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ELUCIDATION OF FOODWEB INTERACTIONS IN SOUTH AFRICAN RESERVOIRS USING STABLE ISOTOPES

Report to the
Water Research Commission

by

William R Harding, Robert C Hart & Lynda G Muller
DH Environmental Consulting
South Africa

WRC Report No. 1918/1/12
ISBN 978-1-4312-0299-7

August 2012

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EXECUTIVE SUMMARY

This research project examined the foodweb structure of the Rietvlei Dam (Pretoria, South Africa) in order to determine the possibilities for fishery biomanipulation as a tool for attenuating the impacts of eutrophication. To do this, the study employed stable isotope analysis (SIA) techniques for the first time in a South African reservoir.

The concept of fishery biomanipulation, also termed “top-down control”, entails the deliberate harvesting of fish in order to relieve the impact of zooplanktivorous fishes on the primary consumers (zooplankton), hence enhancing their grazing impact on the phytoplankton and correspondingly reduce algal blooms. This concept presumes that the phytoplankton comprises species that are edible by zooplankton, and that the fishery comprises obligate zooplanktivorous species. Obligate zooplanktivorous fishes are largely restricted to natural lakes ecosystems, with this feeding guild being sparsely represented in reservoir ecosystems, whose ‘indigenous’ fish fauna predominantly derive from the lotic species ‘captured’ by river impoundment. Zooplanktivory by fish in reservoirs is accordingly primarily opportunistic, and largely rests on the influence of juvenile individuals. In this respect, fundamental differences exist between natural lakes and man-made reservoirs.

While the contemporary understanding of South African reservoir lakes indicates the absence of specialized zooplankton-feeding fish, conflicting opinions were obtained from fishery biologists associated with a previous project that assessed the fish harvesting potential for a set of South African reservoir lakes. In order to clarify these conflicting views, as well as to assess the value of SIA-based reservoir assessment in South Africa, the approach was tested over a period of 30 months.

None of the findings gleaned from this study suggested that zooplanktivory has a significant influence on zooplankton community structure and abundance levels. The evidence obtained using SIA indicates that trophic pathways leading to fish primarily follow benthic, rather than planktonic routes. This evidence strongly counters prospects of foodweb biomanipulation as a mechanism to decrease fish predation on zooplankton, and correspondingly increase the grazing impact of zooplankton on phytoplankton. In this respect, the findings of the SIA approach are entirely consistent with the findings made in the parallel ‘conventional’ analysis of zooplankton abundance and composition that provided no indications of any significant influence of zooplanktivorous fish in the shaping of zooplankton assemblage structure or dynamics.

This study accordingly provides the first direct empirical evidence to test the theoretical challenges countering the utility of classical top-down biomanipulation in South African reservoirs (Hart, 2006), (subsequently supported/endorsed by inferences based on zooplankton-phytoplankton biomass ratios (Hart, 2011)), and indeed confirms them convincingly for Rietvlei. While parallel studies on other systems are desirable to ascertain the generality of this conclusion, the congruence of the present findings with fundamental theoretical arguments suggest, very strongly, that such generality is indeed highly unlikely not to apply.

Bottom-up effects are, accordingly, implicated by default as the primary moulding agents and hence of the greatest value as a focus for lake management interventions. We would strongly caution against the notion that fishery-directed biomanipulation provides a tool for eutrophication management in South Africa. At the very least, it should not be attempted without first defining the foodweb linkages.

The use of stable isotope analysis (SIA) to discern the trophic linkages proved extremely useful. This approach provided an easy-to-use method, which allowed elucidation of the foodweb linkages between the benthos, epiphyton and fish and which would not have been possible using conventional approaches. The use of the SIA-approach for obtaining "snapshot" assessments of the trophic structure and linkages in other South African reservoir-lakes is highly recommended, but not without a word of caution. The interpretation of SIA-derived data is not clear-cut and, especially for once-off assessments, requires a robust, classical understanding of lake foodweb interactions, combined with knowledge of the trophic shift between diet and consumer. Variations in shift can be substantial and changes underlying shifts in $\delta^{15}N$ from food source to consumer are, in many cases, only poorly understood.

The aforementioned notwithstanding, if South African reservoirs are to be managed in future as ecosystems, then the use of SIA provides a powerful adjunct to the conventional and singular reliance on water chemistry.

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The Tshwane Municipality's Rietvlei laboratory (Mrs Leanne Coetzee, Linda Leonard and colleagues) together with the Rietvlei Nature Conservancy staff (Riaan Marias and Henri Kevane) provided indispensable logistical support – equipment storage, use of a survey boat and accommodation, as well as chlorophyll data. Mr Ernst Wohlitz (Tshwane Municipality) is thanked for his help with coordinating various aspects of the project).

Dr Harold van Niekerk (DWA) is thanked for assisting with water column profiling and for his general interest in our work. Mr Johan Koekemoer is thanked for undertaking the capture of fish during October 2009. Mr Christo Raubenheimer assisted with providing fish samples during Year 2 of the project. Messrs John Lanham and Ian Newton from the UCT Stable Light Isotope Unit are thanked for their conscientious attention to the analysis of our samples and for their interest in our work.

Mrs Lynda Muller undertook all of the routine collection and field processing for taxonomic and stable isotope samples, with thoughtful proficiency and care, throughout the project.

Dr Steve Mitchell (formerly WRC) is thanked for his involvement and advice, as are all members of the project Reference Group. Additionally, we would like to thank Vladimir Matveev, Michael Sierp and Erik Jeppesen for their contributions to this project. The use of laboratory and library facilities at the University of KwaZulu-Natal is gratefully acknowledged.

ELUCIDATION OF FOODWEB INTERACTIONS IN SOUTH AFRICAN RESERVOIRS USING STABLE ISOTOPES

SECTION 1: BACKGROUND TO THIS PROJECT

This project arose directly from the development of a remediation protocol for the Hartbeespoort Dam, South Africa (Harding *et al.*, 2004a, b). Hartbeespoort Dam is a hypertrophic, urban-impacted impoundment located west of Pretoria. This dam has long been plagued by persistent cyanobacterial blooms and a generally-impoverished biotic diversity as a consequence thereof.

During the Hartbeespoort Dam study, it was suggested that the possibility of augmenting zooplankton grazing on phytoplankton could possibly be enhanced by means of deliberately managing the dam's fishery (see concepts described in **Section 2, Literature Review**). Expert opinion sourced at that time was supportive of this hypothesis and the Hartbeespoort Dam study was duly-amended to include a provisional census of the fishery assemblage (Koekemoer and Steyn, 2005). The findings of the latter investigation led, subsequently, to a wider analysis of the fisheries of six additional eutrophic and hypertrophic impoundments within the same geographic region (Harding and Koekemoer, 2011).

During the wider study, an opinion contrary to that offered during the Hartbeespoort Dam project, namely that an element of relief of eutrophication pressure might be possible via "top-down" control of coarse (rough) fish – i.e. biomanipulation of the fishes in South African dams, arose. This considered that the basis for the top-down relief approach, derived in Europe and elsewhere, conflicted with the conventional understanding of the forcing functions exerted by fish in South African reservoir lakes, viz. a general absence of zooplanktivorous fishes, coupled to the general inedibility and/or low food value of cyanobacterial species dominant in most eutrophic waters (Hart, 2006). This opinion was further supported by opinions from other South African freshwater fishery biologists who were extremely sceptical of the outcomes predicted from the Hartbeespoort Dam work (Koekemoer and Steyn, 2005) and further argued in a second analysis by Hart (2011). This latter work, which analysed zooplankton:phytoplankton biomass ratios from ten South African reservoirs, concluded that, as obligate visually-feeding zooplanktivorous fishes are scarce or absent, biomanipulation as a management tool is unlikely to be effective.

The aforementioned notwithstanding, the general lack of information and data for whole foodweb studies in South African dams prompted support and funding for this project. Accordingly, therefore, the project focussed on the perceived central role of zooplankton as a trophic level in the lake foodweb. Furthermore, the previously limited use of Stable Isotope Analysis (SIA), as a diagnostic tool in South African dams, supported the need for a comprehensive examination of the value of this approach as an alternative to the more time consuming and hence costly 'classical' approaches.

Funding was obtained to undertake a two-year investigation at a single dam, this a reduction of the original proposal to undertake the work on at least two reservoirs. The original intention was to combine this work with the implementation of the Hartbeespoort Dam Remediation Project – for which specific purpose this project was originally proposed. Regrettably, this offer was curiously declined by the Hartbeespoort Dam Metsiame Project (Dr S Mitchell, pers. comm.) and the project was duly relocated to the Rietvlei Dam, with the kind permission of Tshwane Municipality.

The relocation of the project to Rietvlei was not an ideal choice but was necessitated by budget constraints already embodied in the approved project proposal. Rietvlei may be described as an atypical eutrophic waterbody with, historically, a very low phytoplankton biomass relative to the measured availability of nutrients. The dam is known to contain elevated levels of endocrine disrupting compounds (EDCs) (e.g. Burger, 2008; Bornman et al., 2007, Harding and Koekemoer, 2011) but it is not known whether these contribute directly to limitations of phytoplankton development. Additionally, at the time this project commenced and unbeknown to the project team, Tshwane Water was in the process of installing 16 solar-powered epilimnetic mixers into the dam. As there was no prior mixing or benchmarking study, this deprived this project of a true baseline of conditions occurring before the artificial mixing was initiated.

SCOPE AND AIMS

The central aim of this project was to discern any foodweb linkages that would indicate a 'fish-zooplankton-phytoplankton' ~~'phytoplankton-zooplankton-fish'~~ pathway, i.e. provide merit for top-down intervention via the reservoir fishery. Alternatively, should this not be the case, to define the foodweb linkages extant in this dam, as well as the value of the SIA approach for this purpose.

The project extended, ultimately, over thirty months, from June 2009 to December 2011.

The monitoring protocol included a combination of nearshore and offshore habitats – located per the findings of the previous WRC1643 investigation – i.e. habitats where fish activity was found to be highest (Harding and Koekemoer, 2011). The study combined both classical (i.e. conventional microscopic identification and enumeration of phytoplankton and zooplankton), as well as SIA-analysis of sediments, macrobenthos, diatoms (used as a proxy for phytobenthos), macrophytes, phytoplankton, zooplankton, invertebrates and fish. Water chemistry information was gathered on each sampling occasion, with additional data obtained from the Tshwane Rietvlei Laboratory located at the dam.

Alterations to lake mixing regime

This project unintentionally coincided with a decision to install epilimnetic water mixers into Rietvlei Dam, with the intention of creating a physical environment unfavourable to cyanobacterial development. This intervention in Rietvlei was applied in the apparent absence of a prior benchmarking of the lake's condition, as well as the lack of rigorous scientific evaluation of the perceived efficacy of these mixers for controlling undesirable algal blooms.

In order to establish the pre-intervention aolian mixing conditions, a brief screening assessment of wind-induced mixing was included in this project.

Conceptual background to the use of Stable Isotope Analysis (SIA)

Stable isotope analyses (SIA) are widely used in various lines of ecological research, often to cast light on foodweb structures and on the sources of materials and their fluxes through ecosystems (Karasov & Del Rio, 2007). Their increasing use in freshwaters has been noted by Grey (2006), in a review that cautions on a range of limitations and caveats in their application and interpretation. Foodweb structure and dynamics are conventionally traced using stable isotopes of carbon and nitrogen. Fundamentally, the ratio of the carbon isotopes ^{13}C and ^{12}C ($^{13}C/^{12}C$ – denoted as $\delta^{13}C$, in units of ‰) provides a signature of a given food type that reflects its origin (primary production source) and allows it to be identified and tracked as it flows through its subsequent consumers. The corresponding ratio of the nitrogen isotopes ^{15}N and ^{14}N ($^{15}N/^{14}N$ – $\delta^{15}N$) provides a parallel indication of the number of 'steps' through which a particular food (identified by its $\delta^{13}C$ value) has been transferred in the food web, i.e. the trophic level of the consumer involved.

Despite variability within and between ecosystems, the successive step-wise isotopic shifts (trophic fractionation values) between a particular food and its consumer generally average between +0‰ and +1‰ for $\delta^{13}C$ and +3.0‰ for $\delta^{15}N$ (Grey, 2006), (although Karasov & Del Rio (2007) suggest a slightly higher mean value of +3.4‰ for $\delta^{15}N$, while a range of +3 to +5‰ is commonly applied in marine food webs). The underlying physiological and biochemical processes causing the change in $\delta^{15}N$ remain poorly understood, but largely involve differential fractionation of ^{15}N and ^{14}N during amino acid synthesis during food assimilation, leading to the retention of isotopically-heavier ^{15}N and the excretion of isotopically-lighter ^{14}N. Differences in trophic shifts of $\delta^{15}N$ are accordingly affected by dietary nitrogen content (Adams & Sterner, 2000) and amino acid composition (McClelland & Montoya, 2002), along with differences in the underlying excretory mechanisms of the organisms involved and corresponding biochemical forms of nitrogenous waste (Vanderklift & Ponsard, 2003).

In reality, application of the commonly assumed 'standard' $\delta^{13}C$ and $\delta^{15}N$ fractionation values requires caution. From extensive literature analyses, McCutchan et al. (2003) found that $\delta^{13}C$ rose by 0.5 ± 0.13‰ on average between successive consumers (varying from +0.3 ± 0.14‰ for consumers analyzed whole to +1.3 ± 0.30‰ for consumer muscle), while increases in $\delta^{15}N$ varied according to food type (+1.4 ± 0.21‰, +3.3 ± 0.26‰ and +2.2 ± 0.30‰ respectively for consumers raised on invertebrate diets, other high-protein diets, and plant and algal diets). Logic dictates that enrichment levels in animals, feeding on a

mixed diet of plant and animal matter, should be intermediate between fractionation levels in 'pure' herbivores and 'pure' carnivores, and should reflect the ratio of plant and animal food consumed. Contrary to McCutchan et al. (2003), an extensive meta-analysis by Vanderklift & Ponsard (2003) revealed no significant differences in $\delta^{15}N$ enrichment between animals feeding on plant food, animal food, or manufactured mixtures, although enrichment was significantly lower for detritivores. Instead, their analysis identified a predominant influence of the consumer's basic underlying excretory mechanisms on $\delta^{15}N$ enrichment, which was lower for ammonotelic organisms than ureotelic or uricotelic organisms, accordingly introducing potential taxonomic contrasts.

The current investigation was undertaken as a simple ecological pilot study of foodweb structure, with no mandate to pursue the underlying physiological and biochemical influences detailed above. Accordingly, while cognizant of the inherent complexities they raise in using SIA for the intended purpose, the following account is based on the simplifying assumption that $\delta^{13}C$ signatures lying within ±1‰ of each other reflect a common food source, while trophic level enrichment of $\delta^{15}N$ amounts to +3‰. A conventional $\delta^{13}C/\delta^{15}N$ bi-plot using these isotopic shift values (**Figure 1.1**) schematically illustrates a plausible lake food web structure.

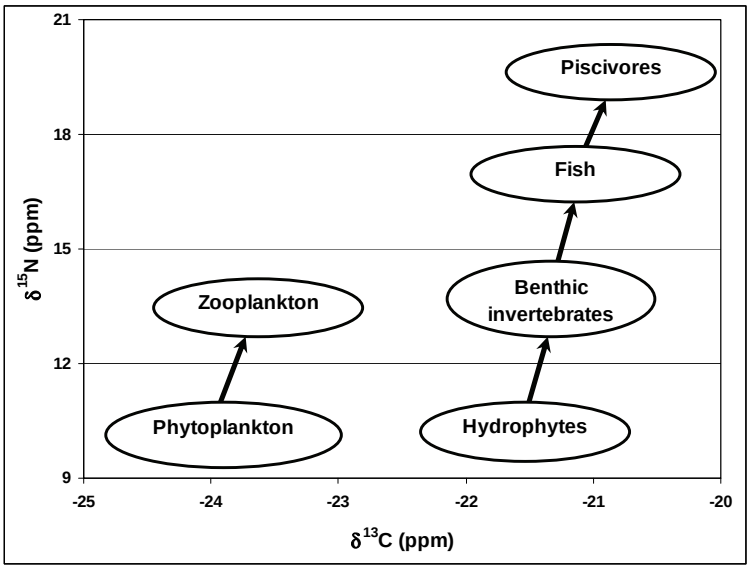

Figure 1.1. Schematic illustration of food web structures in a lake where trophic transfers lead the food chain to fish via benthic rather than planktonic assemblages (as will be exemplified for Rietvlei)

The central role of zooplankton

Zooplankton is globally recognized as a biotic component that plays a fundamentally central and pivotal role in the structure and functioning of lake food-webs. A general overview of features that determine its composition and abundance is given in Section 3. Within the overarching objective of the overall project, to assess the potential for top-down biomanipulation of the Rietvlei food web, zooplankton was studied with two concurrent aims. Firstly, to assess the temporal (and spatial) variations in its composition, coupled with the abundance of its component taxa in order to evaluate impacts of potential zooplanktivory. And secondly, to undertake a parallel assessment of its functional role in the food-web dynamics of the Rietvlei reservoir ecosystem, using stable isotope analysis. The procedures and findings relevant to the first aim are reported in Section 4, while Section 5 reports the findings of the SIA.

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SECTION 2: LITERATURE REVIEW

BACKGROUND

It has been suggested and, in several cases, demonstrated, that the deliberate manipulation of the fish populations of eutrophic waters leads to improvements in water quality (Harding and Koekemoer, 2011). Such improvements may be imparted by alterations (biomanipulation) to top-down control exerted by fish on lower trophic levels (e.g. piscivory, zooplanktivory), alternatively – or in combination with the foregoing, by relief of bottom-up impacts (e.g. sediment bioturbation, increased rates of nutrient availability through excretion, nest disturbance).

Recent studies conducted at a number of South African dams has shown that, in general, the fish assemblages of enriched waters are dominated by a variety of 'coarse' or undesirable fish species – species such as sharptooth catfish, common carp and canary kurper (Harding and Koekemoer, *ibid*). The same research has also indicated that a substantial portion of the coarse fish biomass could be removed, ostensibly relieving both a measure of top-down and bottom-up pressure on the foodwebs of these waters. The degree to which this might occur is impossible to predict without an adequate understanding of the nature of the foodwebs of these dams and the flow of energy through them. The intention of WRC 1918 is to test the value of SIA-based monitoring to rapidly and cheaply describe impoundment foodwebs – in order to provide a prediction of the extent to which these dams will respond to foodweb reshaping.

While fish-directed biomanipulation has been successful in many north-temperate waters, this may not be the case in the warmer waters of South Africa or Australia (e.g. Hart, 2006; Hart and Hart, 2006; Jeppesen et al., 2010), although there are indications from Australia that specific benefits may accrue (Sierp, 2009). Whether or not these concerns are valid or, indeed, the degree to which they may be valid, cannot be confirmed without a close examination of foodweb linkages in South African waters. The examination and elucidation of foodweb linkages using conventional (e.g. gut contents) approaches is expensive, tedious and fraught with constraints. By contrast, SIA-based approaches offer a rapid and cheap means of achieving the same end.

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1. THE ROLE AND VALUE OF SIA-BASED FOODWEB ANALYSES

The stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta\ ^{15}N$) are commonly-used in a variety of terrestrial and aquatic applications to identify foodweb interactions and energy flow (e.g. Rojo *et al*., 2008). The ratios of nitrogen isotopes increase upwards in the foodweb, allowing trophic levels to be discerned. Concomitantly, carbon signatures indicate habitat – with only very small differences between trophic levels. Importantly, the technique allows differentiation between pelagic and littoral habitats as phytoplankton show a greater degree of discrimination for carbon, vs. that shown by benthic algae – with carbon isotope signatures being conserved through consumer levels (Jeppesen *et al*., 2002).

Stable isotopes in an organism reflect an integration of food resources accumulated over time (Kilham *et al*., 2009). Accordingly, larger organisms will tend to have less-variable isotopic signatures compared with their prey. The use of SIA-based interpretations of foodwebs has become known as the "who eats whom and where" approach (e.g. Roux *et al*., 2008). The technique is increasingly being utilized to discern patterns within structurally-complex foodwebs (Roux *et al*., *ibid*). Similarly, it is obvious that this technique may reveal changes in habitat preferences, as well as differences between adult and juvenile feeding and foraging behaviours.

Standardized protocols have been formulated for the use of SIA in aquatic foodwebs (e.g. Smyntek *et al*., 2007). Such protocols include consideration of the need to remove lipids prior to isotope analysis.

Oneida Lake (New York State, USA) has become invaded by zebra mussels (*Dreissena polymorpha*). This raised the concern that the filter-feeding mussels could shift the foodweb carbon flow from the pelagic to the benthos – i.e. this would have a negative effect on the zooplankton. SIA-based examination of the lake foodweb showed that Daphnia was using a distinct source of carbon, whereas the mussels utilized the entire seston (Mitchell *et al*., 1996). The use of SIA has been utilized by Jeppesen *et al*. (2002) to describe fish feeding and foraging behaviour in 5 Faroese lakes.

In Chany Lake (western Siberia), the use of SIA was employed to examine the role of omnivorus cyprinid fish in a shallow, eutrophic lake (Kanaya *et al*., 2009). In this particular case, species such as common carp (*Cyprinus carpio*) were found to depend more on macrophytic than microalgal production. This finding was in contrast to the expected

microalgal – zooplankton – carp pathway that was anticipated. This finding is insightful as common carp constitute one of the three typically-dominant coarse fish in South African reservoirs.

Because enrichment of the $\delta^{15}N$ isotope is enriched by human or animal feeding processes, it is possible to identify natural or anthropogenic sources of carbon and nitrogen. Accordingly, pollution by wastewater or other land-based sources reflects as elevated $\partial^{15}N$ signatures in the biota (Xu and Xie, 2004). With respect to carbon, the differences between C3 ($\delta^{13}C$ = -24 to -34‰) or C4 (and CAM) plants ($\delta^{13}C$ = -6 to -19‰) can be discerned. Carbon signatures in the littoral are also known to vary with season (e.g. Cremona *et al*., 2008; Asaeda *et al*., 2008). This fact both strengthens the use of the method and precludes against the combination of samples.

In order to understand the movement of contaminants or pollutants through a foodweb, it is essential to understand the underlying foodweb itself. Once the flow of energy is known, it becomes a relatively simple matter to track where a known contaminant in one of the biota will move and possibly become biomagnified. Using this approach, Poste *et al*. (2008) successfully tracked the movement of mercury through the foodweb of Lake Bosomtwe in Ghana.

A constraint to obtaining composite analyses of aquatic foodwebs occurs due to difficulties in sampling the phytobenthos. Recently, however, research conducted as part of the integration of diatoms into the biomonitoring protocols of the European Union (EU) Water Framework Directive (WFD) (Kelly *et al*., 2008) has shown that diatoms may be used as proxies for phytobenthos when ecological status is being assessed. The use of diatom samples in SIA analyses – sourced off macrophytes – thus provides a useful link in the foodweb picture.

SIA analyses, while offering valuable insight into foodweb structure, cannot be effectively applied without knowledge of the factors that incorporate variation into the process (e.g. Kilham *et al*., 2009). Thus, SIA cannot be used in ignorance of the driving forces and cause and effect pathways that govern foodweb interactions.

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2. THE PHYTOPLANKTON AND EPIPHYTON OF RIETVLEI DAM

Studies on the phytoplankton of Rietvlei Dam have been few and limited to work carried out as long as 30 years ago. This paucity of information is in stark contrast to recent (2009) decisions to install a fleet of artificial mixers in the dam, ostensibly to offset cyanobacterial development. This decision was apparently taken also in the absence of any estimations of the prevailing hydrodynamic environment.

The only published work on the Rietvlei phytoplankton centres on two publications dealing with nitrogen fixation (Ashton, 1979 and 1981). This work was conducted in response to the development of nitrogen-fixing *Anabaena circinalis* in the dam during the mid-1970s – in an attempt to aid future management procedures. Both of these papers provide no insight as to the phytoplankton assemblages occurring in the dam during the study period.

In a later paper by the same author (Ashton, 1985), generalized seasonality data for 1976 and 1977 are provided – but without any species lists. The data nonetheless provide useful comparative numerical and biomass-based temporal trends for a complete hydrological year.

Unpublished data, collected during 1973/4 by Seaman and Schoeman, dealt with the testing of isolation mesocosms in Rietvlei Dam. This manuscript (Seaman and Schoeman, 1979) provides some data on phyto- and zooplankton assemblages and lists the following diatom species: *Melosira granulata* Ehrenberg Ralfs var *angustissima* O. Muller (dominant diatom encountered at the time, = *Aulacoseira granulata* (Ehr) Simonsen var *angustissima* (O.M. Simonsen); *Thalassiosira pseudonana* Hasle et Heimdal; *Cyclotella meneghiniana* Kutzing; *Cyclotella kuetzingiana* Thwaites, (= *Cyclotella distinguenda* A.K.S.K Prasad); *Stephanodiscus astrae* Ehrenberg (plus var. *minutula* (Kutzing) Grunow); *Epithemia sorex* Kutzing; *Cocconeis placentula* Ehrenberg and *C. pediculus* Ehrenberg; *Synedra tabulata* (Agard) Kutzing, (= *Tabularia tabulata* (C.A. Agardg) Snoeijs); *Nitzschia palea* (Kutzing); *Navicula pelliculosa* (Brebisson ex Kutzing); *Nitzschia amphibia* (Grunow); *Nitzschia silica* Archibald; *Rhopalodia gibba* (Ehrenberg).

Raw data for algal counts for this dam exist within the Department of Water Affairs database. These counts are, however, only to genus level and largely exclude diatoms.

THE ZOOPLANKTON OF RIETVLEI DAM

Historical information regarding zooplankton in Rietvlei Dam is meagre. Following indiscriminate institutional disposal of all historical reports and records (PJ Ashton, pers. comm., 2009), the data from some early studies undertaken by the National Institute of Water Research (NIWR) in the 1970s and 1980s are no longer available. The only report that remains accessible (Seaman & Schoeman, unpublished) provides data derived from small (2 litre) water samples collected from depths of 0 m, 3 m, and just above the bottom, and combined for collective filtration through 6 μm mesh. As a result of this fine mesh size, the species list includes small taxa, like the rotifer *Keratella*. The report lists the presence of *Keratella cochlearis* and *Hexarthra mira* (Rotifera), *Metadiaptomus colonialis* and *Thermocyclops oblongatus* (Copepoda), *Daphnia pulex*, *D. longispina*, *Pleuroxus* spp. and *Alona* spp. (Cladocera) and *Chaoborus* sp. (Insecta). It provides no indication of the relative abundance nor of the temporal variation of these individual taxa, merely showing (their Fig. 16) that total zooplankton numbers varied between ~ 200 and 7000 individuals per m^3 between December 1973 and November 1974. These densities appear surprisingly low, given that they include rotifers which are generally far more abundant than crustacean zooplankters.

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SEAMAN MT AND SCHOEMAN FR (Unpublished) Aspects of the effect of isolation columns in Rietvlei Dam (Transvaal, Republic of South Africa). 14pp plus Figures and Tables.

3. ZOOPLANKTON AND THEIR FUNCTIONAL ROLE

Contextual overview

1. General composition of zooplankton in freshwater pelagia

Zooplankton is a collective term for a taxonomically-mixed assemblage of predominantly small bodied invertebrate animals (aka 'zooplankters'), ranging in size from < 0.2-5 mm, but mostly <1 mm, which generally occupy open water pelagic habitats in lakes and reservoirs, and other standing waters. Recently, however, their importance in vegetated littoral regions of shallow lakes in particular has been identified, and increasing attention has focused on this subset, especially in relation to mitigating eutrophication of shallow lakes through biomanipulation (e.g. Moss, 1998; Van Donk et al., 1990; Burks et al., 2001; Hietala et al., 2004; DeClerck et al., 2005; Meerhoff et al., 2007; Moss, 2008). In larger and deeper lakes, the littoral zone is generally proportionately smaller, and its significance as a habitat that provides physical refuges for zooplankton is reduced commensurately.

The taxonomic composition of the zooplankton varies between (and temporally within) different ecosystems, with the freshwater zooplankton generally dominated numerically by various 'protozoan' taxa, rotifers and crustaceans (copepods and cladocerans). Various other taxa, including insects and arachnids, commonly occur, generally in far lower numbers. Zooplankton is often distinguished into size categories, commonly (but not ubiquitously) into the following size ranges:

- Macro-zooplankton > 500 μm (mostly large-bodied copepods and cladocerans)
- Meso-zooplankton < 500 μm > 200 μm (mostly small bodied crustaceans)
- Micro-zooplankton < 200 μm (mostly rotifers and juvenile crustaceans)

Assessments of abundance and composition of zooplankton within the size-fractions listed above are reasonably simple, and allow some broad functional insights into the community. Samples are commonly collected in vertical or horizontal hauls with a fine-mesh net, or by filtration of water samples collected at defined depths through a mesh sieve. However, as the most commonly used nets are constructed of mesh >50 μm (simply because finer nets clog too rapidly and/or severely), smaller zooplankton (<50 μm) are invariably under-represented in net samples. This fine fraction commonly contains numerous smaller species of rotifers, and often extremely abundant protozoan ciliates and heterotrophic

nanoflagellates (HNFs), the latter two having highly important influences on energy and material fluxes, especially in the decomposition of dead organic particles (detritus).

2. General functional role in pelagic ecosystems

Ecologically, most zooplankters serve as pivotal functional links between the primary producers and higher consumers in pelagic ecosystems; their role is effectively equivalent to that of grazers and browsers in terrestrial ecosystems. Although such notional 'herbivores' (primary consumers) predominate zooplankton assemblages, the community also includes various secondary consumer elements ('carnivores'). The 'herbivores' mostly consume single-celled and/or small colonial algae (eukaryote protists) and cyanophytes (prokaryote bacteria) – both functional analogues of higher plants, while the 'carnivorous' elements are typical predators that consume other components in the zooplankton. However, importantly in terms of their ecological functioning, **all** zooplankters, including the notional 'herbivores' (or grazers) that dominate the assemblage, effectively operate as genuine predators, in that their consumption of autotrophic algae and/or cyanobacteria typically results in the 'death' of these prey items. This outcome contrasts fundamentally with that applicable to terrestrial herbivores (grazers and browsers) whose consumption of plants, while generally detrimental, is seldom directly lethal to the food item consumed (Begon et al., 2006). This issue is not merely of academic interest – it is crucial in understanding the potential role of zooplankton in reducing primary producers stimulated by nutrient enrichment. 'Herbivorous' zooplankters do not merely maim their food items – they kill them – although exceptions related to 'protective' structures such as indigestible enveloping mucilage developed by some algae do exist.

As pivotal central players in pelagic food webs, zooplankters effectively represent small nutritious 'protein packages' of high quality living food, that are consumed by many fish species, ranging from specialized obligate zooplanktivores to facultative or opportunistic zooplankton feeders. The high nutritional quality of zooplankton results in many, if not most young of year (YOY) and 1+ juvenile fish, opportunistically consuming zooplankton if available, even if adults operate in totally different feeding guilds (e.g. herbivores, piscivores, benthivores or detritivores). Specialized zooplanktivorous fishes are largely restricted to natural lake ecosystems; this feeding guild is sparsely represented in reservoir ecosystems, whose 'indigenous' fish fauna predominantly derive from the lotic species 'captured' by river impoundment. Zooplanktivory by fish in reservoirs is accordingly primarily opportunistic, and largely rests on the influence of juvenile individuals, especially among the YOY cohort. In this respect, fundamental differences exist between natural lakes and man-made reservoirs.

Here it is also important to realize that South Africa has only one or two natural lakes, with the surface water landscape dominated by hundreds of large, bulk-storage reservoirs.

The taxonomic composition and abundance of animals in zooplankton assemblages is determined, jointly, by the type/nature and abundance of their food resources and their predators – essentially bottom-up and top-down controls. An immense literature examining these features exists, but is entirely impractical to review in the mandate of this project. However, the central tenet hinges on zooplankton body size, as briefly elaborated below. Seminal findings in this regard are attributable to Hrbáček (1962), whose work pre-empted the now familiar Size Efficiency Hypothesis (SEH) paradigm of Brooks & Dodson (1965), first evaluated by Hall et al. (1976). More contemporary controversies and debating points are synthesized by Jones & Jeppesen (2007), while Hart & Bychek (2011) provide an extensive review of the topic.

From the above brief overview, the significance of zooplankton in considerations of food-web biomanipulation should be self-evident. They are jointly influenced by top-down predatory controls, while themselves effecting top-down regulatory controls on their food resources, as encapsulated in the title of Lampert's (2006) review of *Daphnia*, frequently a dominant component in freshwater assemblages: '*Daphnia*: Model herbivore, predator and prey'. The zooplankton community accordingly plays a critically important central and pivotal role in the determination and regulation of food-web structures and associated trophic transfers in pelagic subsystems. As such, biomanipulation (in its classical sense of manipulating top-down controls) hinges fundamentally on the composition and abundance of the zooplankton assemblage, essentially attempting to maximize both the abundance of 'grazing' zooplankton and the size of its individual components – especially large-bodied cladocerans, in view of their disproportionately elevated feeding rates. While these are the central tenets underlying the SEH, there are intrinsic limits to the magnitude of grazing pressure that can be exerted by zooplankton (Lampert, 1988), in effect determined by bottom-up influences – resource limitations that regulate growth and reproduction of the community (Gliwicz, 2002).

These effects and interactions are schematically summarized in Figure 1.2; their origins, contributory influences, effects and consequences are elaborated in the following descriptive synopsis. Collectively, they contribute to predictable seasonal changes in planktonic assemblages encompassed in the PEG model of seasonal succession (Sommer et al., 1986, Sommer, 1989).

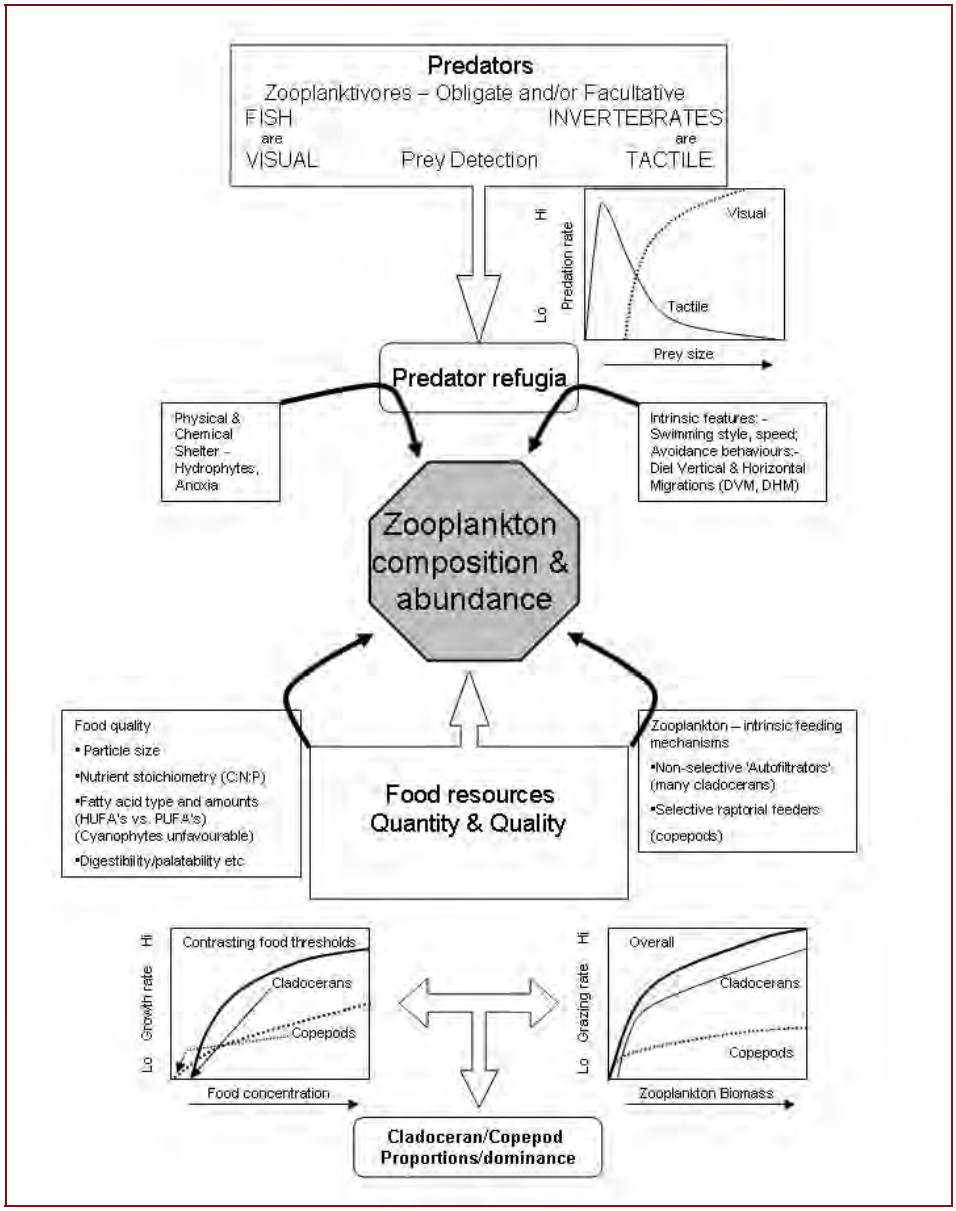

Figure 1.2 Schematic synopsis of key biotic factors and interactions that influence and/or determine the composition and abundance of zooplankton in fresh waters. Original compilation from a wide array of sources, only some of which are cited in the reference list and bibliography. See text below for elaboration/explanation, and selected key references.

3. Top-down control of zooplankton by predators

General considerations

The open-water pelagic environment provides little or no physical cover or shelter from predators. Zooplankton is preyed upon by 'visual' predators (predominantly fish) that locate and/or identify individual prey organisms by sight, and by 'tactile' predators (mostly invertebrates) that perceive and/or discern prey by detecting the hydrodynamic disturbances created by movement (e.g. Zaret, 1980; Kerfoot & Sih, 1987). It is a misplaced notion that fish feed on zooplankton 'automatically' by simply swimming open-mouthed through the water like a mobile sieve. Most rely on visual detection of individual prey items (see the following discussion of features that determine vulnerability). However, 'automatic' feeding does occur in marine ecosystems and can apparently be used by some freshwater fish – catfishes (*Clarias*) being an example relevant to local reservoirs.

Susceptibility to visual predation is influenced by the broad array of factors that influence visibility. Light intensity is primary, and some avoidance behaviours such as diel vertical migration (see below) are strategies evolved to maximize occupancy of deeper darker waters. However, an array of intrinsic features of the individual zooplankters also determines or contributes to their 'visibility'. Among these, size is the most important. All else being equal, large organisms are obviously more easily seen. Movement of potential prey is also critical – small moving animals are more readily discerned than larger stationary ones. Different types of movement also affect visual detection – slow gliding movements (copepods) are less conspicuous than rapid and especially jerky rapid movements (cladocerans). Visual contrasts (dark body parts, including pigmented ephippia, full alimentary tracts) further influence visibility.

Most tactile predators are invertebrates, effectively unable to handle larger prey items. Accordingly, prey vulnerability to tactile detection does not necessarily translate into capture. Nonetheless, tactile cues (magnitude and characteristics of hydrodynamic disturbance) are also linked to body size, swimming speed, and swimming style of potential prey. Detection vulnerability increases with body size and swimming speed, and with irregular movement patterns generated by prey movement.

Two fundamentally different types of tactile predators occur – 'sit and wait' ambush predators like larvae of the phantom midge, *Chaoborus*, and more commonly, 'searching' predators like cyclopoid copepods. For ambush predators, encounter probability generally

rises as the swimming speed of prey items increases – simply because prey are more likely to swim into their detection zone. For searching predators, encounter probabilities depend *inter alia* on relative speeds of movement of predator and prey (e.g. Gerritsen & Strickler, 1977).

Avoidance mechanisms

All else being equal, differences in active avoidance behaviour of prey organisms can profoundly affect their susceptibility to predation. In this regard, copepods and cladocerans differ greatly. Once located, fish capture individual prey items by very rapid expansion of the pharyngeal chamber to generate a powerful suction vortex which draws the prey into the mouth. Copepods tend to respond to such pharyngeal suction with a powerful escape 'jump', greatly reducing predator success rates compared to cladocerans which readily succumb to this feeding mechanism (Drenner & Strickler, 1978). Using a suction pipette, experimental capture rates for live cladocerans and inert bubbles were comparable, and much higher than for live copepods.

Diel Vertical Migration (DVM) – the daytime occupancy of deeper, darker waters, and migration into upper trophogenic zone waters during hours of darkness – is widely explicable as a logical (if energetically costly) strategy to avoid visual predation. DVM incurs both direct and indirect energetic costs – direct expenditure on locomotion, and limitations on food intake while 'displaced' from optimal feeding grounds of the illuminated trophogenic zone during daylight. Accordingly, the ascent into upper waters at night is commonly driven by food requirements, and often coincidentally results in exposure to warmer temperatures (which are metabolically preferable in some, but not all respects). Potential individual and population growth rate and success both relate directly to food availability and temperature, thereby negating a permanent or continuous occupancy of deeper dark waters simply to avoid visual predators. The underlying maxim is that while it is better to be hungry than dead (eaten by a predator), hunger potentially also results in death of individuals and ultimately, of populations. Zooplankters are literally caught between the devil and the deep blue sea.

In shallow lakes, where macrophytes may be extensive, zooplankton may also show Diel Horizontal Migration (DHM) as a predator avoidance strategy. The structural habitat provided by submerged hydrophytes can serve as a refuge from visual predators by day (although conversely it may increase susceptibility to tactile invertebrate predators which commonly abound in this region). In common with vertical ascent in DVM, lateral horizontal movements

offshore during darkness are undertaken for feeding purposes (and perhaps also avoidance of tactile predators during active feeding).

In waters that stratify thermally, dissolved oxygen depletion commonly arises below the thermocline. Accordingly, deep waters can offer a 'chemical' refuge for prey organisms that are tolerant of low oxygen levels (notably *Chaoborus* – which tolerates complete anoxia) against predators that are sensitive to oxygen depletion – as in the case of most fish.

4. Bottom-up control of zooplankton by food resources

Food quantity and food quality

It is self-evident that food availability is a primary constraint on the development of zooplankton, and determines zooplankton biomass – a major state variable in pelagic food web structures (e.g. Gliwicz, 2002). However, the quality of this food resource continues to attract increasing attention (e.g. Hessen, 2008).

A good 'balanced' diet is as important for invertebrate zooplankton 'herbivores' as it is for any vertebrate herbivore (or other feeding guilds). Nutrient stoichiometry (elementary C: N: P ratios), and fatty acid composition are major determinants of food quality, which in turn strongly influences zooplankton success (abundance) and composition. Food resources relatively depleted in P are known to adversely affect the demographics of their consumers, and differential susceptibility to this influence exists between taxa. Increasing evidence points to the importance of diets containing sufficient highly unsaturated fatty acids (HUFAs), a subset of polyunsaturated fatty acids (PUFAs), which are critical for maintaining high growth, survival and reproductive rates and high food conversion efficiencies for a wide variety of freshwater (and marine) organisms (e.g. Brett & Müller-Navarra, 1997; Müller-Navarra, 2008). Although animals can convert PUFAs from one form to another through elongation and desaturation, very few can synthesize PUFAs *de novo*; accordingly, they rely on dietary sources. High quality algal food species are rich in HUFAs, whereas low quality algal food species are poor in HUFA. Cyanophytes, which commonly predominate in eutrophic waters, are generally PUFA-impoverished, and thereby restrict their potential top-down control by zooplankton predators (if pre-emptive size constraints do not preclude their edibility by zooplankton at the outset – see next section). Intrinsic differences in fatty acid composition between major zooplankton taxa affect their respective reproductive rates and generation times (Smyntek et al., 2008).

Eutrophic waters are characteristically P-enriched, and show commensurately lowered N: P ratios. Despite the relative P-enrichment of eutrophic waters, dietary P-limitation of *Daphnia* is paradoxically common (Urabe & Watanabe, 1992; Urabe et al., 1997; Main et al., 1997; Sterner & Schulz, 1998; Boersma, 2000). Cyanophytes that commonly proliferate in such waters generally have an intrinsically low P content, in addition to a characteristically low HUFA content, while a filamentous or colonial morphology of many renders them simply too large for zooplankton to consume. Cyanophytes accordingly tend to be low quality food.

Zooplankton feeding mechanisms

The mechanisms of food particle collection by 'herbivorous' zooplankton, along with correspondingly different food selection abilities (e.g. DeMott, 1986), play a major role in determining the relative success of different taxa in different nutritional conditions. Cladocerans like *Daphnia* filter water 'automatically' – allowing them to process large volumes of water and collect particles suspended therein at a high rate. If the particles are of collectable size and good nutritional quality, this rapid feeding rate allows *Daphnia* to monopolize the food resource, and respond positively in terms of demographic performance. However, faced with poor quality food resources, energetic returns on feeding are disproportionately low in relation to expenditure costs.

On the other hand, copepods employ highly selective raptorial feeding, based on a remarkably sensitive chemosensory capability, which enables their location of (and discrimination between) individual food particles, facilitating their selective 'grappling' and ingestion of desirable elements. For example, they can distinguish the nutritional status of individual food particles, selecting less nitrogen limited cells (e.g. Butler et al., 1989), as well as distinguishing between cells identical in all respects apart from P content, as in the case of the same algal species grown in different culture media – high or low P.

Threshold food concentrations, the minimum food level above which positive individual (and population) growth is possible, are significantly lower for copepods (Hart, 1996) than cladocerans and rotifers (see Figure 2.2 in Scholten et al. (2005)). This gives copepods a competitive advantage when food is limited in quantity and/or poor in quality. However, this advantage cannot be maintained with rising food quantity, as the volume of medium that can be 'processed' is intrinsically lower for copepods than for automatic filtrators like *Daphnia*. However, maximum reproductive rates are directly converse to food threshold concentrations in these groups (rotifers > cladocerans > copepods) – as illustrated in

Scholten et al.'s (2005) Figure 2.2 – partly offsetting the advantages of lower threshold food concentrations.

5. Size-efficiency as a pivotal determining influence of zooplankton structure and abundance

The now familiar Size Efficiency Hypothesis of Brooks & Dodson (1964), pre-empted by the seminal work of Hrbáček (1962) and co-workers, underpins much of the context of top-down controls on zooplankton (e.g. Zaret, 1980; Kerfoot & Sih, 1986) – the conceptual basis informing biomanipulation theory and practice (at least in terms of 'conventional' (top-down) modifications. However, body size also affects bottom-up influences. Among cladocerans, for example, threshold food concentrations decline with increasing body size (Gliwicz, 1990). In effect, larger body size is selectively advantageous in the pelagic zone, except where visual predation pressure is high.

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SECTION 3: METHODS

Sampling Stations

The location of Rietvlei Dam is shown in **Figure 3.1**. The bathymetry of the dam is provided in **Figure 3.2**. **Figure 3.3** shows the positions of the sampling stations. Two offshore stations, named RV1 and RV1A were located in the deepest water east of the dam wall, while three nearshore stations, RV2-4, were situated in an area of known high fish activity (WRC1643 report, see **Figure 3.3**).

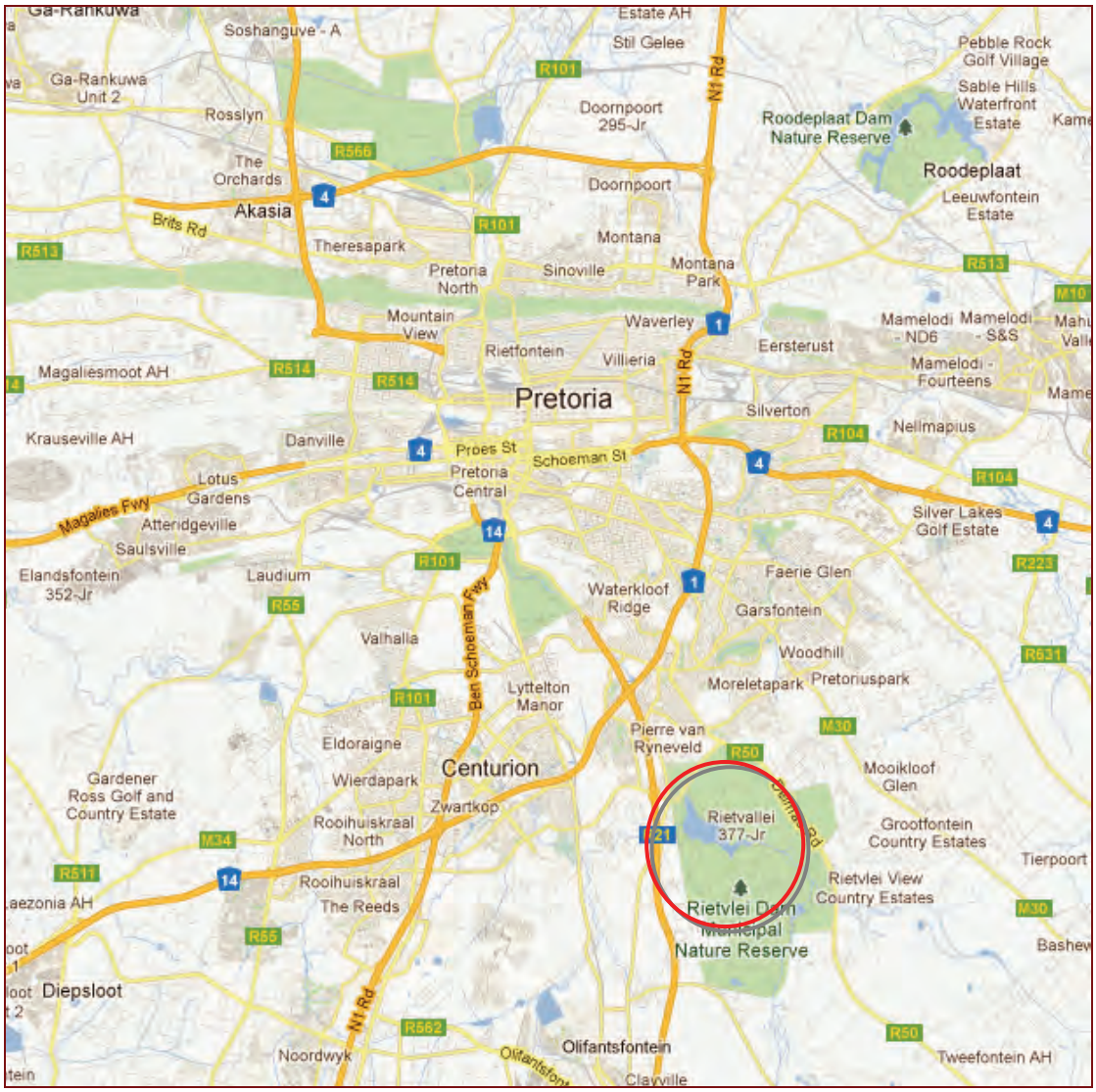

Figure 3.1: Location of Rietvlei Dam (circled) in relation to the City of Pretoria, South Africa (Source: GoogleMaps).

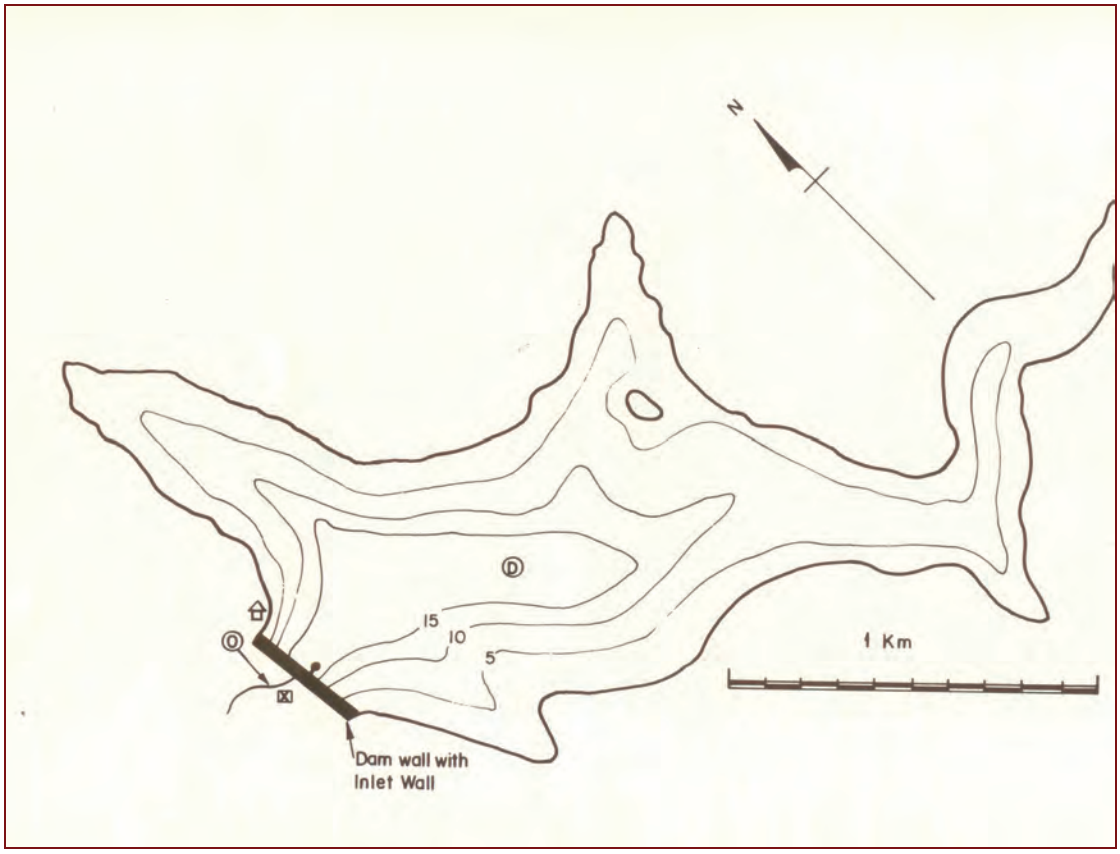

Figure 3.2: Bathymetry of Rietvlei Dam with depths in meters (Source: WRC 1980)

Sampling frequency

The project was initiated on monthly samplings with quarterly reporting of data. During the second year of the project, sampling events were combined into a single exercise with a specific aim, either a more intensive on-site examination of samples or to provide scope for undertaking additional sampling in the catchment. The final outcome was 19 individual sampling occasions for the dam during the 30-month period.

Water quality

The offshore stations (15-18 m deep depending on impoundment drawdown) were profiled at 1-2 meter intervals, surface to bottom, for temperature, electrical conductivity, pH, dissolved oxygen, orthophosphate-P and nitrate-nitrogen. Additional profiling was provided by the DWA on a single occasion. The nearshore stations (three, 3- 5 m deep) were sampled sub-surface for the same parameters.

Figure 3.3: Map of Rietvlei Dam showing the positions of the offshore (1 and 1A) and nearshore (2-4) sampling points.

Water samples were collected at each discrete level using a Van Dorn closing bottle and transferred to a container on board the boat, taking care not to oxygenate the sample during transfer. Measurements of temperature, electrical conductivity, pH and dissolved oxygen were made immediately, while measurements for orthophosphate-P and nitrate-nitrogen were made onshore on completion of each sampling exercise.

Comparative data for the offshore stations were obtained from the Rietvlei Laboratory.

Water transparency was measured using a conventional (Helber-Bios) Secchi Disk. Electrical conductivity, along with temperature, was measured using a Hach Sension5 conductivity meter. pH was measured using a Hach Sension1 pH meter. Dissolved oxygen and oxygen saturation was measured using a YSI 550A meter. Orthophosphate-P and nitrate-nitrogen were determined colorimetrically using Hach phosphate and nitrate colorimeters.

Wind and wave mixing

A screening analysis of wind-induced mixing was undertaken, using wind data collected at the nearby Irene Agricultural Station. Data for the 10-year period preceding this project were utilized. The analysis utilized the Carper and Bachmann (1984) method for the determination of wave-induced mixing depth, based on windspeed and fetch distance for specific wind directions. The windroses generated for the dam are provided in **Appendix B**. The assessment was not extended to include an estimation of the magnitude or duration of internal waves and seiches.

Phytoplankton

Sample collection

Phytoplankton was collected qualitatively in repeated vertical hauls at the designated sampling sites through the study period, using Wisconsin-type nets of 5 μm and 20 μm mesh aperture, with a mouth aperture of 0.2 m. Vertical hauls were made at the selected sampling sites from a depth of 7 m (the deemed stratification depth at the outset of the project) to the surface.

After washing down the net from the outside, the concentrated bucket samples were made up to approximately 0.5 L with 20 μm-filtered lake water and stored in sealed 1 L containers in a cold-box for transport to the field laboratory, where the sample containers were opened with minimal agitation, and allowed to stand for several hours in the dark in a fridge at roughly 4^{o}C to allow sinking of non-motile or non-buoyant algae (or re-flotation of buoyant algae/cyanophytes).

Sample processing

Samples were then separated, decanted and the supernatant water passed through a series of sieves (35, 50 and 80 μm) to remove zooplankters. Sub-samples were removed for microscopic examination and the samples were then further concentrated and dried at ± 60^{o}C, and stored dry pending SIA.

Spot checks for the presence of a phytoplankton component smaller than 20 μm were carried out by means of Lugols-sedimentation of bulk samples, and/or vertical hauls using a 5 μm mesh net.

Phytoplankton samples were examined fresh within 36 hours of collection using conventional light microscopy. All SIA-samples were retained fresh for subsequent drying and analysis (see SIA).

Phytoplankton dry weights were determined after drying to constant mass at 60°C, and then by weighing using a Mettler balance. Dried samples were retained in a desiccator at all times.

The phytoplankton assemblages were assessed against the functional groupings described by Reynolds et al. (2002) and Padisak et al. (2009).

Zooplankton

Zooplankton sample collection

Zooplankton samples were collected using a Wisconsin-type net, incorporating a 'reducing cone' to maximize net filtration efficiency. The net had a mouth diameter of 20 cm and mesh aperture of 80 μm. Parallel samples were collected for 'taxonomic' and stable isotope analyses. Vertical hauls were made at the selected sampling sites from just above the lake bottom to the surface. After washing down the net from the outside, the concentrated bucket samples were made up to approximately 1 L with 80 μm-filtered lake water and stored in sealed 1 L containers in a cold-box for transport to the field laboratory, where the sample containers were opened with minimal agitation, and allowed to stand for several hours in the dark in a fridge at roughly 4^{o}C to allow sinking of non-motile algae (or re-flotation of buoyant algae/cyanophytes). Samples for taxonomic analysis were then decanted and the supernatant water containing active, live zooplankters was sieved through 80 μm mesh, collected quantitatively, and preserved with formalin to a final formalin concentration of about 10%. When necessary, a second-stage separation of algae and motile zooplankton was undertaken, and the > 80 μm retentate combined with that of the first phase separation. SIA samples were treated as described below (Figure 3.4).

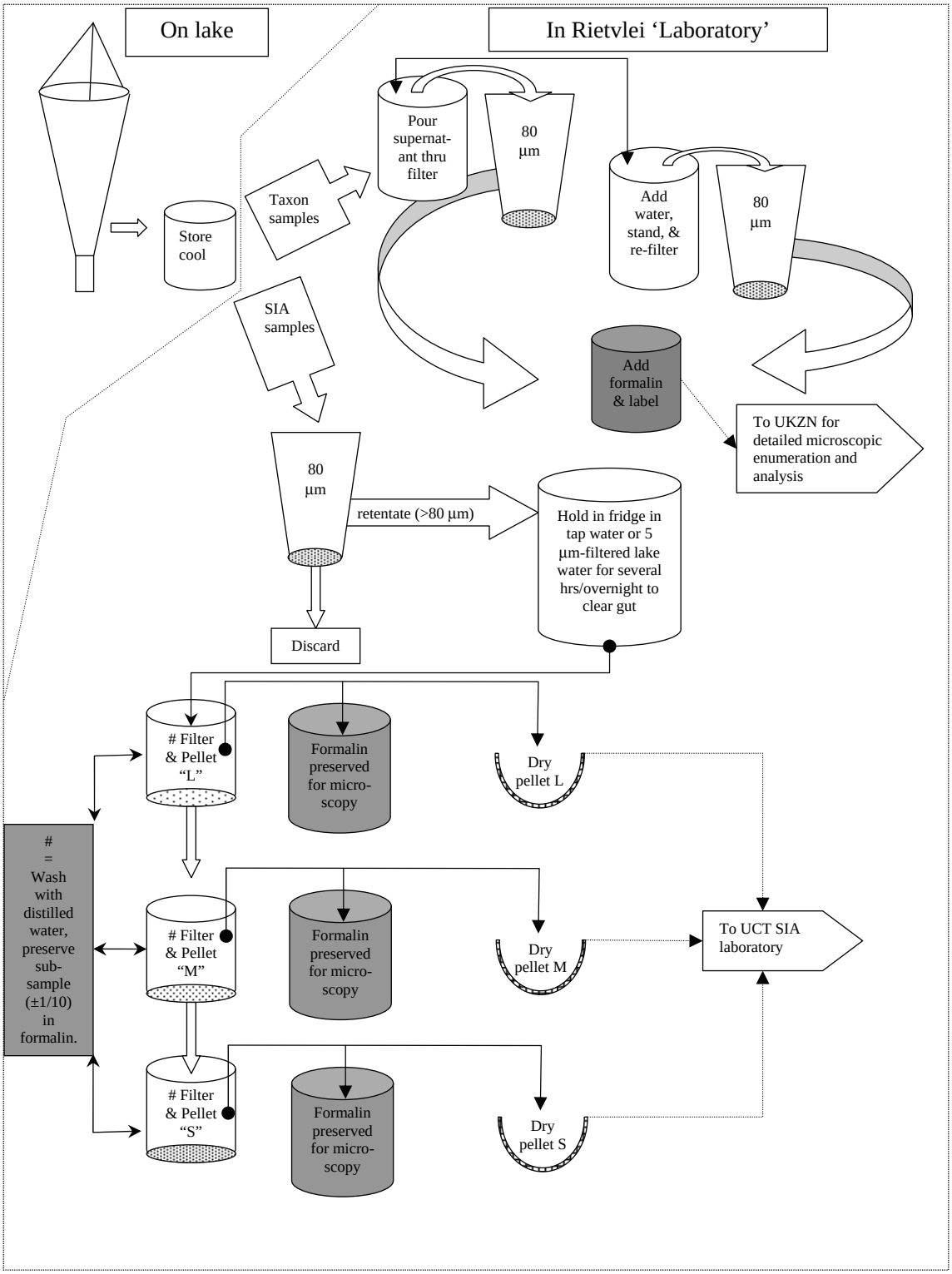

Figure 3.4: Schematic illustration of post-collection treatment procedures used for zooplankton samples in the 'field' laboratory at Rietvlei. "L", "M" and "S" filters for SIA samples are 500 µm, 200 µm and 80 µm respectively.

Various practical constraints limited sampling frequency to intervals of roughly 6 weeks on average – considerably longer than the ideal of 1 to 2 weeks for classical zooplankton

analysis purposes. However, this limitation was unavoidable. Sampling was generally undertaken at four sites, in two areas of contrasting depth and shore-line proximity – three shallow inshore sites along Rietvlei's north-eastern shoreline, and one (sometimes two) deep offshore/mid-lake 'control' site(s).

Taxonomic sample analysis

Preserved samples were decanted into a plankton bucket with a 53 μm mesh-covered window and thoroughly rinsed with tap water. The washed sample was made up to a defined volume (generally 200 mL) in an octagonal sub-sampling vessel, and gently but thoroughly mixed to ensure homogeneity before a volumetrically defined sub-sample (commonly 1 to 5 mL) was withdrawn with a wide-mouthed pipette. This sub-sample was dispensed into a Bogorov counting chamber for microscopic analysis and quantitative enumeration of major taxa and their corresponding size (cladocerans) under a dissecting stereo-microscope at appropriate magnification – generally 25X. Consecutive sub-sample aliquots were counted (with replacement) until around 200 but at least 100 individuals of each major taxon had been enumerated. This generally required the enumeration of between 1/100 and 1/25 of the original sample volume. For less abundant larger organisms like *Chaoborus*, it was often necessary to examine the entire sample in a larger volume counting trough. Systematic analysis of copepod population structure was not attempted, although the density of egg-bearing individuals of all taxa was recorded.

The size-structure of the *Daphnia* population was determined with a conventional measuring eyepiece or optical micrometer (appropriately calibrated against a micrometer slide). Body length was measured as the linear distance between the anterior margin of the head shield and the beginning of the shell spine in a random sample – the first 50 to 55 individuals encountered in the subsample(s).

Sample counts (per net haul) were multiplied by 31.82 to scale them up to numbers per m^2, and then further divided by haul depth to convert them to numbers per m^3. These conversions assume 100% net sampling efficiency. The adjusted counts were applied for all subsequent analyses (except where contextually contra-indicated).

Average individual biomass of *Daphnia* was estimated from the length-mass regression $W=5.2*L^{3.012}$, where W is μg dry mass, and L is geometric mean length (mm) of the measured individuals. This value was applied to the sample counts to obtain a population biomass estimate. Size-frequency distributions for *Daphnia* were derived from all lengths

measured in each sample. The following standard biomass coefficients (μg/individual) were applied to other taxa, disregarding size (cladocerans) or instar stage (copepods): *Bosmina* – 1.5; *Ceriodaphnia* – 2.5; *Chydorus* – 2.0; *Moina* – 2.0; Cyclopoid copepods – 3.0; Calanoid copepods – 7.5; Copepod nauplii – 0.5, and summed to provide an overall estimate of 'total' crustacean biomass.

Samples for SIA

Zooplankton samples destined for SIA were washed with and re-suspended in 20 μm-filtered lake water and stored overnight at ± 4^{o}C. Samples were then fractionated by differential filtration into three size classes – 'Small' (<50 μm), 'Medium' (>50<200 μm) and 'Large' (>200 μm), which were concentrated and dried at ± 60^{o}C (after withdrawing formalin-preserved subsamples for potential microscopic analysis of taxonomic composition), and thereafter stored dry until SIA.

On two occasions, whole zooplankton samples were laboriously sorted by taxon in addition to the routine size-fractionation, to permit the determination of taxon-specific isotope signatures.

Macrophytes

Macrophyte samples were collected at nine locations around the dam (see Figure 3.5). The samples of plant canopy were collected from a boat by cutting leaves and stems at approx 0.3 m sub-surface. Samples were cleaned of sand and silt, transported cooled and fresh prior to drying in air at 60°C prior to SIA analysis. Samples of epiphytic diatoms were collected off the plant surfaces (see below).

Diatoms (epiphyton)

Diatom SIA samples were collected off the stems and leaves of macrophytes, at each of the macrophyte sampling sites, using the "shake in bag" method as described in Taylor et al., 2007. Samples were allowed to settle and then preserved using 95% ethanol in a 80:20 ratio (sample to ethanol) prior to drying and SIA analysis.

Sediments and macrobenthos

Annual collections of sediments and macrobenthos were made adjacent to each macrophyte sampling site (**Figure 3.5**). Samples were collected using a standard Birge-Ekman grab, combining three replicate grabs from each site. The samples were then screened via a 1000 µm mesh screen and the sediments and macrobenthic organisms removed and, respectively, dried or preserved in 95% ethanol for subsequent examination and analysis. Samples for SIA were in air at 60°C prior to SIA analysis.

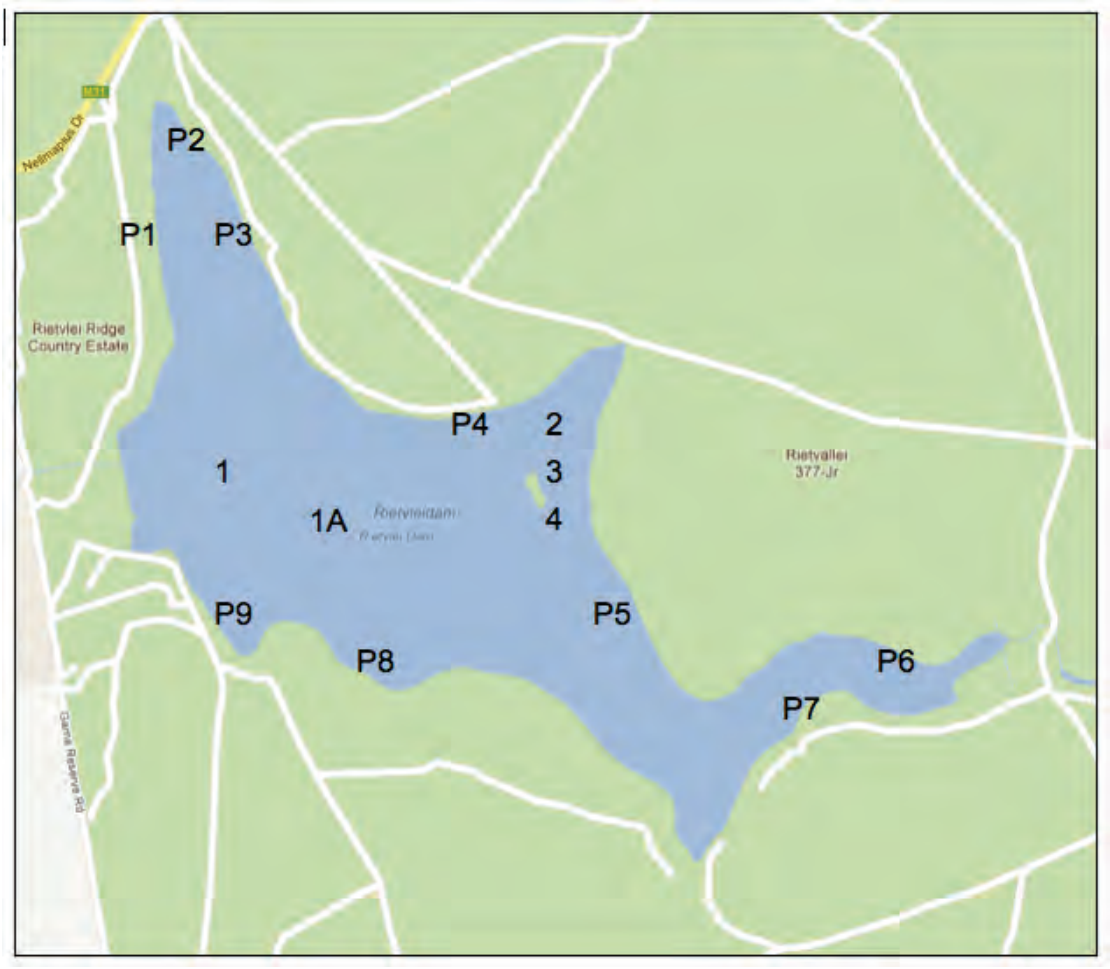

Figure 3.5: Location of the macrophyte and macrobenthos sampling sites (P1-P9).

Fish

Fish samples were collected as a one-off, two-day gill-netting exercise, as well as further collection of samples provided by local fishermen. The procedures followed, as well as the sites netted, are described in Harding and Koekemoer (2011). Samples of dorsal muscle, together with details of each specimen, were removed, wrapped in tin foil and frozen prior to drying in air at 60°C prior to SIA analysis.

Stable isotope analysis

All of the samples were retained frozen prior to air-drying (60°C) and submission for SIA analysis at the University of Cape Town. Samples were weighed into tin cups to an accuracy of 1 microgram on a Sartorius micro-balance. The cups were then squashed to enclose the sample.

The samples were combusted in a Flash EA 1112 series elemental analyzer (Thermo Finnigan, Milan, Italy). The gases were passed to a Delta Plus XP IRMS (isotope ratio mass spectrometer) (Thermo electron, Bremen, Germany), via a Conflo III gas control unit (Thermo Finnigan, Bremen, Germany).

The in-house standards used were:

- Choc – a commercial chocolate/egg mixture;
- Sucrose – "Australian National University (ANU)" sucrose;
- Valine – DL Valine purchased from Sigma;
- MG – Merck Gel – a proteinaceous gel produced by Merck;
- Seal – a seal bone, crushed, demineralized and dissolved in acid, and then reconstituted in gel form;
- Lentil – dried lentils;
- Nastd – Dried nasturtium leaves;
- NH_4Cl – As purchased from a chemical supplier.

All the in-house standards were calibrated against IAEA (International Atomic Energy Agency) standards. Nitrogen is expressed in terms of its value relative to atmospheric nitrogen, while carbon is expressed in terms of its value relative to Pee-Dee Belemnite.

SIA results were pooled for all assessed components of the system except fish and zooplankton. Fish were assessed on an individual (species) basis, with the results within the species pool being grouped. Zooplankton were assessed on the basis of the size fraction (see Zooplankton methods), as well as on a species basis on two occasions, as described both in the Methods as well as in the Results section. SIA data for macrophytes and epiphyton were not assessed on a spatial scale, although the data are available should this be of interest in future.

Statistical analyses

Conventional general parametric statistical tests were undertaken with routines available within Microsoft Excel 2003, employing the 'simplest' test possible (Murtaugh, 2007). Dedicated analysis of differences in zooplankton composition and abundance between inshore and offshore sites was undertaken using non-parametric multivariate analysis routines in the PRIMER package (Version 6.0, Plymouth Marine Laboratory). Zooplankton abundance estimates were log (X + 1) transformed, and a Bray-Curtis similarity matrix was calculated for each group of sites for two dedicated sampling runs. Resulting data were converted into an ordination using non-metric multidimensional scaling (MDS). Analysis of similarity (ANOSIM) was carried out on the data to determine whether zooplankton assemblages differed significantly between inshore and offshore sites, and the relative contributions of taxa to the differences was evaluated using the SIMPER routine.

Unless contextually or specifically indicated to the contrary, the results presented represent average values obtained across all sites sampled on a given sampling dates. Although these 'site-mean' values disregard spatial differences, they integrate and consolidate an otherwise intractably complex data set for presentation purposes. Linear regressions ('trend lines') were fitted to certain data series in order to show/examine longer term temporal trends. The intrinsic limitations of this approach (potential biases introduced by irregular sampling intervals and uneven temporal coverage) are, however, recognized.

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REYNOLDS CS, HUSZAR V, KRUK C, NASELLI-FLORES L AND MELO S (2002) Towards a functional classification of the phytoplankton. *Journal of Plankton Research* **24**:417-428.

SECTION 4: RESULTS AND DISCUSSION

PHYSICO-CHEMICAL CONDITIONS

Water column stability and the influence of wind mixing

From the windroses provided in **Appendix B**, it is apparent that the Rietvlei wind regime, for the 10 years preceding this survey, comprised six distinct orientations, as follows:

- December/January: Winds predominantly from the north/north-east and east/south-east;
- February/March: Winds predominantly from the east/south-east;
- April: Winds predominantly from east to south, with sub-dominant components from the north-west and north;
- May to July: Winds predominantly from south-east to south;
- August: Winds predominantly from north and south;
- September to November: Winds predominantly from the north/north-east.

Observations made on each of the 38 days that the dam was visited indicated that, apart from brief early morning periods of calm, light breezes arise by mid-morning, reaching moderate to fresh conditions by midday and through the afternoon. Strong wind conditions, of sufficient strength to preclude sampling, were encountered on several occasions.

The onset of lake overturn occurs during February-March of the annual cycle, this corresponding to the strengthening of winds primarily from the south to south-east (see **Appendix B**). The lake remains in the mixed state from April to October.

Based on the foregoing directional analysis, wave depths were calculated for two hypothetical mixing points on the lake (see **Figure 4.1**), using wind directions from the north, south and south-east. The effective fetch distances, calculated from a set of radials either side of the directional axis, are shown in **Table 4.1**. The summarized wind conditions are presented in **Table 4.2**. These show the median, minimum, maximum and percentile values for each month.

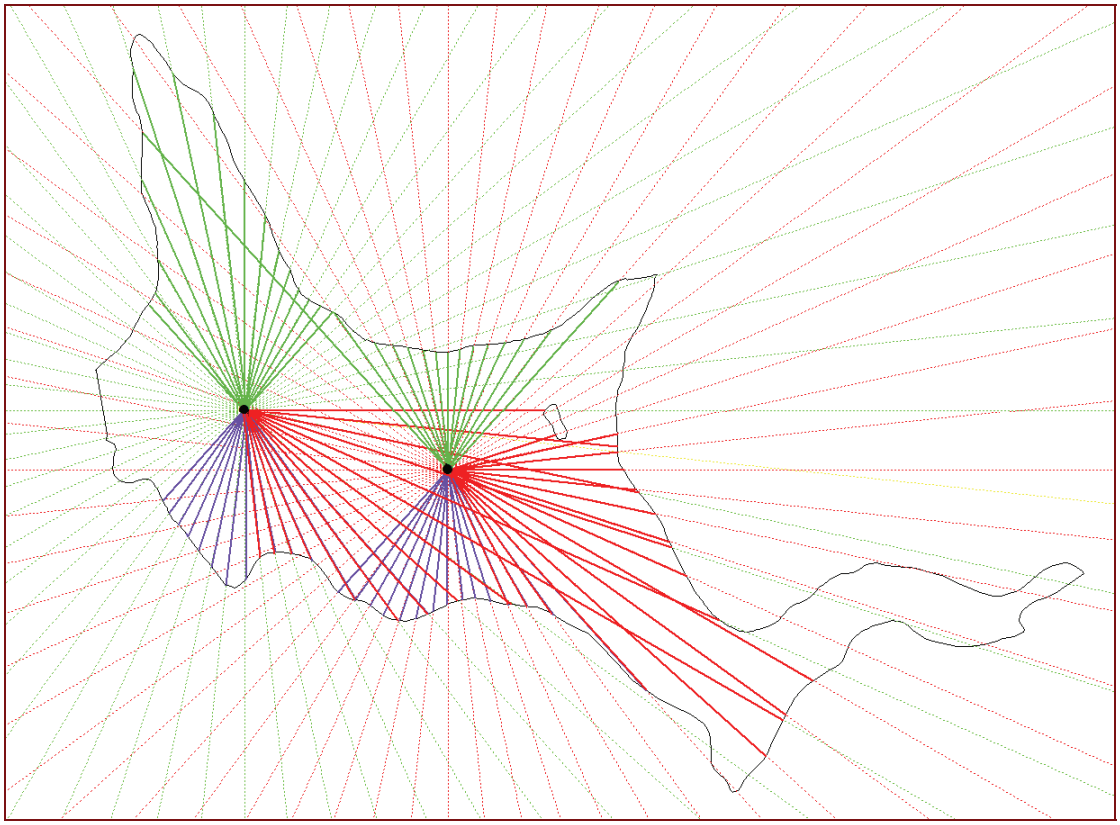

Figure 4.1: Outline of Rietvlei Dam showing the two hypothetical mixing points and their associated wind arcs for winds from the north (green), south (purple) and south-east (red). Dotted lines represent computer-generated 7° radials for each point.

Table 4.1: Windspeeds for Rietvlei, expressed in meters per second (m s^{-1})

Value	J	F	M	A	M	J	J	A	S	O	N	D
Median	2.9	2.9	2.7	2.6	2.6	2.7	2.6	2.8	3.3	3.4	3.3	3.1
Min	0	0	0	0	0	0	0	0	0	0	0	0
Max	12.2	10.2	11.5	10.5	10.5	11.8	11.8	14.7	12.1	13.5	12.4	11.1
25%ile	2.1	2.1	2	1.9	1.8	1.9	1.9	1.9	2.2	2.3	2.3	2.2
75%ile	3.9	3.9	3.7	3.5	3.5	3.6	3.6	4.1	4.6	4.6	4.5	4.3
90%ile	5	5	4.8	4.5	4.5	4.8	4.8	5.6	6	6	5.8	5.4

Table 4.2: Fetch distances for the indicated sites, in meters (m)

Site/Fetch	North	South	South-east
Site 1	604	605	895
Site 2	597	452	698

Using a median windspeed of 3 m s^{-1} for all directions and sites, the depth of mixing, measured as the depth of wave passing the indicated point, was determined. Resulting

values are shown in **Table 4.3**. These data show that for this illustrative, relatively-low, windspeed, wave-induced mixing occurs to a depth of at least 0.5 m on a routine, daily basis. Wave depths will increase downwind of the indicated point and create internal return waves after impinging on the downstream shoreline.

Table 4.3: Waves depths for the indicated sites, based on a windspeed of 3 m s^{-1}.

Site/Fetch	North	South	South-east
Site 1	0.6	0.6	0.7
Site 2	0.6	0.5	0.6

Water quality

The water quality data are summarized for months 1-30 as the depth profiles for the offshore station (Station 1, Figure 3.3).

Water transparency

A temporal representation of the measured Secchi depth, vs. chlorophyll-a (data for chlorophyll obtained from the Rietvlei laboratory) is shown in **Figure 4.2**. Two issues of importance are discernible from these data. Firstly, the water transparency declined progressively throughout the project, as indicated by the trend line overlain on the data. Given the observed general absence of inorganic turbidity, the implication is that phytoplankton biomass increased during the study period.

The second aspect is the lack of any correlation between chlorophyll, as reported by the Rietvlei laboratory and the measured water transparency. As the chlorophyll measurements are made on the raw potable water entering the treatment works, this discrepancy may be attributed to the use of a deep, sub-epilimnion, drawoff point.

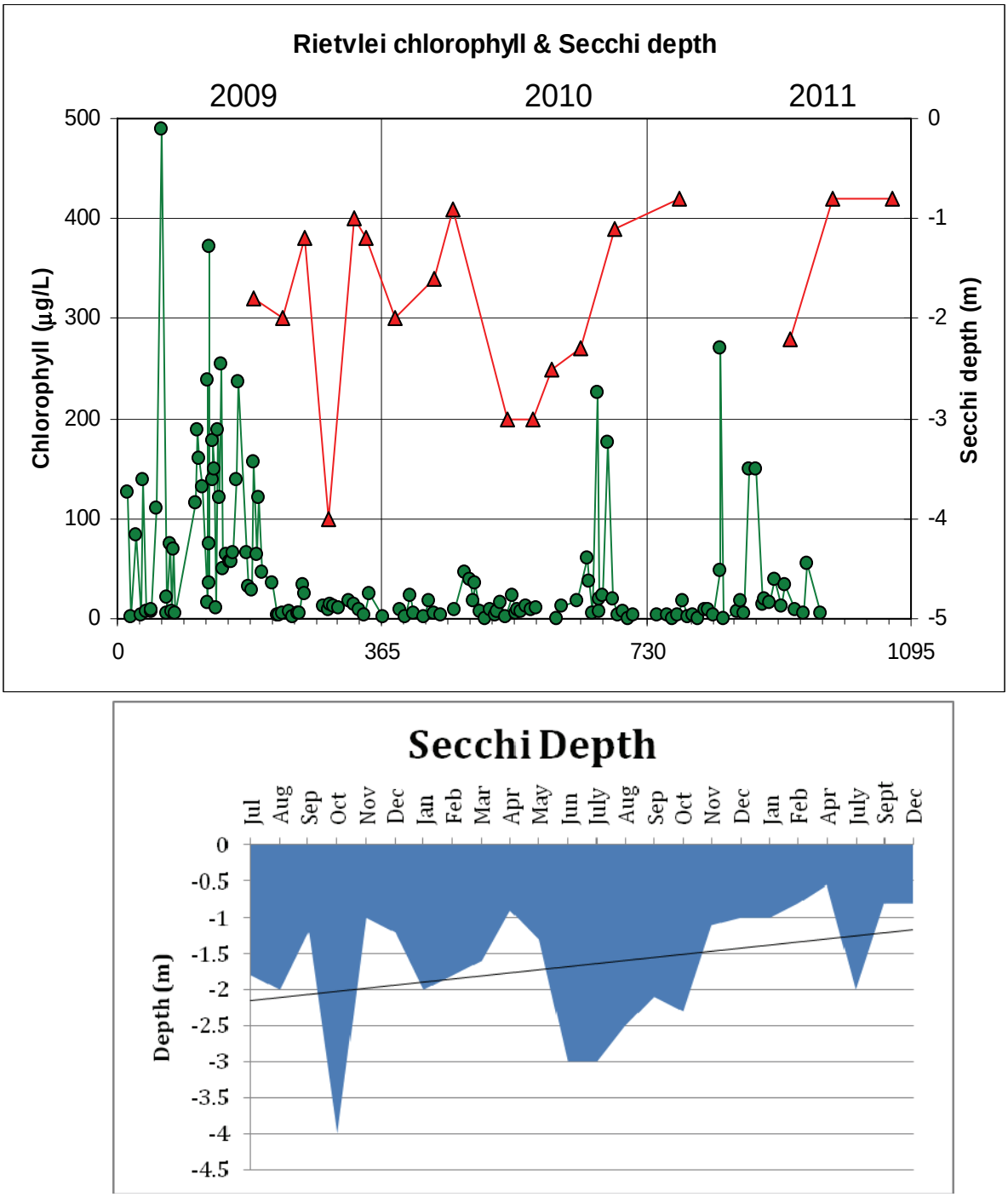

Figure 4.2: Data for chlorophyll and Secchi Depth as measured during this project. The time scale on the upper graph is in Days from commencement of the project in July 2009.

Water temperature

Water temperature profiles (**Figure 4.3a**) show that the dam is mixed between March and August of each annual cycle, with marked stability (stratification) in the upper layers, to a depth of approximately 6 m, between September and February.

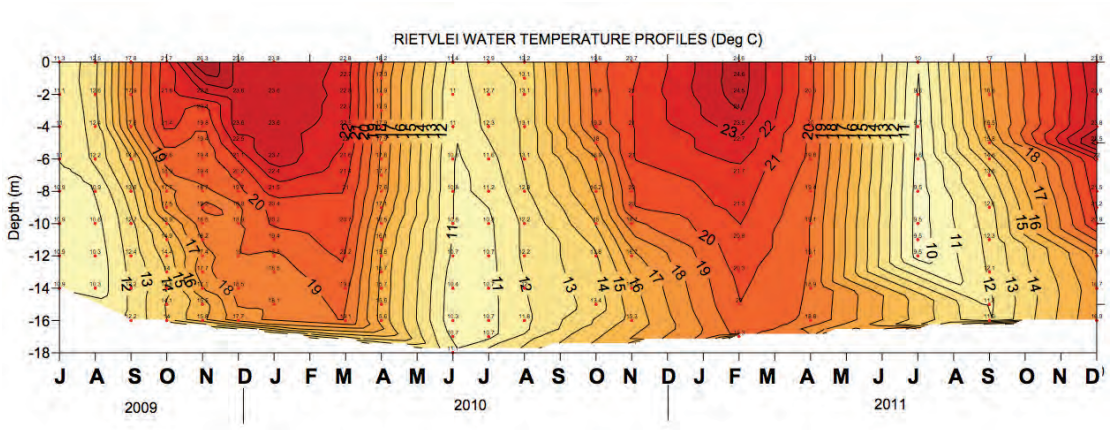

Figure 4.3a: Water temperature profile (Station 1).

This profiling accords closely with data from 1976, shown in **Figure 4.3b**. The 1976 data do, however, show an extended mixing cycle consistent with that measured during 2011:

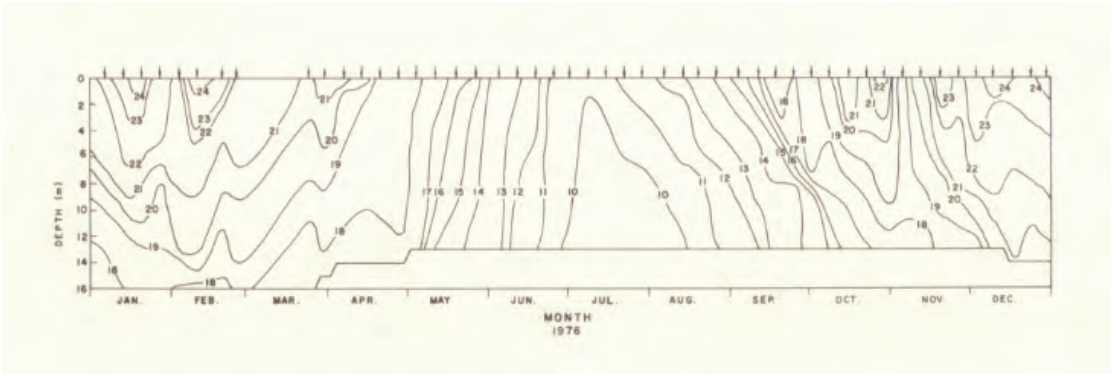

Figure 4.3b: Rietvlei isotherms for 1976

Electrical conductivity

Variations in electrical conductivity were unremarkable and varied by less than 10 mS m^{-1} – both temporally and in the water column, during the 30-month period (see **Figure 4.3c**).

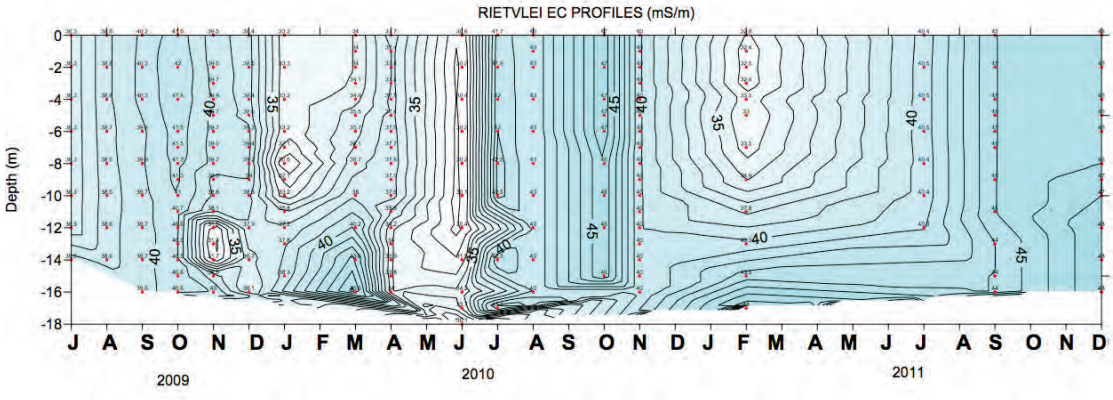

Figure 4.3c: Electrical conductivity profiles (Station 1).

pH

Variation in pH was similarly muted – with pH stratification corresponding to temperature as would be expected (see **Figure 4.3d**). pH values of 8.3-8.8 typified the stratified phases, with values approximately 1 pH unit lower during the periods of mixing.

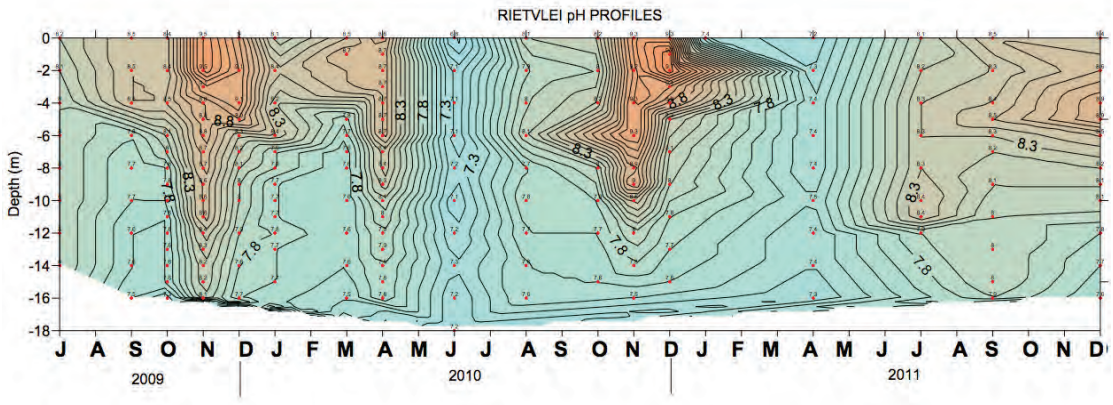

Figure 4.3d: pH profiles (Station 1).

Dissolved oxygen and oxygen saturation

Oxygen stratification corresponded to the thermal profiles (see **Figure 4.4e**). Oxygenation in the upper 6 m of the water column exceeded 50% throughout the period examined (**Figure 4.3f**).

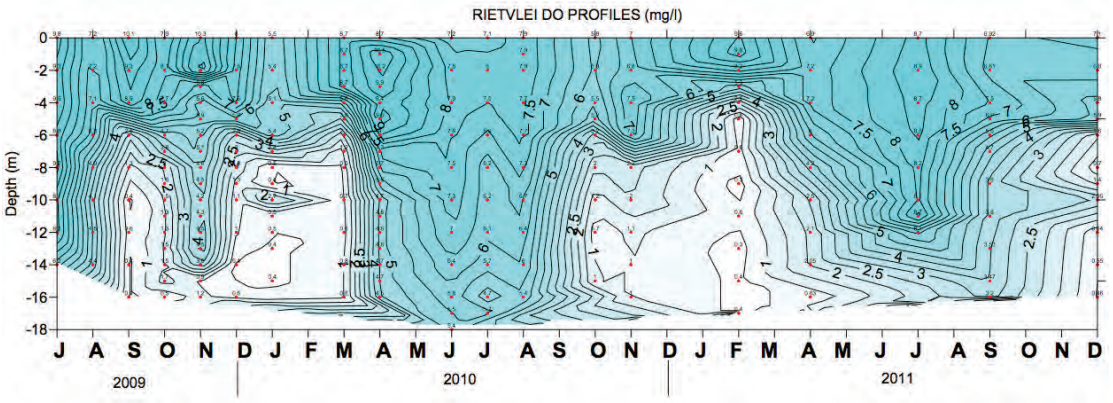

Figure 4.3e: Dissolved oxygen profiles (Station 1).

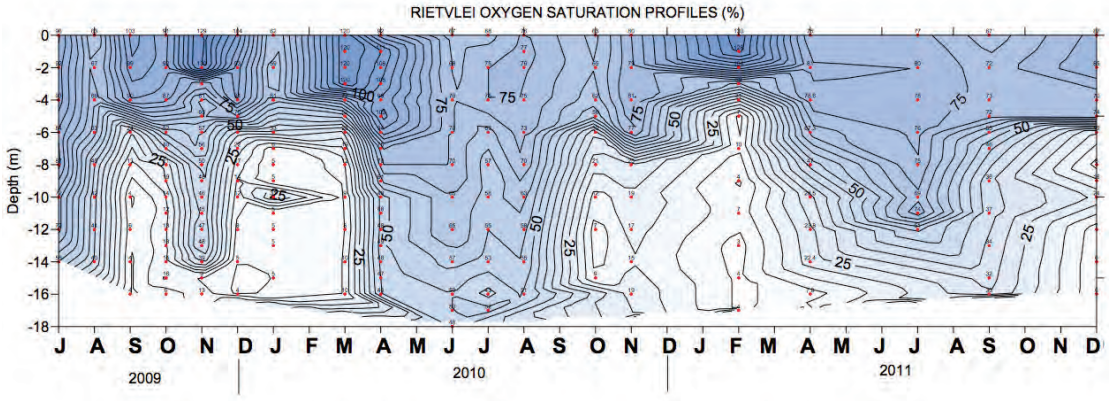

Figure 4.3f: Oxygen saturation profiles (Station 1).

Soluble reactive phosphorus (orthophosphate-P)

The availability of biologically-available phosphorus, as orthophosphate-P, was generally high and in the hypertrophic range (see **Figure 4.3g**), with marked pulses of phosphorus being released from the sediments during the periods of hypolimnion anoxia, as well as at the onset of turnover (cf. Figure 4.3a).

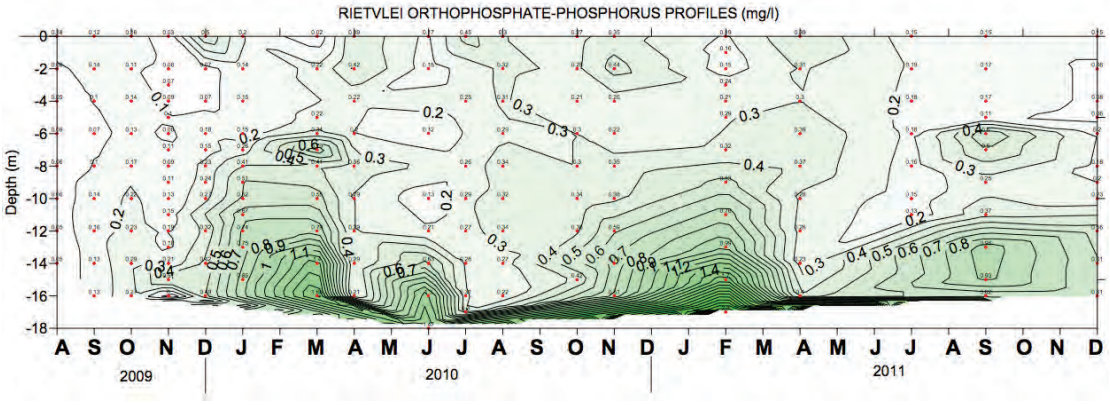

Figure 4.3g: Orthophosphate- phosphorus profiles (Station 1).

Nitrate-nitrogen

Inorganic nitrogen, measured as nitrate-N, was > 0.8 mg L^{-1} throughout the study, with the highest levels evident at the time of complete mixing (cf. Figures 4.3a and 4.3h).

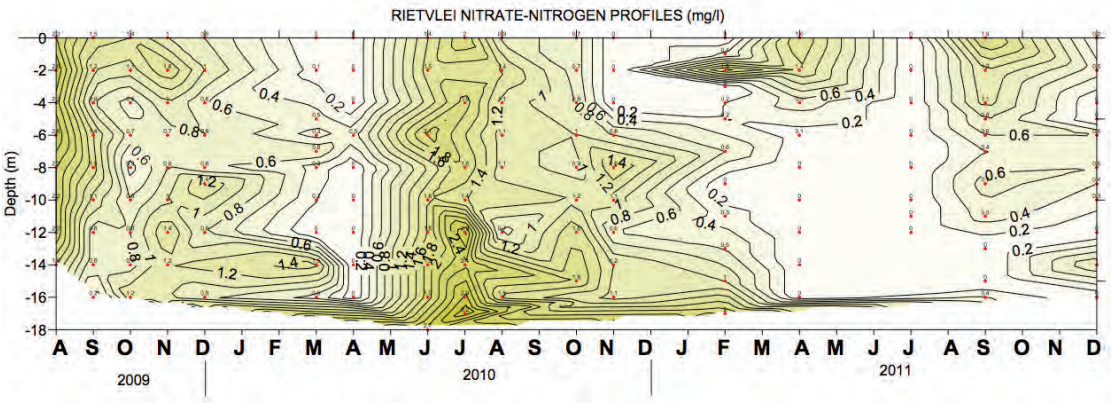

Figure 4.3h: Nitrate-nitrogen profiles (Station 1).

Stability profiles proximal to the water circulators

The Department of Water Affairs assisted with a once-off monitoring exercise to determine whether the effect of the epilimnetic mixers could be discerned using a high vertical resolution of measurements. The profiles provided by DWA, for Site 1, plus a site immediately adjacent to the solar-powered mixer nearest to Site 1, are provided in **Figure 4.4**. These profiles were recorded on 30 April 2010, i.e. during a period of lake mixing. Although not a focus of this project, the effect of the epilimnetic mixers could not be discerned from either of the profiling sets – but would be assumed to be so given the mixed conditions and the shallow lift-depth at which the mixers are set.

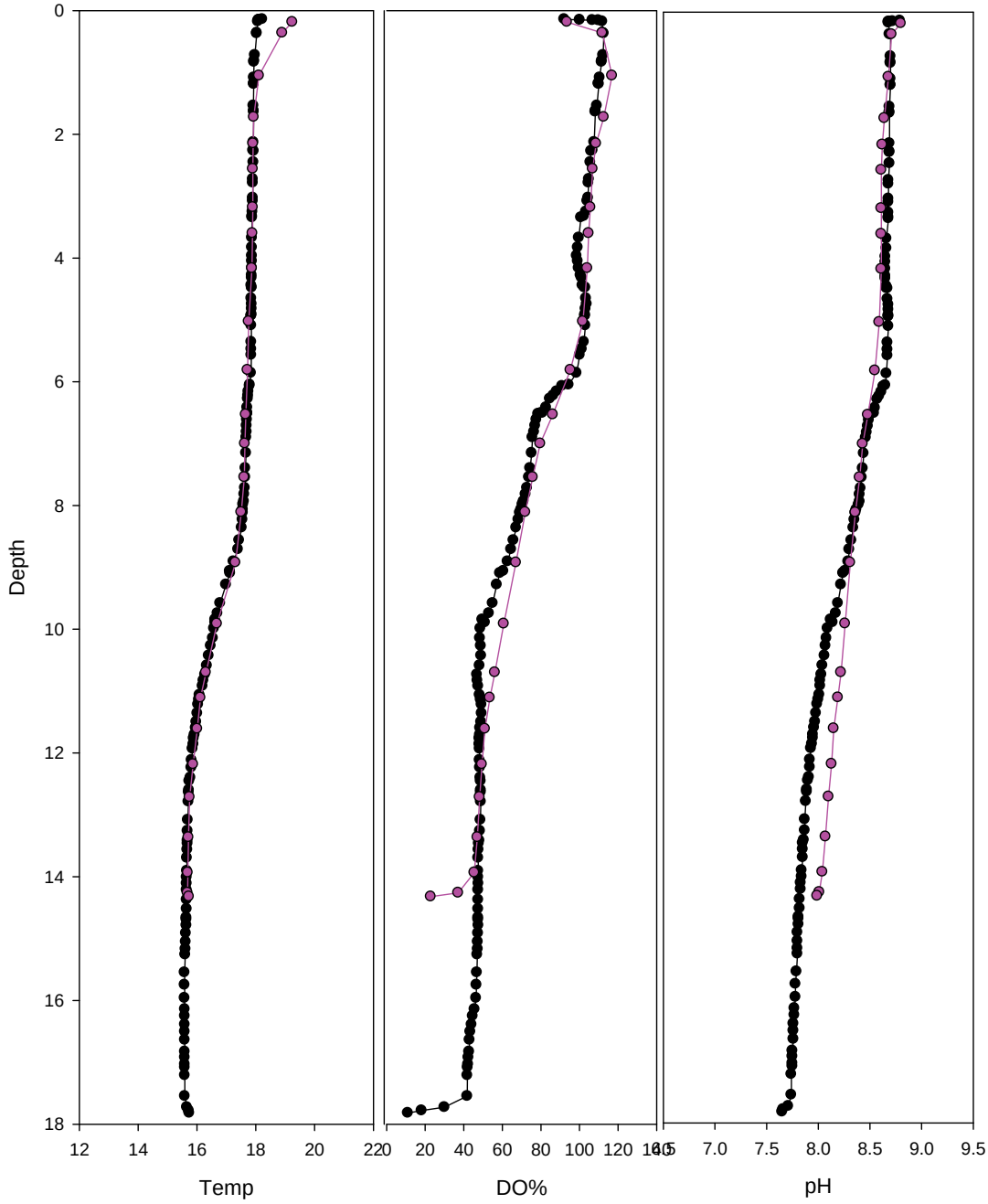

Figure 4.4: Comparative profiles for temperature, oxygen saturation and pH, measured at Station 1 (black circles) and 20 m from the nearest mixer (pink circles) on 30 April 2010 (Source: Dr H de Villiers, Department of Water Affairs).

PHYTOPLANKTON

An underlying precept to the basis for this project, i.e. that the level of algal biomass in the Rietvlei Dam is elevated as a consequence of reduced grazing by zooplankton, is that the algal assemblage contains a predominance of edible algal species. It is a commonality of higher trophic waters, though not a fixed rule, that the phytoplankton become dominated by genera and species that are mostly inedible, with inedibility a function of colony size (cyanobacteria and some chlorophytes), filament length (cyanobacteria and diatoms) or cellular ornamentation (e.g. dinoflagellates) (See Section 3, Central Role of Zooplankton).

Phytoplankton assemblage

The Rietvlei Dam was found to be depauperate in phytoplankton diversity, with less than 10 species recorded on each sampling occasion, and with clearly-defined successional and periodic components, as well as consistent presence of sub-dominant species (see **Table 4.4** and **Appendix C**). The phytoplankton were dominated by species that are typically inedible by zooplankton (e.g. *Ceratium hirundinella, Aulacoseira granulata, Pediastrum duplex, Staurosira pinnata, Staurastrum leptocladium* and *S. pingue*, and *Microcystis aeruginosa*). This suggested, from an early stage of the project, that the zooplankton in Rietvlei were likely to be feeding via a microbial loop pathway (see Zooplankton).

Although phytoplankton data for this dam are extremely rare, the present assemblage is considerably less diverse than that reported in the 1970s (see **Section 2, Literature Review**), although common, central dominants such as *Aulacoseira granulata*, persist.

There was no difference, based on species assemblage, between the inshore and offshore stations (see Appendix C).

The phytoplankton seasonality and periodicity was observed to follow the state of mixing in the reservoir closely, as described below.

Antecedent phytoplankton assemblage and periodicity

An analysis of the phytoplankton assemblage and its periodicity during the ten years prior to this project was made, utilizing data sourced from the Department of Water Affairs' Resource

Quality Objectives (RQS) Directorate. These data are compiled based on samples collected using a 5 m hosepipe sampler, followed by preservation and microscope examination.

A total of 190 sampling occasions were included in the dataset, ranging from January 2001 to December 2011. From these data the following dominant species, out of a total of 48 recorded, were identified based on their frequency of occurrence:

- *Aulacosira granulata*: 58 samples (31%)
- *Ceratium hirundinella*: 96 samples (51%)
- *Cryptomonas* sp: 55 samples (29%)
- *Microcystis aeruginosa*: 70 samples (37%)

When examined on a temporal scale, the data for the dominant species provides an interesting picture of a changing system. **Figure 4.5** shows the dominance of peaks of *M. aeruginosa* and *C. hirundinella*, the two strongest dominants in the ten year dataset. The peaks reveal that *M. aeruginosa* was generally dominant between January 2001 and mid-2007, where after *C. hirundinella* becomes a stark dominant between early 2008 and September 2009, and again thereafter from mid-2010 until the end of 2011. This indicates quite clearly that a major change in phytoplankton dominance had taken place some eighteen months prior to this project commencing, or the epilimnetic mixers being installed.

In the case of the two sub-dominants, *A. granulata* and *Cryptomonas*, the data indicate an increasing frequency of the benthic diatom over the period examined, with short periods of *Cryptomonas* blooms, possibly reflecting organic pollution events.

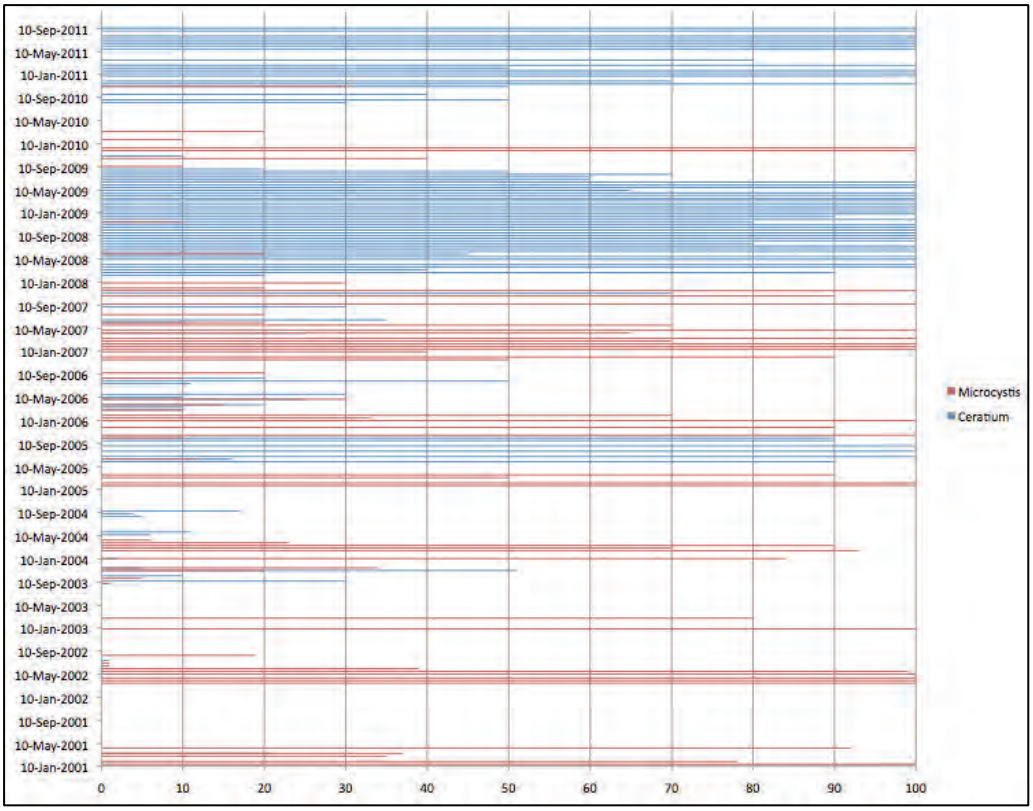

Figure 4.5: Periodicity of dominant phytoplankton during the period January 2001 to December 2011 (Data Source: DWA, RQS).

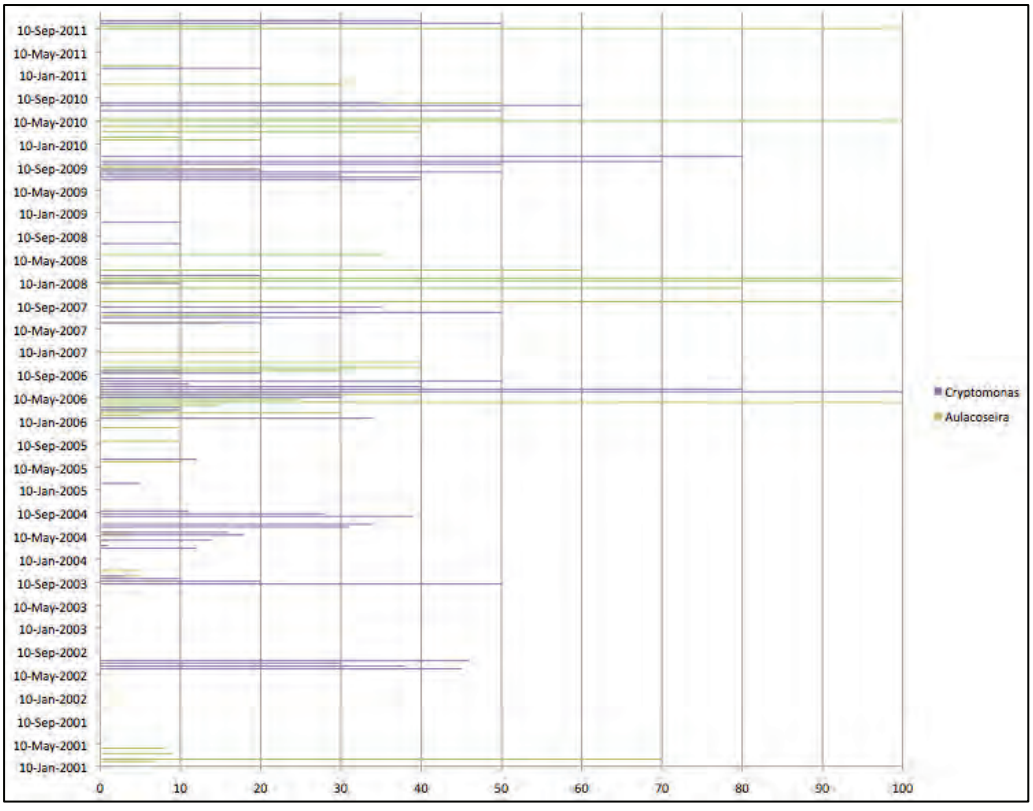

Figure 4.6: Periodicity of sub-dominant phytoplankton during the period January 2001 to December 2011 (Data Source: DWA, RQS).

Phytoplankton assemblage in relation to water column stability

The project commenced in June 2009, with the lake in a mixed state. The phytoplankton assemblage was dominated by *Ceratium hirundinella* and *Aulacoseira granulata*, i.e. with respect to the diatom *A. granulata*, typical of a semi- to continuously-mixed eutrophic to hypertrophic epilimnion. While *C. hirundinella* is typically-associated with stable conditions or slow-flowing rivers in north-temperate regions, it appears to be able to tolerate all mixing conditions occurring in Rietvlei (see below). This is in accordance with findings from other studies (e.g. Perez-Martinez and Sanchez-Castillo, 2002). Overturn may move cysts from the sediments into the water column, along with phosphorus from the hypolimnion, *C. hirundinella* being an alga that responds rapidly to nutrient enrichment (e.g. Matsumura-Tundisi *et al.*, 2010).

The onset of stratification, in October, was marked with the immediate appearance of *Microcystis aeruginosa*. The stratified condition persisted until March 2010, with a rapid decline of *M. aeruginosa*, replaced by *Schroederia setigera*. *S. setigera*, a distinct yet cosmopolitan species typically-associated with stable water columns, dominated thereafter until March 2010, but with *M. aeruginosa* reappearing between January and March 2010.

The onset of mixing in April 2010 saw the rapid disappearance of *M. aeruginosa*. *A. granulata* became dominant, in turn replaced by *Staurastrum leptocladium*. *Coelastrum microporum* appeared as a sub-dominant just prior to the onset of stratification in October 2010.

The phytoplankton assemblage of the newly-stratified lake was rapidly dominated by *C. hirundinella*, together with *Pediastrum duplex* and *A. granulata*. The stratified phase was marked by a brief appearance of *M. aeruginosa*, just prior to lake turnover.

Lake turnover occurred in April 2011, but with *C. hirundinella* remaining dominant throughout the period of mixing. Following the third onset of stratification towards the end of 2011, *M. aeruginosa* resurged, together with three species of *Anabaena*, including *Anabaena circinalis*, as well as co-dominance by the eutrophication-tolerant diatom *Fragilaria ulna*. The presence of cyanobacterial hepatotoxins, microcystins, was detected, using an Abraxis test kit, in the samples collected during the December 2011 site visit. The re-appearance of *M. aeruginosa* occurred together with a substantial increase in macrophyte growth to a level not observed during the previous 30 months (see Macrophytes). During the 1970s,

A. circinalis was a dominant problem alga in Rietvlei (see Section 2) and was linked to low N:P ratios favouring selection for nitrogen-fixing algae.

Microcystis aeruginosa is considered to be sensitive to disturbance in deep lakes, but not in shallow, polymictic environments. In the latter, regularity of mixing, coupled with high irradiation levels, has been shown to boost biomass development (e.g. Harding, 1997).

The filamentous diatom, *Aulacoseira granulata*, dominant during the mixed phases in Rietvlei, benefits from a high disturbance regime and high levels of nutrients.

In summary, Rietvlei's phytoplankton assemblage and its periodicity/seasonality, are reflective of the natural hydrodynamic stability of the lake, coupled with its elevated trophic state. Co-dominance by *Microcystis aeruginosa* with *Ceratium hirundinella* is a common feature of these K-strategist algae in small to medium, eutrophic to hypertrophic lakes. Being sensitive to disturbance, the timing of the appearance of *Microcystis* in the phytoplankton assemblages appears to be linked to other forcing factors. *C. hirundinella,* described as physiologically-flexible (Ho Baek *et al*., 2007) appears to be able to tolerate both states of lake mixing, suggesting that deliberate alterations to mixis in the epilimnion may favour this potentially-problematical (potable water treatment) alga in lakes of elevated trophic state. Sustained epilimnion mixing may also result in increased development of *A. granulata*, a species that can be extremely problematical for the treatment of raw potable water.

In conclusion, the phytoplankton assemblage in Rietvlei is considered to be largely inedible for zooplankton, i.e. that top-down controls of the fishery are likely to have limited benefits for lake management. By contrast, control via "bottom-up" relief, i.e. reduction of nutrient loads to the reservoir, encompass significantly-better prospects. Additionally, no changes in the phytoplankton assemblage or periodicity could be attributed to anything other than the natural forcing-functions impinging on the lake, i.e. no influence of the epilimnetic mixers could be discerned.

Table 4.4: Dominant phytoplankton genera in relation to the state of mixing in Rietvlei.

YEAR 1	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June
Month	1	2	3	4	5	6	7	8	9	10	11	12
Mixing	**Mixed**			**Stratified**							**Mixed**	
Dominant phytoplankton	*Ceratium Aulacoseira*	*Ceratium Aulacoseira*	*Aulacoseira*	*Microcystis*	*Microcystis Schroederia*	*Schroederia*	*Schroederia*		*Schroederia Microcystis Aulacoseira*	*Aulacosira*		*Staurastrum*
Biomass												

YEAR 2	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June
Month	13	14	15	16	17	18	19	20	21	22	23	24
Mixing	**Mixed**					**Stratified**			**Mixed**			
Dominant phytoplankton	*Staurastrum*	*Staurastrum Coelastrum*	*Aulacoseira Ceratium Pediastrum*	*Ceratium Pediastrum Aulacoseira*	*Ceratium*			*Ceratium*		*Ceratium*		
Biomass (o/n)		0.251/0.224		0.126/0.111	0.222/0.734			0.687/1.178		0.148		

YEAR 3	July	Aug	Sept	Oct	Nov	Dec
Month	25	26	27	28	29	30
Mixing	**Mixed**					**Stratified**
Dominant phytoplankton	*Ceratium*		*Ceratium*			*Microcystis Aulacoseira Anabaena Fragilaria*
Biomass (o/n)						

Notes: Biomass = dry weight algal biomass in mg per litre. (o/n) = offshore/nearshore samples

MACROPHYTES

Rooted, submerged macrophytes present in the dam during this survey comprised *Potamogeton pectinatus, P. crispus* and *P. schweinfurthii*. Occurrence of these plants, as per the sampling locations shown in Figure 3.4, was as follows:

Site 1: *P. pectinatus* / *P. crispus*
Site 2: *P. pectinatus* / *P. crispus*
Site 3: *P. crispus*
Site 4: *P. crispus*
Site 5: *P. schweinfurthii*
Site 6: *P. crispus*
Site 7: *P. crispus*
Site 8: *P. pectinatus* / *P. crispus*
Site 9: *P. pectinatus* / *P. crispus*

Macrophyte stands were present in the dam for the first 14 months of the project, i.e. from July 2009 to August 2010. Thereafter, the stands virtually disappeared until Month 20, February 2011. During the last three months of the project (September to end November 2011), the extent and density of all of the stands increased dramatically, to a degree not previously observed during this project.

ZOOPLANKTON

Temporal changes in zooplankton abundance and composition

Site-mean abundance fluctuated considerably through the course of the study – as clearly reflected in changes in total crustacean community biomass (**Figure 4.7**). Overall, average biomass values centered around ~ 500 µg/L, with an apparent decline through the study suggested by the fitted trend-line. This long-term trend is discussed below in relation to potential impacts of SolarBee mixers on food resources.

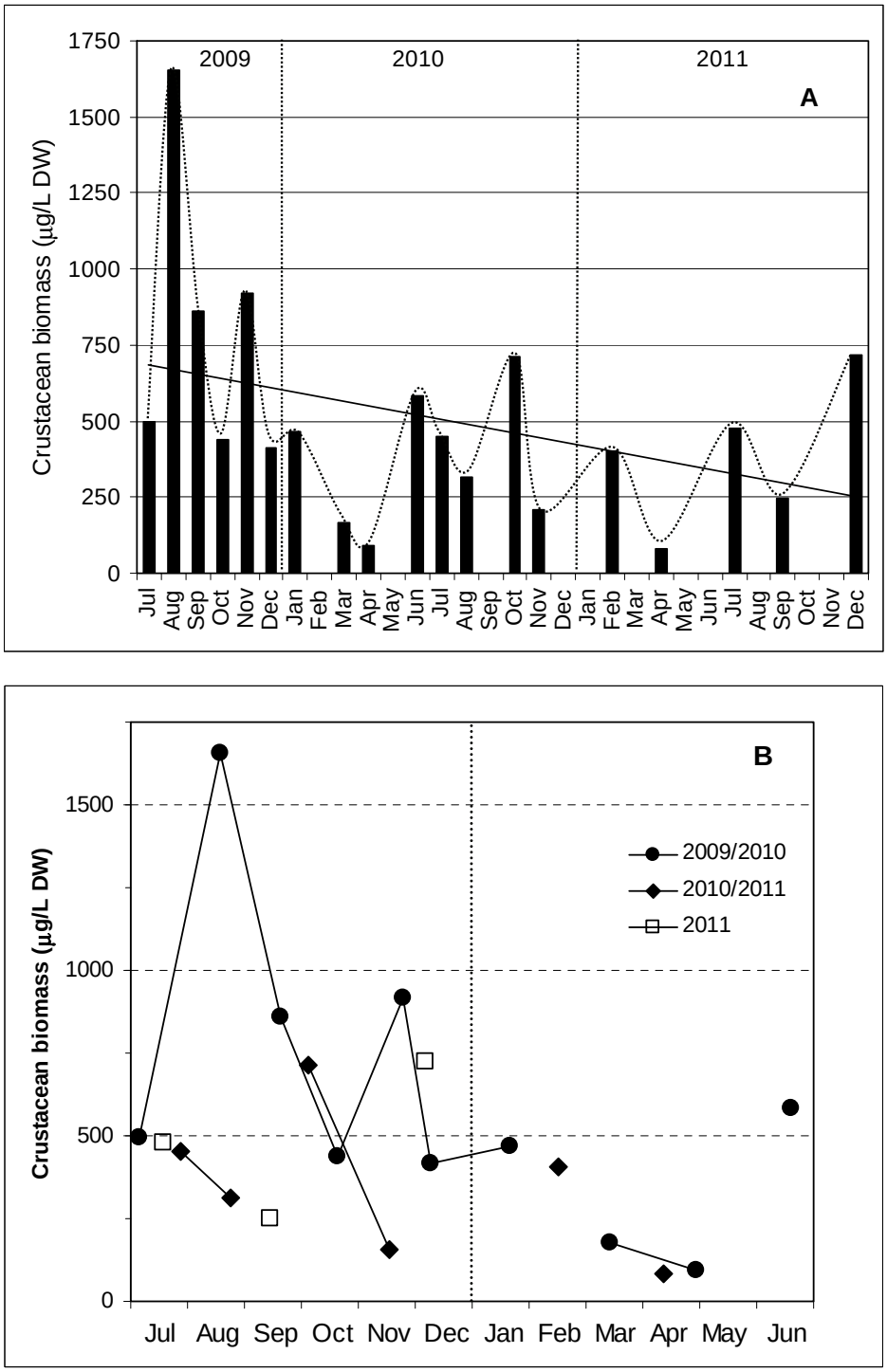

Figure 4.7: (A) Sequential changes in average crustacean zooplankton biomass at all sites sampled on given collection dates, with the overall trend shown by the solid line, and inferred annual patterns of seasonal change indicated by the dotted line. (B) Seasonal changes on an annualized basis.

Irregular sampling intervals make inter-annual comparisons and seasonal trends difficult to discern. However, biomass was generally higher during cool periods of the year and lower during summer when stratification developed in the single deep offshore site (**Figure 4.7A**).

However, this reduction is not directly attributable to stratification *per se*, since the date-specific site-mean values shown are numerically weighted (3:1) in favour of values that prevailed at shallow inshore sites that did not stratify.

On an annualized basis (**Figure 4.7B**), indications exist of a broadly repeatable seasonal trend involving a spring/early summer decline (October/November), followed by early/mid-summer resurgences (November/ December) prior to subsequent declines through high summer (January/February) to annual lows in late summer/autumn (March/April/May). Notably, this pattern is broadly consistent with the 'PEG model' of plankton succession (Sommer et al., 1986; Sommer, 1989) in eutrophic waters.

Temporal changes in biomass are obviously driven by and/or related to fluctuations in density (and average size) of the individual prevailing taxa, which fluctuate in accordance with seasonal changes in bottom-up and top-down drivers (food quantity and especially quality (resource types), and predation) as described in the PEG model.

Figure 4.8A shows changes in mean abundances (individuals/L) of major cladoceran taxa. The single highest density (51.4 ind/L) was exhibited by the small bodied *Bosmina* in March 2010, although the average density of this taxon when present during the study was considerably lower (9.0 ind/L). *Bosmina* was more abundant during the first year (2009/2010) than subsequently, and appeared commoner during autumn, winter and early spring than during summer months. *Ceriodaphnia*, another small-bodied taxon, appeared in low densities (maximum = 27.5/L, mean = 3.3/L) at various times of the year, broadly mirroring the seasonal changes exhibited by *Bosmina*. *Moina* only occurred in April 2010 and December 2011, and is not included in Figure 4.8A.

Large-bodied *Daphnia* were far and away the most common cladoceran taxon overall (average = 20.5/L, maximum = 42.0/L); they were generally most abundant during late winter and early spring, declining strongly during late summer months (**Figure 4.8B**). During the final December (2011) samples, they were virtually absent. Figure 4.8A hints at a decline through the overall study period, in line with that apparent for total biomass (Figure 4.7A). Their decline in warmer months is attributed to (inferred) adverse changes in food quality rather than an increase in fish predation, based on the evidence (given below) regarding their sustained high relative contribution to total zooplankton abundance and large size structure.

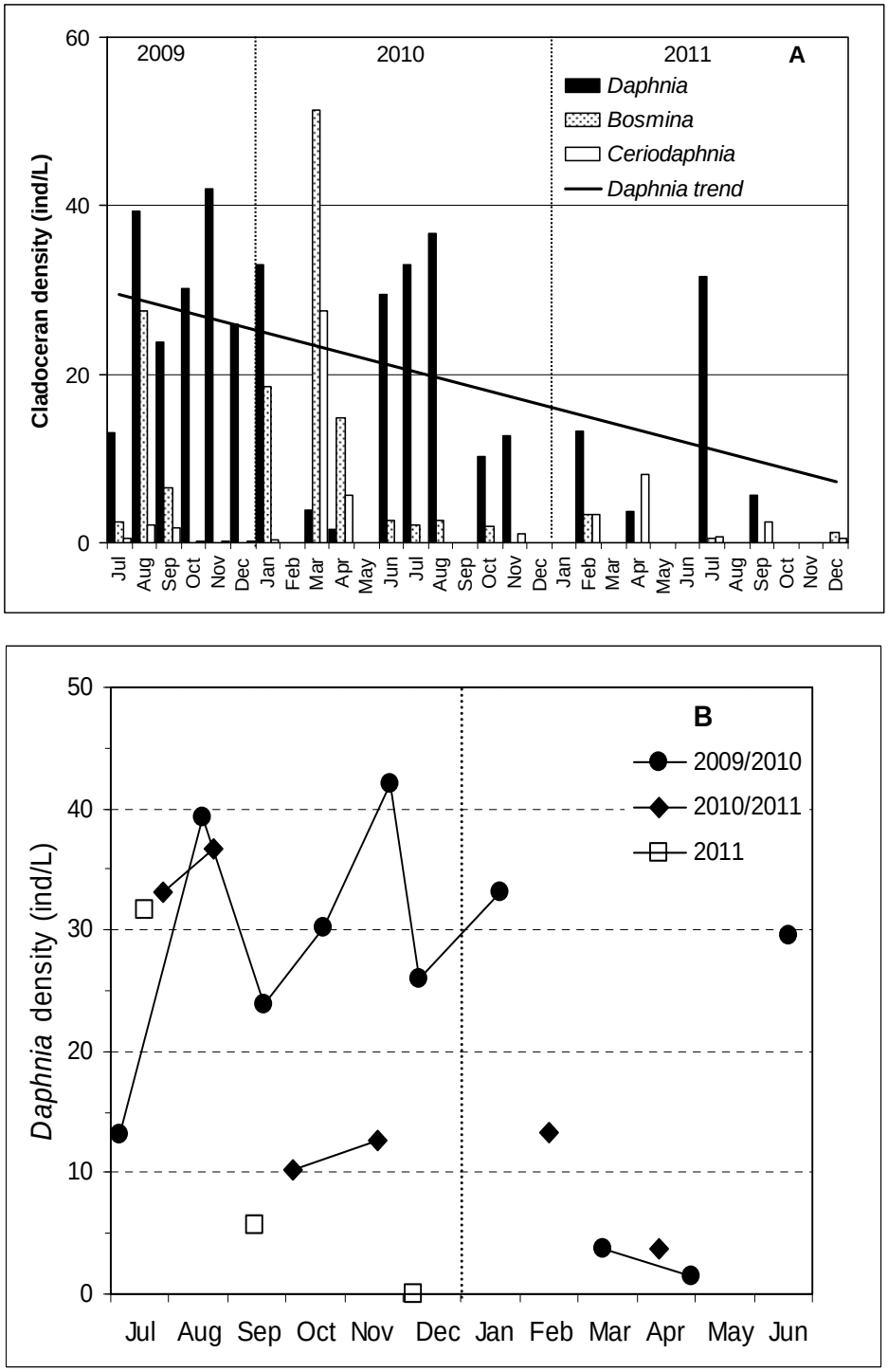

Figure 4.8: (A) Sequential changes in average density of cladocerans at all sites collected on given sample dates. (B) Seasonal changes in *Daphnia* abundance on an annualized basis.

Fluctuations in abundance of copepods, and their naupliar larval stages, are shown in **Figure 4.9**. Cyclopoid copepods numerically dominated the zooplankton overall, with densities roughly five-fold greater than any cladoceran taxa. They were commonest during late winter and spring, and generally declined during summer, broadly in line with cladoceran

densities. A clear exception to this emerged in the final samples (December 2011), when dense populations were present.

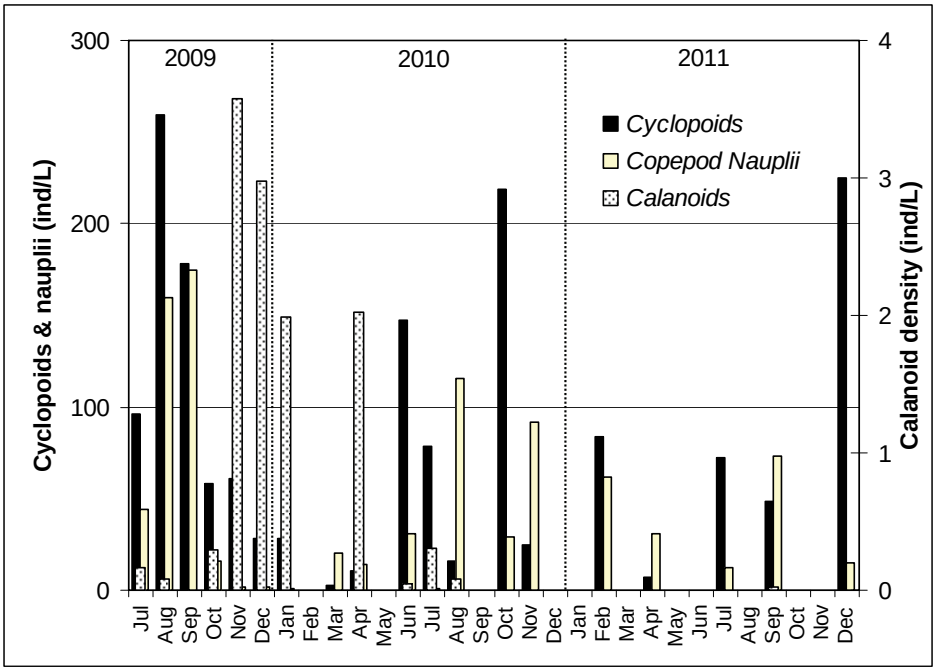

Figure 4.9: Changes in average density of copepods and their naupliar larvae at all sites collected on given sample dates. Note the different axes for cyclopoids and calanoids.

Calanoid copepods were numerically insignificant; their densities were some two orders of magnitude lower than those of cyclopoid copepods. They were at their most abundant during the 2009/2010 summer months when cyclopoid copepods declined (Figure 4.9), but thereafter they declined and effectively disappeared during the remainder of the study. Densities of naupliar larvae understandably varied significantly in line with corresponding total densities of cyclopoid copepodites (r = 0.389, P < 0.01), but were also influenced by cannibalism and predation, notably by the large rotifer *Asplanchna*, which attained very high densities at times during the study.

By virtue of its large size, *Daphnia* contributed strongly to total zooplankton standing stocks, accounting on average for 38% (range = 0 to 76%) of overall crustacean zooplankton biomass for the lake as a whole during the study (**Figure 4.10**), with closely similar values (average = 40% and range = 0 to 89%) for individual sampling sites. This finding is indicative of negligibly low levels or an absence of visual planktivory in Rietvlei, and has particularly important and negative implications regarding the feasibility of biomanipulation.

However, the trend line in **Figure 4.10** indicates a general decline in the proportional contribution of cladocerans to total zooplankton abundance, with a parallel trend line (not shown) for *Daphnia* (but see Figure 4.8A). Essentially, this reflects the progressive increase in copepods (consistently and overwhelmingly of cyclopoid elements) in the community, a shift plausibly-attributable to the installation of SolarBee mixers, and accompanying changes in phytoplankton composition. Circulation strongly favors large, ruderal phytoplankton taxa, and the large dinoflagellate *Ceratium*, which are inedible to *Daphnia* but favour raptorial copepods (see account of 'zooplankton feeding mechanisms' in Section 3). Precisely such a change in zooplankton composition following the onset of *Ceratium* blooms has been documented in another South African reservoir (Hart & Wragg, 2009).

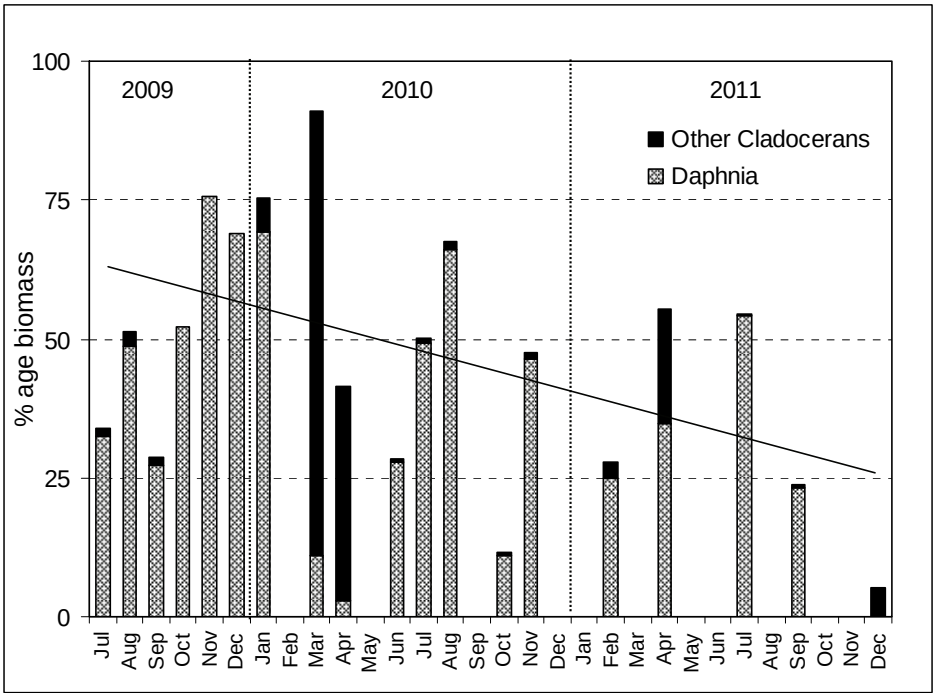

Figure 4.10. Average percentage contributions of *Daphnia* and other (small-bodied) cladocerans to planktonic crustacean standing stocks during the study. Trend line is for total % cladoceran biomass.

Spatial differences in abundance and composition

Average densities per unit area (ind/m^2) through the entire study tended to be higher offshore at Site 1 than at the near-shore Sites 2 to 4. For example, *Daphnia* averaged 191300, 102900, 76907 and 92500 ind/m^2 at Sites 1 to 4, respectively. However, its higher density at Site 1 is largely an artifact of the roughly four-fold deeper water column there

relative to the near-shore Sites 2 to 4. Standardized for haul depths, its corresponding volumetric densities were conversely lower at Site 1 (12.6 ind/L), than the comparable and higher densities at Sites 2 to 4 (28.7, 24.0 and 25.6 ind/L). This comparison is also misleading, however, since volumetric densities at Site 1 are effectively lowered by the inclusion in samples there of a seasonally variable volume of anoxic hypolimnetic water that is generally uninhabitable, introducing a bias that cannot be corrected systematically. Disregarding this sampling bias, one-way ANOVA nevertheless only revealed significant inter-site differences in density for cyclopoid copepods (F = 4.476, P = 0.007, df = 59) and total zooplankton (F = 4.371, P = 0.008, df = 59), although this comparative analysis is confounded by temporal variations in density.

Between-site comparisons, using two-way ANOVA, showed clear influences of both site and time on the abundance of total crustacean zooplankton, as well as on its component taxa (**Table 4.5**). Densities at Sites 2 to 4 were at least 2-fold higher on average than corresponding values at Site 1 in all major taxa apart from *Ceriodaphnia*, and differences were significant ($P \leq 0.05$) in all component groups apart from *Ceriodaphnia* and *Bosmina*. As discussed above, however, values at Site 1 are largely underestimates attributable to seasonally variable habitat constraints imposed by anoxia or micro-aerophylia linked to thermal stratification at this offshore location. Along with the PRIMER findings presented below, inter-site differences are accordingly deemed largely unimportant.

Table 4.5: ANOVA statistics for between-site and between-time differences in density (ind/m^3) of total crustacean zooplankton and the individual components listed. The final column indicates the average and maximum magnitude of difference between inshore Sites 2 to 4 relative to Site 1 through the study.

Variable	Factor	*F*	*P*	*df*	Inshore means and maxima
Total density	Site	3.493	0.0008	3	3.83; 15.90
	Time	7.095	0.0006	14	
Daphnia	Site	3.334	0.028	3	2.04; 5.32
	Time	3.331	0.001	14	
Bosmina	Site	2.141	0.114	3	3.74; 30.86
	Time	3.611	0.002	11	
Ceriodaphnia	Site	0.602	0.618	3	1.10; 7.84
	Time	4.635	0.0003	11	
Cyclopoids	Site	5.894	0.0019	3	7.06; 41.56
	Time	2.267	0.021	14	
Calanoids	Site	1.471	0.242	3	2.86; 16.83
	Time	3.382	0.005	10	
Nauplii	Site	3.630	0.020	3	2.56, 13.72
	Time	9.442	< 0.0001	13	

Comprehensive replicate sampling of zooplankton at the routine offshore and inshore sites and an additional offshore site (1A) was undertaken in April and July 2010 when the lake was unstratified, providing unbiased estimates of density for statistically rigorous comparisons of inshore and offshore locations. Using non-parametric PRIMER analysis, an inshore/offshore difference was confirmed in both months. In April, despite very low zooplankton densities (especially of *Daphnia*), non-metric multidimensional scaling analysis (NMDS) revealed clear inshore/offshore separation (**Figure 4.11**) with Cluster Analysis indicating the differences to be statistically distinct ($P < 0.05$). Using ANOSIM (Analysis of Similarities) with 999 random permutations, the probability of the differences being due to chance was extremely low (0.1%, i.e. $P = 0.001$). SIMPER analysis (Similarity Percentages) identified *Chaoborus* as contributing nearly 30% of the difference (not unexpectedly, given this predator's preference for deep, hypoxic water); along with *Bosmina*, it accounted for almost half the dissimilarity (**Table 4.6**). Collectively with *Metadiaptomus* (the calanoid copepod) and *Daphnia*, these four taxa accounted for nearly 75% of the observed inshore/offshore differences in abundance per unit volume. Despite the statistical significance, Table 4.6 indicates that the scale of the abundance differences is very slight.

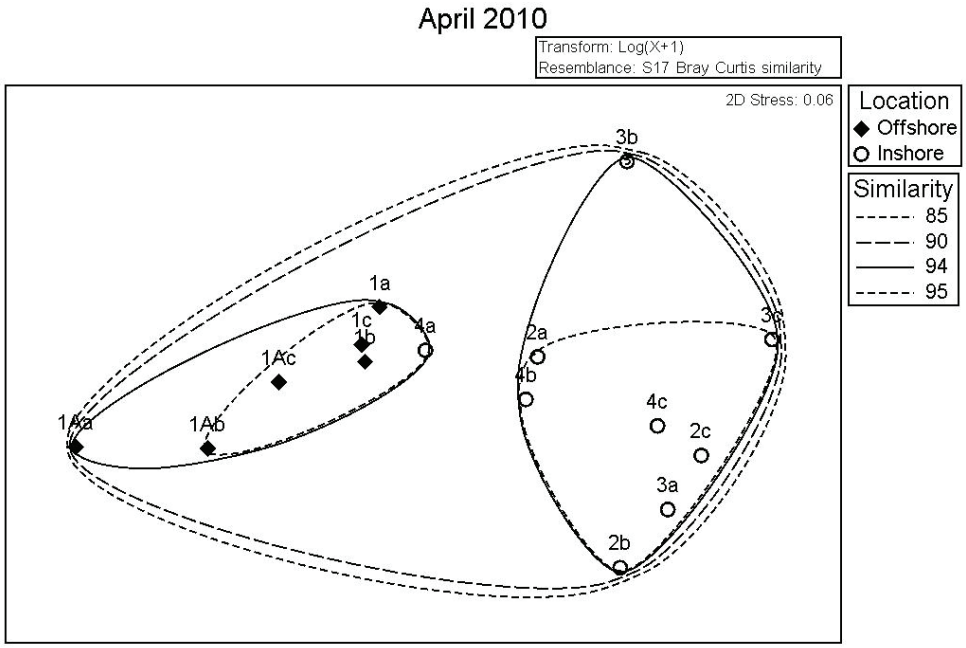

Figure 4.11: NMDS distinction between zooplankton abundances (in April 2010) at the routine inshore sampling sites (Sites 2 to 4) and the regular offshore station (Site 1) with the additional offshore site 1A. Three replicate samples at each site are denoted as a, b and c. Only sample 4a lies outside the inshore cluster of 94% similarity. The stress value of 0.06 indicates an ordination that is most unlikely to lead to misinterpretation.

Table 4.6: SIMPER analysis showing the % contribution (%Contrib) made by each taxon to the inshore/offshore dissimilarity in April 2010. Emboldened values show four taxa that individually contributed roughly between > 10% and nearly 30% of the overall dissimilarity. Two of these cumulatively (Cum.%) accounted for almost half of the overall dissimilarity, nearly 75% of which is explained by four taxa.

Species/taxon	Average Abundance (Ln (X+1))		Av.Diss	Diss/SD	%Contrib	Cum.%
	Offshore	Inshore				
Chaoborus	3.70	1.19	2.10	1.77	**28.93**	**28.93**
Bosmina	8.20	9.86	1.39	2.34	**19.10**	**48.03**
Metadiaptomus	6.52	7.86	1.12	2.41	**15.39**	**63.42**
Daphnia	6.55	7.37	0.84	1.30	**11.51**	**74.93**
Moina	6.85	7.58	0.70	1.36	9.59	84.52
Cyclopoid copepods	8.96	9.44	0.53	1.39	7.34	91.86

In July, when the community was strongly-dominated by two taxa – *Daphnia* and cyclopoid copepods, NMDS revealed a comparable but weaker (Cluster Analysis: $P > 0.05$) inshore/offshore separation (Figure 4.12). SIMPER identified the dissimilarity as being largely attributable to inshore/offshore differences in copepod nauplii, *Bosmina* and *Metadiaptomus* densities, with *Daphnia* differences being negligible (results not included in Table 4.6) (Hart, 2012).

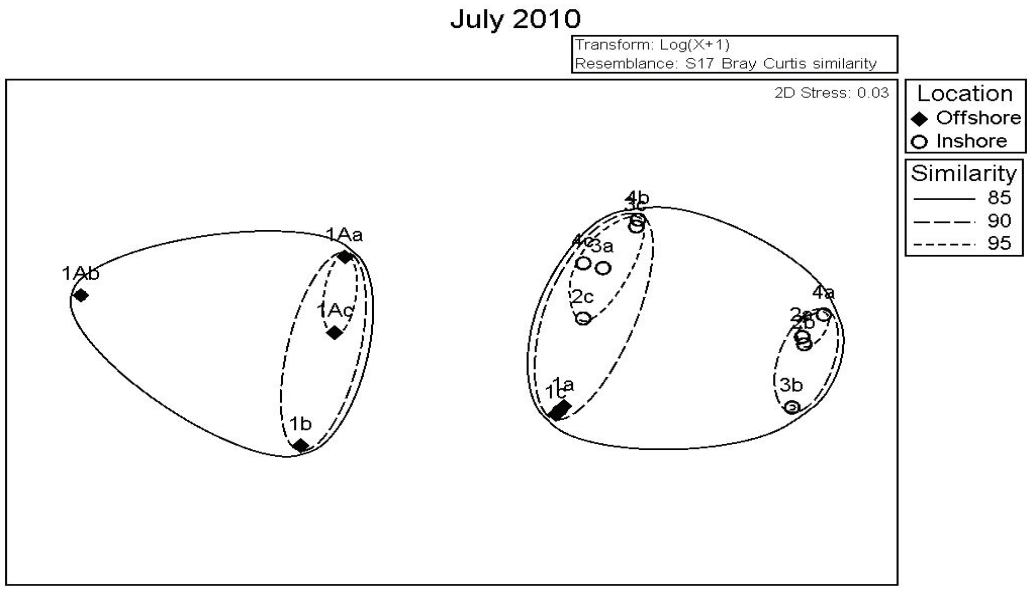

Figure 4.12: NMDS distinction between zooplankton abundances at inshore and offshore sites in July 2010. See legend to Figure 4.11 for further details. Two offshore samples (1a, 1c) lie outside the 85% similarity offshore cluster.

The existence of only slight differences in *Daphnia* density (Table 4.6) is again relevant in the context of biomanipulation, since visual planktivory is expected to be stronger inshore, where opportunistically zooplanktivorous juvenile fish largely occur. However little discernible effect on *Daphnia* abundance is evident in the analyses.

Daphnia size structure

Spatial differences in size structure are directly relevant in the context of predation. In this regard, the generally large average and maximal sizes of *Daphnia* throughout the study (**Figure 4.13**) are notable. Population geometric mean values (n = 50-55 offshore and n = 150-165 inshore per sampling date) which reflect the proportional contributions of the full

size range of juvenile and adult stages present in prevailing conditions populations generally varied around 1.5 mm, while the 10 largest individuals averaged around 2.0 mm. Such large average size in particular strongly contraindicates visual predation, which generally restricts prey items to below 0.5 mm in body size. The general consistency in size structure between inshore and offshore sites further reinforces the improbability of significant visual zooplanktivory (as reasoned in the final paragraph of the preceding section on "Spatial differences…").

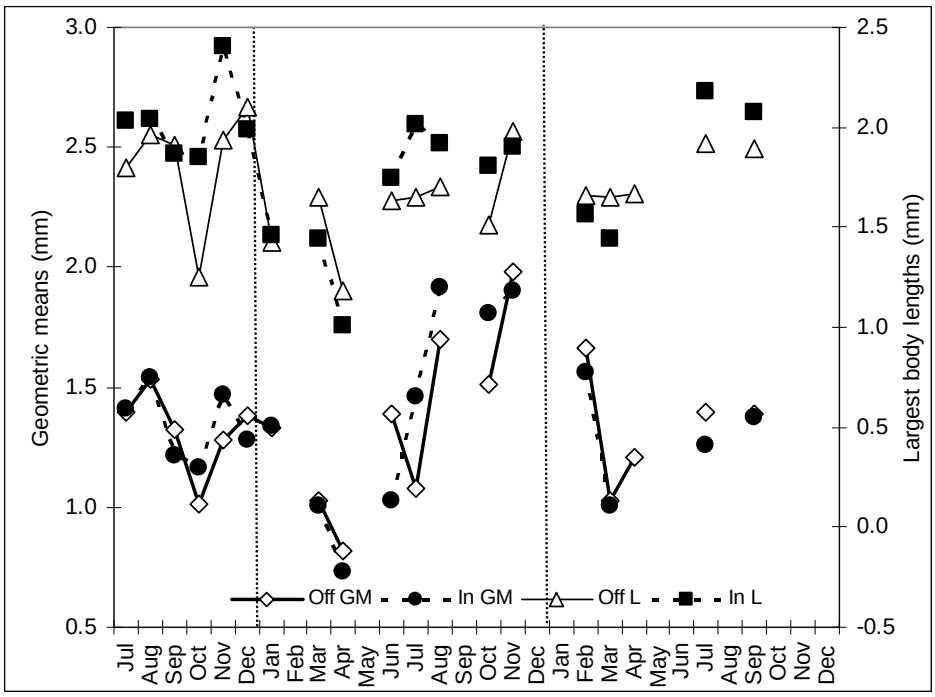

Figure 4.13: Temporal variations in mean body size of *Daphnia* populations at inshore and offshore sites. December 2011 values are not included owing to inadequate sample sizes.

Zooplankton abundance in relation to food resources

Using chlorophyll values (kindly provided by Tshwane Water) as a crude index of food availability, the temporal inter-relationship between zooplankton and the potential food of its predominantly 'herbivorous' component (notably *Daphnia* – see Figure 4.10) is shown in **Figure 4.14**. Notwithstanding the fact that data were not determined concurrently, several clear reciprocities are discernible in the data. For example, low zooplankton biomass levels in July 2009 coincided with high chlorophyll levels, indicating that there were insufficient grazers to 'control' the food supply, while converse values in August 2009 indicated severe 'down-grazing' of the food supply by an abundance of consumers; parallel indications of

predator-prey reciprocity were evident through the study (e.g. January and June 2010, February, April and December 2011).

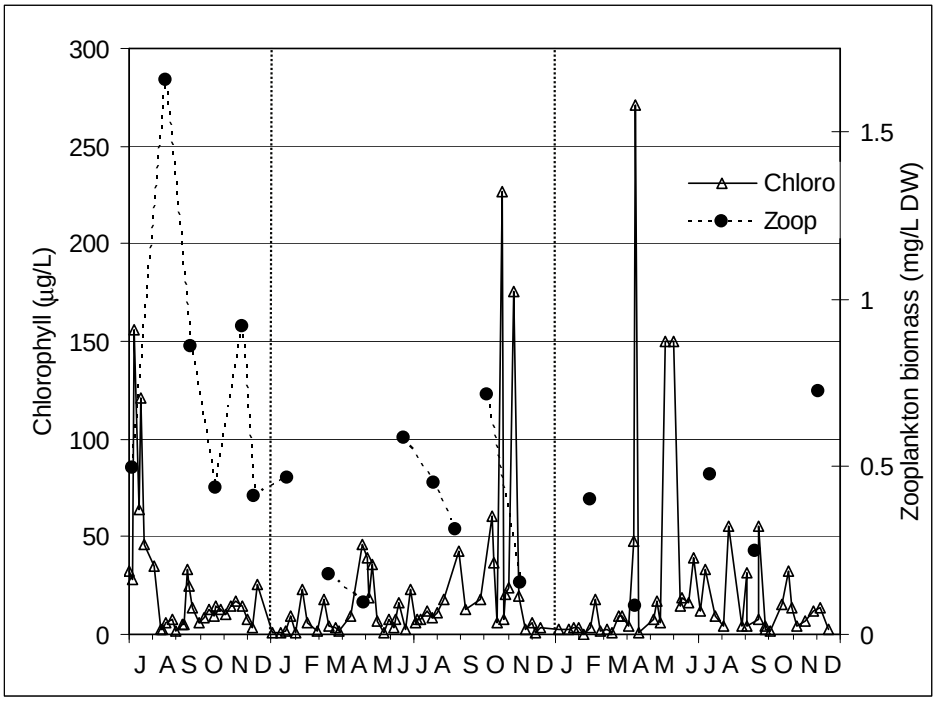

Figure 4.14: Temporal variation in zooplankton abundance in relation to potential food supply.

This trend is clearer in a scatter-plot (using actual or interpolated chlorophyll values) of temporally paired data (**Figure 4.15**), which reflects a negative relationship between zooplankton and chlorophyll. Chlorophyll levels were low when zooplankton abundance was high and vice versa, although the correlation (Pearson's r = -0.333) is not significant. The fitted power regression (see Figure 4.15) is significant ($p < 0.05$), but only on account of one extremely elevated chlorophyll value.

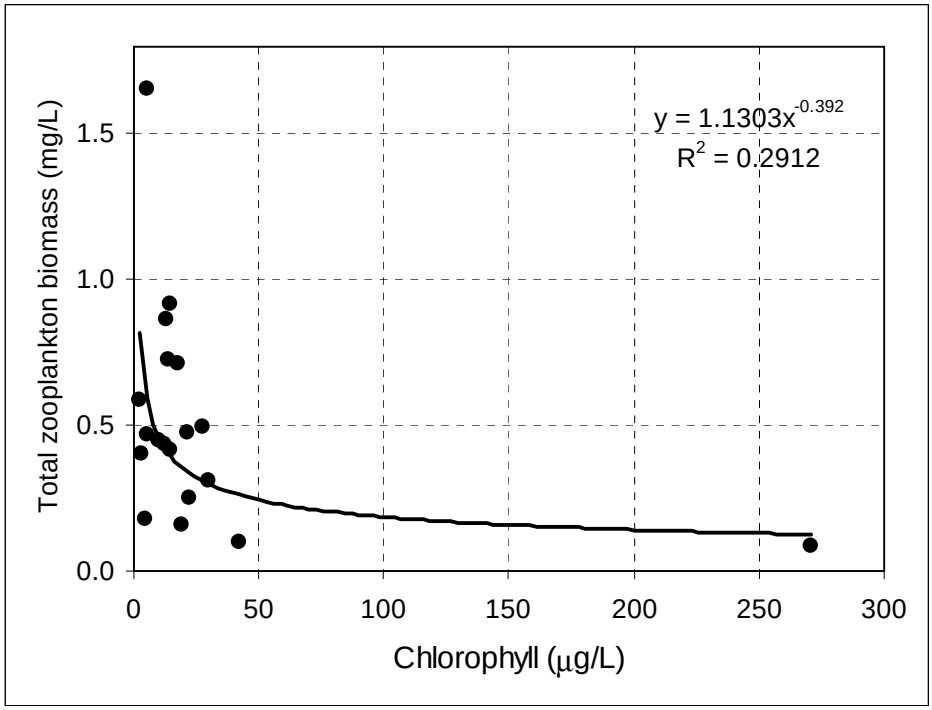

Figure 4.15: Zooplankton abundance in relation to concurrent chlorophyll levels.

Interpreted simplistically, the negative relationship shown in Figure 4.15 implies that chlorophyll is strongly controlled by 'top-down' 'grazing' impacts rather than that zooplankton abundance reflects food availability, a 'bottom-up' influence. However, the 'simplistic' nature of this interpretation must be stressed. Chlorophyll is an integrated measure of all primary producers, not all of which are edible or nutritious. And similarly, not all zooplankton are herbivores, although as noted above, 'herbivorous' *Daphnia* is often a predominant component.

Conclusions

None of the findings reported above suggests that zooplanktivory has a significant influence on zooplankton community structure and abundance levels. Bottom-up effects are accordingly implicated by default as the primary moulding agents. The seeming contradiction between this conclusion and the interpretation provided for Figure 4.15, above, regarding bottom-up influences is an apparent rather than a real one, resolved by the 'simplistic' caution noted therein. Changes in food composition (quality) rather than quantity *per se* are of primary importance, as reflected in the seasonal dynamics observed in cladoceran taxa in particular.

FISH

The capture data are provided in **Table 4.7**. The fish were netted in the vicinity of sampling sites 2-4, i.e. the locations which previously (Harding and Koekemoer, 2011) showed the highest diversity of species caught.

Table 4.7: Fish samples for SIA analysis (October 2009 survey).

Fish sampled in Rietvlei Dam – October 2009				
Sample number	**Site sampled**	**Species**	**Length (cm)**	**Weight**
1.1	R 4	*Clarias gariepinus*	68	3.0 kg
1.2	R 4	*Clarias gariepinus*	52	1.2 kg
1.3	R 4	*Clarias gariepinus*	72	3.3 kg
1.4	R 4	*Clarias gariepinus*	62	2.6 kg
1.5	R 4	*Clarias gariepinus*	66	2.6 kg
2.1	R 4	*Pseudocrenilabrus philander*	6.5	4 g
2.2	R 12	*Pseudocrenilabrus philander*	8	8 g
2.3	R 12	*Pseudocrenilabrus philander*	9	11 g
2.4	R 12	*Pseudocrenilabrus philander*	8.5	11 g
2.5	R 12	*Pseudocrenilabrus philander*	9	10 g
3.1	R 4	*Chetia flaviventris*	15	46 g
3.2	R 4	*Chetia flaviventris*	10.5	13 g
3.3	R 12	*Chetia flaviventris*	20	100 g
3.4	R 12	*Chetia flaviventris*	19	91 g
3.5	R 12	*Chetia flaviventris*	18.5	80 g
4.1	R 10	*Labeobarbus polylepis*	50	2.536 kg
4.2	R 10	*Labeobarbus polylepis*	48	2.300 kg
4.3	R 10	*Labeobarbus polylepis*	46	2.112 kg
4.4	R 10	*Labeobarbus polylepis*	46	1.956 kg
4.5	R 10	*Labeobarbus polylepis*	47	2.073 kg
5.1	R 4	*Tilapia sparrmanii*	15.5	75 g
5.2	R 12	*Tilapia sparrmanii*	10	21 g
5.3	R 12	*Tilapia sparrmanii*	14	56 g
5.4	R 12	*Tilapia sparrmanii*	14	60 g
5.5	R 12	*Tilapia sparrmanii*	11.5	31 g
6.1	R 12	*Barbus paludinosus*	7	5 g
6.2	R 12	*Barbus paludinosus*	6.5	4 g
6.3	R 12	*Barbus paludinosus*	7	6 g
6.4	R 12	*Barbus paludinosus*	7.5	6 g
6.5	R 12	*Barbus paludinosus*	7	5 g

Additional fish samples, collected during Year 2 of the project, comprised:

- 8 carp specimens (*Cyprinus carpio*)
- 2 barbel (*Clarias gariepinus*)
- 1 small-scale yellowfish (*Labeobarbus polylepis*)
- 1 canary kurper (*Chetia flaviventris*)

Although Rietvlei is a lake rich in common carp (Rietvlei is a trophy angling venue for this species), no apparent reason could be identified for the absence of carp from the October 2009 netting exercise. This is further confusing in that the exercise was undertaken by the same team responsible for the targeted capture of carp from Hartbeespoort Dam during the aforementioned WRC1643 research project. Carp were easily captured using rod and reel gear during Year 2 of the project.

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SECTION 5: STABLE ISOTOPE ANALYSIS

PLANKTON

Considerable temporal variation and a wide range in absolute values were evident in both the $\delta^{13}C$ and $\delta^{15}N$ signatures (all values as per mille (‰)) of net phytoplankton and all zooplankton size components (**Figures 5.1 and 5.2**).

On most sampling dates, median $\delta^{13}C$ values for the four planktonic components were generally within 5‰, although much wider disparities also occurred (Figure 5.3). However, $\delta^{13}C$ values, considered collectively for all planktonic components, were almost consistently lower (higher negative values) during the first than the second half of the study centering respectively on around -27.5 ‰ and -20 ‰ (Figure 5.1). This is further exemplified in the clearly positive overall trend line fitted to the phytoplankton values. No definitive causal explanation can be advanced to account for this 'inter-annual' disparity.

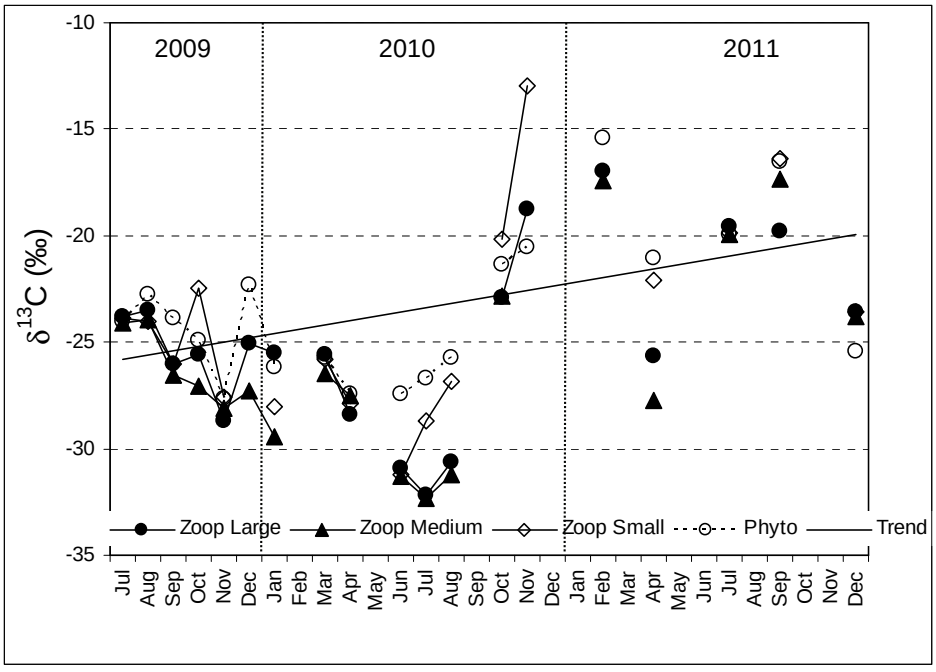

Figure 5.1: Temporal changes in median $\delta^{13}C$ values of different planktonic components, with trend line fitted to phytoplankton median values.

Clear variation in $\delta^{15}N$ values between consecutive sampling dates was also evident, with July/August 2010 values reaching markedly lower levels than at any other time (**Figure 5.2**). Median $\delta^{15}N$ values tended to be slightly lower during the later phase of the study, as reflected in the negative trend line for phytoplankton (Figure 5.2). However, plankton

signatures collectively remained centered around +12.5‰ throughout the study, without as marked longer-term changes as reflected in the $\delta^{13}C$ values (compare trend lines in Figures 5.1 and 5.2).

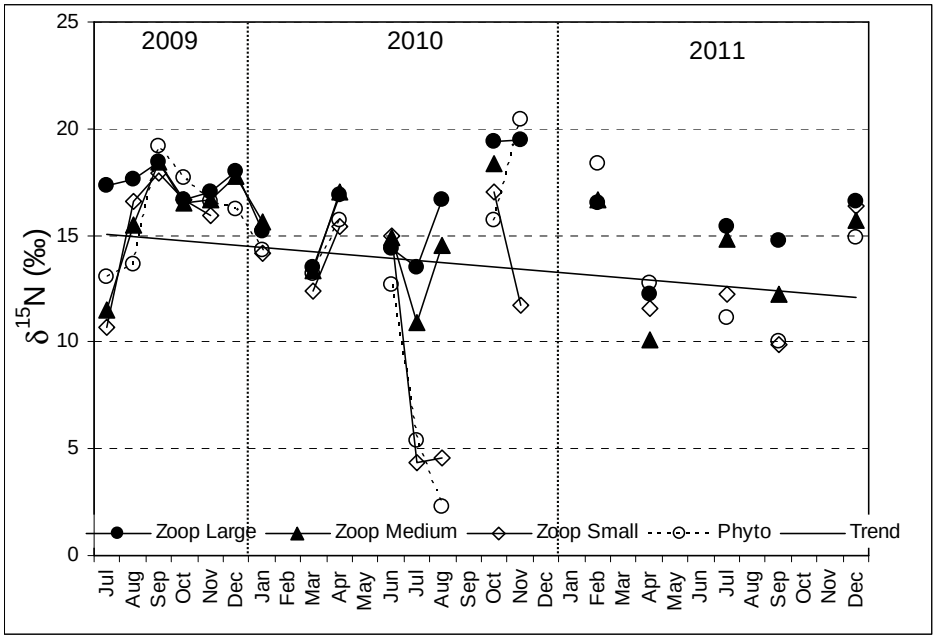

Figure 5.2: Time line of changes in median $\delta^{15}N$ values of different planktonic components

Differences between the $\delta^{13}C$ signatures of phytoplankton and their putative zooplankton consumers varied quite widely in magnitude, but also in direction (Figure 5.1) – with cases of both concordance or slight enrichment (as expected from trophic fractionation theory), to cases of isotopic depletion. This is most clearly evident in a correlation scatter-plot (**Figure 5.3**) based on all individual paired data points obtained during the study. Points lying above and below the isoline in this diagram represent cases of isotopic depletion and enrichment, respectively. Cases of depletion were less numerous and of slighter magnitude than cases of enrichment, but did occur in all zooplankton size fractions. Considered overall, however, consumer signatures tended to track those of their putative food, as reflected in the highly significant ($P \leq 0.001$) correlation coefficients (see the linear regression equations embedded in Figure 5.3) of phytoplankton values and all zooplankton size fraction values. Respective average (± SD) fractionation values of +1.75 (± 2.19), +2.13 (± 2.09) and +0.33‰ (± 2.31) for large, medium and small zooplankton, suggest general $\delta^{13}C$ enrichment for large and medium, but not small zooplankton (although the SD values indicate wide variability).

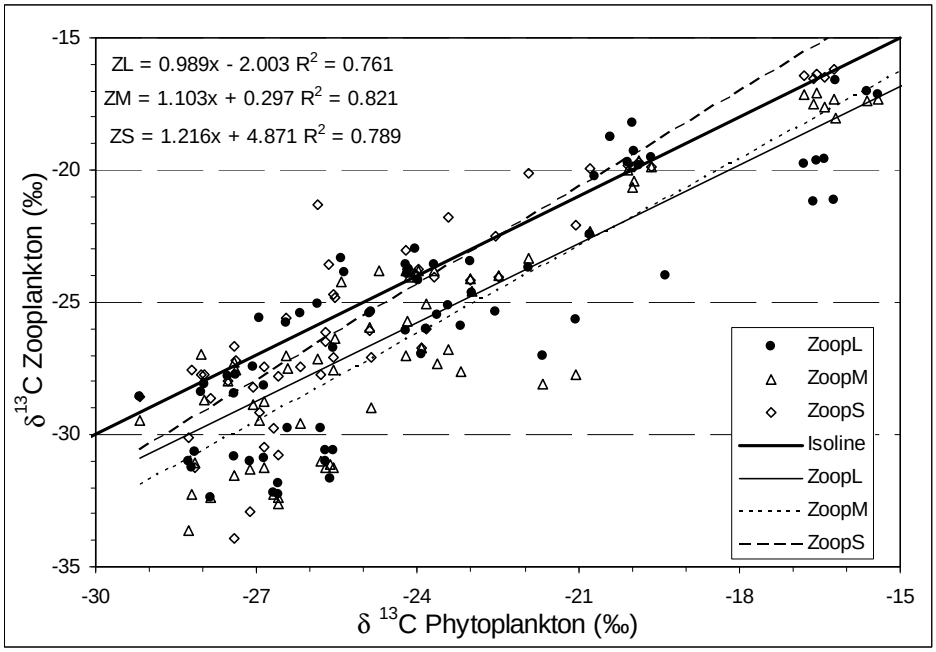

Figure 5.3: Carbon signatures of zooplankton size fractions in relation to concurrent phytoplankton signatures, with associated linear regression relationships and correlation coefficients.

A similar examination for nitrogen (**Figure 5.4**) indicates far less consistency in its isotopic fractionation. This is reflected in the lower (but nevertheless all highly significant, $P \leq 0.001$) correlation coefficient values returned for nitrogen than for carbon, and particularly in the low regression slopes (< 0.4) for large and medium zooplankton fractions. The scatter-plot indicates that the relationships were strongly leveraged (biased) by the consumer signatures obtained when phytoplankton $\delta^{15}N$ values were low (< 10 ‰), notably during the 2010 winter (Figure 5.2).

Overall, fractionation values for $\delta^{15}N$ averaged (± SD) +3.07 (± 4.18), +1.86 (± 3.54) and -0.19 ‰ (± 2.71) for large, medium and small zooplankton respectively, suggesting general $\delta^{15}N$ enrichment for large and medium, but not small zooplankton (in line with the corresponding trend for $\delta^{13}C$), but with correspondingly wide variability reflected in the high SD values.

Signatures of small zooplankton were frequently enigmatic, showing higher percentage isotopic depletion than large or medium zooplankton; respectively 52 vs. 27 and 15% for $\delta^{13}C$, and 62 vs. 21 and 25% for $\delta^{15}N$. This anomaly is attributed to unavoidable and inconsistent 'contamination' of the small zooplankton fraction samples by sedimented phytoplankton, leading to mixed and unreliable signatures. This is especially apparent in

their proximity to the isoline when phytoplankton $\delta^{15}N$ values were < 6 ‰ (**Figure 5.4**). The small zooplankton fraction is accordingly largely disregarded in subsequent considerations of overall food web structure.

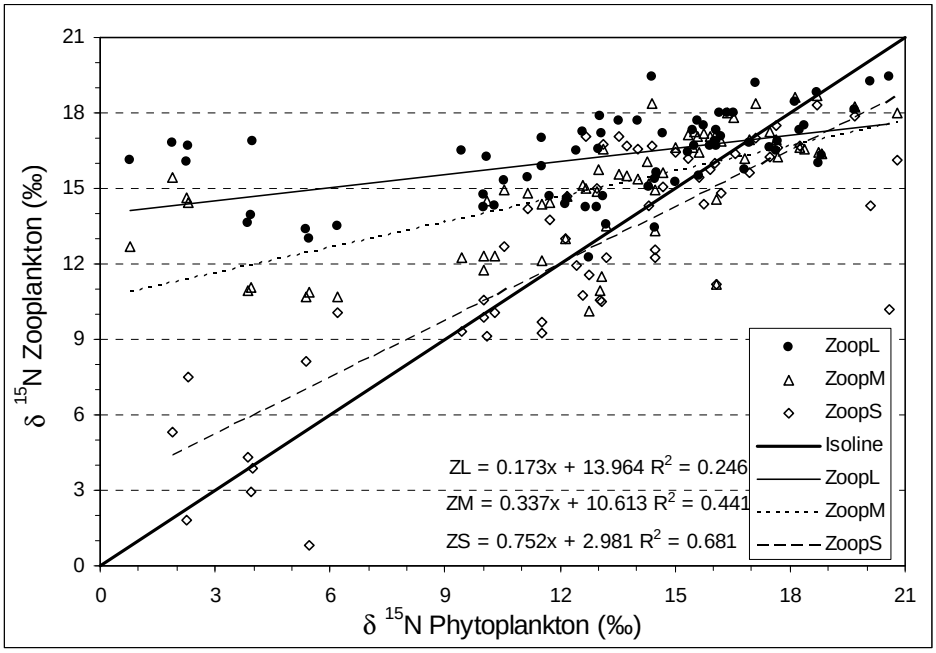

Figure 5.4: Nitrogen signatures of zooplankton size fractions in relation to concurrent phytoplankton signatures, with associated linear regression relationships and correlation coefficients.

TAXON-SPECIFIC DETERMINATIONS

SIA of individual zooplankton taxa was undertaken along with the routinely determined plankton components in March and October 2010, and yielded various contrasting results. In March (**Figure 5.5**), $\delta^{13}C$ values ranged overall between roughly -29‰ and -23‰ (Figure 5.5), and between roughly -25‰ and -20‰ in October (**Figure 5.6**). Substantially greater contrasts were evident in $\delta^{15}N$ values, which increased from between 10‰ and 16‰ in March to between 14‰ and 26‰ in October (a span of four levels). Despite some overlap, phytoplankton $\delta^{15}N$ values in March were relatively higher than those of large, medium and small zooplankton fractions, as well as for the cladoceran consumer taxa assayed – *Daphnia*, *Bosmina* and *Ceriodaphnia* (Figure 5.5). Along with calanoid copepods, all the above consumers (apart from one batch of *Ceriodaphnia*) were broadly aligned with phytoplankton in terms of $\delta^{13}C$, whereas cyclopoid copepods along with one *Ceriodaphnia* sample were $\delta^{13}C$ depleted (left-shifted).

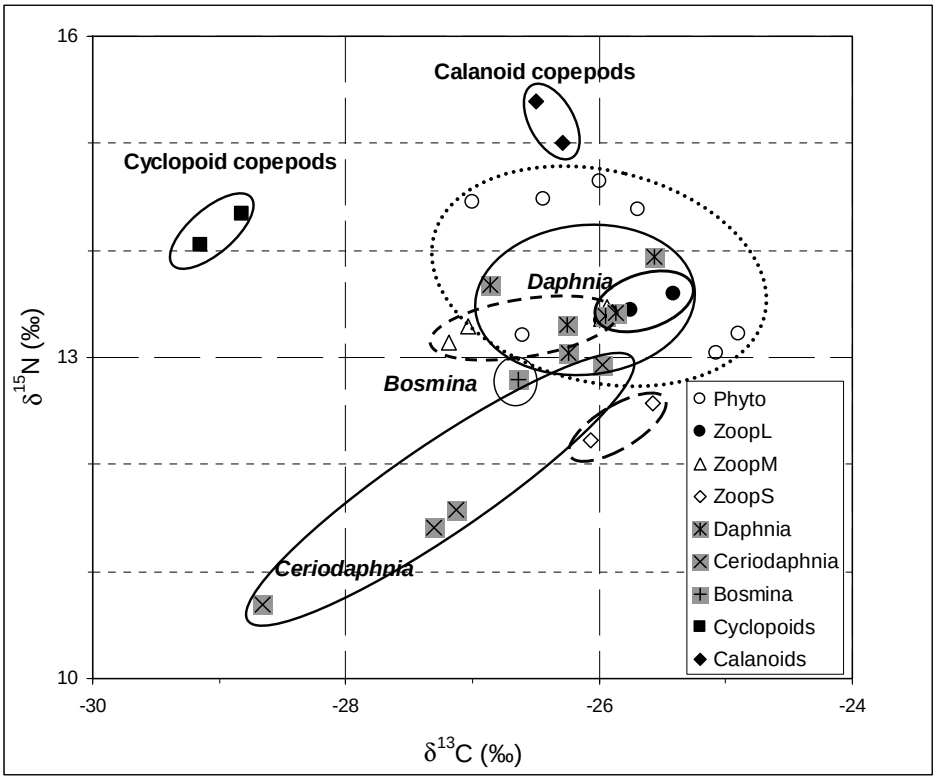

Figure 5.5: Stable isotope signatures of individual taxa in relation to concurrent values of routinely analyzed components in March 2010. Envelope clusters embrace all values.

By contrast, in October (Figure 5.6), virtually all consumer taxa or size fractions showed strong $\delta^{15}N$ enrichment (particularly in *Daphnia*) along with high $\delta^{13}C$ depletion, relative to phytoplankton. The predominantly carnivorous predator *Chaoborus* occupied a surprisingly low position in the food web (well below *Daphnia*, but broadly at the level of the large and medium zooplankton size fraction clusters). However, predatory hydrachnellids (watermites) occupied an expected apical position.

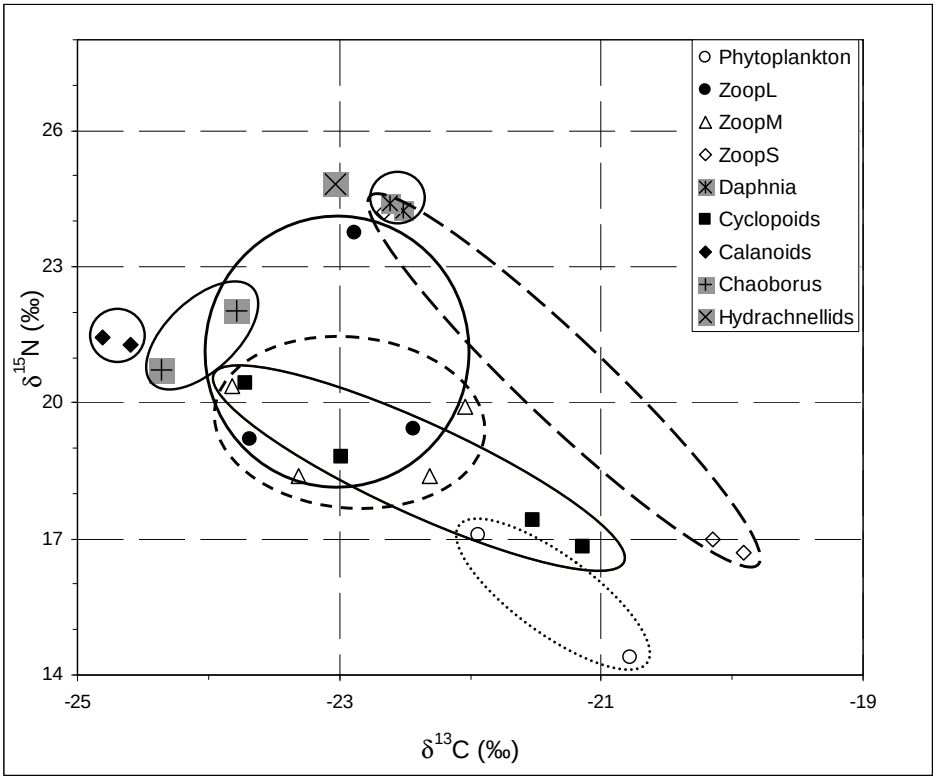

Figure 5.6: Stable isotope signatures of individual taxa in relation to concurrent values of routinely analyzed components in October 2010

BENTHOS

Benthic hydrophytes, along with their epiphytic diatoms, were collected for SI assay in most temporal samplings. Temporal variations in median isotope signatures of macrophytes and diatoms are plotted in **Figures 5.7 and 5.8**.

As in the phytoplankton, considerable temporal variability was apparent in both C and N signatures of the benthic primary producers. Values of $\delta^{13}C$ were generally lower for macrophytes than epiphytes, with respective overall averages (± SD) of -20.23 ± 3.57 and -18.37 ± 4.33, while the trend lines in Figure 5.7 indicate that both tended to increase during the study, in common with trend noted for phytoplankton and other components of the plankton (Figure 5.1).

Overall, average $\delta^{15}N$ values were closely similar for macrophytes (14.96 ± 2.75) and epiphytes (15.33 ± 2.24), in line with their functional correspondence as primary producers. No distinct temporal trend in $\delta^{15}N$ values was apparent for either group (Figure 5.8), again consistent with the findings made for planktonic components (Figure 5.2).

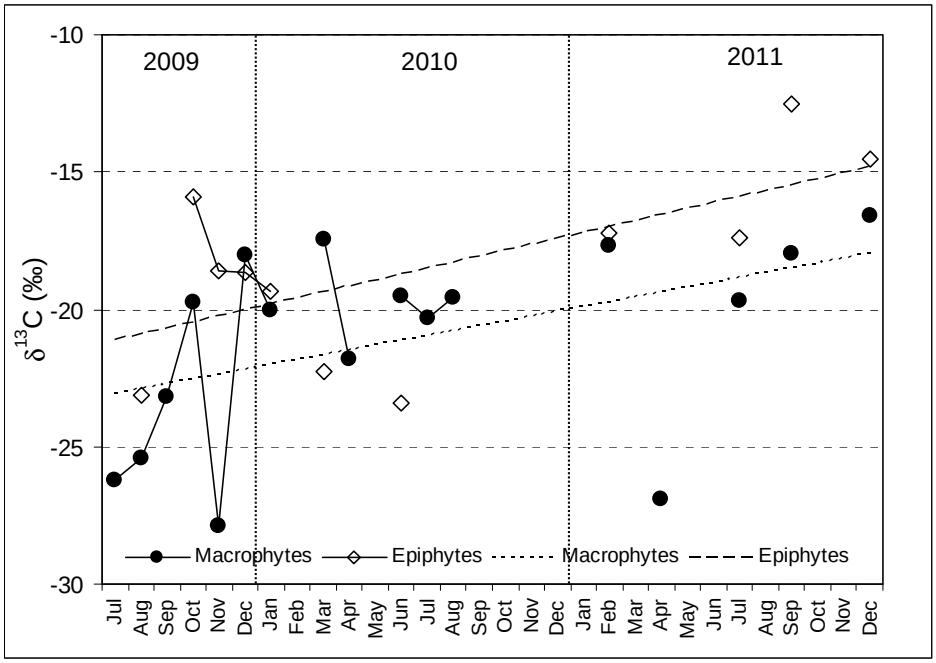

Figure 5.7: Temporal variation in median $\delta^{13}C$ signatures of macrophytes and epiphytic diatoms

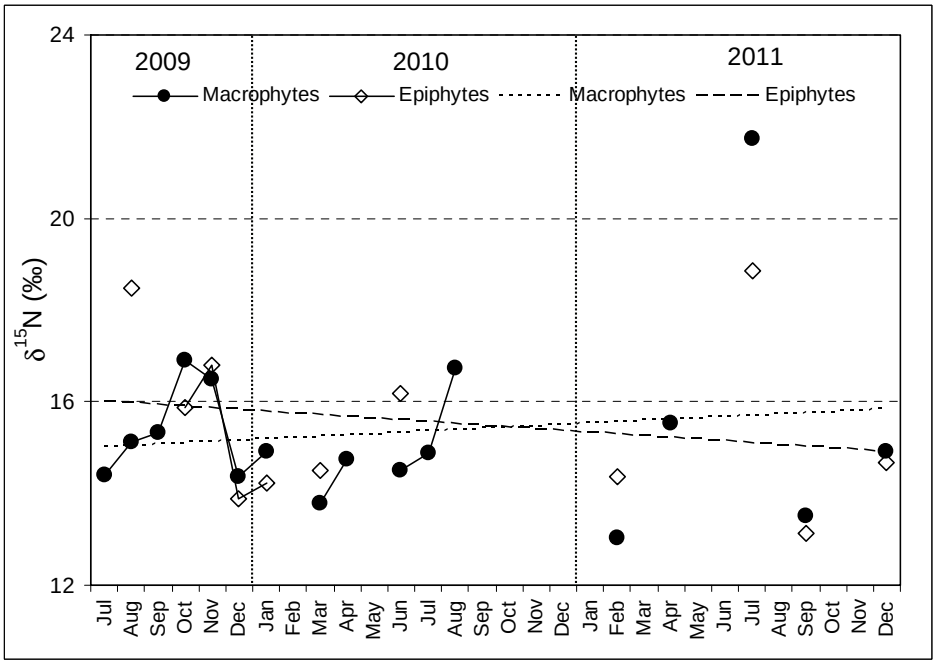

Figure 5.8: Temporal variation in median $\delta^{15}N$ signatures of macrophytes and epiphytic diatoms

Benthic macro-invertebrate and sediment substrate samples were collected annually, and accordingly no evaluation of temporal changes can be made. However, the available data

are illustrated in **Figure 5.9** and are included in the consolidated foodweb biplot shown in **Figure 5.11**.

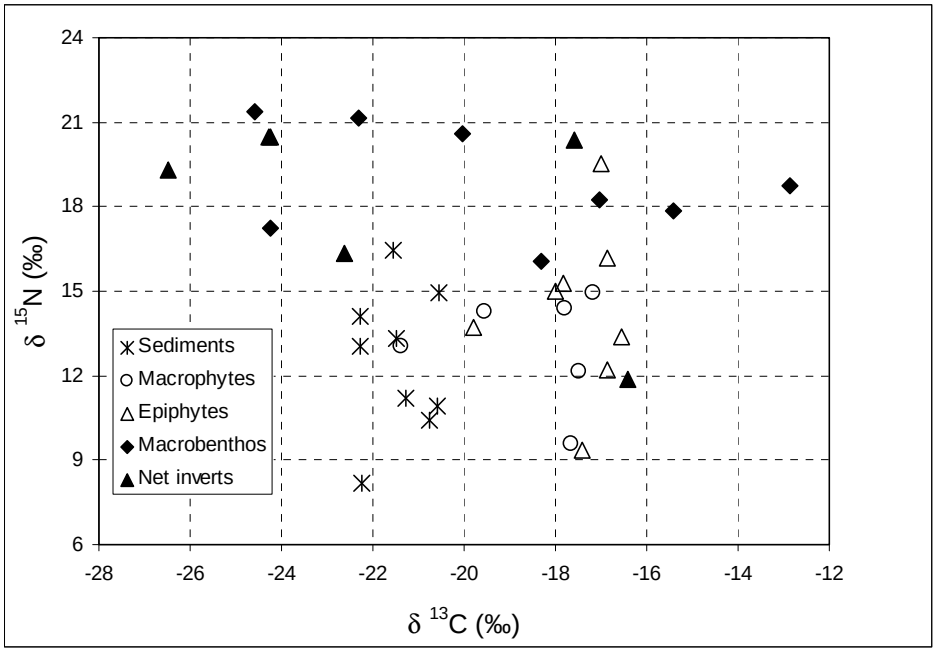

Figure 5.9: Isotope signatures for benthic macroinvertebrates (macrobenthos) and littoral invertebrates (net inverts) in November 2010 in relation to macrophyte and epiphyte producer values in February 2011 and bottom sediment values in April 2011.

Benthic consumer values exhibited a wide range of food sources (reflected in the wide range of $\delta^{13}C$ values), but were generally enriched by around one trophic level ($\delta^{15}N$ = ~ + 3‰) relative to benthic producers (macrophytes and epiphytes). The variability in both $\delta^{13}C$ and $\delta^{15}N$ signatures is partly attributable to the lack of taxonomic uniformity in bulked samples, and the corresponding inconsistency in mixtures of herbivores, carnivores, detritivores and omnivores. An increased frequency of benthos sampling, coupled to taxonomic separation prior to SIA analysis, is recommended for future studies of this nature.

FISH

Species-specific isotope signatures of fish are shown in **Figure 5.10**, along with concurrent signatures obtained for planktonic producers and consumers in October 2009 and the benthic producers (macrophytes) in August 2009, the temporally closest samples. The $\delta^{15}N$ values show that all fish species were considerably elevated in trophic position (nominally one to two trophic levels higher) than both the primary producer elements and the notionally primary consumer zooplankton, while their $\delta^{13}C$ signatures were clearly enriched

(right-shifted), relative to all planktonic components apart from some values for phytoplankton and small zooplankton. Such $\delta^{13}C$ enrichment was most pronounced for *Chetia flaviventris* (canary kurper) the most likely visual planktivore, while two other taxa perceived to feed on zooplankton – *Cyprinus carpio* (common carp) and *Clarias gariepinus* (catfish) reported to feed on zooplankton, show little indication of doing so on the basis of the results obtained. The difference in isotopic signatures between fish species is attributable to corresponding intrinsic differences in their feeding biology (as summarized in Skelton, 1993), clearly exemplified in the concordance of $\delta^{13}C$ values in macrophytes and *Tilapia sparrmanii*, an omnivore feeding on plants and their associated fauna.

The relatively high trophic positions of *Labeobarbus*, *Pseudocrenilabrus*, *Clarias* and *Chetia* accord with their (general or seasonal) predatory feeding habits; the lower positions of C*yprinus* (which tends to bottom-grub) and *Tilapia* reflect their prevailing omnivory, while the somewhat intermediate position shown by *Barbus* is in accordance with its diverse range of mostly animal foods (Skelton, 1993).

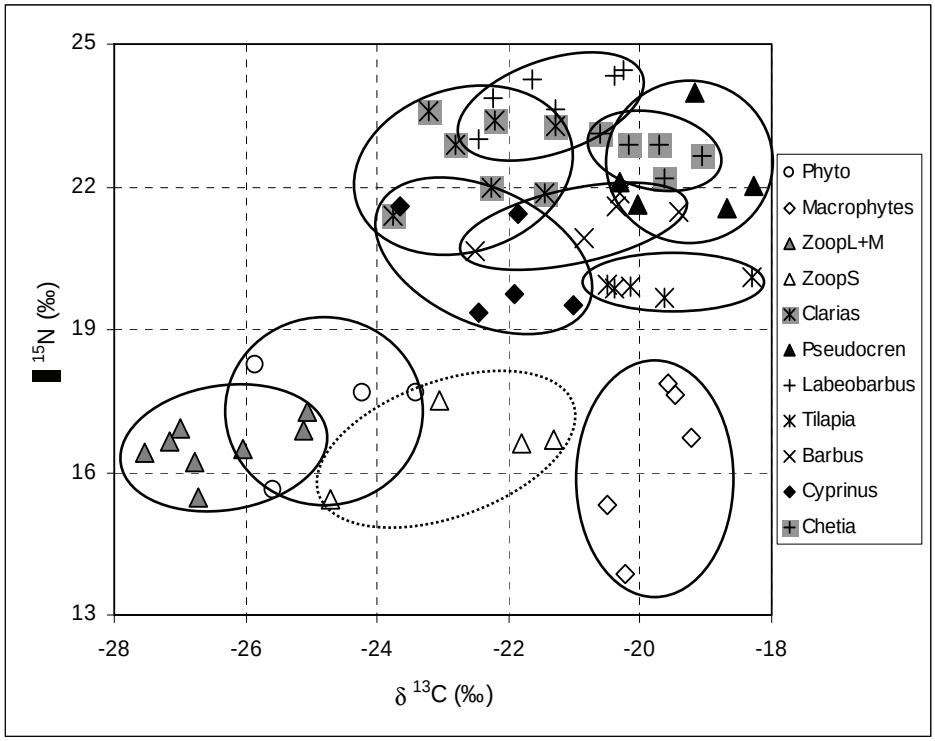

Figure 5.10: Isotope signatures for fish and planktonic components in October 2009, and macrophyte values in the preceding August. Fish taxa are listed by genera, abbreviated to Pseudocren in the case of *Pseudocrenilabrus philander*. Full identities of the other taxa listed are (sequentially) *Clarias gariepinus*, *Labeobarbus polylepis*, *Tilapia sparrmanii*, *Barbus paludinosus*, *Cyprinus carpio* and *Chetia flaviventris*

CONSOLIDATED FOOD WEB

A consolidation of all isotope signatures, obtained for notionally-different functional elements in Rietvlei during the overall study, is provided by **Figure 5.11**. It is stressed that the term 'notional' is a constraining simplification. 'Fish' for example clearly do not all feed equivalently. Nonetheless, this approach permits a general overview of foodweb structure in the system, despite the limitation arising from the wide range of values obtained, particularly in components which were sampled most frequently – notably the planktonic elements in general, and the benthic producers (macrophytes and epiphytes). As described in the preceding respective accounts, much of this variation arose from temporal changes, which can clearly confound an integrated analysis of foodweb structure. This, in turn, suggests the value of single 'snap-shots' of 'time-frozen' analyses (as reflected in Figure 5.10), although these conversely disregard potentially important temporal effects, a constraint noted by Grey (2006).

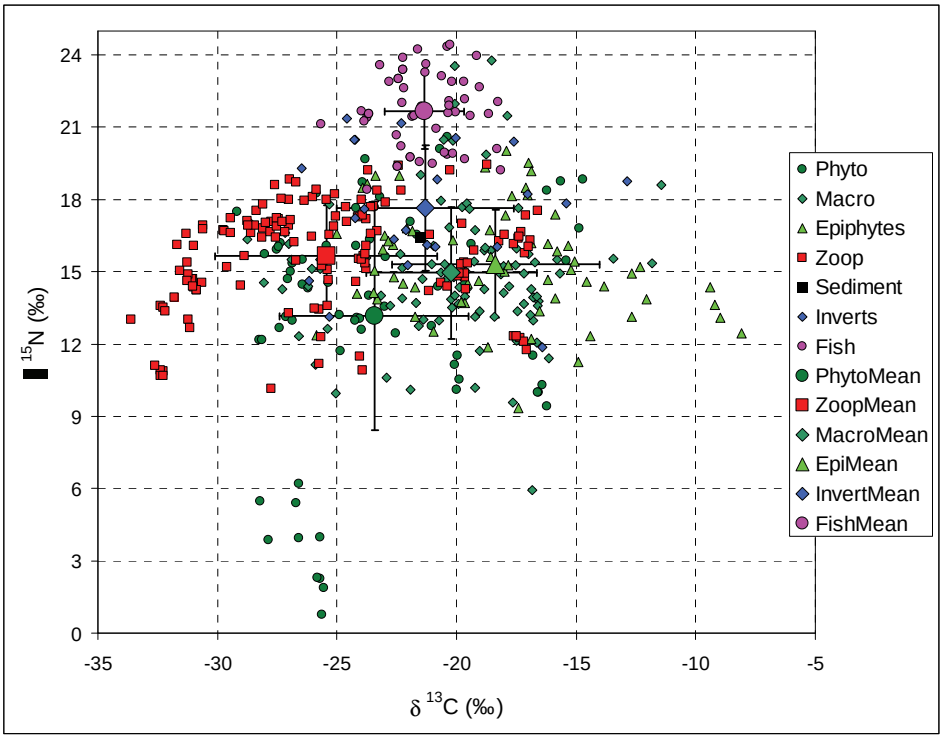

Figure 5.11: Consolidated biplot of all isotopic signatures obtained for designated components. 'Zooplankton' combines the values of large and medium size fractions, but excludes the small fraction values. Symbol shadings designate primary producers (open), and notionally primary (grey) and secondary (black) consumer components

Disregarding the confounding influence of temporal variability, Figure 5.11 nevertheless indicates trophic alignments in respect of types or sources of food as well as trophic level advances. This is more clearly evident using overall mean values (± SD – Figure 5.12, or ± 95% CI – Figure 5.13) to remove the symbol 'clutter' and expose general trends and patterns. From Figure 5.13 it is apparent that while zooplankton lie approximately one trophic level above the predominantly large 'net' phytoplankton, they are significantly ($P < 0.05$) carbon depleted by roughly 3 ‰ from this putative food. This plausibly reflects their consumption of or reliance on other food types, variously including a) edible phytoplankton predominantly comprising small taxa not represented in the samples of 'net' phytoplankton; b) mixtures of algae and other fine particulate organic material, both living (bacteria) and dead (detritus); c) microbial loop components such as nanoflagellates and ciliates; and d) other planktonic invertebrates such as rotifers. The examination of spot bulk samples of phytoplankton sedimented with iodine or collected using a 5 µm mesh net, indicated that for this study, option a) above was unlikely.

Benthic primary producers were significantly carbon-enriched ($P < 0.05$) compared to phytoplankton, more markedly in the case of epiphytes than macrophytes. Benthic primary consumers showed trophic enrichment by roughly one level, but also showed (non-significant) carbon depletion relative to their presumed food source, thus mirroring the trends apparent for both carbon and nitrogen within planktonic components. Taxonomic dietary differences and temporal discords in sample coverage appear likely to contribute to the carbon 'misalignment' evident between benthic producers and consumers, although the difference was significant only between benthic invertebrates and epiphytes.

Despite embracing different feeding guilds, the fish carbon signatures align well with those of the benthic primary consumers, while their nitrogen signatures are sufficiently enriched to indicate their functional trophic position more than one level above the benthic macro-invertebrates, while being two levels above zooplankton, with whose carbon signatures they are totally discordant. This carbon discordance is reflected most robustly in Figure 5.10, which reflects data for which the confounding influence of temporal variability is largely reduced or excluded.

Overall, the data reflect no significant overlap between planktonic and benthic food chains, with fish clearly aligned with the latter.

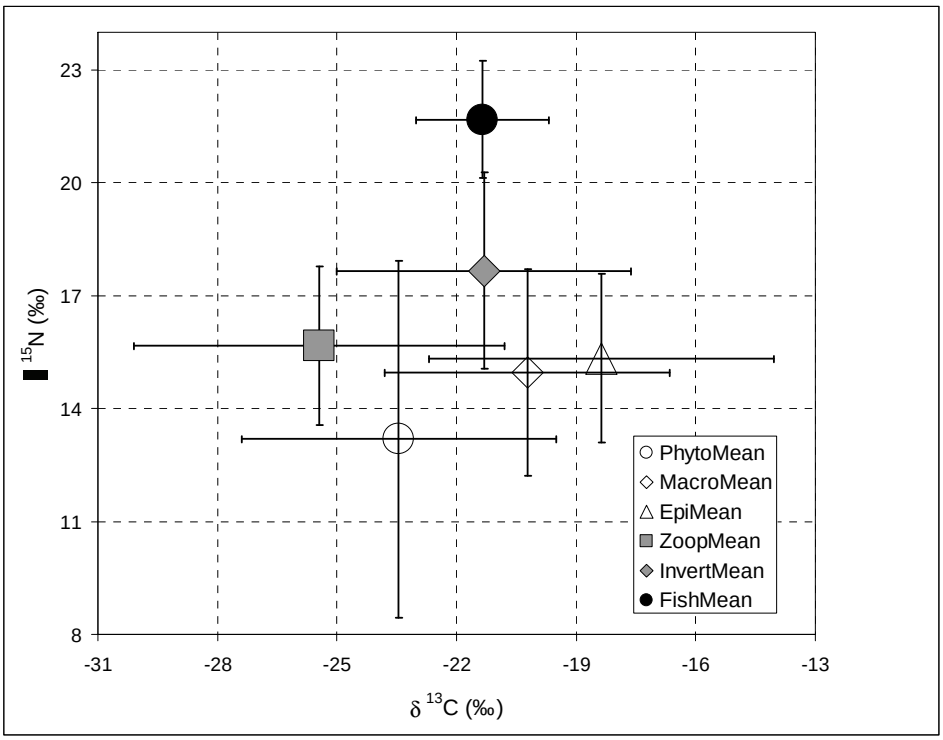

Figure 5.12: Biplots of overall average isotopic signatures obtained for the designated food web components in Rietvlei. The error bars reflect standard deviations in the upper panel and 95% confidence intervals in lower panel

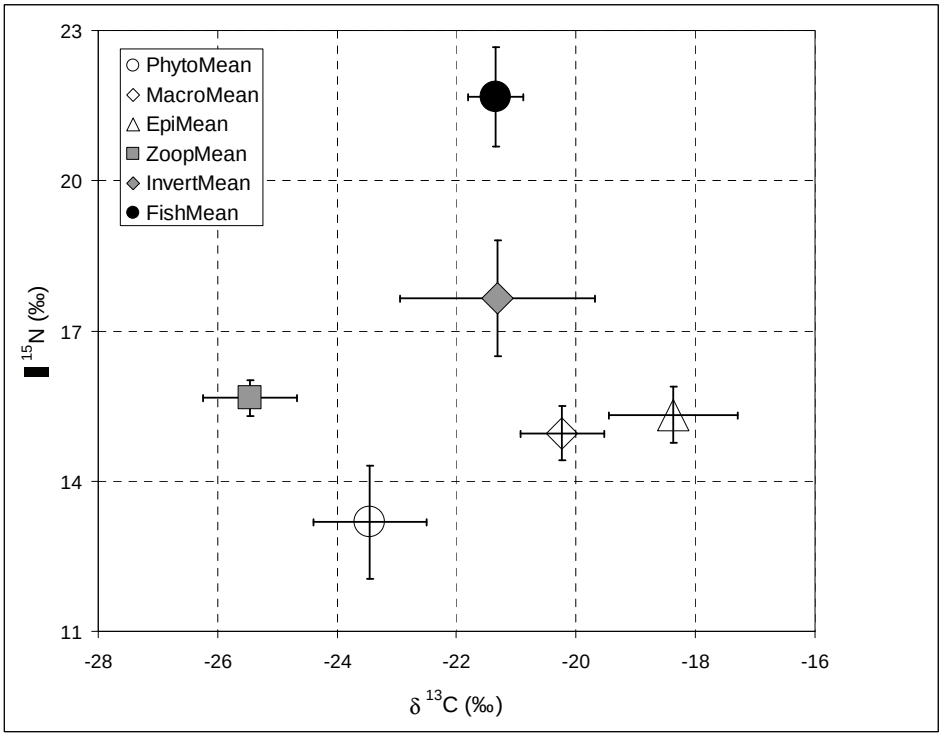

Figure 5.13: Biplots of overall average (± 95% CI) isotopic signatures obtained for the designated food web components in Rietvlei. Note that zooplankton $\delta^{13}C$ signatures differ distinctly from all other named components, including phytoplankton.

CONCLUSIONS

The evidence obtained using SIA indicates that trophic pathways in Rietvlei leading to fish primarily follow benthic rather than planktonic routes. This evidence strongly counters prospects of top-down food web biomanipulation as a mechanism to increase fish predation on zooplankton, and correspondingly increase the grazing impact of zooplankton on phytoplankton in this ecosystem. In this respect, the findings of the SIA approach are entirely consistent with the findings made in the parallel 'conventional' analysis of zooplankton abundance and composition that provided no indications of any significant influence of zooplanktivorous fish in the shaping of zooplankton assemblage structure or dynamics (see Section 4).

This study accordingly provides the first direct empirical evidence to test the theoretical (Hart, 2006) and inferential (Hart, 2011) challenges countering the utility of classical top-down biomanipulation in South African reservoirs, and indeed confirms them convincingly for Rietvlei. While parallel studies on other systems are desirable to ascertain the generality of this conclusion, the congruence of the present findings with fundamental theoretical arguments suggest, very strongly, that such generality is indeed highly unlikely not to apply.

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