviving tadpoles occurring below waterfall have assumedly outgrown the gape of resident trout. *H. natalensis* tadpole populations, occurring below the selected waterfalls, might be advantaged by the cover provided by the complex nature of the stream bottom in respect of gravel, rounded stones and boulders, typical of a headwater stream, but because the mean condition factor (CF) of tadpoles occurring below the waterfalls is comparatively lower than those of sampling sites occurring above, presence of trout may affect growth rate. The observed movement by tadpoles at night may also limit predation.

5.2.3 Niche overlap and habitat preferences between *H. natalensis* tadpoles and trout.

H. natalensis tadpoles have a significant preference for shallow, fast flowing, riffle water associated with gravel, stones and boulders that are free of sedimentation by sand and silt. The tadpoles selected runs in the absence of riffles in the low flow spring sampling period. In contrast, trout prefer deeper, slower flowing pools and runs. The greatest degree of habitat overlap occurred in runs dominated by gravel, stone and boulders, which was more likely to occur during spring in the general absence of riffle habitat. This suggests that this is potentially the seasonal period when predation is greatest, since trout populations are at their highest, and the abundance of *H. natalensis* populations at its lowest below waterfalls. Grid based modeling, relative to specific preferences of *H. natalensis* tadpoles for specific river biotope, benthic structure and depth indicated that the highest proportion of suitable habitat occurs above the selected waterfalls at Injesuthi and Monk's Cowl, with proportionally comparatively less favourable habitat conditions occurring below the waterfalls.

5.3 Limitations of the research project.

Limitations to the project included combining both species of trout into a single unit and referring to them as trout relative to the moderately low abundance of specimens sampled (n=83) sampled in the survey. Full site details, representing the geo-physical environmental conditions existing at all 12 sampling sites, was only collected at four sampling sites, each located furthest upstream and downstream of the two selected waterfalls. Considering a normal river continuum, geo-physical differences between sampling sites is likely to increase the greater the distance each site is located from the waterfall midpoint access point. This essentially weakens the argument in favour of trout predation being primarily accountable for the decline of *H. natalensis* populations occurring below each selected waterfall.

5.4 Ecological importance of waterfalls relative to the conservation of *H. natalensis* populations in the UDPWHS in association with trout.

Results conclusively indicate that waterfalls which function as a barrier to the upstream migration of predatory fish (alien trout, in this instance) are vitally important for the continued conservation of *H. natalensis* populations in the UDPWHS. This study also indicates that smaller waterfalls, which partially limit the upstream migration of trout, might also contribute to a greater abundance of *H. natalensis* occurring below larger waterfalls. This study also confirms findings of earlier field surveys conducted (Karssing & Craigie, 2004a, 2004b; Karssing & Mickleburgh, 2005) indicated that *H. natalensis* populations in the UDPWHS mainly occur as isolated populations above waterfalls. The fragmentation of *H. natalensis* populations in the UDPWHS consequently increases their vulnerability to stochastic events such as floods, droughts and runaway fires. These threats might be exacerbated by poor catchment management that potentially compromise favourable *H. natalensis* riffle habitat through sedimentation.

5.5 Management recommendations for controlling trout populations within the UDPWHS.

In theory, the best long term remedial option is the complete eradication of alien trout from streams in the UDPWHS. However, this management option is difficult to achieve in practice due to the large size and aggressive nature of many of the UDPWHS rivers. For these reasons, electrofishing is not considered to be a suitable eradication tool in the UDPWHS. Field surveys I have conducted (Karssing & Craigie, 2004a,b; Karssing *et al*., 2004; Karssing & Mickleburgh, 2005; Karssing *et al.* 2007) have also indicated an inherent lack of suitable sites in the UDPWHS in which trout can be eradicated between waterfalls, thus extending the effective downstream range of *H. natalensis* tadpoles. I currently propose that a catch and kill policy be adopted for trout in all rivers of the UDPWHS with no limitations imposed upon the type of recreational fishing tackle used.

5.6 **Future research.**

On a global scale, future research needs to identify cost-effective methodologies that can selectively eradicate alien species such as trout without causing further harm to non-target indigenous fauna and associated ecosystems. Future research derived from this project needs to establish whether down-stream drift contributes to the overall abundance of *H. natalensis* populations occurring below waterfalls in the UDPWHS, whether adults voluntarily spawn in these lower areas, and what proportion of the downstream drift, if any, is lost to trout predation. Research needs to be conducted on the perceived vulnerability of *H. natalensis* tadpoles to trout predation respective to the body lengths of both predator and prey. The analyses of the gut contents of captured trout by fisherman could reveal the length preferences of tadpole prey as well as the upper size limits. It might also point to the vulnerability of the earliest larval stages.

REFERENCES

Allen, K.R. 1951. The Horokiwi stream: a study of a trout population. *N. Z. Mar. Dep. Fish. Bull.* 10:1-238.

Altig, R. & Brodie, E.D. 1972. Laboratory behaviour of *Ascaphus truei* tadpoles. *J.Herpetol.* 6:21-24.

Anon. 2000. Conservation Assessment and management Plan for Southern African Frogs (2000). Cape Town, South Africa. <http://www.cbsg.org/reports/reports/exec.sum/so_africa_frog_camp.pdf.> Accessed July 2009.

Anstis, M. & Littlejohn, M.J.1996. The breeding biology of *Litoria subglandulosa* and *L.citropa* (Anura: Hylidae), and a re-evaluation of their geographic distribution. *Trans.R.Soc.S.Aust.*122:33-34.

Aronsson, S & Stenson, J.A.E. 1995. Newt-fish interactions in a small forest lake. *Amphibia-Reptilia.* 16:177-184.

Avery, A. 2007. Rachel Carson Syndrome Case 1: The Disappearance of Yellow –legged Frogs. <http://www.cfgi.org/materials/key_pubs/rachel-carson-syndrome-yellow-legged-frogs.ttm>. Accessed July 2009.

Bachman, R. 1984. Foraging behavior of free-ranging wild and hatchery brown trout in a stream. *Trans.Amer.Fish. Soc.* 113:1-32.

Balon, E.K. & Bruton, M.N. 1986. Introduction of alien species or why scientific advice is not heeded. *Env. Biol. Fish.* 16:225-230.

Berger, L., Speare, R., Daszak, P., Green, D.E., Cunningham, A.A., Goggin, C.L., Slocombe, R., Ragan, N.A., Hyatt, A.D., Mc Donald, K.R., Hines, H.B., Lips, K.R., Marantelli, G., Parkes, H. 1998. *Chytridiomycosis causes amphibian mortality associated with population declines in the rainforests of Australia and Central America.* Ed. R. May. University of Oxford, U.K. National Academy of Sciences.

Blaustein, A.R., Hokit, D.G, O'Hara, R.K. & Holt, R.A. 1994. Pathogenic fungus contributes to amphibian losses in the Pacific Northwest. *Bio.Cons.*67:251-254.

Blaustein, A.R. & Kiesecker, J.M. 2002. Complexity in conservation: lessons from the global decline of amphibian populations. *Eco.Lett.* 5:1-2.

Boussu, M.F. 1954. Relationship between trout populations and cover on a small stream. *J. Wild.Mangt.* 18:229-239.

Boustead, N.C. 1982. Fish diseases recorded in New Zealand, with a discussion on potential sources and certification procedures. *N. Z .Min Agric. Fish., Res. Div, Occ. Publs.* 34:1-19.

Boycott, R.C. 2004. Natal Ghost Frog *Heleophyrne natalensis* Hewitt, 1913. Pp.100-101 in Minter, L.R., M.Burger, J.A.Harrison, H.H.Braack, P.J.Bishop, and D.Kloepfer, eds. A*tlas and Red Data Book of the frogs of South Africa, Lesotho and Swaziland.* SIMAB Series #9. Smithsonian Institute, Washington, DC.

Bradford, D.F. 1989. Allotropic distribution of native frogs and introduced fishes in high Sierra Nevada lakes: implication of the negative effect of fish introductions. *Copeia.*1984 (4):966-976.

Bradford, D.F. 1991 Mass mortality and extinction in a high-elevation population of *Rana mucosa*. *J.Herpetol.* 25:174-177.

Bradford, D.F., Graber, D.M. & Tabatabai, F. 1993. Isolation of remaining populations of the native frog, *Rana muscosa,* by introduced fishes in Sequoia and Kings Canyon National Parks, California. *Conserv.Biol.* 7:882-888.

Bradford, D.F., Cooper, S.D., Jenkins, T.M., Kratrz, K., Sarnelle, O. & Brown, A.D. 1998. Influences of natural acidity and introduced fish on faunal assemblages in Californian Alpine Lakes. *Can. J. Fish. Aquatic Sci*. 55:2478-2491.

Braňa, F., Frechilla, L, Orizaola, G. 1996. Effect of introduced fish on amphibian assemblages in mountain lakes of northern Spain. *Herpetol. J.* 6:145-148.

Branch, W.R. 1994. Let their songs be heard: frogs – jewels of the wetlands. *Africa, Environment and Wildlife.* 2:40–47.

Brattstrom, B.H. & Warren, J.W. 1955. Observations on the ecology and behaviour of the Pacific Treefrog, *Hyla regilla. Copeia.*1955:181-191.

Bright, C. 1995. Bio-invasions: the spread of exotic species. *World Watch.* 1995:10-19.

Brodie, E.D. (Jnr), Formanowicz, D.R. (Jnr), & Brodie, E.D. 1978. The development of noxiousness of *Bufo americanus* tadpoles to aquatic insect predators. *Herpetol. J* 34:302-306.

Brown, L.R., Gardner, G. & Halweil, B. 1999. *Beyond Malthus: Nineteen dimensions of the population challenge*. Washington, DC: Worldwide Institute.

Bruton, M.N. 1986. Life history stages of invasive fishes in Southern Africa. In: *The ecology and management of biological invasions in southern Africa.* I.A.W. Mc Donald, F.J. Kruger & A.A. Ferrar (Eds). A.A.Balkema, Cape Town, South Africa. pp. 201-209.

Bruton, M.N. 1995.Have fishes had their chips? The dilemma of threatened fishes. *Enviro.Bio.Fishes Journal.* 43:1-27.

Bull, E.L and Marx, D.B. 2002. Influence of fish and habitat on amphibian communities in high elevation lakes in north-eastern Oregon. *Northwest Sci.* 76:240 - 248.

Bump, G. 1951. Game introductions, when, where, how? *Trans. 16th N. American. Wildl. Conf.* pp.316-325.

Burger, W.L. 1950. Novel aspects of the life history of two *Ambystomas species. J.Tenn. Acad. Sci.* 25:252-257.

Burnet, A.M.R. 1970. Seasonal growth of brown trout in two New Zealand streams. *N. Z. J. Mar. Freshwat. Res.* 4:55-62.

Burton, T.M & Likens, G.E. 1975. Salamander populations and biomass in the Hubbard Brook experimental forest, New Hampshire. *Copeia.* 1975:541-546.

Cambray, J.A. 1997. Eco-terrorist Trout. *Mail & Guardian*, May issue. p 27.

Cambray, J.A. 2003. Impact on indigenous species biodiversity caused by the globalization of alien recreational freshwater fishes. *Hydrobiologia.* 500:217-230.

Carruthers, V. 2001.Frogs and Frogging in Southern Africa. Cape Town. Struik Publishers (Pty) Ltd.

Carter, D. 1997. Maintaining wildlife naturalness in wilderness. *Int. J. Wilderness* 3(3):1-21.

Carufel, L.H. 1980. Construction and use of a velocity head rod for measuring stream velocity and flow. BLM/AK Technical Report 5. U.S. Department of the Interior. Bureau of Land Management. Alaska State Office.

Cassey, P., Blackburn, T., Duncan, R.P. & Gaston, K.J. 2005. Causes of exotic bird establishment across oceanic islands. *Proc. R. Soc. London,* Ser B.272

(1576):2059- 2063.

Channing, A. 2001.Amphibians of central and southern Africa. Cornell University Press, New York.

Chapman, D.W. 1966a. Food and space as regulators of salmonid populations in streams. *Amer. Nat.* 100:345-357.

Chapman, D.W.1966b. The relative contribution of aquatic and terrestrial primary producers to the trophic relations of stream organisms. *Pymatuning Lab. Field Biol.* 4:116-130.

Claska, M.E. & Gilbert, J.J. 1998. The effect of temperature on the response of *Daphnia* to toxic cyanobacteria. *Freshwater Biol.* 39:221-232.

Clements, J.1988. *Salmon at Antipodes*. John Clements, Ballarat.

Cockrum, E.L. 1962. *Introduction to mammalogy*. The Ronald Press Company, New York.

Cole, D.N. & Landres, P.B. 1996. Threats to wilderness ecosystems: impacts and research needs. *Eco.Appl.* 6:168-184

Cory, L. 1963. Effects of introduced trout on the evolution of native frogs in the high Sierra Nevada. J.A.Moore, (ed). *Proceedings of the XVI International Congress of Zoology*, 20-27 August 1963, Washington, D.C.

Cottam, C. 1950. The effects of uncontrolled introductions of plants and animals. *Proc. Pop. Int. Conf. Prot. Nat.* pp.408-415.

Cowx, I.G. 2002. Analysis of threats to freshwater conservation: past and present challenges. In: *Conservation of Freshwater Fishes*: *Options for the Future*. M.J. Collares-Pereira, M. Coelho & I. Cowx (Eds.). Oxford, Blackwell Science. pp 443-452.

Crass, R.S. 1964. Freshwater Fishes of Natal. Shuter & Shooter, Pietermaritzburg. McMillan South Africa (Publishers) (Pty) Ltd. P.O.Box X19, Northlands, Johannesburg, 2116.

Crass, R.S. 1986. Trout in South Africa. Mc Millan South Africa (Publishers) (Pty) Ltd,

Crawley, M.J. 1986. The population biology of invaders. *Phil. Trans. R. Soc. Lond., series* B. 314:711-731.

Crawley, M.J. 1987. What makes a community invasible? In: *Colonization, succession and stability.* A.J.Gray, M.J. Crawley & P.J.Edwards (Eds.). Oxford University Press, London. pp. 429-454.

Crawley, M.J. 1989. Chance and timing in biological invasions. In: *Biological invasions; a global perspective.* J.A. Drake, H.A. Mooney, F.di Castri, R.H. Groves, F.J. Kruger, M. Rejmanek & M. Williamson (Eds.) John Wiley and Sons, New York, pp. 407-423.

Crowder, L.B. & Cooper, W.B. 1982. Habitat structural complexity and the interactions between bluegills and their prey. *Ecology*. 63:1802-1813.

Crowell, K.L. 1973. Experimental zoogeography: introduction of mice to small islands. *Amer. Nat.* 107:535-58.

Crowley, P.H. 1978. Effective size and persistence of ecosystems. *Oceololgia.* 35:185-195.

Daszak, P., Berger, L., Cunningham, A.A., Hyatt, A.D., Green, D.E., Speare, R. 1999. Emerging infectious diseases and amphibian declines. *Emerg.Infect.Dis.* 5:735-748

De Angelis, D.L. 1975. Stability and connectance in food web models. *Ecology*. 56:238-243.

Diamond, J.M & Case, T.J. (Eds) 1986. In: *Community Ecology*. Overview: introductions, extinctions, exterminations, and invasions. Harper & Row, New York.

Dickens, C & Graham, M. 2002.South African Scoring System (SASS) version 5. Rapid Bio-assessment Method for Rivers. *South.Afr.J.Aquat.Sci*. 27:1-10.

Dickman, M. 1968. The effect of grazing by tadpoles on the structure of a periphyton community. *Ecology*. 49:1188-1190.

Drake, D.C. & Naiman, R.J. 2000. An evaluation of restoration efforts in fishless lakes stocked with exotic trout. *Conserv.Bio.* 14:1807-1820.

Drost, C.A. & Fellers, G.M. 1996. Collapse of a Regional Frog Fauna in the Yosemite Area of the Californian Sierra Nevada USA. *Conserv. Bio.* 10(2):414-425.

Duellman, W.E. & Trueb, L. 1986. *Biology of amphibians*. McGraw-Hill, New York.

Duff, D.A.1995. Fish stocking in U.S. federal wilderness areas: challenges and opportunities. *Int. J. Wilderness.* 1:17-19.

du Preez, L. & Carruthers, V. 2009. A Complete Guide to the Frogs of Southern Africa. Struik Nature, 80 McKenzie Street, Cape Town, 8001 South Africa. Pp 196-199.

Eaton, J.G. & Scheller, R.M. 1996. Effects of climate on fish thermal habitat in streams of the United States. *Limn. Oceanogr.* 41(5):1109-1115.

Ebenhard, T. 1989. Bank vole *Clethrionomys glareolus* Schreber 1780. Propagules of different sizes and island colonization. *J.Biogeogr.* 16:173-80.

Edwards, D.J. 1978. Salmon and trout culture in Norway. Fishing Books Limited, Farnham, Surrey, England. p.195.

Ehrlich, P.R. 1989. Attributes of invaders and the invading process. In: *Biological invasions; a global perspective.* J.A.Drake, H.A.Mooney, F.di Castri, R.H.Groves, F.J.Kruger, M.Rejmanek & Williamson (Eds). John Wiley and Sons, New York. pp.315-29.

Elton, C.S. 1958. *The ecology of invasions by animal and plants.* John Wiley, New York.

Endangered Wildlife Trust News, (2004). Flying in the Face of Conservation. The Fly Fishing Debate < http://www.ucsusa.org/ssi/invasive species/science-of-invasive-species.html>Date accessed 01/07/07.

Everest, F.H.1969. Habitat selection and spatial interaction of juvenile Chinook salmon and steelhead trout in two Idaho streams. Ph.D. Dissertation. University of Idaho, Moscow.

Fausch, K.D. 1988. Tests of competition between native and introduced salmonids in streams: what have we learned? *Can.J.Fish.Aquat.Sci.* 38:1220-1227.

Feminella, J.W. & Hawkins, C.P. 1994. Tailed frog tadpoles differentially alter their feeding behaviour in response to non-visual cues from four predators. *J. N. Am. Benthol. Soc.* 13(2):310-320.

Frigiel, C.R. & Semlitsch, R.D. 1990. Populations variation in survival and metamorphosis of larval salamanders (*Ambystoma maculatum*) in the presence and absence of fish predation. *Copeia* 1990(3):818-826.

Fox , M.D. & Fox, B.J. 1986. The susceptibility of natural communities to invasion. In: *Ecology of biological invasions: an Australian perspective.* R.H.Groves & J.J.Burdon (Eds.). Australian Academy of Science, Canberra, pp.57-67.

Fraley, J. 1996. Cooperation and controversy in wilderness fisheries management. *Fisheries.* 21:16-21.

Frost, W.E & Brown, M.E. 1967. *The Trout.* Collins, London.

Funk, W.C. & Dunlap, W.W. 1999. Colonization of high elevation lakes by long-toed salamanders (*Ambystoma macrodactylum*) after the extinction of introduced trout populations. *CanJ.Zool.* 77:1759-1767.

Gamradt, S.C., Kats, L.B. & Anzalone, C.B.1997. Aggression by non-native crayfish deters breeding in California newts. *Conserv. Bio.*11:793-796.

Gerber & Gabriel. 2002. Aquatic Invertebrates of South African Rivers Field Guide. Institute for Water Quality Studies. Department of Water Affairs and Forestry. Private Bag X313. Pretoria, 0001.

Giles, N. 1984. Development of the overheads fright response in wild and predator naïve three-spined sticklebacks, *Gasterosteus aculeatus. Anim. Behav.* 32:276-279.

Gillespie, G.R. 2001. The role of introduced trout in the decline of the spotted tree frog *Litoria spenceri* in south-eastern Australia. *Biol.Conserv.*100:187- 198.

Gillespie, G.R. & Hines, H.B.1999. Status of temperate riverine frogs in south-eastern Australia. In *Declines and Disappearances of Australian Frogs*. Campbell, A. (Ed.). Environment Australia, Canberra, pp.109-130.

Gillespie, G.R. & Hollis, G.J. 1996. Distribution and habitat of the spotted tree frog *Litoria spenceri* (Anura:Hylidae), and an assessment of potential causes of population declines. *Wildlife.Res.* 23:49-75

Glaw, F. and Kohler, J. 1998. Amphibian species diversity exceeds that of mammals. *Herpetol.J.* 29:11-14.

Global Invasive Species Programme. 2000. 100 of the World's Worst Invasive Alien Species. Lowe, S.J., M.Browne & S.Boudjelas. Published by the IUCN/SSC Invasive Species Specialist Group (ISSG), Aukland, New Zealand.

Gould, S.J. & Vrba, S. 1982. Exaptation: a missing term in the science form. *Paleobiology*. 8:4-15.

Grande, M., Muniz, I.P & Anderson, S. 1978. Relative tolerance of some salmonids to acid waters. *Theor. Appl. Limnol.* 20:2076-2084.

Grinnell, J. & Storer, T.I. 1924. Animal life in the Yosemite. University of California Press, Berkeley.

Gow, C.E. 1963. Notes on the habitat of *Heleophyrne rosei. Afr.Wildlife.* 17:113-116.

Gulve, P.S.1994. Distribution and extinction patterns within a northern metapopulation of the pool frog, *Rana lessonae. Ecology* 75:1357-1367.

Hamman, K. 2002. Let’s play the ball not the man. *Flyfishing.* 15:9-10.

Hanski, I. 1989. Metapopulation dynamics: Does it help to have more of the same? *Trend.Ecol.Evo.*4:113-115.

Hanski, I & Gilpin, M. 1991. Metapopulation dynamics: Brief history and conceptual domain. *Bio.J.Linn.Soc*. 42:3-16.

Hanski, I. & Simberloff, D. 1997. The metapopulation approach, its history, conceptual domain, and application to conservation. In: *Metapopulation biology: ecology, genetics, and evolution*. I.A.Hanski & M.E.Gilpin (Eds.). Academic Press, San Diego. pp. 5-26.

Hartman, G.F.1963. Observations on behaviour of juvenile brown trout in a stream aquarium during winter and spring. *J. Fish. Res. Board. Can.* 20(3):769-787.

Hayes, M.P. & Jennings, M.R. 1986. Decline of ranid species in western North America; are bullfrogs *Rana catesbeiana* responsible? *J.Herpetol.* 20:490-509.

Hayes, J.W. 1988. Mortality and growth of juvenile brown and rainbow trout in a lake nursery stream, New Zealand. *N. Z. J. Mar. Freshwat. Res.* 22:169-179.

Hawkins, C.P., Gottschalk, L.J. & Brown, S.S.1988. Densities and habitat of tailed frog tadpoles in small streams near Mt.St.Helens following the 1980 eruption. *J.N. Am.Benthol.Soc.* 7:246-252.

Hazzard, A.S. 1946. Warm water fish management. *Calif. Fish. Gam.* 32(1):19-26.

Heacox, C.E. 1974. The complete brown trout. Winchester Press, New York, 182 pp.

Herbst, E. 1997. "Eco-terrorist trout" swims against flow. *Mail & Guardian.* p.25.

Hecnar, S.J. & Mc Closkey, R.T. 1997. Spatial scale and determination of species status of the green frog. *Conser.Biol.* 11:670-682.

Hero, J-M, 2001. Antipredator Defenses Influence the Distribution of Amphibian Prey Species in the Central Amazon Rain Forest. *Biotropica.* 33(1):131-141.

Hey, S. 1926. Preliminary Report on the Inland Waters of South Africa with Regard to the Suitability for the Introduction of Edible Fish. Cape Town: Department of Mines and Industries. p.140.

Heyer, W.R., McDiarmid, R.W. & Weigmann, D.L. 1975. Tadpoles, predation and pond habitats in the tropics. *Biotropica.* 7:100-111.

Hobbs, D.F. 1955. Do newly introduced species present a separate problem? *Proc. N. Z. Ecol. Soc.* 2:12-14.

Holomuzki, J.R.1995. Oviposition sites and fish-deterrent mechanisms of two stream anurans. *Copeia.* 1995:607-613.

Holomuzki, J.R. 1986. Predator avoidance and diel patterns of use of larval tiger salamanders. *Ecology.* 67:737-748.

Holway, D.A & Suarez, A.V. 1999. Animal behaviour: an essential component of invasion biology. *Trend.Ecol.Evo.* 14:328-330.

Honegger, R.E. 1978. Amphibiens et retiles menaces en Europe. Conseil de I'Europe, Strasbourg.

Hunt, R.L. 1971. Responses of brook trout population to habitat development in Lawrence creek. *Wisconsin Dept. Nat. Res. Tech. Bull. 48,* Madison. 33 pp.

Hunter, C.J., Palmer, T. & Meloy, E. 1990. Better Trout Habitat – A Guide to Stream Restoration and Management. Island Press, Washington , D.C.

Hunter, D. & Gillespie, G.R.1999. The distribution, abundance and conservation status of riverine frogs in Kosciuszko National park. *Australian Zoologist.* 31:198-209

Hutchinson, R.P. 1971. The effect of fish predation on the zooplankton of ten Adirondack Lakes, with particular reference to the alewife, *Alosa pseudoharengus. Trans. Amer. Fish. Soc.*100:325-335.

Idyll, C. 1942. Food of rainbow, cutthroat and brown trout in the Cowichan River System, British Columbia. *J. Fish. Res. Board Can.* 5:448-458.

Ikuta, K., Shikama, T., Oda, S. & Okumoto, N. 1992. Acid tolerance of eyed embryos and larvae in salmonid fishes. *Bull. Nat. Res. Inst. Aquaculture* (*Japan*). 21:39-45.

Impson, N.D., 2001. *When yellowfish become an invasive alien species – mistakes of the past*. In: Proceedings of the 5th Yellowfish Working Group Conference. P.M.Arderne & M.Coke. (Eds). Himeville, KwaZulu-Natal. pp.68-62.

IUCN 2004. The world Conservation Union. ROSA. Regional office for southern Africa. The Ghost Family (2004): Six Amphibians Exclusive to Southern Africa.. Third IUCN World Conservation Congress, 17-25 November, Bangkok, Thailand. < http://www.iucnrosa.org.zw/news/ghost_frogs.htm .> Date accessed 01/07/07.

IUCN Invasive Species Specialist Group (ISSG).< http://www.issg.org/ >. Accessed 9 May 2010

Jackson, P.D. 1981. Trout introduced into south-eastern Australia: their interaction with native species. *Victorian Naturalist.* 98:18-24.

Jameson, D.L. 1956. Growth, dispersal, and survival of the Pacific tree frog. *Copeia.* 1956:25-29.

Janzen, D.H.1986. *The eternal external threat*. In:. Conservation Biology: the science of scarcity and diversity. M.E.Soulé, (Ed). Sinnauer Associates, Sunderland, Massachusett. pp. 286-303.

Jennings, W.B., Bradford, D.F & Johnson, D.F. 1992. Dependence of the garter snake *Thamnophis elegans* on amphibians in the Sierra Nevada of California. *J. Herpetol.* 26:503-505.

Jörgen I. J, Carlsson, M & Sundström, F.L. 2000. Habitat Preference Increases Territorial Defence in Brown Trout (*Salmo trutta*). *Behav.Ecol.Sociobiol.* 48(5):373-377.

Kalleberg, H. 1958. Observations in a stream tank of territoriality and competition in juvenile salmon and trout *Salmo salar* L. and *Salmo trutta* L. *Rep. Inst. Freshwat. Res. Drottninghol.* 39:55-98.

Karlstrom, E.L. 1962. The toad genus *Bufo* in the Sierra Nevada of California; ecological and systematic relationships. *Univ. Calif. Publ. Zool.* 62:1 -104.

Karssing, R.J. & Craigie, J. 2004a. Trout free zones within the UDPWHS. Kamberg Nature Reserve. Unpublished Ezemvelo KZN Wildlife Field Survey Report.

Karssing, R.J. & Craigie, J. 2004b. *Trout free zones within the UDPWHS. Monks Cowl Nature Reserve.* Unpublished Ezemvelo KZN Wildlife Survey Report.

Karssing, R.J., Craigie.J., Howell, A. 2004. *Trout free zones within the UDPWHS, Spitsberg Stream, Wonder Valley, Monks Cowl Nature Reserve*. Unpublished Ezemvelo KZN Wildlife Field Survey Report.

Karssing, R.J. & Mickleburgh, K. 2005. *Trout free zones within the UDPWHS, nDedema River, Cathedral Peak Nature Reserve*. Unpublished Ezemvelo KZN Wildlife Field Survey Report.

Karssing, R.J., Rivers-Moore, N., Nkosi, M. 2007. *Field survey of the Injesuthi River, Giant's Castle Game Reserve.* Unpublished Ezemvelo KZN Wildlife Field Survey Report.

Kats, L.B. & Dill, D.M. 1998. The scent of death: chemosensory assessment of predation risk by prey animals. *Ecoscience.* 5:361-394.

Kats, L.B & Ferrer, R.P. 2003. Alien predators and amphibian declines: Review of two decades of science and transition to conservation. *Diversity and Distributions.* 9:99-110.

Kats, L.E., Petranka, J.W. & Sih, A. 1988. Antipredator defenses and the persistence of amphibian larvae with fishes. *Ecology*. 69(6):1865-1870.

Kiescker, J.M. 2003. Invasive species as a global problem: insights towards understanding the worldwide decline of amphibians. Amphibian Conservation. (Ed. R.D.Semlitsh). Smithsonian Press, Washington D.C. in press.

Kiesecker, J.M., Blaustein, A.R., Miller, C.L. 2001. The transfer of a pathogen from fish to amphibians. *Conserv.Bio.* 15:1064-1070.

Kirby, R. 1997. The sucked thumb is a great storyteller. *Mail & Gaurdian.* May 1997. p.19.

Knapp R.A. 1996. Nonnative trout in natural lakes of Sierra Nevada: an analysis of their distribution and impacts on native aquatic biota. Sierra Nevada ecosystem project: final report to the Congress, Vol III, Centers for Water and Wildlife Resources, University of California, Davis, CA. pp. 363-407.

Knapp, R.A. & Matthews, K.R. 1998. Eradication of non-native fish by gill-netting from a small mountain lake in California. *Restor.Ecol.* 6:207-213.

Knapp, R.A. & Matthews, K.R. 2000. Non-Native Fish Introductions and the Decline of the Mountain Yellow-Legged Frog from within Protected Areas. *Conserv.Biol.* 14(2): 428-438.

Knapp, R.A., Matthews, K.R. & Sarnelle, O. 2001a. Resistance and resilience of alpine lake fauna to fish introductions. *Ecol.Monogr.* 71:401-421.

Knapp, R.A., Corn, P.S. & Schindler, D.E. 2001b. The Introduction of non-native Fish into Wilderness Lakes: Good Intentions, Conflicting Mandates, and Unintended Consequences. *Ecosystems.* 4:275-278.

Kolar, C.S. & Lodge, D.M. 2000. Freshwater non-indigenous species: interactions with other global changes. In: *Invasive species in a changing world.* (H.A.Mooney & R.J.Hobbs(Jnr)(Eds.), Island Press, Washington, D.C. pp. 3-30.

Kruse, K.C. & Francis, M.G. 1977. A predation deterrent in the larvae of the bullfrog, *Rana catesbeiana. Trans. Am. Fish. Soc* 106:248-252.

Kruse, K.C & Stone, B.M. 1984. Largemouth bass *Micropterus salmoides* learn to avoid feeding on toad (*Bufo*) tadpoles. *Anim. Behav.* 32:1035-1039.

L'abée-Lund, J.H.; Langeland, A. & Sægrov, H. 1992. Piscivory by brown trout *Salmo trutta*(L) and arctic charr *Salvelinus alpinus*(L) in Norwegian Lakes. *J.Fish.Biol.* 41(1):91-101.

Lambiris, A.J.L. 1990. Presence, Distribution, Population Size, Survival Requirements, Threats to and Importance of Amphibians in Natal. Natal Parks Board Internal Report. Po.Box 13069. Cascacades, 3202. South Africa.

Lawler, S.P.1989. Behavioural responses to predators and predation risk in four species of larval anurans. *Anim. Behav.* 38:1039-1047.

Lawler, S.P, Dritz, D., Strange, T. & Holyoak, M. 1999. Effects of introduced mosquitofish and bullfrogs on the threatened California red-legged frog. *Conserv.Biol.* 13:613-622.

Lawler, K. & Hero, J-M. 1997. Palatability of *Bufo marinus* tadpoles to a vertebrate fish predator decreases with development.*Wildl. Res.* 24:324-327.

Lawton, J.H. & Brown, K.C. 1986. The population and community ecology of invading insects. *Phil. Trans. R. Soc. Lond.* Series B, 314:607-618.

Leavitt, P.R., Schindler, D.E., Paul, A.J., Hardie, A.K. & Schindler, D.W. 1994. Fossil pigments records of phytoplankton in trout-stocked alpine lakes. *Can. J. Fish. Aquat. Sci.* 51:2411-2423.

Lever, C. 1996. Naturalized fishes of the world. Academic Press, New York.

Lewis, S.L. 1969. Physical Factors influencing Fish Populations in Pools of a Trout Stream. *Trans. Amer. Fish. Soc.* 98:14-19.

Li, H.W. & Moyle, P.B. 1981. Ecological analysis of species introductions into aquatic systems. *Trans. Amer. Fish. Soc.* 110:772-782.

Licht, L.E. 1968. Unpalatability and toxicity of toad eggs. *Herpetologica.* 24:93-98.

Liem, K.F. 1961. On the taxonomic status and granular patches of the Javanese frog, *Rana chalconota*. *Herpetologica.* 17:69-71.

Liss, W.J & Larson, G.L. 1991. Ecological effects of stocked trout on North Cascades Naturally Fishless Lakes. *Park Science.*11:22-23.

Longcore, J.E., Pessier, A.P., Nichols, R.D. 1999. *Batrachochytrium denderobatidis* gen. et sp. Nov., a chytrid pathogenic to amphibians. *Mycologia.* 91:219-227.

Loope, L.L & Mueller-Dombois, D. 1989. Chararcteristics of invaded islands with special reference to Hawaii. In: *Biological invasions; a global perspective.* J.A.Drake, H.A.Mooney, F.di Castri, R.H.Groves, F.J.Kruger, M.Rejmanek & Williamson (Eds.). John Wiley and Sons, New York, pp. 257-280.

Lowe, S.J., Browne, M. and Boudjelas, S. 2000. 100 of the world’s worst alien invasive species. Auckland, new Zealand, IUCN/SSC Invasive Species Specialist Group (ISSG).

Lynch, M.1979. Predation, competition and zooplankton community structure; an experimental study. *Limnol.Oceanogr.* 24:253-272.

Macan, T.T. 1966.The influence of predation on the fauna of a moorland fishpond. *Arch. Hydrobiol.* 61:432-452.

MacAuthur, R. 1955. Fluctuations of animal populations, and a measure of community stability. *Ecology.* 36:533-536.

MacCrimmon, H.R. & Marshall, T.L. 1968. World distribution of brown trout, S*almo trutta. J. Fish. Res. Board Can*. 25:2527-2548.

Maitland, P.S. 1972, A key to the freshwater fishes of the British Isles. *Freshw. Biol. Assoc*. 17:1– 37.

Manteifel, Y. 1995. Chemically-mediated avoidance of predators by *Rana temporaria. J. Herpetol.* 29: 461-463.

Marshall, T.L. & MacCrimmon, H.R. 1970. Exploitation of self-sustaining Ontario stream populations of brown trout S*almo trutta* and brook trout *Salvelinus fontinalis*. *J. Fish. Res. Board. Can.* 27(6):1087-1102.

Martinez-Solano I, Barbadillo L.J. & Lapena, M. 2003. Effect of introduced fish on amphibian species richness and densities at a montane assemblage in the Sieer de Neila, Spain. *Herpetol.J.*13:167-173.

Matthews, K.R., Pope, K.L., Preisler, H.K. & Knapp, R.A. 2001. Effects of non-native trout on Pacific Teefrogs *Hyla regilla* in the Sierra Nevada. *Copeia.* 2001:1130-1137.

Matthews, K.R., Knapp, R.A. & Pope, K.L. 2002. Garter snake distribution in high elevation ecosystems: is there a link with declining amphibians and nonnative trout introductions? *J.Herpetol.* 36:16-22.

McDowall, R.M. 1968. Interactions of native and alien faunas of New Zealand and the problem of fish introductions. *Trans. Am. Fish. Soc.* 97:1-14.

McDowall, R.M. 1984. Exotic fishes: the New Zealand experience. In *Distribution, biology and management of exotic fishes*. W.R.Coutenay & J.R.Stauffer, J.R. (Eds.). Hopkins Univ. Press, Baltimore, pp.200-214.

McDowall, R.M. 1987. Impacts of exotic fishes on the native fauna. In *Inland waters of New Zealand.* ed. A.B.Viner (Ed.). *DSIR Bull.* 241: 333-347.

McDowall, R.M. 1990a. *New Zealand freshwater fishes.* Heinemann Reed, Aukland.

McDowall, R.M. 1990b. When galaxiid and salmonid fishes meet – a family reunion in New Zealand. A.B.Viner (Ed). *DSIR Bull.* 241:333-347.

McDowall, R.M. 2003. Impacts of introduced salmonids on native galaxiids in New Zealand upland streams; a new look at an old problem. *Trans. Amer. Fish. Soc.*132:229-238.

McNeely, J.A. 2000. The future of alien invasive species: changing social views. In *Invasive species in a changing world.* H.A.Mooney & R.J.Hobbs (Eds.). Island Press, Washington, D.C. pp.171-189.

McNaught, A.S., Schindler, D.W., Parker, B.R., Paul, A.J., Anderson, R.S., Donald, D.B. & Agbeti, M. 1999. Restoration of the food web of an alpine lake following fish stocking. *Limno.Oceanogr*. 44:127-136.

Meffe, G.K. 1985. Predation and species replacement in American southwestern fishes: a case study. *Southwest. Nat.* 30:173-187.

Meffe, G.K., Hendrickson, D.A., Minckley, W.L. & Rinne, J.N. 1983. Factors resulting in the decline of the endangered Sonoran topminnow (Atheriniformes: Poeciliidae) in the United States. *Biol. Cons.* 25:135-159.

Meffe, G.K. & Carroll, C.R. 1997. *Principles of Conservation Biology.* Sinauer Associates Inc. Publishers, Sunderland.

Metter, D.E. 1964. A morphological and ecological comparison of the two populations of the tailed frog, *Ascaphus truei*. *Copeia.*1964:181-195.

Metter, D.E. 1967.Variation in the ribbed frog *Ascaphus truei* Stejneger. *Copeia.* 1967:634-649.

Mills, D.1971. Salmon and trout; A resource, its ecology, conservation and management. St Martin's Press, New York. 351 pp.

Minckley, W.L. 1973. Status of the razorback sucker, *Xyrauchen texanus* in the lower Colorado River system. *Southwest. Nat.* 28:165-187.

Mitchell, C.P. 1986. Effects of introducing grass carp on populations of two species of native fish in a small lake. *N. Z. J. Mar. Freshw. Res.* 20:219-230.

Molony, B. 2001. Environmental requirements and tolerances of Rainbow Trout *Oncorhynchus mykiss* and Brown Trout S*almo trutta* with special reference to Western Australia. Fisheries Research Report No. 130, Fisheries Research Division, WA Marine Research Laboratories, PO Box 20, North Beach, Western Australia, 6920.

Mooney, H.A. 1998. The Globalization of Ecological Thought. Oldedendorf, Germany: Ecology Institute.

Morin, P.J.1983. Predation, competition, and the composition of larval anuran guilds. *Ecol. Monogr.* 53:119-138.

Morin, P.J. 1986. Interaction between intra specific competition and predation in an amphibian predator-prey system. *Ecology.* 67:713-720.

Morin, P.J, Lawler, S.P & Johnson, E.A. 1990. Ecology and breeding phenology of larval *Hyla andersonii*: The disadvantage of breeding late. *Ecology.* 71:1 590-1 598.

Moulton, M.P. & Pimm, S.L. 1986. Species introductions into Hawaii. In: *Ecology of biological invasions of North America and Hawaii,* H.A.Mooney & J.A.Drake (Eds.). Springer, New York, pp.231-49.

Moyle, P.B. & Light, T. 1996. Biological invasions of fresh water: empirical rules and assembly theory. *Biol. Conserv*. 78:149-161.

Moyle, P.B. & Mount, J.F. 2007. Homogenized rivers, homogenized faunas. *Proc. Natl. Acad. Sci. USA*.104:5 711 – 5 712.

Myrick, C.A. & Cech. J.J (Jnr). 2004. Temperature effects on juvenile anadromous salmonids in California's central valley: what don't we know? *Reviews in Fish Biology and Fisheries.* 14:113-123.

National Geographic Magazine, April 2009.

National Marine Fisheries Service. 2004. Guidelines for Electrofishing Waters Containing Salmonids Listed Under the Endangered Species Act. NOAA Fisheries Service, 1315 EastWest Highway, Silver Springs, MD 20910.

Needham, P.R. 1969. Trout streams (Revised Edition – Carl F.Bond).Winchester Press, New York. 241pp.

New Hampshire Volunteer Assessment Programme (2007). Glossary of River Ecology Terms. < des.**nh**.gov/organization/commissioner/pip/.../vrap_**glossary**.pdf >. Accessed May 2010.

Newman, M.A. 1956. Social behaviour and interspecific competition in two trout species. *Physiol. Zool.* 29:64-81.

Northcote, T.G. 1988. Fish in the structure and function of freshwater ecosystems: a 'top-down' view. *Can.J.Fish.Aquat.Sci.* 45:361 –-379.

Nyström, P., Svensson, O., Lardner, B., Brönmark, C. & GranEli, W. 2001.The influence of multiple introduced predators on a littoral pond community. *Ecology.* 82:1023-1103.

Olsen, M.T., Lodge, D.M., Gapelli, G.M. & Houlihan, R.J. 1991. Mechanisms of impact of an introduced crayfish *Orconectes rusticus* on littoral congeners, snails and macrophytes. *Can.J.Fish.Aquat.Sci.* 48:1853-1861.

Orians, G.H. 1986. Site characteristics favouring invasions. In: *Ecology of biological invasions of North America and Hawaii.* H.A.Mooney & J.A.Drake (Eds.). Springer, New York, pp. 133-148.

Orr, R.T. 1970. Animals in migration. MacMillan, London, England.

Paine, R.T. 1966. Food web complexity and species diversity. *Am.Nat.*100:65-75.

Passmore, N.I. & Carruthers, V.C. 1995. South African Frogs. A Complete Guide. Published jointly by Southern Books (Pty) Ltd, P.O.Box 3103, Halfway House, 1685 and Witwatersrand University Press, Johannesburg, Private Bag 3, WITS, 2050.

Parker, B.R., Schindler, D.W., Donald, D.B. & Anderson, R.S. 2001. The effects of stocking and the removal of a non-native salmonid on the plankton of an alpine lake. *Ecosystems.* 4:334-345.

Pearl, C.A. & Adams, M.J. 2005. *The Cascades frog Rana cascade*. M.J.Lannoo (Ed.), Status and conservation of US amphibians, University of California, Berkeley. pp.538-540.

Pearsall, W.H. 1959. The ecology of invasion: ecological stability and instability. *New Biology*. 29: 95-101.

Pechmann, J.H.K., Scott, D.E., Semlitsch, R.D., Caldwell, J.P., Vitt, L.J. & Gibbons, W. 1991. Declining amphibian populations: The problem of separating human impacts from natural populations. *Science*. 253:892-895.

Pechmann, J.H.K & Wilbur, H.M. 1994. Putting declining amphibian populations into perspective: natural fluctuations and human impacts. *Herpetoligica.* 50:65-84.

Petranka, J.W., Kats, L.B., & Sih, A. 1987. Predator-prey interactions among fish and larval amphibians: use of chemical cues to detect predatory fish. *Anal.Behav.* 35:420-425.

Petranka, J.W. 1988. Salamanders of the United States and Canada. Smithsonian Institution Press, Washington, D.C.

Petrushevski , G.K. & Shulman, S.S. 1961. The parasitic diseases of fishes in the natural waters of the USSR. In: *Parasitology of fishes*. V.A. Dogiel,, G.K. Petrushevski & Y.I. Polyanski, (Eds.). Oliver Boyd Publishers, London..

Pilloid, D.S., Peterson, C.R. and Ritson, P.I. 2002. Evaluating effects of fish stocking on amphibian populations in wilderness lakes. In: Cole, D.N., Mc Cool, S.F., Borrie, W.T. and O'Loughlin, J. (Eds.). *Wilderness science in a time of change Conference*. Volume 5, Wilderness Ecosystems, Threats, and Management. Proceedings RMRS-P15-VOL-5. Ogden: USDA Forest Service, Rocky Mountain Research Station. pp.328 – 335.

Pimm, S.L.1980. Food web design and the effect of species deletion. O*ikos.* 35:139-149

Pimm, S.L. 1989. Theories of predicting success and impact of introduced species. In *Biological invasions: a global perspective.* J.A.Drake, H.A.Mooney, F.di Castri, R.H.Groves, F.J.Kruger, M.Rejmanek & Williamson (Eds.). John Wiley and Sons, New York, pp.351-388.

.

Pope, K.L & Matthews, K.R. 2001. Movement Ecology and Seasonal Distribution of Mountain Yellow-Legged Frogs *Rana muscosa* in a High-Elevation Sierra Nevada Basin. *Copeia*.2001(3):787-793.

Pope, K.L. 2007. Assessing Changes in Amphibian Population Dynamics Following Experimental Manipulations of Introduced Fish. *Conserv.Biol*. 22(6):1572-1581.

Porter, K.R.. 1972. Herpetology. Saunders, Pennsylvania.

Pough, F.H., Andrews, R.M., Cadle, J.E., Crump, M.L., Savitzky, A.H. & Wells, K.D. 1998. Herpetology. Prentice-Hall, New Jersey.

Power, M.E., Matthews, W.J & Stewart, A.J. 1985. Grazing minnows, piscivorous bass, and stream algae: dynamics of a strong interaction. *Ecology*. 66:1448-1456.

Reh, M.E. & Seitz, A. 1990. The influence of land use on the genetic structure of populations of the common frog *Rana temporaria.Biol.Conserv.* 54:239-249.

Resetaris, W.J & Wilbur, H.M. 1989. Choice of oviposition site by *Hyla chryoscelis*: role of predators and competitors. *Ecology* 70:220-228.

Reshetnikov, A.N. 2003. The introduced fish, rotans (*Perccottus glenii*), depresses populations of aquatic animals (macro invertebrates, amphibians, and a fish). *Hydrobiologia* 510:83-90.

Relyea, R.A. & Mills, N. 2001. Predator-induced stress makes the pesticide carbaryl more deadly to grey treefrog tadpoles *Hyla versicolor*.*P.Natl.AcaSci-Biol.* 98: 2 491-2 496.

Riley, S.P.D, Shaffer, H.B., Voss, S.R. & Fitzpatrick, B.M. 2003. Hybridization between a rare, native tiger salamander and its introduced congener. *Ecol.Appl.*13:1263-1275.

Rivers-Moore, N.A., Jewitt, G.P.W., Weeks, D.C. & O'Keefe, J.H. 2004. Water temperature and fish distribution in the Sabie River system: Towards the development of an adaptive management tool. WRC report no. 1065/1/04. Water Research Commission, Pretoria, South Africa.

Ross, S.T. 1991. Mechanisms structuring stream fish assemblages: are there lessons from introduced species? *Environ. Biol. Fish.* 30:59-68.

Roughgarden, J., Pacala, S. & Rummel, J.A. 1984. Strong present-day competition between the *Anolis* lizard population of St Maarlen Neth Antilles. In: *Evolutionary ecology*. B. Shorrocks (Ed.). Blackwell Scientific Publications, Oxford, pp.203-220.

Rowan, D.E. 1996. Effects of fish and habitat factors on benthic communities in high elevation lakes of the Sierra Nevada in California. Unpublished master's thesis. Las Cruces, N.M: New Mexico University.

Rowntree, K.M & Wadeson, R.A. 1999. A hierarchical geomorphological model for the classification of selected South African rivers. Water Research Commission Report 497/1/99, Pretoria. 334 pp.

Rummel, J.D. & Roughgarden, J. 1985. Effects of reduced perch height separation on competition between two *Anolis* lizards. *Ecology.* 66:430-44.

Runn, P.& Milbrink, G. 1977. Early development of three salmonid species in acidified water. *Zoon.* 5:127-132.

Russel, I.A. 1999. Freshwater fish of the Wilderness National Park. *Koedoe.* 42:73-78.

Russel, I.A. 2001. Freshwater fishes of Bontebok National Park. *Koedoe*. 44:71-77.

Schuck, H.A. 1943. Survival, population density, growth and movement of the wild brown trout in Crystal Creek. *Trans. Am. Fish. Soc.* 73(1):209-230.

Scott, W.B. & Crossman, E.J. 1973. Freshwater fishes of Canada. *Fish. Res. Bd. Canada. Bull.* 14.

Schaub, D.L. & Larson, J.H. 1978. The reproductive biology of the Pacific treefrog *Hyla regilla*. *Herpetologica.* 34:409-416.

Seale, D.B. 1980. Influence of amphibean larvae on primary production, nutrient flux, and competition in a pond ecosystem. *Ecology.* 61:1531-1550.

Seghers, B.H. 1974. Geographic variation in the responses of guppies *Poecilia reticulata* to aerial predators. *Oceologia.*14:93-98.

Semlitsch, R.D. 1987. Interactions between fish and salamander larvae. *Oecologia.* 72:482-486.

Semlitsch, R.D.& Gibbons, J.W.1988. Fish predation in size-structured populations of treefrog tadpoles. *Oecologia* . 75:321-326.

Semlitsch, R.D & Reyer, H.U. 1992. Modification of anti-predator behaviour in tadpoles by environmental conditioning. *J. Anim. Ecol.* 61:353-360.

.

Simberloff, D. 1981. Community effects of introduced species. In: *Biotic crises in ecological and evolutionary time*. M.H.Nitecki (Ed.). M.H. Academic Press, New York. pp. 53-81.

Simberloff, D. 1990. Community effects of biological introductions and their implications for restoration. In: *Ecological restoration of New Zealand island.* D.R.Towns, C.H.Daugherty & A.E.Atkinson (Eds.). *N.Z.Conserv. Sci. Publs*. No.2. pp. 61-76.

Simon, K.S. & Townsend, C.R. 2003. Impacts of freshwater invaders at different levels of ecological organisation, with emphasis on salmonids and ecosystem consequences. *Freshwater. Biol*. 48:982 – 994.

Sinsch, U. 1990. Migration and orientation in anuran amphibians. *Ethol.Ecol.Evol.*2:65-79.

Skelton, P.H., 1987. South African Red Data Book. Fishes. South African National Scientific programmes report No.137. Pretoria: CSIR.199 pp.

Skelton, P.H., 2001. A complete Guide to the Freshwater Fishes of Southern Africa. Struik Publishing, , Cape Town, South Africa.

Solomon, D.J.& Templeton, R.E. 1976. Movements of brown trout S*almo trutta* in a chalk stream. *J. Fish. Biol.* 9:411-423.

Soulé, M.E. & Sanjayan, M.A. 1998. Conservation targets-do they help? *Science.* 279:2060-2061.

Southern, H.N. 1979. The stability and instability of small mammal populations. In: Ecology of small mammals. D.M.Stoddart (Ed.). Chapman and Hall, London, England. pp.103-134.

Sredl, M.J.& Collins, J.P.1992. The interaction of predation, competition, and habitat complexity in structuring an amphibian community. *Copeia.* 1992:607-614.

Stebbins, R.C., 1985. Western reptiles and amphibians. Houghton Mifflin Company, Boston.

Stebbins, R.C & Cohen, N.W. 1995. A natural history of amphibians. Princetown University Press, New Jersey.

Stein, B.A., Kutner, L.S & Adams, J.S. 2000. Precious heritage: the status of biodiversity in the United States. Oxford University Press, Oxford.

Storer, T.I. & Usinger, R.L.1963. Sierra Nevada natural history. Univ.of California Press, Berkeley.

Stuckenberg, B.R. 1969. Effective temperature as an ecological factor in southern Africa. *Zoologica Africana.* 4:145-197.

Sullivan, K., Martin, D.J, Cardwell, R.D., Toll, J.E. & Duke, S. 2000. An analysis of the effects of temperature on salmonids of the Pacific Northwest with implications for selecting temperature criteria

<http://www.sei.org/downloads/reports/salmon/2000.pdf > Accessed on July 2009.

Swincer, D.E, 1986. Physical characteristics of sites in relation to invasions. In: *Ecology of biological invasions: an Australian perspective.* R.H.Groves & J.J.Burdon (Eds.). Australian Academy of Science, Canberra. pp.67-76.

Taylor, J.T. 1983. Orientation and flight behaviour of a neotenic salamander *Ambystoma gracile* in Oregon. *Am.Midl.Nat*. 109:40-49.

Taylor, J.T.1984. Comparative evidence for competition between the salamanders *Ambystoma gracile* and *Taricha granulose. Copeia.*1984:672-683.

Thoen, C., Bauwens, D. & Verheyen, R.F. 1986. Chemoreceptive and behavioural responses of the common lizard *Lacerta vivipara* to snake chemical deposits. *Anil.Behav.* 34:1805-1813.

Thorp, J.H. 1986. Two distinct roles for predators in freshwater assemblages. *Oikos.* 47: 75-82.

Tilzey, R.D.J. 1977. The key factors in the establishment and success of trout in Australia. *Proc. Ecol. Soc. Aust.* 10:97-105.

Townsend, C.R. 1991. Exotic species management and the need for a theory of invasion ecology. *New.Zeal.J.Ecol.* 15:1-3.

Townsend, C.R. 2003. Individual, Population, Community, and Ecosystem Consequences of a Fish Invader in New Zealand Streams. *Conserv.Bio.* 17(1):38-47.

Townsend, C.R. & Crowl, T.A. 1991. Fragmented population structure in a Native New Zealand fish: an effect of introduced brown trout? *Oikos.* 61:347-354.

Townsend, C.R. 1996. Invasion biology and ecological impacts of brown trout *Salmo trutta* in New Zealand. *Bio.Conserv.*78:13-22.

Tyler, M.J.1997. The Action Plan for Australian Frogs. Biodiversity Group, Environment Australia, Canberra.

Tyler, T., Liss.W.J., Ganio, L.M., Larson, G.L, Hoffman, R., Deimling, E. & Lomnicky, G. 1998. Interaction between Introduced trout and Larval Salamanders *Ambystoma macrodactylum* in High-Elevation Lakes. *Conserv.Biol.* 12(1):94-105.

US Wildlife Report. 1986. Habitat suitability Index Models and Instream Flow Suitability Curves:Brown Trout. Biological Report 82 (10.124). Natural wetlands research Centre, US Fish and Wildlife Service, 700 Cajundome Boulevard, Lafayette, LA, 70506.

Vannote, R.L. & Sweeney, B.W. 1980. Geographic analysis of thermal equilibria: A conceptual model for evaluating the effect of natural and modified thermal regimes on aquatic insect communities. *Am.Nat*. 115:667-695.

Vaughn, T.A. 1986. Mammalogy. W.B. Saunders, New York.

Voris, H.K & Bacon, J.P. (Jnr.). 1966. Differential predation on tadpoles. *Copeia.*1966: 594-598.

Wadeson, R.A. 1994. A geomorphololological approach to the identification and classification of instream flow requirements. Southern African Journal of Aquatic Sciences. 20(1/2):38-61.

Wager, V.A. 1965. Frogs of South Africa. Purnell & Sons. Cape Town.

Waldman, B .1982. Sibling association among schooling tadpoles; field evidence and implications. *Anim. Behav.* 30:700-713.

Wassersug, R.J. 1971. On the comparative palatability of some dry-season tadpoles from Costa Rica. *A.Midl.Nat.*. 86:101-109.

Watson, G.F., Littlejohn, M.J., Hero, J-M., Robertson, P. 1991. Conservation Status, ecology and Management of the Spotted Tree Frog *Litoria spenceri*. Authur Rylah Institute Technical report Series No. 116. Department of Conservation and Environment: Australia.

Wedekind, H., Hilge.V. & Steffens, W. 2001. Present status, and social and economic significance of inland fisheries in Germany.*Fisheries.Manag.Ecol*. 8(4-5):405-414.

Weithman, A.S. & Haas, M.A. 1984. Effects of dissolved-oxygen depletion on the rainbow trout fishery in Lake Taneycomo, Missouri. *Trans. Am. Fish. Soc.* 113:109-124.

Weldon, P.J. & Burghardt, G.M. 1979. The ophiophage defensive response in crotaline snakes: extension to new taxa. *J.Chem.Ecol.*, 5:141-151.

Welty, J.C., & Baptista, L. 1988. The life of birds. Saunders, New York

Wesche, T.A. 1980. The WRRI trout cover rating method. Water Resour. Res. Inst., Laramie, Wy. Water Resourc. Ser. 78. 46 pp.

Williamson, M. 1989. Mathematical models of invasion. In: *Biological invasions: a global perspective.* J.A.Drake, H.A..Mooney, F.di Castri, R.H. Groves, F.J.Kruger, M.Rejmanek & M.Williamson (Eds.). John Wiley and Sons, New York, pp. 329-350.

Williamson, M. & Fitter, A. 1996. The characters of successful invaders, *Biol. Conserv.* 78:163-70.

Wilson, E.O. 1965. The challenge from related species. In *The genetics of colonizing species.* H.G.Baker & E.Stebbins (Eds.).New York, Academic, 588 pp.

Wilcox, B.A.1980. Insular Ecology and Conservation.. In: *Conservation biology*. M.E.Soule & B.A.Wilcox (Eds.). Sinauear associates, Massachusetts. pp 97-117.

Wingfield, C.A. 1940. The effect of certain environmental factors on the growth of brown trout *Salmo trutta* . *Exp. Biol.* 17:435-448.

Woodward, B.D. 1983. Predator-prey interactions and breeding-pond use of temporary-pond species in a desert anuran community. *Ecology*. 64:1549-1555.

Wootton, J.T. 1994. The nature consequences of indirect effects in ecological communities, *Ann. Rev. Ecol. Syst.* 25:443-466.

Wydoski, R.S. & Whitney, R.R. 1979. Inland fishes of Washington. Univ. of Wash. Press, Seattle, WA.

Young, M.K. and Harig, A.L. 2001. A Critique of the recovery of greenback Cutthroat Trout. *Conserv.Biol..* 15(6):1575–1584.

Zaret, T.M. & Paine, R.T. 1973. Species introduction in a tropical lake. *Science.* 182:445-449.

Zwiefel, R.G. 1955. Ecology, distribution and systematics of frogs of the *Rana boylei* group. *University of Calif*

APPENDICES

Appendix A: GIS layers used in a desktop study to identify geo-physical features occurring at Injesuthi and Monk's Cowl sampling sites.

Accessed: Ezemvelo KZN Wildlife, Biodiversity Conservation and Planning Division, Queen Elizabeth Park, PO Box 13069, Cascades, 3202, South Africa. Telephone 033-8451999.

GIS Layers	Scale	Format	Reference/ Source
Geology		Arcview vector	Environmental Management Framework, KwaZulu-Natal, DEAT
Soil Type		Arcview vector	Environmental and Tourism Potential Atlas 2001, KwaZulu-Natal Province, DEAT
KZN Mean Annual Temperature		Raster	Schulz, R.E. and Maharaj, M. 2006. Mean Annual temperature. *In:* Schulze, R.E. (Ed). 2006. **South African Atlas of Climatology and Agrohydrology.** Water research Commission, Pretoria, RSA, WRC Report 1489/1/06, Section 7.2.
KZN Mean Annual Precipitation		Raster	Schulz, R.E., Lynch, S.D. and Maharaj, M. 2006. Annual Precipitation. *In:* Schulze, R.E. (Ed). 2006. **South African Atlas of Climatology and Agrohydrology.** Water research Commission, Pretoria. RSA, WRC Report 1489?1/06, section 6.2.
KZN Slope		Raster	Kasseepursad, B. 2001. Derived from KZNDEM file obtained

			from Compumap
KZN Aspect		Raster	Kasseepursad, B. 2001. Derived from KZNDEM file obtained from Compumap
KZN Vegetation and Forest Types		Arcview Vector	KwaZulu-Natal Vegetation Map. Ver.2 21 September 2006. Ezemvelo KZN Wildlife, Conservation Planning, P.O.Box 13053, Cascades, 3202, Pietermaritzburg.
KZN Water Yield		Raster	Created by: N.A. Rivers-Moore N.A. 2006. Data Source: Schulze, R. E. (Ed). 2006. South African Atlas of Climatology and Agrohydrology. Water Research Commission, Pretoria, RSA, WRC Report Schulze, R.E. 1982. Agrohydrology and Climatology of Natal. ACRU report no. 14, Department of Agricultural Engineering, University of Natal, Pietermaritzburg.

:

Appendix B: Data source, frequency of collection, parameters, sampling sites, datasets collected and equipment used in data collection

Data source	Frequency of data collection	Parameters	Sampling sites	Datasets collected	Field sampling equipment
Water temperature	Recorded daily at 90 minute intervals	Daily maximum Daily minimum Daily average	IN1 IN4 IN6 M1 M4 M6	1 Oct 07- 31 Dec 07 1 Jan 08 - 31 Mar 08 1 Apr 08- 30 Jun 08 1 Jul 08- 30 Sep 08	Dallas Thermochron –iButtons Protective 20 cm water pipe casing plus 1.5 m X 3 mm stainless steel cable
Physical features	September 07	River width River length Riverbank type	IN1 IN6 M1 M6	September 2007	30 m tape measure 30 m rope knotted at 3 m intervals

		Bank structure River depth River biotope type Benthic structure type			20 m rope knotted at 5 m intervals 1.5 m stainless steel ruler calibrated in cm
Water quality	September 07 (spring) February 08 (summer) May 08 (autumn) July 08 (winter)	Temperature pH Electrical conductivity TDS DO	IN1 IN2 IN3 IN4 IN5 IN6 M1 M2 M3 M4 M5	September 07 (spring) February 08 (summer) May 08 (autumn) July 08	Hanna HI 9143 DO meter Hanna HI 991300 pH/EC/TDS/Temp meter

			M6	(winter)	
River health	September 07 (spring) February 08 (summer) May 08 (autumn) July 08 (winter)	Average score per taxon (ASPT)	IN2 IN5 M2 M5	September 07 (spring) February 08 (summer) May 08 (autumn) July 08 (winter	30 X 30 cm 1000 micron net Laboratory sampling Tray Laboratory dissecting kit Pipette Magnifying glass Small sampling jars with 100 % ethanol 5 L Collapsible bucket

Electro-fishing	September 07 (spring) February 08 (summer) May 08 (autumn) July 08 (winter)	Number of tadpoles/fish captured Length of tadpoles/ fish captured Mass of tadpoles/fish captured Biotope type/ benthic structure type/ depth and flow rate preferences of tadpoles and fish recorded at floating markers	IN1 IN2 IN3 IN4 IN5 IN6 M1 M2 M3 M4 M5 M6	September 07 (spring) February 08 (summer) May 08 (autumn) July 08 (winter	Honda 700 portable generator Electro-fishing probes and 30 m electrical cord 30 X 30 cm 1000 micron net 30 cm hand net Collapsible 5 L bucket 1.5 m stainless steel ruler 50 m Tape measure Aquarium hand net 200 x 20 g Spring balance

					100 x 1 g Spring balance
Flow-velocity	September 07 (spring) February 08 (summer) May 08 (autumn) July 08 (winter)	Wetted width of cross section Active channel width of cross section Depth at 0.5 m intervals at river cross section using open and closed ruler measurements	IN1 IN4 IN6 M1 M4 M6	September 07 (spring) February 08 (summer) May 08 (autumn) July 08 (winter	20 m ski-rope knotted at 0.5 m intervals 1 m stainless steel drawing ruler 30 m tape measure Roll 0.40 mm monofilament Spirit level

Appendix C: GPS Waypoints and altitude of sampling sites.

Site code	Locality	Latitude	Longitude	Altitude
IN1TOP	Injesuthi	-29.16446	29.42170	1727
IN1BOT		-29.16342	29.42136	1719
IN2TOP		-29.16150	29.42116	1707
IN2BOT		-29.16041	29.42040	1680
IN3TOP		-29.15850	29.41922	1674
IN3BOT		-29.15726	29.41881	1647
IN4TOP		-29.15600	29.41859	1653
IN4BOT		-29.15479	29.41885	1631
IN5TOP		-29.15360	29.42017	1625
IN5BOT		-29.15020	29.42146	1637
IN6TOP		-29.15142	29.42119	1615
IN6BOT		-29.15020	29.42146	1594
M1TOP	Monks Cowl	-29.04697	29.39215	1483
M1BOT		-29.04680	29.39329	1417
M2TOP		-29.04462	29.39640	1407
M2BOT		-29.04419	29.39764	1407
M3TOP		-29.04137	29.39985	1370
M3BOT		-29.04018	29.40032	1373
M4TOP		-29.03912	29.40324	1327
M4BOT		-29.03903	29.40477	1341
M5TOP		-29.03734	29.41062	1317
M5BOT		-29.03709	29.41224	1317
M6TOP		-29.03645	29.41467	1294
M6BOT		-29.03589	29.41604	1292

* TOP = Finish and BOT = Start

Appendix D: Physical Landscape-scale PCA Variables

```
********* PRINCIPAL COMPONENTS ANALYSIS -- Plots    in Site att space **********
PC-ORD, Version 4.17
7 Apr 2009, 10:55

Cross-products matrix contains CORRELATION COEFFICIENTS among Site att

VARIANCE EXTRACTED, FIRST 10 AXES
---------------------------------------------------------------
Broken-stick
AXIS    Eigenvalue   % of Variance  Cum.% of Var.  Eigenvalue
---------------------------------------------------------------
1        5.939       59.387       59.387        2.929
2        2.648       26.481       85.868        1.929
3        1.413       14.132      100.000        1.429
4        0.000        0.000      100.000        1.096
5        0.000        0.000      100.000        0.846
6        0.000        0.000      100.000        0.646
7        0.000        0.000      100.000        0.479
8        0.000        0.000      100.000        0.336
9        0.000        0.000      100.000        0.211
10        0.000        0.000      100.000        0.100
---------------------------------------------------------------
FIRST 6 EIGENVECTORS
--------------------------------------------------------------------------------
Eigenvector
Site att        1         2         3         4         5         6
--------------------------------------------------------------------------------
Precipit     0.3669    -0.0872    -0.3571    -0.3371     0.2759     0.2229
Gradient     -0.0027     0.5108     0.4677     0.2374     0.3968    -0.3097
Aspect       0.3302     0.3435     0.1680     0.2019    -0.2997     0.7382
Altitude    -0.3865     0.0465    -0.2754    -0.3326     0.1226     0.0871
Geologic      0.3967    -0.1260    -0.1281     0.1728    -0.2555    -0.3263
Soil Typ     0.3967    -0.1260    -0.1281     0.1728    -0.2555    -0.3263
Vegetati     0.3967    -0.1260    -0.1281     0.1755     0.6836     0.0617
% Forest    -0.3096    -0.3946    -0.1138     0.6736     0.1781     0.2625
```

```
% Mixed      0.0566     0.5387    -0.3878     0.0019     0.1097    -0.0140
% Grass     0.1887    -0.3433     0.5806    -0.3665     0.1412     0.1261
---------------------------------------------------------------------------
COORDINATES (SCORES) OF Plots
-------------------------------------------------------------------------------------------
Axis (Component)
Plots              1          2          3          4          5          6
-------------------------------------------------------------------------------------------
1 IN1         -3.1851    -1.8097    -0.2786     0.0000     0.0000     0.0000
2 IN6         -1.5272     2.4769     0.6407     0.0000     0.0000     0.0000
3 M1           2.0605     0.3590    -1.7778     0.0000     0.0000     0.0000
4 M6           2.6518    -1.0262     1.4157     0.0000     0.0000     0.0000
-------------------------------------------------------------------------------------------

*********************************** End of PCA ***********************************
```

Correlation matrix between physical landscape-scale variables.

	Precipitation	Gradient	Aspect	Altitude	Geological type	Soil Type	Vegetation type	% Forest cover	% Mixed veld cover	% Grass over
Precipitation	1.00									
Gradient	-0.36	1.00								
Aspect	0.56	0.57	1.00							
Altitude	-0.71	-0.11	-0.78	1.00						
Geological type	0.96	-0.26	0.63	-0.88	1.00					
Soil Type	0.96	-0.26	0.63	-0.88	1.00	1.00				
Vegetation type	0.96	-0.26	0.63	-0.88	1.00	1.00	1.00			
% Forest cover	-0.53	-0.60	-0.99	0.71	-0.58	-0.58	-0.58	1.00		
% Mixed veld cover	0.19	0.47	0.51	0.09	0.02	0.02	0.02	-0.60	1.00	
% Grass cover	0.20	-0.08	0.20	-0.70	0.45	0.45	0.45	-0.08	-0.74	1.00

Appendix E: Geomorphological PCA Variables

```
********* PRINCIPAL COMPONENTS ANALYSIS -- Sites    in Site Att space **********
PC-ORD, Version 4.17
7 Apr 2009, 12:32

Cross-products matrix contains CORRELATION COEFFICIENTS among Site Att



VARIANCE EXTRACTED, FIRST 10 AXES
------------------------------------------------------------
Broken-stick
AXIS    Eigenvalue   % of Variance  Cum.% of Var.  Eigenvalue
------------------------------------------------------------
1        7.741        64.508        64.508        3.103
2        3.259        27.158        91.666        2.103
3        1.000         8.334       100.000        1.603
4        0.000         0.000       100.000        1.270
5        0.000         0.000       100.000        1.020
6        0.000         0.000       100.000        0.820
7        0.000         0.000       100.000        0.653
8        0.000         0.000       100.000        0.510
9        0.000         0.000       100.000        0.385
10        0.000         0.000       100.000        0.274
------------------------------------------------------------
FIRST 6 EIGENVECTORS
-----------------------------------------------------------------------------
Eigenvector
Site Att          1          2          3          4          5          6
-----------------------------------------------------------------------------
Run         -0.3253     0.2144     0.1762     0.0631     0.3284     0.0229
Riffle       0.2500    -0.3977    -0.0276    -0.1359     0.2963     0.5909
Glide       -0.3273    -0.1921     0.2247     0.4162    -0.0720    -0.0399
Pool         0.3200    -0.1336    -0.3862    -0.1148    -0.0905    -0.4406
Back-edd     -0.3444    -0.1476    -0.1029     0.1011     0.4426    -0.4257
Backwate      0.3223     0.1385     0.3653    -0.3517     0.2644    -0.2034
Exposed      -0.2681     0.3673     0.0619    -0.3847    -0.3961    -0.1256
```

```
Bedrock      0.3008    -0.0348    0.5438    0.2624    0.1977    -0.2933
Boulder     -0.3280    -0.1409   -0.3200   -0.3136    0.4198    -0.0568
Stone        0.0632    -0.5421   -0.1069    0.1287   -0.2832    -0.2909
Stone/Gr    -0.3117    -0.2470    0.2214    0.0435   -0.2468     0.1796
Gravel       0.1690     0.4351   -0.4026    0.5688    0.1088     0.1000
----------------------------------------------------------------------------

COORDINATES (SCORES) OF Sites
------------------------------------------------------------------------------------------
Axis (Component)
Sites            1          2          3          4          5          6
------------------------------------------------------------------------------------------
1 IN1          3.2633    -0.6913    1.2156    0.0000    0.0000    0.0000
2 IN6         -4.1794    -1.3942    0.3835    0.0000    0.0000    0.0000
3 M1          -0.6437     3.0970   -0.0590    0.0000    0.0000    0.0000
4 M6           1.5597    -1.0115   -1.5401    0.0000    0.0000    0.0000
------------------------------------------------------------------------------------------
********************************** End of PCA **********************************
```

Correlation matrix between geomorphological PCA variables

.

	% Run	% Riffle	% Glide	% Pool	% Back-eddy	% Back-water	% Exposed	% Bedrock	% Boulder	% Stone	% Stone /Gravel	% Gravel
% Run	1.00											
% Riffle	-0.91	1.00										
%Glide	0.73	-0.39	1.00									
% Pool	-0.97	0.80	-0.81	1.00								
% Back-eddy	0.75	-0.47	0. 94	-0.75	1.00							
% Backwater	-0.65	0.43	-0.82	0.60	-0.96	1.00						
% Exposed	0.94	-1.00	0.46	-0.85	0.53	-0.48	1.00					
% Bedrock	-0.69	0.61	-0.62	0.55	-0.84	0.93	-0.63	1.00				
% Boulder	0.67	-0.44	0.85	-0.63	0.98	-1.00	0.49	-0.92	1.00			
% Stone	-0.56	0.83	0.16	0.43	0.10	-0.13	-0.79	0.15	0.12	1.00		
% Stone/Gravel	0.65	-0.29	0.99	-0.75	0.93	-0.81	0.36	-0.58	0.83	0.26	1.00	
%Gravel	-0.19	-0.23	-0.79	0.38	-0.62	0.47	0.15	0.13	-0.50	-0.64	-0.85	1.00

Appendix F: Hydrological PCA Variables.

```
********* PRINCIPAL COMPONENTS ANALYSIS -- Sites    in Attribut space **********
PC-ORD, Version 4.17
7 Apr 2009, 16:07

Cross-products matrix contains CORRELATION COEFFICIENTS among Attribut

VARIANCE EXTRACTED, FIRST 10 AXES
------------------------------------------------------------
Broken-stick
AXIS    Eigenvalue   % of Variance  Cum.% of Var.  Eigenvalue
------------------------------------------------------------
1        6.193       51.607       51.607        3.103
2        3.466       28.887       80.494        2.103
3        2.341       19.506      100.000         1.603
4        0.000        0.000      100.000        1.270
5        0.000        0.000      100.000        1.020
6        0.000        0.000      100.000        0.820
7        0.000        0.000      100.000        0.653
8        0.000        0.000      100.000        0.510
9        0.000        0.000      100.000        0.385
10        0.000        0.000      100.000        0.274
------------------------------------------------------------

FIRST 6 EIGENVECTORS
----------------------------------------------------------------------------
Eigenvector
Attribut         1          2          3          4          5          6
----------------------------------------------------------------------------
Av.Depth      0.2950    -0.3316    -0.1848    -0.1097    -0.0267    -0.1657
Max.Dept      0.3707    -0.0005    -0.2525     0.2294    -0.1850     0.0791
%Exposed     -0.3374    -0.0361     0.3524     0.4465    -0.1311    -0.4316
Av.Wet.W      0.2570    -0.3707     0.2211     0.5076     0.2907     0.0730
Act.Chan      0.2315    -0.3958     0.2311    -0.2484    -0.0033     0.3235
Sep07.Fl     -0.3405    -0.0679    -0.3371     0.4627     0.2969     0.5465
May08.Fl     -0.2635     0.0021    -0.4935    -0.2293     0.0155     0.0907
Jul08.Fl    -0.1243     0.4388     0.3181    -0.0278    -0.1714     0.3375
```

```
%U/C Ban     0.3876    0.1398    0.0277   -0.0234    0.1458    0.0981
%V.Bank     -0.2922   -0.2369    0.3439   -0.3821    0.5276    0.0734
%S.Bank     -0.2462   -0.3551   -0.2830   -0.0442    0.0078   -0.3510
%B.Bank      0.2122    0.4424   -0.1347    0.0246    0.6678   -0.3334
----------------------------------------------------------------------------

COORDINATES (SCORES) OF Sites
-----------------------------------------------------------------------------------------
Axis (Component)
Sites            1          2          3          4          5          6
-----------------------------------------------------------------------------------------
1 IN1         0.4516     1.3407    -2.3940     0.0000     0.0000     0.0000
2 IN6        -1.7587    -2.9278    -0.2548     0.0000     0.0000     0.0000
3 M1         -2.5574     1.8511     1.4954     0.0000     0.0000     0.0000
4 M6          3.8645    -0.2641     1.1534     0.0000     0.0000     0.0000
-----------------------------------------------------------------------------------------

********************************** End of PCA **********************************
```

Correlation matrix between hydrological PCA variables.

	Mean Depth (cm)	Max. Depth (cm)	% Exposed substrata	Mean Wet Width (m)	Active Chan. Width (m)	Sep. Flow (m/s)	May. Flow (m/s)	Jul Flow (m/s)	% U/C Bank	% Vert. Bank	% Sloped Bank	% Boulder. Bank
Mean Depth (cm)	1											
Maximum Depth (cm)	0.79	1.00										
%Exposed benthos	-0.73	-0.98	1.00									
Mean Wet.Width (m)	0.80	0.46	-0.31	1.00								
Act.Chan.Width (m)	0.78	0.40	-0.24	1.00	1.00							
Sep 07.Flow (m/s)	-0.40	-0.58	0.44	-0.63	-0.58	1.00						
May 08.Flow (m/s)	-0.27	-0.31	0.14	-0.68	-0.65	0.94	1.00					
Jul 08.Flow (m/s)	-0.87	-0.47	0.47	-0.60	-0.61	-0.09	-0.16	1.00				
% Undercut Bank	0.54	0.87	-0.80	0.45	0.38	-0.87	-0.66	-0.07	1.00			
%VerticalBank	-0.41	-0.87	0.92	0.02	0.09	0.40	0.08	0.12	-0.79	1.00		
% Sloped Bank	0.08	-0.40	0.33	-0.08	-0.02	0.83	0.73	-0.56	-0.78	0.51	1.00	
% Boulder Bank	-0.06	0.57	-0.61	-0.30	-0.38	-0.45	-0.19	0.41	0.72	-0.86	-0.78	1.00

Appendix G: Water Quality PCA Variables

```
********* PRINCIPAL COMPONENTS ANALYSIS -- Sites    in Attribut space **********
PC-ORD, Version 4.17
7 Apr 2009, 14:52

Cross-products matrix contains CORRELATION COEFFICIENTS among Attribut



VARIANCE EXTRACTED, FIRST 10 AXES
--------------------------------------------------------------
Broken-stick
AXIS    Eigenvalue   % of Variance  Cum.% of Var.  Eigenvalue
--------------------------------------------------------------
1        7.527       62.728       62.728        3.103
2        3.557       29.641       92.370        2.103
3        0.916        7.630      100.000        1.603
4        0.000        0.000      100.000        1.270
5        0.000        0.000      100.000        1.020
6        0.000        0.000      100.000        0.820
7        0.000        0.000      100.000        0.653
8        0.000        0.000      100.000        0.510
9        0.000        0.000      100.000        0.385
10        0.000        0.000      100.000        0.274
--------------------------------------------------------------
FIRST 6 EIGENVECTORS
------------------------------------------------------------------------------
Eigenvector
Attribut         1          2          3          4          5          6
------------------------------------------------------------------------------
pH_Sep07     -0.2612      0.3261      0.3439      0.8279      0.1098     -0.0224
pH_May08      0.2120      0.4290      0.0878     -0.1661      0.2408     -0.5490
pH_Jul08    -0.1137      0.4624     -0.3941      0.0011     -0.0716      0.0494
TDS_Jan0    -0.3423     -0.1287      0.2538     -0.2113      0.5987      0.0120
TDS_May0     -0.3429     -0.1793     -0.0288     -0.0198     -0.1438     -0.3856
TDS_Jul0    -0.3556     -0.1090      0.0790     -0.1191      0.2865     -0.0592
DO_Sep07      0.2326     -0.3993     -0.1680      0.3494      0.0641      0.1871
DO_May08      0.3029      0.2140      0.3998     -0.2077      0.1289      0.2099
```

```
DO_July0     0.2648    0.3641   -0.0280   -0.0070    0.0597    0.1696
Av.Temp_     0.3371   -0.1903   -0.1314    0.1988    0.0891   -0.6419
Av.Temp_    -0.2546    0.1968   -0.6395    0.0197    0.2940    0.0646
Av.Temp_     0.3440   -0.1500   -0.1791    0.1502    0.5873    0.1389
------------------------------------------------------------------------------
COORDINATES (SCORES) OF Sites
-------------------------------------------------------------------------------------------
Axis (Component)
Sites            1          2          3          4          5          6
-------------------------------------------------------------------------------------------
1 IN1        -2.9639    -0.4864     1.2718     0.0000     0.0000     0.0000
2 IN6        -1.9514    -1.1744    -1.3888     0.0000     0.0000     0.0000
3 M1          0.8089     3.2003    -0.1756     0.0000     0.0000     0.0000
4 M6          4.1064    -1.5396     0.2926     0.0000     0.0000     0.0000
-------------------------------------------------------------------------------------------

******************************End of PCA ******************************
```

Correlation matrix between water quality PCA variables.

	pH Sep 07	pH May 07	pH Jul 08	TDS Jan 08	TDS May 08	TDS Jul 08	DO Sep 07	DO May 08	DO July 08	Mean Temp Jan 08	Mean Temp Jul 08	Mean Temp Sep 08
pH_Sep 07	1.00											
pH_May 08	0.11	1.00										
pH_Jul 08	0.64	0.49	1.00									
TDS_Jan 08	0.60	-0.72	-0.01	1.00								
TDS_May 08	0.46	-0.82	0.01	0.96	1.00							
TDS_Jul 08	0.60	-0.73	0.10	0.98	0.99	1.00						
DO_Sep 07	-0.97	-0.25	-0.80	-0.46	-0.34	-0.48	1.00					
DO_May 08	-0.22	0.84	-0.05	-0.79	-0.93	-0.86	0.16	1.00				
DO_July 08	-0.11	0.98	0.38	-0.86	-0.91	-0.85	-0.05	0.87	1.00			
Mean Temp_Jan 08	-0.92	0.24	-0.55	-0.81	-0.75	-0.84	0.88	0.58	0.43	1.00		
Mean Temp_Jul 08	0.53	-0.16	0.77	0.42	0.55	0.56	-0.63	-0.66	-0.24	-0.70	1.00	
Mean.Temp_Sep 08	-0.91	0.31	-0.48	-0.86	-0.79	-0.88	0.84	0.60	0.50	1.00	-0.66	1.00