Adaptability and vulnerability of Riverine Biota to Climate Change – Developing Tools for Assessing Biological Effects

Report to the
Water Research Commission

by

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Executive Summary

Background and motivation

Freshwater ecosystems are amongst the most vulnerable in the world with respect to global climate change. Southern Africa has been identified as a 'critical region' of water stress. Existing anthropogenic stresses on freshwater ecosystems are substantial and global climate change exacerbates this stress. Water temperature is a key component of aquatic ecosystems and understanding the role of temperature in these systems and the ecological consequences of changes in water temperature, is thus of critical importance.

The establishment of thermal guidelines that adequately protect aquatic ecosystems and their biota is dependent on an understanding of a river's thermal signature and the vulnerability of its biota to changes in water temperature. This study focuses on the development of biological temperature thresholds for one ecological component of freshwater ecosystems, the aquatic macroinvertebrates. Ultimately these thresholds will be incorporated into the water temperature component of the ecological Reserve. This information will facilitate the evaluation of current thermal stress, together with scenarios of likely future stress given global climate change.

The research undertaken during the preceding WRC projects has seen the development of a protocol for establishing water temperature guidelines for incorporation into the ecological Reserve. This proposed method is based on sound science and encompasses both a statistical and biological threshold. The transfer of this knowledge to appropriate implementing bodies such as the Resource Directed Methods Directorate of the DWS is seen as key.

Aims and objectives

The specific aims of the project are as follows:

- To investigate the influence of thermal history, acclimation temperature and rate of temperature change on upper thermal limits of thermally sensitive aquatic macroinvertebrates.
- To undertake preliminary investigation of sublethal effects of water temperature on selected aquatic macroinvertebrates.
- To investigate regional variation in thermal sensitivity of selected aquatic macroinvertebrates.
- To develop biological temperature thresholds for selected aquatic macroinvertebrates.
- To examine adaptability/resilience to climate change in rivers using a connectivity index as an indicator of potential risk.

The project attempted and successfully achieved all of these aims.

Methodology

Hourly water temperature data collected from 18 rivers across five regions in South Africa (Western Cape, Southern Cape, Eastern Cape, KwaZulu-Natal and Mpumalanga) facilitated the generation of thermographs for each river and enabled comparisons to be made amongst rivers. Thermal load (as mean annual temperature and summer temperatures) and variability (including predictability) best explained the grouping of rivers, which ranged from cool headwater streams to warmer lowland rivers.

Laboratory experiments were undertaken to determine the upper thermal limits of a number of aquatic macroinvertebrate taxa. These were undertaken for all five regions and, for the Western Cape, for all seasons. Sublethal effects were also examined. Lastly, to establish catchment resilience, a connectivity index that incorporates longitudinal (in-stream barriers), lateral (catchment transformation) and temporal (function of changes in flow and water temperature regimes) thermal aspects was developed. This is critical in resource conservation and development planning.

Summary of key findings and discussion on the thermal experiments and generation of biological temperature thresholds

Upper thermal limits, expressed CT_{max} values (Critical Thermal maximum) and 96h ILUT (Incipient Lethal Upper Temperature) varied spatially amongst regions, with differences most evident amongst winter versus summer rainfall regions. Within-region variation was also apparent in the Western Cape, where both CT_{max} values and 96h ILUT varied by approximately $2^{o}C$ amongst sites. CT_{max} values and 96h ILUT varied temporally with distinct differences between summer (highest) and winter (lowest), and less distinct and more variable differences in spring and autumn. The influence of acclimation temperature on thermal limits was unclear with amphipods exhibiting a trend of increasing CT_{max} as acclimation temperature increased, although this was not evident for mayflies. Similarly the effect of the rate of temperature change varied amongst taxa, with amphipods showing no response, while CT_{max} values for mayflies decreased as rate of temperature change increased. CT_{max} values varied significantly amongst genera within families for five of the seven comparisons undertaken. Maximum Weekly Allowable Temperature (MWAT) thresholds, which serve as an integrator of physiological effects on aquatic organisms, varied amongst taxa and rivers, ranging from $15.8^{o}C$ to $20.9^{o}C$.

From these thermal experiments it is clear that both spatial and temporal variation in upper thermal limits is evident across a range of taxa. Some broad observations regarding MWAT thresholds were noted as follows.

For all regions, the period of maximum thermal stress is similar and occurred during the warmer months (September to April) although the extent and duration varied amongst regions, river zones and in some instances sites within a region. Taxa from sites in upper catchments generally have lower MWAT thresholds than taxa from sites lower down in the catchment. Taxa from a site below a dam that released warmer surface water had higher MWAT thresholds than a site on the same river above the dam. The MWAT threshold has

correlated with Mean Annual Temperature (MAT), which means that in the absence of thermal experimental data, it is feasible to use MAT as a surrogate to derive a biological temperature threshold at a medium level of confidence. Undertaking thermal experiments on a site-specific basis would increase the confidence of this MWAT threshold, but logistical constraints may not always allow for this. Generalisations in terms of thermal guidelines made at a broad nationa scale for the ecological Reserve are inappropriate and should at the very least need to be conceived at a regional or even local scale.

Sublethal effects of water temperature on aquatic macroinvertebrates

Life-history traits were determined for selected indicator species from the EPT taxa (namely Ephemeroptera: *Lestagella penicillata,* Plecoptera: *Aphanicercella scutata* and Trichoptera: *Chimarra ambulans*). Overall, life-history responses appeared to be finely tuned to the thermal regimes, whilst the hydrological regime was noted as a driver determining population size and mortality. This confirms the importance of water temperature as a driver of biotic responses.

The thermal optimum for growth of *L. penicillata* was between 16°C and 18°C. Successful egg development and hatching occurred between 10°C and 20°C for *L. penicillata* and *Aphanicercella scutata*. For *C. ambulans*, successful hatching occurred at a wider range of temperatures from 10°C to 25°C. Preliminary thermal preferences were estimated for 11 families of aquatic macroinvertebrates and recommendations for improving the experimental system were provided.

The development of a connectivity index to reflect resilience of rivers to climate change

A connectivity index that incorporates longitudinal (in-stream barriers), lateral (catchment transformation) and temporal (function of changes in flow and water temperature regimes) connectivity was developed using rivers in KwaZulu-Natal. The resultant Disconnectivity layer was overlayed with the areas of conservation importance as developed for the provincial freshwater conservation plan. The combination of these data show areas of high conservation value relative to degree of connectivity, and when plotted together allow conservation planners to make choices between areas of high conservation value when resources are limited. Ultimately, the utility of the connectivity index will be a function of the combination of vulnerability and connectivity as a measure of resilience. This connectivity index may be used for ranking catchments in terms of vulnerability and resilience.

Conclusion and recommendations for further research

This study has generated experimental data on thermal limits of aquatic macroinvertebrates, including both lethal limits and sublethal limits. This research has encompassed five geographic regions, 18 rivers and nine thermally sensitive taxa. These thermal limits have been used to generate biological temperature thresholds. A protocol for incorporating water temperature into the ecological Reserve has been developed.

The project has developed a connectivity index for the rivers of Kwazulu-Natal, which may be used in subsequent validation of National Freshwater Ecosystem Priority Areas (NFEPAs) in South Africa. In addition, the protocol for which may be applied in other provinces.

Establishment of long term water temperature monitoring sites

Strategic selection of long term monitoring sites for installation of water temperature loggers should receive the highest priority. To date water temperature monitoring has not been undertaken on a regular basis in South Africa, although the importance of monitoring water temperature in rivers was recognised as early as the late 1980s. With global climate change exacerbating existing stresses on aquatic ecosystems, and given the expected increase in air temperature and change in precipitation across the country, it is no longer adequate to talk about monitoring water temperature, rather implementing.

Collection of water temperature data will facilitate the generation of thermal signatures for all rivers, from which thermal thresholds and guidelines may be established. Over time such data would allow for an estimation of long term trends associated with climate change, and enable comparisons to be made between actual thermal changes versus predicted thermal changes.

As a starting point it is recommended that the 40 DWS gauging stations for which a reliable flow record is available (based on previous research) be prioritised for installation of water temperature loggers. Given the importance of protected areas from the perspective of reference or natural rivers, it is further recommended that national (SANParks) and regional (e.g. CapeNature, Mpumalanga Tourism and Parks Agency, Eastern Cape Department of Economic Development, Ezemvelo KZN Wildlife) authorities be consulted. Including long term monitoring sites in such protected areas would greatly benefit the monitoring programme.

Generation of MWAT exceedances for South Africa

The potential exists to use the correlations between MWAT and water temperature metrics as well as other spatial variables to model MWAT spatially. On this basis, a next step would be to produce spatial maps of MWAT exceedances for different genera or species and seasons for current and future scenarios. This could be readily linked to global change scenarios using mean annual air temperatures, and the relationship between MWAT and air temperature, etc. The implementation of a water temperature monitoring programme as recommended above would greatly enhance the reliability of this spatial map. It would be further enhanced by the extension of the thermal experimental research to other regions, which would allow for the determination of MWAT values for more regions and taxa.

Further studies on biotic responses to water temperature (and flow)

The present study has provided some of the first information in South Africa on the responses of biota to water temperature and thermal stress. Since global climate change is likely to have "winners" (e.g. thermally sensitive taxa with limited geographic range) and "losers" (e.g. warm water pest species), further studies on additional species and regions would greatly advance our understanding of the biotic responses to elevated water temperature and allow for further predictions in terms of these "winners" and "losers". This could be done for aquatic invertebrates as well as other taxonomic groups including periphyton, fish and amphibians, in both riverine and wetland environments. Such studies could include aspects related to both the lethal and sublethal effects of water temperature, which would allow for enhanced understanding of, for example, life histories and environmental cues for critical activities such as emergence and spawning.

Understanding the relationship between groundwater and water temperature

The importance of groundwater in rivers and thermal buffering properties of groundwater is well recognised. However the potential consequences of groundwater abstraction on water temperatures in rivers that are largely groundwater dependent is not yet fully understood. A study focusing on this aspect would be beneficial and improve our understanding of the links between surface water, groundwater, water temperature and biological consequences.

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Pfananani Ramulifho: An MSc thesis on developing a connectivity index by Pfananani Ramulifho, Dept. Biological Sciences, School of Bioresources Engineering and Environmental Hydrology, University of KwaZulu-Natal, titled; “Development of a connectivity index to assess aquatic macroinvertebrate species vulnerability to thermal change: a case study in KwaZulu-Natal province”.

Jody-Lee Reizenberg: A one year internship with the Freshwater Research Centre and an Honours degree project by Jody-Lee Reizenberg, Department of Environmental and Geographical Sciences, University of Cape Town titled; “Spatial variation in upper thermal limits of aquatic macroinvertebrates in the Liesbeek River, Cape Town”.

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List of Acronyms

7-D	Seven-day
CTE	Critical thermal endpoint
CTM	Critical thermal method
CT_{max}	Critical thermal maximum
CT_{min}	Critical thermal minimum
CV	Coefficient of variation
DWS	Department of Water and Sanitation
ELOHA	Ecological limits of hydrological alteration
ELOTA	Ecological limits of thermal alteration
IHA	Indicators of Hydrologic Alteration
ILT	Incipient lethal temperature
ILUT	Incipient lethal upper temperature
ITA	Indicators of Thermal Alteration
LT_{50}	Lethal temperature where 50% mortality achieved
MAT	Mean Annual Temperature
MWAT	Maximum weekly allowable temperature (chronic threshold)
OT	Optimal temperature
PCA	Principal Components Analysis
SD	Standard deviation
WT	Water temperature

Chapter 1. Introduction

Prior to 2008 knowledge on water temperature in South African rivers was extremely limited. Recent research (K5/1799) on water temperature in lotic ecosystems advanced our understanding and resulted in the development of preliminary thermal guidelines for the ecological Reserve (Dallas et al. 2012, Rivers-Moore et al. 2013a). Hourly water temperature data were analysed to generate temperature metrics that characterise the magnitude, frequency, duration and timing of thermal events (Rivers-Moore et al. 2012). Taking spatial differences into account, thermal regions were proposed for the Western and Eastern Cape (Dallas et al. 2012). The project provided insight into the links between water temperature and biotic responses (Dallas and Ketley 2011, Dallas and Rivers-Moore 2012, Eady et al. 2013, Eady et al. 2014, Ross-Gillespie 2014) and thermal characteristics (Dallas and Rivers-Moore, 2011).

Thermally sensitive aquatic macroinvertebrate taxa that were suitable bioindicators of thermal alteration were identified (Dallas and Ketley 2011, Dallas and Rivers-Moore 2012) and methods for estimating lethal limits, including Critical Thermal Method (CTM) and Incipient Lethal Temperature (ILT) method, were evaluated and used to establish upper lethal limits of a range of taxa and for determining the relationship between the two methods. This research demonstrated the value in measuring thermal tolerance in a controlled laboratory environment and generated preliminary data on biological temperature thresholds based on acute effects. In undertaking this research, Dallas and Rivers-Moore (2012) noted that the issue of thermal sensitivity and the derivation of upper thermal limits for the setting of biological temperature thresholds is complex. Several aspects that would improve our understanding of spatial and temporal variation in thermal limits of aquatic macroinvertebrates warranted further examination. These included the influence of thermal history, acclimation temperature, and rate of temperature change on upper thermal limits. In addition, variation in thermal tolerance amongst species or genera within a macroinvertebrate family was unknown and would allow for the refinement of family level biological thresholds of bioindicators of thermal alteration. Examination of the extent to which upper thermal limits within a species vary spatially amongst rivers, would increase understanding of site-specific biological thresholds and their importance in refinement of temperature criteria and assist in the development of thermal guidelines.

These guidelines are important for effective protection and management of aquatic ecosystems. Ever increasing anthropogenic pressure on aquatic resources related to hydrological (e.g. river regulation and water abstraction), land use and global climate changes, make thermal guidelines critical. In order to manage anthropogenic changes to aquatic ecosystems, resource managers commonly set guidelines based on maximum allowable changes (e.g. Nelitz et al. 2007). Identification of appropriate temperature criteria that inform these “allowable changes” whilst still protecting aquatic biota and thus ecosystems, is complicated by the highly variable nature of temperature in rivers, coupled with the differing temperature requirements of aquatic species (Sullivan et al. 2000). Understanding these aspects forms the basis of the current project.

1.1 Aims and objectives

The overall objective of the project was to refine the upper thermal limits of aquatic macroinvertebrates to facilitate the formulation of biological temperature thresholds for incorporation into the water temperature component of the ecological Reserve. This knowledge is considered important for the protection of aquatic ecosystems and will provide insight into the adaptability and vulnerability of biota to climate change and allow for the generation of recommendations linking biota and climate change. The specific aims of the project are as follows:

- To investigate the influence of thermal history, acclimation temperature and rate of temperature change on upper thermal limits of thermally sensitive aquatic macroinvertebrates.
- To undertake preliminary investigation of sublethal effects of water temperature on selected aquatic macroinvertebrates.
- To investigate regional variation in thermal sensitivity of selected aquatic macroinvertebrates.
- To develop biological temperature thresholds for selected aquatic macroinvertebrates.
- To examine adaptability/resilience to climate change in rivers using a connectivity index as an indicator of potential risk.

The results of this project have been compiled in this technical report as follows:

- Chapter 1: An introduction to the topic of water temperature and riverine organisms, outlining the aims of the project and outline of the report.
- Chapter 2: A review of the ecological consequences of global climate change for freshwater ecosystems in South Africa.
- Chapter 3: An overview of the approaches, metrics and methodologies for examining adaptation to climate change in freshwater ecosystems.
- Chapter 4: An overview of experimental methods for determining upper thermal limits of aquatic macroinvertebrates, including both lethal and sublethal effects.
- Chapter 5: A synthesis of experimental data for estimating lethal limits for aquatic macroinvertebrates based on two methods, CTM and ILT.
- Chapter 6: A description of the experiments undertaken to estimate sublethal effects, including the effect of temperature on growth rate, emergence and egg development.
- Chapter 7: The development of a connectivity index to reflect resilience of rivers to climate change.
- Chapter 8: An overview of the development of biological temperature thresholds for aquatic macroinvertebrates.
- Chapter 9: Conclusions and recommendations.

Chapter 2. Literature review on the ecological consequences of global climate change for freshwater ecosystems in South Africa

2.1 Introduction

Freshwater ecosystems are considered to be among the ecosystems most vulnerable to global climate change. Observational records and climate projections provide abundant evidence that freshwater resources have the potential to be strongly impacted by climate change, with wide-ranging consequences for human societies and ecosystems. Southern Africa has been identified as a 'critical region' of water stress and South Africa is a water-stressed country with a mean annual precipitation of 500 mm per annum, with 65% of the country, especially the arid and semi-arid interior and western regions, receiving on average <500 mm per annum.

Global and regional climate change models predict likely trends in the magnitude and amplitude of event-driven systems, primarily rainfall and air temperature. Changes include shifts in mean condition, variance and frequency of extremes of climatic variables, which result in changes in water quantity, especially in arid and semi-arid regions.

Historically, focus on the consequences of global climate change trends has tended to be on terrestrial ecosystems, with less attention given to aquatic ecosystems. In the last decade, focus has shifted to freshwater ecosystems and the number of studies published annually has increased dramatically. This shift follows the recognition that freshwater ecosystems are vulnerable to climate change that aquatic organisms are highly sensitive to climate change and that climate change is expected to worsen freshwater conditions, especially in Mediterranean regions. Several climate model projections warn of widespread biological invasions, extinctions and the redistribution and loss of critical ecosystem functions.

This review explores the likely impacts of climate change on freshwater ecosystems, focusing on lotic (rivers) ecosystems, although several issues are also relevant for lentic (lakes, wetlands) systems (Dallas and Rivers-Moore 2014). It builds on information gleaned during an interactive workshop of climate change and freshwater specialists. It summarises key issues related to climate change and freshwater ecosystems within the context of South Africa, but with reference to international research. It discusses the abiotic drivers of climate change and the ecological consequences of climate change to freshwater ecosystems. These consequences are separated into those affecting water quantity, water quality, physical habitat and aquatic biological assemblages. Several guiding principles aimed at minimising the potential impact of climate change on freshwater ecosystems are discussed, including those focused on water quantity and the maintenance of appropriate environmental flows, integration of global climate change into water quality management, conservation planning for freshwater biodiversity, and the promotion of ecosystem resilience. Although specific scientific literature on climate change and freshwater ecosystems in South Africa is limited, relevant studies have been consulted and links have been made to the potential ecological consequences of climate change.

2.2 Setting the stage – abiotic drivers of global climate change

General circulation models (GCMs) are a class of computer-driven models for weather forecasting; those that project climate change are commonly called global climate models. GCMs simulate the most important features of the climate (i.e. air temperature and rainfall) reliably at a large scale, although, as uncertainties are inherent in GCMs, predictions for rainfall intensity, frequency and spatial distribution have a lower confidence. CGMs are commonly downscaled to enable their outputs to be made relevant to regional- or local-scale climate change scenarios be made relevant to regional- or local-scale climate change scenarios.

In South Africa, regional models have developed to a stage where pattern changes at a sub-national scale are made with confidence, while confidence for the magnitude of change is weaker. According to these models, predictions are not uniform within South Africa and climate change is likely to impact most strongly on the western regions, with less of an impact as one goes eastwards. Certain areas are likely to become 'winners' in light of certain projected changes, while other areas are likely to become 'losers' as more water-related stresses are experienced. 'Hotspots' of concern are the southwest of the country, the west coast and, to a lesser extent, the extreme north of South Africa. The responses of rainfall and temperature as predicted by global climate change models are summarised in Appendix 1, with summer and winter rainfall regions given separately where relevant.

2.3 Ecological consequences of global climate change

Primary climate change drivers are precipitation, air temperature and evaporative demand. Ecological consequences of global climate change on freshwater ecosystems may be grouped into effects that relate to water quantity, water quality, habitat and biological assemblages (Appendix 2).

2.3.1 Water quantity

Global climate change drivers directly affect the quantity of water in freshwater ecosystems by changing run-off patterns, increasing the frequency and intensity of extreme events, and changing groundwater recharge rates. In terms of runoff patterns, much of South Africa is projected to have increases in annual streamflows by 20% to 30%, regardless of whether it is a year of median flows or a year with the 1:10 year low or high flows. The exception is the southwestern Cape which will have reduced streamflows especially in the wet years. Interannual variability is projected to increase (20-30%) in most of the country, with the exception of the southwestern Cape where variability is projected to decrease. Researchers have projected that most parts of South Africa are likely to experience reduced frequency, duration and intensity of droughts, with the exception being the west coast and northwest, which will exhibit marked increases in annual droughts. Floods and stormflow, i.e. water generated from a specific rainfall event, are projected to increase across South Africa, particularly in the central west where both magnitude and variability of stormflow will increase.

2.3.2 Water quality

Higher water temperatures, increased precipitation intensity, and longer periods of low flows are projected to exacerbate many forms of water pollution, including sediments, nutrients, dissolved organic carbon, pathogens, pesticides, salt and thermal pollution. The quality of water in many South African rivers and wetlands is already widely compromised and climatic drivers therefore act as additional stresses on these ecosystems. Water quality changes, including water temperature, affect the solubility of oxygen and other gases, chemical reaction rates and toxicity, and microbial activity. A reduction in the concentration of dissolved oxygen, particularly under the combined effects of high temperature and low flows, is particularly deleterious to aquatic organisms. The effects of water quality variables on aquatic ecosystems have been widely documented, with specific studies focusing on specific variables, including water temperature. Recent studies on the link between air and water temperature and the effect of elevated water temperature on aquatic organisms have included experimental laboratory work which together with field-based studies, has allowed for the development of tools for assessing water temperature in river ecosystems and scenario prediction for elevated temperatures. Estimating the likely increase in water temperature from predicted changes in air temperature is, however, complex and dependent on insulators and buffers such as solar radiation, groundwater input and shading. One of the key issues is how lapse rates (change in water temperature degrees with every 100 m altitude) will change.

Other water quality variables likely to increase in response to more intense rainfall events include turbidity and nutrients, with sediment washed in from the catchment or, in the case of nutrients, mobilised from the riverbed (e.g. phosphorus). Changes in nutrient loads and nutrient cycles (and carbon cycling) may result in increased algal growth, changes in eutrophic condition, as well as increased incidences of cyanotoxins, which affect human health negatively. Other adsorbed pollutants such as metals may also be mobilised, together with increased transport of dissolved pollutants such as pesticides and pathogens. The synergistic and antagonistic interactions of several water quality variables make it especially difficult to predict the likely consequences of climate change on receiving water bodies; suffice it to say that these consequences are likely to be significant given the levels of stress already imposed on these systems.

2.3.3 Physical habitat

Changes in the amount, seasonal distribution and intensity of rainfall may affect channel geomorphology, longitudinal and lateral connectivity, and aquatic habitat, through changes in run-off. Likely consequences of changes in flow on the geomorphology of river systems depend on the direction of change with increased discharge (e.g. in the eastern region) likely resulting in channel enlargement and incision, greater channel instability and sinuosity, and increased bank erosion, while decreased discharge (e.g. in the western region) may result in channel shrinkage, greater channel stability, vegetation encroachment, and sedimentation in side channels. Loss of longitudinal and lateral connectivity can lead to isolation of populations, failed recruitment and local extinction; the maintenance of natural

connectivity patterns is thus essential to the viability of populations of many riverine species and for maintaining instream integrity.

2.3.4 Biological

Thermal and hydrological regimes are master variables driving river ecosystems. Temperature is a primary climate change driver, while flow has been shown to change substantially in response to changes in rainfall patterns. Potential biological consequences of climate change include changes in aquatic biodiversity, changes in individual life-history patterns, changes in communities, changes in species distribution and range, extinction of vulnerable species, increase in the number and spread of invasive and pest species, and an increase in waterborne and vector-borne diseases.

2.4 Discussion

Global climate change is recognised as an additional, amplifying driver of system variability and cannot therefore be viewed in isolation from other stressors. Many non-climatic drivers affect freshwater resources at a global scale and the quantity and quality of resources are influenced by, for example, land-use change, construction and management of reservoirs, pollutant emissions, and water and wastewater treatment. South Africa is fortunate in having established widely recognised approaches for determining the 'ecological Reserve' – a South African term used to describe what are globally referred to as environmental water requirements. In spite of provisions of the *National Water Act (Act 36 0f 1998),* there is widespread agreement among scientists that South Africa's aquatic ecosystems have significantly deteriorated. A key reason given for this deterioration is the widespread failure to operationalise, monitor and enforce ecological reserves – a legislated framework for securing water quality and quantity for the environment. Understanding the likely consequences of climate change for freshwater ecosystems is therefore of critical importance to the future well-being of the resource and society.

Several guiding principles, or proactive response options, aimed at minimising the potential impact of climate change on freshwater ecosystems, were formulated during a workshop. These guiding principles include those focused on water quantity and the maintenance of appropriate environmental flows, integration of global climate change into water quality management, conservation planning for freshwater biodiversity, the promotion of ecosystem resilience, and extending climate change science into policy and public discourse. The adoption of a proactive, 'no-regrets' policy with respect to climate change has been widely endorsed in South Africa and elsewhere; such a policy calls for proactive management which includes actions such as restoration, land purchases, and measures that can be taken now to maintain or increase the resilience of rivers. The alternative – reactive management – which involves responding to problems as they arise by repairing damage or mitigating ongoing impacts, has been shown to be inadequate and short-sighted and results in considerable ecological, social and economic consequences and costs in the longer term.

2.5 Conclusions

Proactive assessment and monitoring are critical as these would allow for the identification of ecological triggers and thresholds, including thresholds of vulnerability, which may be used to monitor and inform decisions, as well to improve the ability to forecast based on this knowledge. Identification of ecological reference sites for long-term monitoring and routine monitoring of these and other impacted sites within a framework of established biomonitoring programmes are critical. This monitoring would facilitate detection of change, both in response to non-climate as well as climate change induced effects, although the ability of the various monitoring tools to facilitate this may still need to be validated. One of the key challenges facing freshwater ecologists is to develop a suite of tools for detecting the impacts of climate change in complex natural systems that can be applied across multiple spatio-temporal scales and levels of organisation. Integration of long-term, empirical survey data with models and manipulative experiments will facilitate the development of mechanistic, and hence predictive, understanding of responses to future change.

Chapter 3. Approaches, metrics and methodologies to examine adaptation to climate change in freshwater ecosystems

3.1 Introduction

Global climate changes amplify existing stressors on ecological systems, and often have cascading effects. Because the impacts of climate change, and its rate of change, are added to already significant impacts and degradations caused by humans, the effects of climate change "could be particularly profound for aquatic ecosystems" (Rieman and Isaak 2010, p. 1). The two fundamental drivers (air temperatures and rainfall) of climate change that impact on freshwater systems will affect water temperatures and hydrological regimes (timing, frequency and extent of extreme events). "Current rates of climate change are unprecedented and biological responses to the changes have also been rapid at the levels of ecosystems, communities and species" (Heino et al. 2009, p. 39). Fundamental changes in climate could lead to fundamental changes in physiology, behaviour and growth of individuals, as quantified through a series of 90 metrics reflecting a hierarchical level of impacts from molecular to organism to ecosystem (Geyer et al. 2011). Changes in water temperatures are likely to have a cascading and magnified impact at all three levels, and changes in community structure as a result of trophic cascades (as noted through disturbances of trout on freshwater ecosystems – Townsend 2003).

Global climate change causes major changes in spatial configuration of habitats, and static conservation planning approaches are not adequate to deal with such changing environmental gradients (Turak et al. 2011). Such concerns are already being raised in regions which are comparable to South Africa's hydrological landscape. Australia's Murray-Darling Basin is noted as having an uncertain future in response to climate change, where flows in most of its rivers are already over-allocated (Pittock and Finlayson 2011). Such systems have evolved based on all naturally available water and so reductions in flows typically cause a loss of diversity. Climate change will exacerbate existing stresses, where return intervals of hydrologic events which drive aspects of ecosystem functioning, will change (Pittock and Finlayson 2011).

While a lot of uncertainty exists, certain "knowns" are emerging, such as that in the Rocky Mountains the climate will become warmer and drier. There are already observed/measured/confirmed changes in hydrological regimes, linked to changes in runoff, viz. changes in timing of runoff by 1-3 weeks, winter flood frequency, decreasing summer flows and flood frequency probabilities (all in the United States). However, underlying geology means that blanket statements can't be made, as certain geology types with greater groundwater influence, will have a buffering effect (Rieman and Isaak 2010). Rising temperatures, coupled with changes in hydrology, will make instream environments unsuitable for many species. Temperature is a crucial habitat feature, as an axis in the multidimensional niche (Elliott and Elliott 2010). Climate change is expected to worsen freshwater conditions (higher temperatures and longer droughts) in Mediterranean areas (Dallas 2013). Rivers-Moore et al. (2013b) used a conservative 2°C increase in water temperatures. This figure is in accordance with values used in current studies elsewhere,

which list increases of 1-3°C (Lester et al. 2011, Turak et al. 2011), with California's Mediterranean-montane climate expected to warm by as much as 2-6°C (Viers and Rheinheimer 2011). Even in the more popular literature, an average warming of 1.1°C is presented as a given, conservative value, with rises of up to 6.4°C in the next century under a "business as usual" scenario. It is further noted that we are already committed to a 1°C increase, and probably also 2°C. The problem/area of uncertainty rests with a likely positive feedback system, where initial increases are likely to trigger additional increases in temperatures (Caldecott 2008). Temperature rises will be especially deleterious to high altitude, fast-flowing streams where cold stenotherms lose their thermal refuges (Turak et al. 2011 citing various authors). Similar effects of climate change are expected for fish, with research showing that habitat for cold water species is likely to shrink with increases in air temperatures, while warm water species will increase their relative abundance and expand into new upstream habitats (Almodovar et al. 2010).

In recognising the magnitudes of potential impacts of climate change on freshwater systems, and confronting the uncertainties associated with these changes, three useful questions to pose are: What is changing, what are the implications for aquatic organisms, specifically fish and aquatic macroinvertebrates, and what can we do about it (Rieman and Isaak 2010)? We propose that answering these questions is most pragmatically achieved using methods contextualised within the Ecological limits of hydrological alteration (ELOHA) framework (Poff et al. 2009). This framework is a method for assessing flow needs for streams and rivers to support regional flow management. It is a holistic approach in that it integrates hydrological and ecological data, including the development of empirical models that directly predict ecological responses to various types and degrees of flow alteration. This provides the mechanism for posing testable hypotheses, informed by published studies and expert knowledge that document the response of ecological processes and patterns to a range of flow conditions, both natural and altered. These in turn potentially form the basis for interactions between scientists, environmental managers and stakeholders in enabling a decision-making process of choosing desired flow patterns based on societal values, and linking hypothesis-driven monitoring programmes to an adaptive management cycle (Poff et al. 2009). Integration of long-term, empirical survey data with models and manipulative experiments will facilitate the development of mechanistic, and hence predictive, understanding of responses to future change (Woodward et al. 2010).

The aim of this chapter is to identify approaches, metrics and methodology to examine adaptation to climate change in freshwater ecosystems. Focus is on methods for assessing the level of departure from reference conditions in flows and water temperatures (since both are linked), how to link this to biotic response, and through this to assess the capacity for adaptation of freshwater biota to climate change impacts.

3.2 Assessment of methods

Water temperatures and flows are sensitive to the impacts of climate change, in response to changes in air temperatures and changes in precipitation patterns (Rieman and Isaak 2010). These responses have important implications for aquatic macroinvertebrate community structure, because flow and water temperature regimes interact together to influence many

physical and biological processes (Olden and Naiman 2010). While both variables interact with each other, temperatures may be more constraining than flows (Olden and Naiman 2010). Flow controls the volume of habitat, while together with water temperatures (and other variables); these form the resource axes that affect spawning, foraging, migration and refugia. Changes in the flow and temperature resources to which a species is adapted can affect timing of life histories, such as timing of migration in salmon (Hodgson and Quinn 2002).

3.2.1 Time series and metrics

To begin any analyses of the impacts of these primary drivers (i.e. flows and water temperatures) requires time series data, which may be either observed or simulated. Ideally, sub-daily time series data are required for water temperatures to be able to describe daily means, minima and maxima. Data for water temperatures are generally less freely available and for shorter periods, than flow data. A first approach in using time series is to test long-term data for trend. One approach is time series analyses (autoregressive integrated moving average or ARIMA) to find trends in warming (Almodovar et al. 2012). Even with limited data, generalisations can be made; for example, data for the US indicate that there are statistically significant trends in warming (increases in mean summer temperatures across a network of sites in the US) and in cumulative heat units (degree days) (Rieman and Isaak 2010). There is a recognised need for a wider network of monitoring sites across different stream types and latitudes, because different streams are more (or less) sensitive to warming trends than others, because different factors in different streams (such as buffering by groundwater) interact in different ways to make generalisations difficult (Poole and Berman 2001). Small streams may be affected more than larger rivers due to the close relationships between air temperature and water temperature of small streams (Heino et al. 2009).

The next step is to break these time series down into ecologically relevant metrics. Approaches to describing time series generally attempt to measure variability by either agglomerating or disaggregating time series data. Agglomerative approaches make use of techniques such as duration curves, while disaggregation approaches make use of indices that focus on state and threshold values using descriptive statistics, and attempt to understand the links between timing, duration and magnitudes of different system states. Already nearly twenty years ago, methods were derived to describe flow time series in terms of magnitude, frequency, duration and timing of events (Richter et al. 1996), with the initial approaches being conceptually refined over time (Richter 2009).

While streamflow is perceived as a ‘master variable’ or ‘maestro’ shaping many fundamental ecological characteristics of riverine ecosystems (Poff and Zimmerman, 2010), temperature metrics have been shown to be no less important than discharge metrics in explaining differences in invertebrate community structure (Jackson et al. 2007). In South Africa, a similar conceptual approach has been used to successfully describe water temperature time series (Rivers-Moore et al. 2012).

These methods would be incomplete without also using ecological metrics. Decreased values of ecological metrics typically mirror changes in flow and temperature metrics (Jackson et al. 2007, Poff and Zimmerman, 2010), although it may be difficult to define generic/unambiguous quantitative relationships between flow alteration and ecological response, in part because alteration of flow regimes is typically confounded with other environmental factors (Poff and Zimmerman, 2010).

3.2.2 Measuring deviation from reference conditions

Describing time series data based on metrics allows sites to be grouped in multivariate statistical space and classified based on flow/thermal similarities, i.e. regime types. A key reason for doing this is that conceptually, the form and direction of an ecological response should be similar within same types. This allows scientists to measure deviation from reference conditions (in terms of statistical departures of variables), based on the premise that increasing degrees of flow or temperature alteration correspond with increasing degrees of ecological change. This approach has been used, with some success, to define thermal regions in the southern Cape region of South Africa (Rivers-Moore et al. 2013a). Ideally, this approach would need to be extended over a wider geographical area and with improved predictive power before it can be viewed as a robust spatial classification.

3.2.3 Ecological implications – habitat modelling and scenario analyses

There is growing evidence that in terrestrial systems, responses to climate change are reflected through changes in distributions, invasions, local extinctions and changes to community structures and food webs. While there is no reason to doubt that these trends, and changes in expression of life histories, also hold true for freshwater systems, there is less evidence available in the literature to support this for freshwater systems (Rieman and Isaak 2010, citing various authors). However, what is known is that climate change is expected to have significant impacts on hydrologic regimes and freshwater ecosystems (Aldous et al. 2011). Few basins have adequate numerical models to guide the development of freshwater climate adaptation strategies (Aldous et al. 2011), and the need exists to objectively assess alternative plans on the basis of their relative ecological merits, and models allow for this and provide a useful tool to support management (Lester et al. 2011).

A number of modelling approaches can be used, all of which essentially link a biotic response to abiotic conditions. These include cause-and-effect models, referred to as deterministic models; and species distribution models. Both approaches integrate the steps described previously, and provide the capability to undertake scenario analyses.

3.2.3.1 Deterministic models

Ecological modelling in the context of assessing climate change can be as simple or as complex as the research question requires. Such approaches can be either species-specific or focus on groups of species. Species-specific models are often statistical, where a common approach is to link biota to environmental conditions using empirical relationships, such as those used by Lester et al. (2011). Susceptibility of species to climate change is likely

to vary amongst species and will in part depend on their biological traits. Those species with specialized habitat and/or microhabitat requirements; narrow environmental tolerances or thresholds that are likely to be exceeded at any stage in the life cycle; dependence on specific environmental triggers or cues; dependence on inter-specific interactions; and poor ability to disperse to or colonize a new or more suitable range; are likely to be more susceptible (Desta et al. 2012). The purpose of these models is to define flow/temperature alteration-ecological response relationships (Poff et al. 2009). Such models provide a useful context for posing plausible hypotheses, and are particularly useful when there are clear threshold responses, or where inflection points in probabilities in finding a species based on temperature exceedances can be defined (Poff et al. 2009). Thermal tolerances help explain the broad patterns of species occurrence, and may be inferred from behavioural observations or laboratory experiments (e.g. Dallas and Ketley 2011, Dallas and Rivers-Moore 2012). Threshold responses can describe either lethal limits or chronic warming leading to increased mortalities and shifting habitat distribution (Rieman et al. 2007, Rieman and Isaak 2010).

Inputs to these models may come from a number of sources, such that instead of a single model, a suite of coupled models is used for scenario analyses, where one model's output provides input to another model. An inescapable problem with this modelling approach is that they magnify systematic errors through the modelling sequence (Aldous et al. 2011, Lester et al. 2011). Another problem is that it may be difficult to tease out the exact role of temperature when other confounding variables exist (Poff et al. 2009, Rieman and Isaak 2010). In many cases, it is to be expected that predictive capacity of these models varies, because of differences in species responses to thermal stress, and differential response to flow regime changes (Wengar et al. 2011, Sheldon 2012). Other factors include the inability to include fine-scale variables for regional predictive models (Wenger et al. 2011); that laboratory measures of thermal tolerance exclude other stressors (Almodovar et al. 2012), that species may tolerate greater temperature extremes in situ than those to which they are exposed, and behavioural responses to avoid thermal extremes may not be accounted for (Sunday et al. 2012).

When flow and water temperature data are available, and threshold can be identified, models linking biotic response to abiotic drivers have been successfully used for pest species and species of conservation importance (Rivers-Moore et al. 2013b). However, it has already been noted that data, either as direct data records or as stream-scale environmental data to estimate water temperatures (Rieman et al. 2007), are not always available, and in these cases surrogates can be used. Like Rivers-Moore et al. (2013b), Almodovar et al. (2010) used moving average models (MWAT) linked to thermal thresholds to predict thermal habitat for brown trout.

In cases where both water temperature and threshold data were missing, successful models were still developed. For example, Rieman et al. (2007) regressed the lower elevation limits of fish against latitude and longitude using relevant raster images (DEM, longitude and latitude). Another approach was used by Sheldon (2012), who converted air temperature increases to altitudinal shifts using an elevational lapse rate. These data were used to develop logistic multiple regression models to predict stonefly responses to temperature changes. Wenger et al. (2011) used air temperatures from downscaled general circulation

models coupled with a hydrologic model to forecast effects of altered flows and increased temperatures on four interacting species of trout in the interior western US. By linking 12 abiotic variables as predictors of species distributions and multiple logistic regression models, and use of estimated future temperature and flow metrics, Wenger et al. (2011) were able to model forecasted species suitable habitat, and assess losses.

3.2.3.2 Species distribution models

Freshwater biodiversity is particularly vulnerable to climate change, and extinction rates are equal to or higher than for terrestrial systems. Climate warming is likely to have considerable effects on the geographical distributions of freshwater organisms. In broad terms, cold water species (and higher latitude species at their lower physiological limits) will be negatively affected while warm water species will be positively affected. Various studies have documented range shifts in species although different taxonomic groups may not show similar responses to climate change (i.e. effects will be species- and ecosystem-specific). This is also partly a function of dispersal ability and strategy (Heino et al. 2009). While this makes predictions difficult (Heino et al. 2009, and citing various authors), such information is important, inter alia, in assessing capacity of protected areas to accommodate future changes.

Even without absolute fine-scale data, coarse-grained occurrence data can be used to detect climatic niche shifts, despite criticism on the basis that species distributions are not only a function of climatic variables but also biotic factors and stochastic events (Lauzeral et al. 2010). Various habitat distribution models are available, including Maxent (Phillips et al. 2006, Phillips and Dudik 2008, downloadable from www.cs.princeton.edu/~schapire/maxent). This model approach is suitable in cases of limited data as Maxent performs well using presence data only where sample sizes are small. Using this approach, geospatial data about major habitat conditions can be linked to species present data to derive species distribution maps, as has been shown to be successful by other authors in conservation planning exercise in rivers (for example Esselman and Allan 2011).

3.2.3.3 Scenario analyses

In the face of uncertainty it will become increasingly necessary to develop catchment scenarios (Rieman and Isaak 2010), particularly scenarios of extreme conditions (high temperatures and low flows). Body weight, fecundity and water temperatures are intimately linked (Vannote and Sweeney 1980), and therefore, from both conservation and pest management perspectives, it is useful to model potential interactions between these variables under different thermal scenarios. A useful approach is to assign levels of risk (high, medium or low, where risk is proportional to area of habitat patches lost with climate change) associated with different levels of warming, and evaluate relative changes in habitat (Rieman et al. 2007).

Simulation modelling is useful in highlighting restoration alternatives and eliminating alternatives which provide less environmental benefits (Null et al. 2010). A limitation of scenario analyses is that they only consider potential habitat without considering other impacts on distribution (Sunday et al. 2012). Declines in freshwater biodiversity have been

associated with multiple anthropogenic drivers, with climate-induced changes in water temperature and hydrological regimes typically ranking amongst the most influential (Heino et al. 2009). It may also be necessary to consider synergistic relationships between climate change and acidification (de Moor, pers. comm. 2012), and eutrophication, increased algal production and biomass, and land cover alterations. Additionally, feedbacks between climate change and anthropogenic impacts (changes to land use) have been noted (Heino et al. 2009).

Another useful element for scenario modelling is the use of bioclimatic envelopes. Here, different warming scenarios (for example, 0.5 to 3°C increases in water temperatures measured over 0.5°C increments, Almodovar et al. 2010) could be used to predict changes in thermal habitat. This provides a spatial representation of bioclimatic envelopes, highlighting areas of local extinction risk and how potential progressive patterns of habitat fragmentation are likely to occur.

3.2.4 What can be done?

"Deliberate and strategic design of resilient systems...is now recognised as a major social-scientific challenge of the 21st century" (Palmer at al. 2004 in Poff et al. 2009), i.e. system management (or reduced system mismanagement) towards enhancing resistance and resilience (Rieman and Isaak 2010). Two common themes in the literature towards achieving this are application of "adaptation" and "mitigation" measures (Rieman and Isaak 2010). Barriers to adaptation include the degree of river connectivity and directional orientation, the legacy of previous disturbances (such as past pollution), and identifying non-climatic stressors on freshwater systems (Covich 2009).

Broad approaches could include steps to reduce non-climate stresses such as habitat degradation, and complemented through conservation and expansion of critical habitat (see National Freshwater Ecosystem Priority Areas, NFEPA – Driver et al. 2011). Because difficult choices are likely to have to be made, criteria for prioritization will be required; these could include ecological and evolutionary significance (Rieman and Isaak 2010). Given that management decisions are likely to be taken within a context of great uncertainty, tools to assist in assessing the likelihood of success of different strategies are increasingly being used in water resources management. One such approach is the use of influence diagrams/Bayesian networks (Rieman and Isaak 2010) (See Appendix 3).

Implementing these principles for river systems will require multiple, integrated approaches. These include planning of dispersal corridors, minimising anthropogenic impacts (plus habitat restoration and management), taking a catchment perspective (planning protected area networks), designing long-term monitoring programmes and networks (Heino et al. 2009), institutional approaches, and promoting system adaptability or connectivity. These last two options are both discussed in the sections below.

3.2.4.1 Institutional approach

It follows from the nature of freshwater systems (longitudinal systems which are dependent on upstream and surrounding catchment conditions) that problem-solving will need to

occur across a range of organisations. This implies that institutionally, the challenge will be to coordinate efforts, which necessitates a move away from a competitive mindset towards a more cooperative mindset between stakeholders. Considerable work has already been undertaken towards achieving this, both through an understanding of cross-policy objectives in the freshwater sector (Roux et al. 2006), and in practice through the cross-sectoral buy-in of the NFEPAs in South Africa (Driver et al. 2011). Key challenges in maintaining the momentum of these processes is to continue discussion between stakeholders on evaluating acceptable risk (i.e. perceived value versus scientific uncertainty) of different management interventions. This should form the basis for an adaptive process (i.e. learning by doing). It is not the purpose of this document to discuss design of monitoring programmes, beyond the point that they should be designed with a hypothesis-testing framework, and are very much a function of institutional willpower. While the challenge of merging science and management remains difficult, with much remaining to be learned, we know enough to start (Poff et al. 2009).

3.2.4.2 Promoting system adaptability and connectivity

"The interaction of temperature with flow and other physical processes may lead to new patterns of disturbance that will influence the resilience and persistence of broader habitat and population networks" (Rieman and Isaak 2010, p. 16). Because the rate of change and the capacity for resilience in many populations is highly likely to be different from the geological past, the capacity for adaptation (and conversely the barriers to adaptation) needs to be understood (Rieman and Isaak 2010). Rieman et al. (2007) note that a warming climate could profoundly affect distribution and abundance of freshwater biota, and that climate warming will increase fragmentation of existing habitats (smaller and more isolated patches) and accelerate decline of species. This is because loss in connectivity impacts on the capacity to adapt and disperse (Rieman and Isaak 2010), and because reductions in population size and loss of inter-population connectivity increase risk of local extinctions by increasing vulnerability to demographic and environmental stochastic events (Almodovar et al. 2012). It is important to recognise that fish and aquatic macroinvertebrates respond very differently to the same environmental gradients, resulting in different turnover patterns between both groups (Rivers-Moore 2012). Reducing vulnerability therefore requires both an understanding of metapopulation theory for key freshwater species, and recognition that persistence of a species is linked to size and connectivity of patches.

While it is thus difficult to apply generic rules on how different communities of aquatic biota will adapt to changing environmental gradients, one useful approach is to consider the effects of climate change on migration patterns. Few examples of this exist for aquatic macroinvertebrates, with more examples in the literature on fish. Temperature and flow impact on migratory corridors (Hodgson and Quinn 2002), and migrations are a good system to test hypotheses on changes in migration timing, since migration timing is assumed to be an adaptation to long-term average conditions experienced by migrating adults across the whole migration route (including water temperatures – 19°C for sockeye salmon and flows) (Hodgson and Quinn 2002). In the United States, alternative migration strategies of salmon have been observed to avoid stressful summer temperatures. These include premature migration versus delayed migration of up to 20-60 days (Hodgson and Quinn 2002).

For systems to be able to respond to thermal changes requires connectivity. Disruptions to the river continuum may be spatial (physical barriers) or temporal (altered flow or water temperature signatures). In the former case, practical steps towards increasing system connectivity include removal of barriers to dispersal: i.e. to facilitate colonisation of species better suited to new environments and which still provide ecological function and value when indigenous species are not able to persist. However, implementing this is not without moral or ethical issues, since removal of barriers might also be a negative in facilitating alien invasions (Rieman and Isaak 2010). For example, in the Drakensberg Mountains of KwaZulu-Natal, natural barriers prevent upstream migration of trout, which have been shown to have a negative impact on certain amphibians (Karssing et al. 2012). Describing temporal disruptions to connectivity (for example, timing and magnitude of thermal and/or flow events linked to spawning) requires use of metrics, and describing departure from a known reference condition, as described earlier. The development of a connectivity index to reflect resilience of rivers to climate change is described in chapter 7 of this report.

3.3 Conclusions

This chapter has highlighted that a number of methods and tools available are for assessing the vulnerability of biological communities to the effects of climate change. Many of these approaches have been applied to fish, with little available literature discussing aquatic macroinvertebrates, especially in southern Africa. While these tools can be used in isolation, it is useful to apply them within a heuristic framework. We recommend applying a similar framework – the "Ecological Limits of Thermal Alteration" (ELOTA) for assessing impacts of climate change on aquatic macroinvertebrates in southern Africa. Assessments of vulnerability and adaptability should be guided by hypothesis generated by local scientists and stakeholders, as the start of a process. Implementation, the ultimate goal of such a process, should be informed by what can be realistically achieved with respect to water temperatures and river connectivity, represented as a matrix of impacts versus achievable actions.

Chapter 4. Experimental methods for determining upper thermal limits of aquatic macroinvertebrates

4.1 Introduction

Water temperature is an important abiotic driver of aquatic ecosystems (e.g. Caissie 2006, Dallas 2008, Webb. et al. 2008) influencing many aspects of an organism's existence including its feeding, metabolic and growth rates, fecundity, emergence, behaviour and ultimately survival. All organisms have a range of temperatures, often termed the 'optimum thermal regime' (Vannote and Sweeney 1980), at which optimal growth, reproduction and other measures of "health" are greatest (Dallas and Day 2004). This range is bounded by the thermal tolerance range (i.e. the range over which a species can survive) with upper and lower tolerance limits demarcating the thermal extremes. Organisms of a given species can survive on either side of this optimal range but as the tolerance limits are approached, more and more abnormalities become evident. The first signs of thermal stress are usually behavioural with avoidance of suboptimal conditions. Beyond this, physiological stress may become evident: respiratory, metabolic or excretory rates may increase, for instance. All of these changes may be accompanied by a decrease in egg and/or sperm production and hence in fecundity. Further, as tolerance limits are approached, organisms become more susceptible to parasites and pathogens and to food shortages. Very often adults are able to survive apparently unaffected by suboptimal conditions, although breeding success may be greatly reduced if eggs cannot hatch or larvae cannot grow. Juveniles are frequently far more sensitive than adults and while adult populations of fish survive and grow under suboptimal conditions there is no recruitment to the population because the juveniles do not hatch or grow.

Determining thermal limits experimentally is useful for the setting water temperature guidelines. The approach adopted by Rivers-Moore et al. (2013a) for setting water temperature guidelines for the ecological Reserve in South Africa is based on the seven-day (7D) moving average of mean, maximum and minimum daily temperatures, and exceedances. A second element, which relates to a threshold temperature that signals when adverse biological responses are likely to occur, is linked to the first. These threshold temperatures are determined via laboratory studies with lethal endpoints most commonly estimated using the Critical Thermal Method (CTM) or the Incipient Lethal Temperature (ILT) method; and sublethal endpoints over longer periods – typically physiological and metabolic effects (e.g. growth rates, respiration), phenological effects (e.g. voltinism, emergence), effects on reproductive success and fitness (e.g. fecundity, hatching success), behavioural effects (e.g. migration), and broad scale ecological effects (e.g. density, competitive interactions).

This chapter outlines methods used for estimating upper thermal limits of aquatic macroinvertebrates, and includes a discussion of lethal and sublethal methods and responses, and draws on published local and international studies. It provides an overview and a basic background of some of the major effects of sublethal temperatures on aquatic

organisms and provides insight into some of the methodologies as well as experimental procedures currently used in both field and laboratory studies.

4.2 Methods for estimating lethal and sublethal effects

In general terms, measurements of growth rates, age to maturity, fecundity, etc., all provide information on the immediate effects of toxins (or temperature) on individual organisms. While it is possible to undertake such investigations in the field, the organisms need to be large enough (e.g. fish) to be tagged, measured, released, re-caught and re-measured at appropriate intervals. Such measurements are valuable because they are performed on individual organisms that have been subjected to the totality of conditions in the stream from one measuring period to the next (Dallas and Day 2004). The disadvantages are essentially two-fold. Firstly, although one can determine the effects of those conditions on the organisms being measured, one has little idea of the actual conditions that caused the measured effects. Secondly, such techniques are only useful for large or sessile (attached) organisms. In practice such investigations are seldom achieved and most analyses of the effects of physico-chemical parameters (including temperature) on individuals are carried out in the laboratory. Numerous laboratory studies have been conducted on the effects of individual water quality constituents on individual organisms of a variety of species. The process employed is commonly termed "toxicity testing"; whether or not the constituents or attributes to be tested are indeed toxic, such as with water temperature; and effects range from sublethal to lethal.

Toxicity tests may be conducted using standard laboratory organisms such as water fleas (*Daphnia*) and guppies (*Poecilia reticulata*) or using indigenous organisms from rivers and may be based on one or more species. Toxicants tested may be single substances or complex mixtures. Regardless of the approach followed, the results of toxicity tests link chemical concentrations to biotic responses, providing an interpretive link between information on water chemistry (provided by routine chemical monitoring) and information on ecosystem health (provided by biomonitoring). Biological indicators that are commonly used in toxicity testing, and which could be used for estimating the effects of temperature changes in aquatic ecosystems and their biotas, are listed in Table 4.1.

4.2.1 Lethal effects of temperature on organisms

Ectotherms are dependent on their surrounding environment and external sources (i.e. sunlight or water temperature) for the regulation of body temperature which in turn controls physiological and metabolic processes (Cabanac 1975). As such the body temperatures of aquatic ectotherms (e.g. aquatic invertebrates and aquatic insects) are very closely related to the water temperatures to which they are exposed. Body temperature is arguably the most important ecophysiological variable affecting the functioning and performance of ectotherms as it directly relates to almost all aspects of behavioural and physiological responses including; locomotion, immune function, sensory input, foraging ability and rates of feeding and growth (Angiletta et al. 2002). While ectotherms have evolved an array of behavioural and physiological responses to regulate body temperature given the thermal heterogeneity of certain environments, specifically the lotic environment,

such thermoregulatory responses are not always possible at certain times of the day or in some environments (Angiletta et al. 2002). In nature when aquatic ectotherms are exposed to water temperatures that exceed the thermal tolerance limits to which they have adapted and evolved, the initial behavioural responses exhibited are normally attempts to escape unfavourable conditions by swimming, migrating or drifting away to more suitable refugia (Lagerspetz 2003). Where escape is impossible normal physiological process such as locomotive capacity, feeding or respiration, begin to shut down, ultimately leading to mortality (Terblanche et al. 2007). Such are the lethal effects of temperature on aquatic ectotherms. The lethal temperature (i.e. the temperature that will result in death of an organism) is affected by several factors (Figure 4.1, from Dallas 2008, after Langford 1990) including the rate of change of temperature, acclimation (i.e. an organism's temperature history), duration of exposure (i.e. acute versus chronic), life history stage (e.g. eggs and larvae are often more susceptible than adult stages), multiple stresses (e.g. water quality impairment), and adaptive strategies that allow for behavioural thermoregulation or physiological adaptations.

Table 4.1. Some biological indicators that can be used to estimate the effects of temperature on aquatic ecosystems and their biotas (modified from Dallas and Day 2004)

Attribute of	Biological Indicator	Method
Individual species	Behaviour Growth rate Metabolic rate Sensitivity to pathogens Condition Fecundity Age to maturity Survival rate Abundance Biomass Recruitment and turnover	Used in toxicity testing (usually laboratory-based)
Biotic communities	Species composition Biodiversity (e.g. number of species) Complexity of interrelationships Community succession Alteration in key species Resilience to change Sensitivity to change	Field data and community-level toxicity tests
Natural processes	Rate of photosynthesis Rate of nutrient cycling Rate of decomposition	Field data

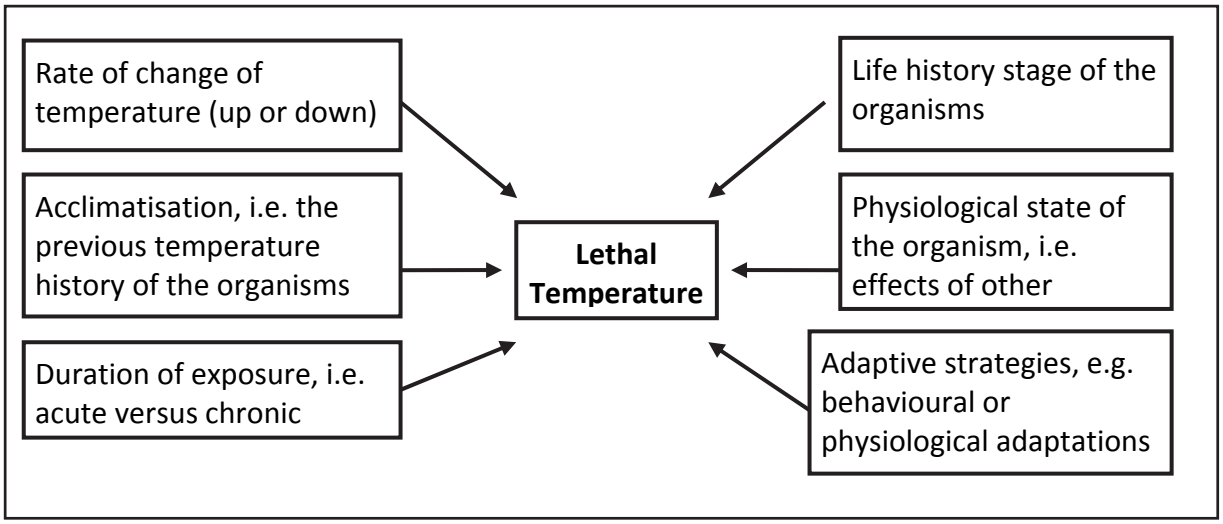


Figure 4.1. Factors affecting the lethal temperature for aquatic organisms (Dallas 2008, adapted from Langford 1990)

Lethal temperatures are typically determined experimentally within a controlled laboratory environment. They are commonly based on one of two methods; the dynamic method (e.g. Critical Thermal Method – CTM) or static method (Incipient Lethal Temperature – ILT) (Lutterschmidt and Hutchison 1997, Dallas and Ketley 2011, Dallas and Rivers-Moore 2012). Both these methods rely on the experimental generation of a response temperature that provides an estimate of lethality.

4.2.1.1 Critical Thermal Method (CTM)

The dynamic method involves changing temperature at a constant rate until a predefined sublethal endpoint, which is used to estimate lethality, is reached. The CTM is used to determine a critical thermal endpoint (CTE), expressed as either critical thermal maxima (CT_{max}) or minima (CT_{min}). CTE is a behavioural stress response and is defined as the "arithmetic mean of collected thermal points at which locomotor activity becomes disorganised to the point at which the organism loses its ability to escape conditions that will promptly lead to its death" (Cox 1974 cited by Ernst et al. 1984). In fish, it often includes loss of equilibrium and onset of muscle spasms (Beitinger et al. 2000). The analogous behavioural stress response of aquatic macroinvertebrates varies amongst taxa but generally includes either a loss of grip and inability to remain attached to a substrate or increased movement, both followed by immobility, with a lack of response when stimulated with a jet of water. In aquatic insects CTM has been widely used for a range of organisms, is comparatively quick and requires a relatively small sample size of test organisms (Dallas and Rivers-Moore 2012).

4.2.1.2 Incipient lethal temperature (ILT)

The static method, which is equivalent to the incipient lethal temperature method (Beitinger et al. 2000), involves holding duration constant while temperature is varied, with assessments based on survival of a proportion of a sample (Terblanche et al. 2007). The resultant lethal temperature (LT_{50}) is the temperature at which 50% of the sample survives in a specified time. The experimental time period is of longer duration, normally 4-10 days,

and requires a large number of individuals (approximately n=150). For a given biological stage (eggs, larvae, juveniles, adults), the lethal temperature depends on the acclimation temperature and the period of exposure to warming or cooling; it rises when the acclimation temperature increases, then reaches a threshold value (Souchon and Tissot 2012). This threshold value is generally termed the Ultimate Incipient Lethal Temperature (UILT) while the lethal temperatures in the phase of dependence on acclimation temperature are called the Incipient Lethal Temperature (ILT). Figure 4.2 (Souchon and Tissot 2012, page 7) shows three regions: a) the zone of thermal tolerance, compatible with survival, b) the zone of thermal resistance; c) the zone of instantaneous mortality.

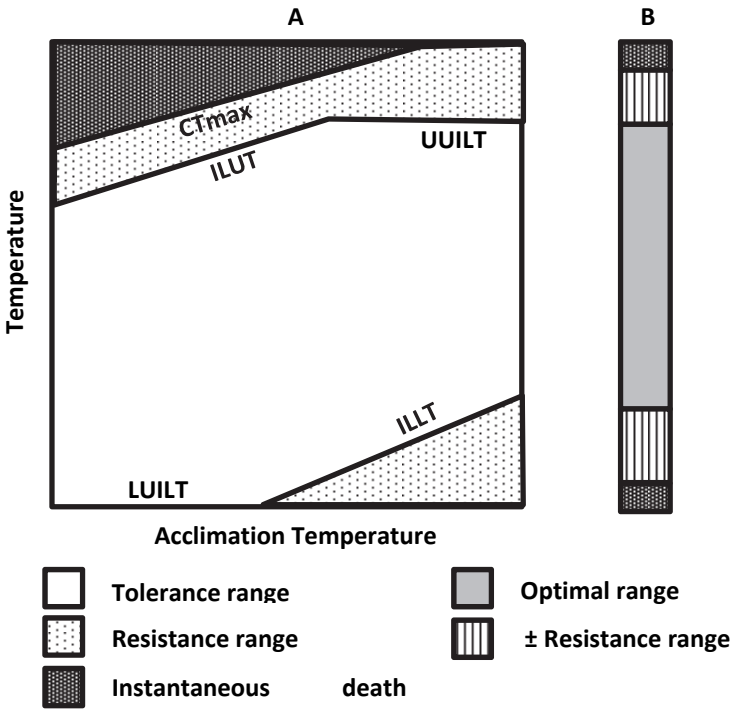


Figure 4.2. Different thermal variables (adapted from Beitinger et al. 2000). (a) Different temperature variables versus acclimation temperature, (b) synthetic range of temperature tolerance. IULT: Incipient Upper Lethal Temperature, ILLT: Incipient Lower Lethal Temperature, UUILT: Upper Ultimate Incipient Lethal Temperature, LUILT: Lower Ultimate Incipient Lethal Temperature, CTmax: Maximum Threshold established by CTM

4.2.2 Sublethal effects of temperature

The sublethal effects (i.e. referring to that which does not kill a cell or organism, but usually forces adaptation for survival) of water temperature on aquatic ectotherms, however, are far more numerous and sometimes quite subtle. As a result, they have received much attention in freshwater ecological studies since the late 1960's (Ward and Stanford 1982, Webb et al. 2008). Generally the sublethal effects of water temperature can broadly be split into five major categories:

- physiological and metabolic effects,

- phenological effects,
- effects on reproductive success and fitness,
- behavioural effects,
- broad scale ecological effects

Each of these categories can be further subdivided into a number of response variables which are affected differently by changes in water temperature and which have been the focus of a large number of freshwater studies (Table 4.2).

While many studies on the sublethal effects of temperature are carried out under natural conditions in the field, through the regular sampling of sites over the period of one or two years (e.g. most phenological studies, ecological studies), several questions related to the sublethal effects of temperature lend themselves more to experimental procedures that can be conducted in the laboratory over shorter time periods (e.g. physiological and metabolic studies, studies on reproductive success and fitness, behavioural studies). The aim of this section is to provide the reader with an overview and a basic background of some of the major effects of sublethal temperatures on aquatic organisms and also to provide insight into some of the methodologies as well as experimental procedures currently used in both field and laboratory studies on the topic.

4.2.2.1 Physiological and metabolic

Studies relating the sublethal effects of temperature to physiological and metabolic processes in aquatic organisms (e.g. foraging rates, performance curves) are more commonly carried out in the laboratory. Organisms are usually kept under a range of constant temperatures or in digitally controlled water baths where temperature can gradually and precisely be increased. Studies relating to growth rates and secondary productivity have also been performed in artificial streams, mesocosms and flow-through systems (e.g. Sweeney and Vannote 1981) or conducted under natural conditions in the field.

Growth and size

It is well established that temperature is a major factor effecting growth rates of aquatic organisms through its influence on rates of ingestion, assimilation, activity, metabolism, etc. and numerous studies have illustrated that lower water temperatures result in decreased growth rates while warmer water temperatures cause an increase in growth rates, shorter development times and smaller adult size at emergence (see for example Hynes 1970, Brittain 1982, Ward and Stanford 1982, Sweeney and Vannote 1984, Giberson and Rosenberg 1992, Sibly and Atkinson 1994, Gillooly et al. 2002, Reynolds and Benke 2005). A large body of literature deals with the theories surrounding the relationship between development time and body size (Stearns and Koella 1986, Roff 1992, Berrigan and Koella 1994, Atkinson 1995, Nylin and Gotthard 1998, Angiletta et al. 2004).

Table 4.2. Summary of the major effects of sublethal water temperatures on aquatic organisms

Major effects	Response variables	General findings summarised from studies of response variables in relation to temperature	Selection of relevant literature
Physiological and metabolic	Growth rates Size at emergence Performance curves Secondary productivity and assimilation Respiration	Growth rates increase with increasing temperature to an optimum, after which they begin to decline and tend to zero as thermal tolerance limits are approached. Temperatures for optimal growth do not translate to temperatures for optimal growth efficiency, emergence success or length of emergence period. Faster growth at warmer temperatures results in smaller size at maturity. Colder temperatures result in slower growth, longer development time and larger size at maturity. Performance is optimal at intermediate temperatures and outside of this range it is reduced. Secondary productivity is increased at warmer temperatures. Respiration is increased at warmer temperatures and oxygen can become a limiting factor at warmer temperatures.	Nebeker 1971a, Heiman and Knight 1975, Brittain 1976, Sweeney and Vannote 1978, Huey and Stevenson 1979, Vannote and Sweeney 1980, Humpesch 1981, Brittain 1983, Benke et al. 1984, Rigler and Downing 1984, Sweeney 1984, Stearns 1989, Rowe and Ludwig 1991, Giberson and Rosenberg 1992, Golubkov et al. 1992, Atkinson 1994, 1995, Abrams et al. 1996, Angilletta et al. 2004, Connolly et al. 2004, Manush et al. 2004, Das et al. 2005, Reynolds and Benke 2005, Rostgaard and Jacobsen 2005, Acuña et al. 2008, Rotvit and Jacobsen 2013, Verberk and Bilton 2013, Verberk et al. 2013.
Phenological	Total development time Voltinism flexibility Timing and length of	Faster growth rates at warmer temperatures leads to shorter development periods and can result in early emergence cues in aquatic insects. Cold temperatures result in slower growth rates, longer development periods and delayed emergences in insects and are normally associated with fewer generations over year (univoltine life cycles). Warmer temps with greater variability promote more	Nebeker 1971a, Southwood 1977, Clifford 1982, Brittain 1982, Sweeney 1984, Sweeney et al. 1986, Perry et al. 1987, Söderström 1988, Southwood 1988, Rader and Ward 1989, Brittain 1990, Giberson and Rosenberg 1992, Sweeney et al. 1995, Harper and Peckarsky 2005, Verberk et al. 2008, Elliott 2009, Resh and Rosenberg 2010.

Major effects	Response variables	General findings summarised from studies of response variables in relation to temperature	Selection of relevant literature
	emergence	generations produced in a year (bi- tri- or multi-voltinism) and more flexible life histories, while more conservative, less flexible life histories are selected for under colder more stable conditions. Extended, unsynchronised emergence periods in aquatic insects at warm temperatures, while more synchronous and shorter emergence at cold temps	
Reproductive success and fitness	Fecundity Rates and success of egg development Juvenile survival and recruitment	Fecundity is directly related to body size of females at maturity and so declines with higher temperatures. Eggs develop faster with increasing temperatures that are within egg development limits. Temperatures that result in fastest egg development are not always those that result in highest hatching success. Hatching success is usually highest at temperatures slightly lower than those that promote fastest development. High temperatures approaching development limits lead to deformed or retarded development and lower hatch success. Low temperatures below species-specific thresholds can induce egg diapause and similarly temperatures above species-specific thresholds can terminate diapause. Increased temperatures result in higher juvenile mortality rates.	Green 1966, Harper and Hynes 1970, Stearns 1976. Keen 1979, Humpesch 1980a, Humpesch 1980b, Humpesch and Elliott 1980, Sweeney and Vannote 1981, Sutcliffe and Carrick 1981, Humpesch 1984, Brittain et al. 1984, Tómasson et al. 1984, Elliott 1984, Brittain and Lillehammer 1987, Elliott 1988, Lillehammer et al. 1989, Brittain 1991, Brittain and Campbell 1991, Jackson and Sweeney 1995, Brittain 1995, Sweeney et al. 1995, Pritchard et al. 1996, Corkum et al. 1997, Gillooly and Dodson 2000, Yoshimura et al. 2006, Knispel et al. 2006.
Behavioural	Migration and movement	Organisms migrate or move to a zone of thermal preference when introduced to a wide range of temperatures, even if this	Hutchison and Manness 1979, Gerritsen 1982, Baker and Feltmate 1989, Richardson et al. 1994, Hernández-Rodríguez and Bückle-Ramirez 1997,

Major effects	Response variables	General findings summarised from studies of response variables in relation to temperature	Selection of relevant literature
	Drift	means foregoing access to resources such as food. Dramatic and sudden increases in temperature can lead to catastrophic drift in aquatic insects to escape. Gradually increased temperatures can lead to increased amplitude of diel drift.	Bury et al. 2000, Burks et al. 2002, Hernández-Rodríguez et al. 2002, Gerald and Spezzano 2005, Rossetti and Cabanac 2006, Tate et al. 2007, McCullough et al. 2009.
Ecological	Density Distribution patterns Species composition Species richness	Richness generally increases with increased annual water temperature variation. Increased variation in diel temperatures favours certain species while negatively affecting others. Species composition is generally more diverse in habitats experiencing a wider range of annual and diel temperatures. Water temperatures control aquatic species distribution patterns. Cool headwaters represent ancestral habitat of many aquatic insects – colonisation of lower reaches and lentic waters involved adaptation to warmer thermal conditions. Cool waters represent thermal refugia for cold adapted stenotherms. While warm stenotherms and eurytherms are able to colonise habitats exhibiting a wide range of temperatures. Latitudes and altitudes promoting optimum temperature ranges for a wider range of species result in greatest densities and abundances of those species.	Cummins 1974, McMahon 1975, Sweeney and Vannote 1978, Vannote et al. 1980, Vannote and Sweeney 1980, Ward and Stanford 1983, Hawkins 1986, Brittain 1989, Townsend 1989, Brittain 1991, Hart and Rayner 1994, Brittain and Bildeng 1995, Hogg and Williams 1996, Williams 1996, Vinson and Hawkins 1998, Gasith and Resh 1999, Jackson et al. 2001, Durance and Ormerod 2007, Ficke et al. 2007, Fjellheim and Raddum 2008, Gustafson 2008, Rahel and Olden 2008, Woodward et al. 2010, Statzner and Dolédec 2011, Sheldon 2012, Filipe et al. 2012.

Growth in univoltine aquatic insects is assessed by monitoring individual organisms over the entire growth period from hatching to adult emergence or over a particular part of the life cycle. At the start of the study period/experiment a subsample of the study organisms are measured (e.g. body length head capsule width), dried and then weighed to obtain a measure of relationship of length to mass. Thereafter organisms are sampled at each interval and a small sacrificial subsample is similarly measured, dried and weighed to provide length-to-biomass relationships for the full range of sizes measured over the study period. For the remaining organisms, sclerotised body parts are measured in conjunction with body length to provide a measure of increasing size. Where body length-to-biomass equations have been calculated for the study organism at a range of all possible sizes, instantaneous growth rates are then calculated by determining the change in initial biomass compared to final biomass from hatching to emergence (Benke 1993) or over the specific time period measured. Instantaneous growth rates as well as rates of body size increase can then be compared across temperature treatments to determine thermal optima for growth.

Similarly, growth rates can be calculated in relation to sex and age to determine if differences in growth exist as a result of the effects of sexual dimorphism or if growth rates vary over different developmental stages (see for example Nylin and Gotthard 1998, Shama and Robinson 2006). In most aquatic organisms (especially aquatic insects) monitored in the laboratory, the number of flagellar segments on the antennae of organisms can be counted upon each successive moult and thus can be quantitatively related to body size and instars to provide an accurate measure of age (e.g. Khoo 1964, Pöckl 1992). Age can then be related to growth rate to determine if growth rate remains constant throughout the entire life cycle or if it changes at different stages or ages. It is not always possible though, to determine the age of natural populations using this method, owing to breakages in the antennae occurring naturally.

Performance curves

By monitoring specific types of performance in aquatic organisms (i.e. any physiological aspect such as locomotion or foraging ability) across a range of temperatures (generally using a dynamic setup such as a water bath where temperature can gradually be increased – see Lutterschmidt and Hutchinson 1997), thermal performance curves can be established (Angiletta et al. 2002, Angiletta 2006). Thermal performance curves are best described by an asymmetric function where maximum performance is achieved at intermediate body temperatures, termed the thermal optimum (Angiletta et al. 2002) (Figure 4.3). The performance breadth is essentially the range of sublethal body temperatures at which performance is greater than or equal to an arbitrary level of performance, for example where performance is greater than equal to 50% of the maximum (Angiletta et al. 2002). The performance breadth is bounded on either side by the critical thermal limits (see Lutterschmidt and Hutchison 1997), or maximum (CT_{max}) and minimum (CT_{min}) body temperatures that permit any performance (Angiletta et al. 2002). By utilising such an approach the sublethal effects of temperature on particular aspects of physiology or behaviour can be determined in aquatic organisms (Huey and Stevenson 1979, Huey 1982).

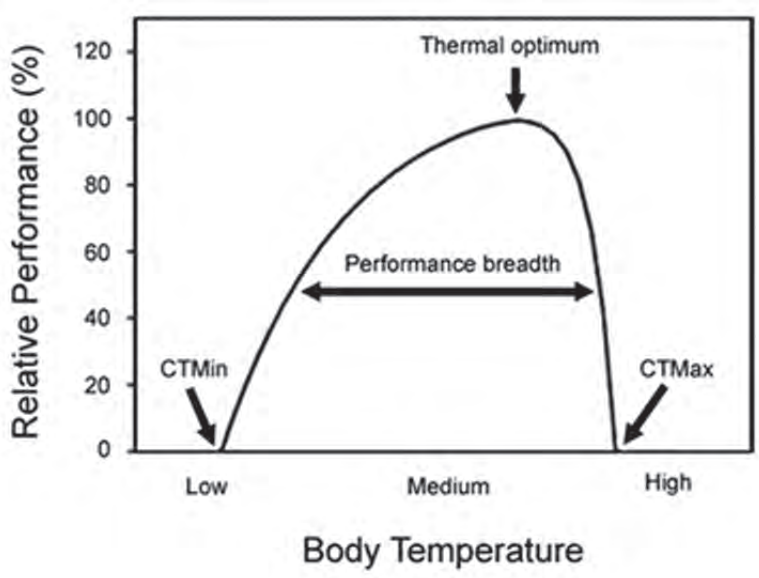


Figure 4.3. Representation of a typical performance curve for an aquatic organism where a specific performance measure is monitored at regular intervals as temperature is increased resulting in increased body temperatures. Modified from Angilletta et al. 2002

Secondary productivity

Secondary productivity of a heterotrophic population can be defined as the biomass accumulated by that population per unit time (Rigler and Downing 1984, Benke and Huryn 2006) which is affected by assimilation efficiency and net production efficiency. For example, Benke and Huryn (2006) describe annual secondary productivity as the sum of all biomass produced by a population during one year, including production remaining at the end of the year and all production lost during this period (possibly through mortality, loss of tissue reserves through moulting and also emigration). Downing (1984) suggests that such information can be used to address a) the transfer of energy or materials within communities and ecosystems, b) the rational management of aquatic resources, c) the detection of the effects of pollution and d) the formation of general theories of biological productivity.

While several methods exist for calculating secondary productivity (see Waters and Crawford 1973, Rigler and Downing 1984, Benke and Huryn 2006) certain common data are required. Either samples of the population are taken at different times during its development, or for the entire growth period the population is split into different size classes (Rigler and Downing 1984). Measures of mean body mass of individuals as well as an estimate of the number of individuals (density) for each time period or size class are then taken (Rigler and Downing 1984). The history of the population or cohort is then reconstructed as a simplified histogram of numbers of individuals against individual mass (Rigler and Downing 1984). Calculations of secondary productivity are usually accomplished in field studies based on regular sampling at a number of natural sites. Laboratory experiments (using living streams, artificial streams or mesocosms) can however be used to obtain insights into selected aspects of production processes for a given time period under different environmental conditions (e.g. differing thermal regimes using a static setup – Lutterschmidt and Hutchinson 1997) (see also Gulati 1974). Though, given the complex

nature of interactions in natural systems, caution should be exercised when interpreting data obtained from such simplified laboratory experiments (Gulati 1974).

Secondary productivity has been shown to increase in relation to increasing water temperature where these water temperatures are within the thermal tolerance limits for the organism in conjunction with increases in biomass levels. As high growth rates and high biomass are not commonly maximised in conjunction with each other, it is often the case that rapid growth rates are concomitant with low biomass and slow to moderate growth rates with high biomass (Huryn and Wallace 2000). Warm temperate streams experiencing frequent disturbance tend to yield unusually high levels of production, as these conditions select for taxa with rapid growth rates, short development periods and smaller sizes at maturity (Huryn and Wallace 2000). In such scenarios production is driven by rapid growth rates rather than biomass accrual (Huryn and Wallace 2000). While water temperature plays a major role in driving secondary production, photoperiod as well as nutrition quantity and quality are additional factors that should be considered in conjunction with temperature when assessing optimum rates of secondary productivity (Savage 1986, Sweeney and Vannote 1981, Giberson and Rosenberg 1992, Reynolds and Benke 2005).

Respiration

At warmer temperatures growth rates are faster, as are metabolic demands for oxygen (Hutchison 1981, Verberk et al. 2011). The ratio of oxygen supply to demand thus decreases and oxygen shortages arise as water temperature increases. The result is that cardiac and ventilatory activities of aquatic organisms may become unable to provide sufficient oxygen at high temperatures, which subsequently leads to a shift from aerobic to anaerobic metabolism to conserve energy status (Frederich and Pörtner 2000, Frederich and Pörtner 2000, Verberk et al. 2013) and also decreased performance. Additionally, algal food sources are in greater supply at warmer temperatures, however oxygen concentration is inversely proportional to temperature and this, combined with the high respiration activity of algae at night, acts to deplete available oxygen resources at warmer temperatures. Also, it has been shown recently for a species of stonefly *Dinocras cephalotes* (Verberk and Bilton 2011) and several other aquatic insect orders (Verberk and Bilton 2013) that heat tolerance can be improved with increased oxygenation of the medium.

For these reasons respiration rates of aquatic organisms can be measured at a range of water temperatures to determine at which threshold temperatures oxygen becomes a limiting factor. More specifically this can involve determining temperatures which trigger the onset of anaerobic metabolism (Verberk et al. 2013), those observed to be detrimental to a performance response (e.g. locomotion, foraging), and those ultimately affecting the survival of the organism under study.

With the introduction of respirometer chambers by McIntire (1964) scientists over the past three decades have been able to successfully determine respiration rates of benthic communities and individual organisms in the laboratory (Bott 2006). These chambers have enabled the researchers to control for individual environmental factors (e.g. water temperature – where chambers can be placed in constant temperature environments) and relate these factors to metabolic parameters or performance responses of the study organisms on different substrata (Bott 2006). Certain challenges exist with regard to the use

of respiration chambers in the laboratory however, such as mimicking natural flow conditions and velocities as well as hyporheic processes and exchanges; maintaining natural nutrient levels; controlling for excess heat generated from pumps; amongst other issues such as the transport of organisms, keeping and acclimatising them (Rostgaard and Jacobsen 2005, Bott 2006). More recently though, simpler setups involving hermetically mounted handheld oxygen probes have been used to good effect in determining respiration rates of aquatic insects in sealed chambers (see Golubkov et al. 1992).

4.2.2.2 Phenological

Phenological effects are those related to the timing of specific life history events (such as hatching or emergence in aquatic insects). It can also encompass the time requirement for the entire developmental period, which in turn relates to voltinism and the specific type of life cycle exhibited (Hynes 1970, Verberk et al. 2008). Life history studies, autecology and descriptive ecology provide fundamental information necessary to conduct virtually all modern evolutionary and applied ecological studies, particularly those relating to freshwater invertebrates (Butler 1984, Verberk et al. 2008, Resh and Rosenberg 2010). This is because only once information about an organism's life-history has been obtained, can one begin to understand and predict the response of that organism to variation and change within and between lotic ecosystems and the factors that induce such change, such as temperature (Power *et al.* 1988, Resh and Rosenberg 2010).

Total development time and voltinism flexibility

As temperature exerts a major control over metabolic processes and growth, it is logical that organisms exhibiting faster growth rates can complete their development in a shorter period of time and thus produce more generations or cohorts in a single year than those exhibiting lower growth rates. Voltinism is the term used to describe the frequency or number of annual broods, generations or cohorts produced by an organism in the space of a year. Univoltine organisms are those that produce a single cohort or generation within a year. Multivoltine organisms are those that produce more than one generation per year and they can be further classified into bivoltine and trivoltine organisms which produce two and three generations respectively, while polyvoltine organisms can produce three or more generations. Semi-voltine organisms are those in which a single generation can take more than a single year to develop (two or three years) (see Hynes 1970, Clifford 1982).

Some species of aquatic insect (viz. the univoltine Palearctic *Leptophlebia* species and Nearctic *Leptophlebia cupida*) exhibit fairly constant development times and voltinism over a wide range of latitudes, climates and environments (Clifford et al. 1979, Brittain 1982). Others, however (e.g. multivoltine Baetidae, Simuliidae as well as the univoltine species *Rhithrogena semicolorata*) have been found to vary the number of generations produced in a year to suit local environmental conditions exhibiting a large degree of flexibility in their life cycle development time and consequently their voltinism over their distributional range (Hynes 1970, Humpesch 1979, Ward and Stanford 1982).

The majority of life history studies are based on regular field observations or samples collected from either several sites or a single site for a period of a year or longer. Organisms collected in each sample for each time period are enumerated (to provide either relative

abundance or density), measured (body length, head capsule width) and then assigned either a life cycle stage or instar (e.g. using antennal counts) where possible. The number and size of organisms can then be tracked through time from hatching to adult emergence to provide insight into the number of cohorts or generations produced in a year (i.e. voltinism), the length of development period and the timing of adult emergence. This information can then be analysed and related to metabolic processes such as growth rate and secondary productivity.

While comparatively few life history studies have been conducted in the laboratory compared to the field, field work however can still leave gaps in the knowledge which are difficult to address without laboratory studies, such as the length of the adult flight period, differential effects of sexual dimorphism, methods of oviposition as well as the duration of egg development, presence of diapause and hatching success. Although laboratory studies are often questioned as to their usefulness and potential for application to complex natural conditions (Hynes 1970, Hogg and Williams 1996), it has been emphasised that ultimately, it is a combination of field work and laboratory studies that is able to yield much more information than either method conducted alone (Hynes 1970, Brittain 1982). Laboratory setups such as flow-through systems, aerated containers or field based mesocosms are useful as they are able to mimic natural conditions in a contained and controlled environment (see for example Humpesch 1979, Sweeney and Vannote 1981, Sweeney et al. 1986, Peckarsky and Cowan 1991, Giberson and Rosenberg 1992, Reynolds and Benke 2005, Harper and Peckarsky 2006, Kendrick and Benstead 2013). In such setups the effect of certain environmental factors, that under natural conditions covary, can instead be decoupled, independently controlled and experimentally tested (e.g. photoperiod, temperature regime, flow rate, oxygen saturation). Generally, more modern systems now allow for either constant or fluctuating thermal regimes with a high level of precision and control over a number of variables (e.g. Clements et al. 2013). While these systems can never truly replicate natural conditions to the same degree of efficiency, they do allow for the complicated interaction of variables affecting the life histories of aquatic insects to be broken down and studied separately. In addition, laboratory studies have been used to explore the effect of temperature on voltinism – especially using organisms that have naturally fast growth rates and short development times (e.g. chironomidae – Reynold and Benke 2005).

Timing and length of emergence

Linked to total development time and voltinism is the timing of adult emergence and the length of the emergence period. Emergence is the transition from the aquatic nymphal stage to the terrestrial sub-imago during which the nymphal skin is shed, normally at the water's surface or on surrounding riparian vegetation (Brittain 1982). Emergence is influenced by variations in the growth rates of the pre-emergent nymphal population and should be considered an integral component of life cycle strategies (Brittain 1980). Irregular timing of emergence in species, owing to altered thermal regimes in rivers, can be lethal to aquatic insects. This is because a) they can emerge in conditions potentially unsuitable for their survival (Nebeker 1971a, Raddum 1985) and b) males might emerge and die before females emerge, preventing successful mating (Nebeker 1971a). As such many aquatic

insects are adapted so that seasonal changes in temperature act as cues for the timing of emergence.

The timing and duration of emergence involves responses mainly to water temperature, often interacting with combinations of photoperiod dissolved oxygen and flow (Nebeker 1971a, Ward and Stanford 1982). Data collected from emergence traps in field studies and also from laboratory studies of mayflies have revealed both diel patterns (largely crepuscular) and seasonal patterns (occurring mostly during the warmer months) of emergence, as well as latitudinal and altitudinal differences resulting in the shifting of the timing of emergence (onset of emergence delayed with increasing altitude and higher latitudes – likely associated with cooler water temperatures) (Brittain 1982, Ward and Stanford 1982, Campbell 1986). Where several aquatic species co-exist (e.g. congeneric species) peak emergence has been observed to be separated in time, with generally either synchronised or dispersed patterns exhibited (Brittain 1982, Ward and Stanford 1982). Though, the same species can also exhibit differences in emergence patterns dependent on abundance and locality as well as from year to year (Brittain 1982, Ward and Stanford 1982).

The sublethal effects of temperature on the timing and duration of emergence are particularly well suited to investigation in laboratory experiments. Immature nymphs of aquatic insects can easily be collected from the field, acclimated and then transferred to flow-through systems, aerated containers or field based mesocosms, in which they can be reared to maturity under different experimental conditions. In particular water temperatures can be manipulated using either static or fluctuating thermal regimes to determine the effect of temperature on the timing and length of emergence. Laboratory studies of this sort have been able to demonstrate that a) specific threshold temperatures can initiate emergence, b) emergence can be hastened or delayed by adjusting experimental water temperatures, c) emergence period is extended in warmer conditions, d) larger size and higher fecundity at emergence is caused by cooler temperatures, e) adult longevity is increased when nymphs are reared at cooler temperatures, and f) the time between emergence of males and females is increased with increasing temperature (Nebeker 1971a,b, Brittain 1976, Sweeney 1978, Sweeney and Vannote 1981, Ward and Stanford 1982, Giberson and Rosenberg 1992, Harper and Peckarsky 2006).

4.2.2.3 Reproductive success and fitness

Fecundity

Adult female body size of aquatic insects has been found to be directly related to fecundity, with females of larger species producing more eggs (Brittain 1982, Ward and Stanford 1982). Even within populations of the same species the same trend holds true. As water temperature has been shown to affect growth rates of nymphs and the final body size at emergence, the trade-off between growth and sexual development under environmental time constraints has evolutionary and ecological implications on fecundity (Ward and Stanford 1982).

Numerous laboratory studies, have therefore investigated the sublethal effects of water temperature on the fecundity of reared female aquatic insect nymphs in conjunction with the size or fitness of reared adults at emergence (e.g. Sweeney and Vannote 1981, Giberson

and Rosenberg 1992, Elliott 2013). For these experiments the general approach is to collect immature nymphs from the field and rear them to maturity in systems similar to those used to obtain information on growth rates, life cycle development times as well as voltinism (i.e. using flow-through systems, aerated containers, mesocosms, and artificial streams). As spermatogenesis and oogenesis are completed in the final nymphal instar of most aquatic insects (Britain 1982), upon emergence, females can be collected, their body size measurements recorded, after which they are dissected in order to count the number of eggs produced.

Rates and success of egg development and juvenile survival

Water temperature is the principal factor determining the rate of development of eggs, hatching success, the length of the hatch period, as well as the induction and termination of egg diapause in aquatic insects (Brittain 1982, Ward and Stanford 1982, Gillooly and Dodson 2000). It has been suggested that specific threshold or critical temperatures determine the onset and breaking of egg diapause in certain aquatic insect species and in some cases hatching success as well as the length of the egg incubation period (Elliott 1972, Humpesch 1980a, b, Lillehammer et al. 1989). Additionally critical thermal limits for egg development also provide an indication of the range of temperatures suitable for the growth of juvenile nymphs, which is useful for determining the effects of water temperature on recruitment.

As aquatic insect eggs do not require nutrition for development (Pritchard et al. 1996), generally have low oxygen requirements, and are unaffected by photoperiod or flow velocities (Brittain 1982) this makes them ideal subjects for simple, cost effective laboratory experiments. Eggs to be used in these experiments are usually collected from mated, gravid adult organisms captured in the field by means of a hand net or aspirator. By submerging containers in pools used as oviposition sites, eggs can also be directly collected from the field, where mated females might be difficult to obtain. In the Ephemeroptera and Plecoptera captured gravid females freely oviposit eggs when placed in vials or jars containing some water. Alternatively, for certain organisms (e.g. the Plecoptera, Trichoptera), collected adults readily mate and oviposit in laboratory conditions (Brittain and Lillehammer 1987, Elliott 1988, Ross-Gillespie unpublished data) providing a simple means of obtaining eggs.

Where these approaches are not possible or prove to be unsuccessful, eggs can further be obtained through artificial fertilization techniques, which have proved successful for many species of Ephemeroptera that do not mate readily in the laboratory (Brittain 1982). For artificial fertilisation, mature nymphs can be collected from the field and placed in aerated containers secured with netting, until they emerge. Following the final moult to the imago stage, the female insects are measured and then carefully decapitated to induce oviposition, or dissected to remove mature eggs (Hunt 1951, Ross-Gillespie and Dallas 2011). Similarly, male insects are measured and then carefully dissected to remove the testes, or the tip of the abdomen is removed and gently crushed to obtain sperm. Eggs and sperm from the insects are then mixed together with a few drops of either water from the collection site (Hunt 1951, Humpesch 1980a, b, Sweeney and Vannote 1987) or standard insect saline solution (Ross-Gillespie and Dallas 2011) and left for 2-4 minutes to ensure fertilisation of eggs (Hunt 1951, Humpesch 1980a, b, Sweeney and Vannote 1987).

Once eggs have been collected using any of the aforementioned processes they can be placed in water-filled containers (e.g. petri dishes, beakers or tanks) and held at either constant or fluctuating temperature regimes. Eggs are then monitored daily for signs of development, diapause and hatching. Upon hatching the number of successfully hatched eggs at each temperature is recorded along with the length of the hatch period and ultimately the egg development period (normally the length of time measured as degree days[1] to median hatch) is calculated (Pritchard et al. 1996). Reaction norms can be calculated and the relationship between temperature and time for egg development can be described (Pritchard et al. 1996). Where successful hatching occurs, hatched organisms can then immediately be used in rearing, growth, physiological and even behavioural experiments or used in lethal experiments to determine juvenile mortality rates.

Experiments of this sort have generated a large amount of literature and have provided valuable insights into the ecological and evolutionary strategies adopted by a range of aquatic organisms to deal with different thermal environments, including aquatic insects (Brittain 1976, Humpesch 1980a, b, Brittain 1982, Elliott 1984,1988,2009, Sweeney and Vannote 1984, Brittain and Lillehammer 1987, Jackson and Sweeney 1995, Pritchard et al. 1996, Reynolds and Benke 2005, Elliott 2013), amphipods (Keen 1979, Sutcliffe and Carrick 1981, Pöckl and Timischl 1990) and fish (Brungs, 1971, Fonds 1979, Mitchell 1989, Crisp 1990, Semmens and Swearer 2011). Some common trends observed in these studies, particularly those focused on aquatic insects, have been that lower water temperatures generally result in longer egg development times, while shorter development times are observed at warmer temperatures and also under fluctuating or diel thermal regimes when compared to constant temperatures (Humpesch 1978, Ward and Stanford 1982). As such the relationship between temperature and time to hatch in many species of the Ephemeroptera and Plecoptera can be described by the power law (Brittain 1982, Lillehammer et al. 1989). For the Ephemeroptera, the observed range in suitable developmental temperatures for species from temperate areas in Europe and North America is large, extending only between 5-10°C in some species, while in others a hatching is observed over a broad range from 12°C to as high as 36°C (Brittain 1982). It has been stated that in general Ephemeropteran eggs hatch between 3°-21°C (Brittain 1982). Similar ranges have been shown for Plecoptera (Harper 1973). When thermal conditions are at an optimum the hatching period of eggs is generally short, however highly variable hatch durations have been observed both within and among species (Ward and Stanford 1982). Extended hatch durations have also been observed in laboratory conditions (Clifford et al. 1979) and at higher temperatures in conjunction with lower hatch success (Ross-Gillespie and Dallas 2011) which may suggest that extended hatches are an adaptive response to unpredictable conditions (Ward and Stanford 1982). Interestingly hatching success is also not necessarily highest at temperatures which permit the fastest development of eggs (Ward and Stanford 1982, Ross-Gillespie and Dallas 2011). Additionally egg diapause (an egg resting stage during which no development occurs – a mechanism for evading unfavourable or stressful conditions) has been observed over both summer and winter periods for several

[1] Degree days can be explained as the cumulative temperature experienced by an organism above a certain threshold temperature for development (in cases where this developmental threshold is not known for a specific organism cumulative temperature above 0°C is normally measured)

species of aquatic insect (Ephemeroptera, Plecoptera, Simulidae – Harper and Hynes 1970, Hynes 1970, Brittain 1975, Pritchard 1996) and Crustaceans (Cladocera – Frey 1982, Yurista 1997, Slusarczyk and Rybicka 2011). Information on thermal limits for egg development in aquatic organisms is still limited and originates largely from Northern Hemisphere studies on aquatic insects (namely the Ephemeroptera, Plecoptera and Trichoptera) (Britain 1982), with few exceptions from the southern Hemisphere (e.g. Brittain 1991a, 1991b, 1995, Ross-Gillespie and Dallas 2011, Ross-Gillespie unpublished data).

4.2.2.4 Behavioural

In addition to affecting physiological processes, phenological traits and reproductive aspects, sublethal temperatures also affect the behaviour of ectotherms (Gerritsen 1982). Specific behavioural responses that enable ectotherms to exploit the thermal heterogeneity of their environments in order to maximise biochemical and physiological processes or enhance survivability, thereby indirectly influencing reproductive and ecological efficiency, are termed thermoregulatory behavioural responses (Hutchison and Maness 1979, Hutchison and Spriestersbach 1986). Mechanisms that control behavioural thermoregulation in ectotherms are more sensitive to temperature change than mechanisms controlling physiological adjustments (Cabanac 1979). Studies on thermoregulatory behaviour have primarily been undertaken on fish and reptiles with comparatively fewer studies conducted on amphibians and aquatic invertebrates (Hutchison and Spriestersbach 1986, Dallas 2008). Locomotion is commonly associated with thermoregulatory behaviour in aquatic ectotherms. This is because in the aquatic environment most organisms have to move significantly further in order to accomplish a significant change in body temperature compared to terrestrial organisms (e.g. lizards) which are better able to control rates of heat gain or loss through changes in posture or orientation (Hutchison and Spriestersbach 1986).

4.2.2.5 Migration, movement and drift

Aquatic organisms, particularly fish, are known to utilise thermal refugia and often thermoregulate by migrating to areas of cooler water when surrounding water temperatures are outside of their preferred range or exceed their upper tolerances (e.g. Torgensen et al. 1999, Elliot 2000, Ebersole et al. 2001, Gardner et al. 2003). Fish possess acute temperature discrimination powers and use behaviour to avoid or rapidly escape thermally hostile areas, if thermally favourable environments are available (Beitinger et al. 2000). The habitat occupied by a particular species (or age class) in the field has been shown to change seasonally and even daily in response to the location of preferred temperatures (Coutant 1987). Thermal refugia protect biotic communities from extreme thermal disturbances and are most numerous in intact riverine systems with riparian vegetation and groundwater (Torgensen et al. 1999). Undercut banks and overhanging vegetation also increase the availability of less thermally stressful habitats (Bell 2006). Coldwater patches were normally associated with lateral seeps, cold side-channels, floodplain tail seeps, floodplain seeps and stratified pools (Mosley 1983, Ebersole et al. 2001). These patches may be 3 to 10°C lower than instream temperatures.

Aquatic crustacean zooplankton *viz.* copepods and cladocerans, have been found to exhibit clear thermoregulatory behaviour in the form of migration, where they swim upwards in

response to sudden increases in temperature and to sink in response to decreases in temperature (Barber 1961, Gerritsen 1982). Similar responses in zooplankton have been found as a result of diel, seasonal, and interannual changes as well as thermal stratification in lakes and dams (Gorski and Dodson 1996, Ryan and Dodson 1998, Burks et al. 2002, Beklioglu et al. 2008, Schalau et al. 2008, Ziarek et al. 2011). The frequency of upward swimming has also been found to be proportional to the rate of change of temperature (Gerritsen 1982). However phototactic responses can complicate matters, where cool animals exhibit positive phototactic reactions and swim upwards towards a light source and warm organisms exhibit a negative phototactic reaction swimming away from a light source (Gerritsen 1982).

For many aquatic insects the hyporheic habitat (particularly coarse substrate, such as cobbles) provides a refuge from fast velocities (boundary layer effect) as it does from both low (geothermal groundwater inputs, buffered water temperatures, protection from underwater ice) and high temperature (from cooler groundwater exchanges, lack of incoming solar radiation) extremes (Hynes 1970, Brown et al. 2005, Caissie 2006). When compared to surface water temperatures, intragravel temperatures within the substratum have been found to be lower during summer and higher over winter (Caissie and Giberson 2003, Brown et al. 2005). As a result many aquatic insects can simply migrate down a relatively short distance to gain shelter from adverse conditions (Hynes 1970); this in turn has an effect on their growth. Similarly the temperature differential in the substratum can also influence fish habitat conditions (Shepherd et al. 1986, Crisp, 1990) and the development of salmonid eggs (Combs, 1965, Alderdice and Velsen, 1978, Beer and Anderson, 2001). The amplitude of diel patterns of invertebrate drift may also be increased at warmer temperatures (Waters 1972, Keller 1975, Brittain and Eikeland 1988) while catastrophic drift responses can result from drastic and sudden temperature changes (Ward and Stanford 1982).

A popular method of investigating thermoregulatory behaviour (particularly migratory responses to temperature preferences) in fish, crustaceans and aquatic insects, in the laboratory, is through the use of either vertical or horizontal thermal gradient tanks (Cherry and Cairns, 1982, Boubée et al. 1991, Richardson et al. 1994). This technique involves the establishment of a temperature gradient (either horizontal or vertical) that is then used to evaluate, by determining movement into different thermal zones and the time spent in these zones, the temperatures preferred or avoided by various organisms. The method facilitates the determination of the effects of gradual temperature changes and allows ontogenetic shifts in temperature preferences to be calculated. For example Boubée et al. (1991), using a thermal gradient tank, showed experimentally that the final preferred temperature of *Galaxias maculates* a resident fish in New Zealand, is about 20^{o}C and that temperatures above 29.5^{o}C were totally avoided.

These setups have been successfully used for larval Odonata (Leggot and Pritchard 1986, Baker and Feltmate 1989), larval Tipulidae (Kavaliers 1981), Palaemonidae (Hernández-Rodríguez and Bückle-Ramirez 1997), Gastropoda (Kavaliers 1980, Rossetti et al. 1989, Gerald and Spezzano 2005), hatchling and yearly freshwater turtles (Bury et al. 2000) and also fish (Boubée et al. 1991, Hernández-Rodríguez et al. 2002).

4.2.2.6 Ecological

As sublethal water temperatures govern so many biological and physiological aspects of aquatic organisms including behavioural responses such as migration to areas of thermal preference, it follows that water temperatures can ultimately determine patterns of distribution, densities, species richness and species composition of aquatic organisms, both within and among aquatic habitats (Coutant, 1977, Brittain 1982, Ward and Stanford 1982, Downes et al. 1993, Wichert and Lin 1996, Jacobsen et al. 1997, Dallas et al. 2012, Rivers-Moore et al. 2012).

Density, distribution patterns, species composition and species richness

Temperature is the abiotic variable most closely related to changes in latitude and altitude which have both, through several studies from tropical and temperate regions, been found to impact aquatic community structure and species diversity (Hynes 1970, Kownacki and Kownacki 1972, Stout and Vandermeer 1975, Ormerod et al. 1994, Jacobsen et al. 1997, Jacobsen 2004). Diversity and species richness have both been found to increase with increasing water temperature and therefore decrease with altitude and latitude (Brittain 1982, Jacobsen et al. 1997). Estimates suggest that tropical lowland streams have on average two to four-fold higher species richness than temperate lowland streams (Jacobsen et al. 1997). Faster rates of evolution and speciation as a result of shorter generation times and elevated mutation rates at increased temperatures have been proposed by Rohde (1992) to explain the greater levels of diversity observed at warmer temperatures and thus further elucidate the potential causes for the latitudinal gradients observed in biodiversity.

Several studies also illustrate a zonation pattern of aquatic organisms along the longitudinal profile of rivers concomitant with thermal differences along the profile (e.g. Brittain 1975, Ward 1981, Jacobsen et al. 2010), however these studies have been thought to obscure the actual effect of temperature (Jacobsen et al. 1997) because habitat type changes along the profile as well and habitat type itself has major effect on species composition, richness, and density (Minshall 1985, Jacobsen and Friberg 1997, Voelz and MacArthur 2000).

Ward and Stanford (1982) citing several other authors, point out that unpredictable thermal environments, particularly those which experience a wide annual temperature range, have been proposed to enhance species diversity in several ways: 1) such conditions are suitable for wide range of organisms (cold stenotherms, warm stenotherms as well as eurytherms), 2) a greater number of species can co-exist especially when temporal segregation occurs as a result of differential thermal responses during different periods of the annual thermal cycle, 3) species can avoid competition during different parts of the year through niche segregation as a result of the specific thermal limits for the induction and termination of dormancy that have evolved at both high and low temperature extremes and also temporal separation of resource use as well as emergence times, 4) wide daily variation in temperature can provide a greater range of thermal optima and allow for increased species packing, despite the fact that suboptimal conditions would be encountered by each species for a part of the diel cycle.

It should be noted here that investigations into the ecological effects of sublethal temperatures on aquatic organisms (e.g. species composition, species diversity, densities)

have generally been undertaken in field studies, where the entire benthic community is considered as opposed to laboratory studies where generally only a single species is focussed on (Vinson and Hawkins 1998). This is largely because of the complexities involved in ensuring that artificial systems afford the same opportunities for colonization, succession and diversity of aquatic organisms that is offered by natural systems along with similar stable chemical and physical properties (Warren and Davis 1971). As such, literature related to this topic is generally limited to field studies. Some of the most insightful field studies relating the effect of water temperature on aquatic species richness, composition, density and distribution have been those where a) hypolimnetic flows are released from dams and reservoirs upstream (e.g. Raddum 1985, Brittain and Saltveit 1989, Brittain 1991, Saltveit et al. 1994, Voelz et al. 1994, Gooseff et al. 2005), b) heated effluents enter the channel (e.g. Langford and Aston 1970, Langford 1990), c) whole in channel manipulations have been undertaken (e.g. Hogg and Williams 1996), d) similar natural systems with differing thermal regimes have been contrasted (e.g. Minshall 1985, Jacobsen et al. 1997, Castella et al. 2001, Haidekker and Hering 2008, Friberg et al. 2009), and e) long term data sets have revealed evidence for effects of global climate change (e.g. Durance and Ormerod 2007).

4.3 Experimental procedures

This section provides an overview of experimental procedures and techniques that may be applied for the evaluation of lethal and sublethal upper thermal limits of aquatic organisms. Methods outlined for the estimation of lethal limits have been extensively applied (see Dallas and Ketley 2011, Dallas and Rivers-Moore 2012), while some methods for the evaluation of sublethal effects have been applied (Ross-Gillespie 2014), although several additional methods are also described.

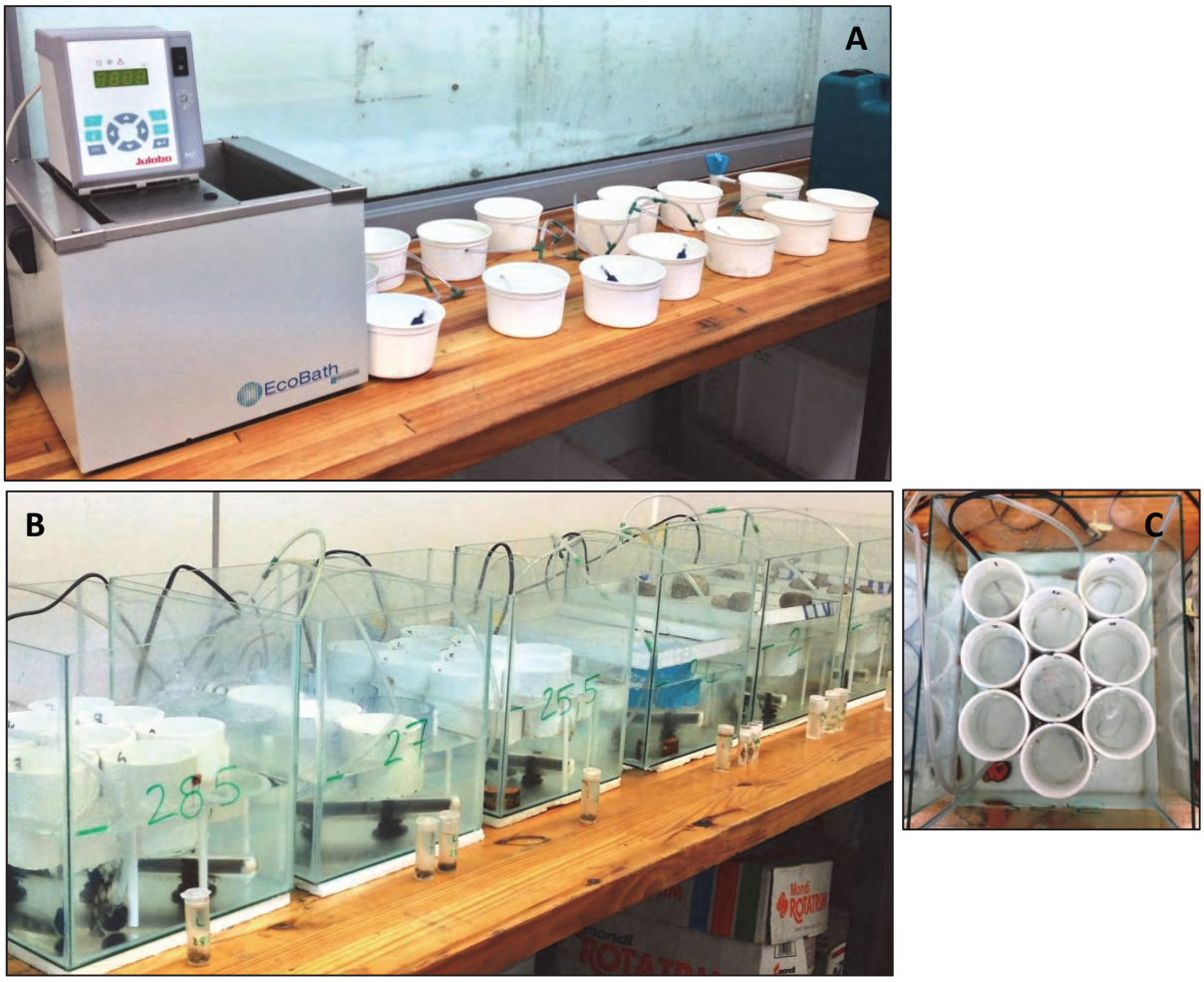
4.3.1 Methods for estimating lethal effects

The two common methods for estimating lethality vary in terms of duration, with CTM taking up to two hours, while ILT method runs for a minimum of 48 hours, with 4 and 10 day common experimental durations. The endpoint temperatures obtained from CTM are generally higher than those found with the ILT method, due to the generally shorter period of exposure to the tested temperatures.

4.3.1.1 Critical Thermal Method (CTM)

For CTM experiments, organisms are placed in an experimental chamber, which is immersed in a water bath in a temperature controlled room maintained at an ambient temperature approximating stream temperature of 16^{o}C (Photo 4.1A). Each experiment is approximately 90 min in duration, which includes a 30 minute control phase, followed by a 60 minute experimental phase during which the water temperature is raised at a constant rate of 0.34^{o}C per minute using a circulating heater (JulaboTM). This rate of increase is considered fast enough to avoid acclimation, but slow enough to ensure that the core temperature of the test animal approximated the ambient temperature (Ernst et al. 1984). The circulating pump ensured that the percentage saturation of dissolved oxygen remains above 70% during the experiments. The reaction to heating is assessed by observing the behavioural response of test organisms, including the pre-experimental behaviour, the PTR (Point of

Thermal Reactivity) and the CTE (Critical Thermal endpoint). Organisms are monitored throughout the experiment and as an organism exhibits behavioural signs of reaching the CTE, the CT_{max} temperature is recorded and the organism is removed from the experimental chamber and placed in aerated tubs at the start temperature. Only those organisms that recover are included in the results, as this is a prerequisite of this method.



Photos 4.1. A: Water bath and immersion heater with recovery tubs for CTM experiments; B: Aquaria tanks, immersion heaters and experimental chambers for LT_{50} experiments; C: Experimental chambers

4.3.1.2 Incipient lethal temperature (ILT)

For the LT_{50} experiments, organisms are placed in experimental chambers immersed in glass aquarium tanks, filled with dechlorinated freshwater, which is heated using water heaters to a minimum of five different temperatures (Photos 4.1 A and B). Constant temperatures are selected to attain a range of mortalities from 0 to 100%. Water temperature is recorded at one hour intervals throughout the experiment using Hobo TidbiTs v2 loggers (Onset Computer Corporation, 2008) placed in each of the tanks. Aquarium tanks are aerated using two air stones per tank to ensure that the percentage saturation of dissolved oxygen remained above 75%. Optimally thirty individuals of each taxon are placed in the

experimental chamber. Starting at the acclimation temperature, the temperature of a housing tank is gradually increased by 2-4^{o}C/h, until the appropriate target temperature is reached. An appropriate number of individuals are then removed from the housing tank and placed in the experimental chamber of the tank at the target temperature. A control temperature, which approximated water temperatures at capture, is included to test potential mortality factors such as influences from handling, air, water or a lack of food. Tanks are checked for survival every 24 h for four to 10 days subsequent to the target temperature being reached. Organisms displaying no movement are either gently prodded or squirted with water from a pipette to check for survival. Dead individuals are removed and preserved in ethanol to confirm their identities.

4.3.2 Methods for estimating sublethal effects

Water temperature and by proxy sublethal water temperatures, influence virtually every aspect of the biology of aquatic organisms (Ward and Stanford 1982). As such, the effects of water temperature on aquatic organisms, for example aquatic insects, are diverse and complicated (Nebeker and Lemke 1968). As a consequence of this, there are numerous laboratory/experimental setups and experimental procedures, along with an equally vast number of modifications to these that can be utilised and followed to measure the wide array of responses. This section is not intended to provide an exhaustive review of all of the laboratory setups and experimental procedures that are used to assess the effects of sublethal water temperatures on aquatic organisms. Rather it is intended to provide a brief description of a few of the most effective laboratory setups for investigating each specific response category as outlined in Table 4.2, together with a short summary of the basic experimental procedures to be followed in a typical experiment.

Certain laboratory setups are well suited for the investigation of several response variables – either simultaneously or in sequence. For instance flow-through systems and aerated containers can be used to determine growth rates, size at emergence, total development time, voltinism, emergence patterns and fecundity, thus incorporating response variables from three major effect categories viz. physiological/metabolic, phenological and reproductive success. For this reason, response variables from each major effect category, their associated experimental setup, experimental time frame and a rough costing have been summarised in Table 4.3. This provides a rough indication of where a single laboratory setup can be used to investigate a number of responses.

4.3.2.1 Water-filled containers

These setups are commonly used for assessing the sublethal effects of water temperature on the development times and hatching success of aquatic insect eggs as well as for assessing the juvenile survival rates of newly hatched organisms but depending on the organism they can also be used to rear organisms in growth experiments (e.g. Reynolds and Benke 2005, Brittain 1976). They are amongst the simplest and cheapest of laboratory setups and involve the use of containers (constructed of a chemically inert substance) of any shape or size that are filled with stream water or dechlorinated mains drinking water that has been circulated in a reservoir for approximately 24 hours. The water in the container is

not required to be aerated, but merely a portion of water manually replaced with fresh water on a regular basis (daily).

In a simple growth experiment conducted by Brittain (1976) on *Leptophlebia vespertina,* newly hatched nymphs were obtained from collected eggs masses which had hatched under laboratory conditions. Ten newly hatched nymphs were transferred to individual petri dishes half filled with lake water where they were reared until emergence (350 days). The petri dishes were placed in a constant environment room that was altered weekly to reflect ambient stream temperatures at the site from which the eggs were collected. Photoperiod was set to a 12 hour regime. Water in the individual petri dishes was replaced each week along with a fresh supply of food in the form of *Littorella* plants. Each week at least six of the nymphs were measured using an ocular micrometer to obtain body length measurements (excluding antennae and cerci). Moults/instar change was recorded when fresh exuvia were observed in the dishes. The relationship between nymphal body length and the number of days after hatching could then be defined.

4.3.2.2 Aerated containers

This laboratory setup generally comprises a set of containers of the same dimensions (e.g. glass aquaria), that are placed within controlled temperature environments. Where possible these environments would also provide full spectrum lighting controlled through a time switch to allow for an adjustable or constant photoperiod. The containers are either filled with normal tap water (normally dechlorinated mains drinking water that has been circulated in a reservoir for approximately 24 hours) or freshly collected stream water that is then circulated and aerated using pumps or compressed air via airstones to maintain oxygen concentration at saturation levels. Where only one controlled environment is available, the temperature of the environment would normally be set to either the coldest temperature treatment to be investigated – the control – or ambient stream temperature on either a constant or fluctuating cycle. Each container would then be heated by calibrated aquarium heaters to provide constant temperatures above the control. Unlike flow-through systems, these systems are generally self-contained. This means that fresh water is not constantly pumped in from a reservoir and the system does not drain via overflow pipes or weirs. As such, at regular time intervals (1-2 weeks) old water is required to be manually replaced with fresh water to prevent any unwanted nutrient build up and to maintain stability of water chemistry and quality. If the equipment is available and or budget permits, this setup could comprise a series of water baths set to constant temperatures. Pumps or airstones could be used to circulate water in these baths.

For this setup if the experimental design necessitates the provision of a standardised food source, pumps placed in each container for circulation purposes can be fitted with simple filtration canisters (filled with filtration media – normally glass fibre mats) to assist in the maintenance of stable water chemistry and quality and to prevent unwanted nutrient/bacterial build up. On the whole these systems tend to be somewhat simpler in design compared to flow-through systems and are therefore more cost effective and widely used.

Table 4.3. Summary of experimental setups, experimental time frame and equipment costing that can be used to determine sublethal effects of water temperature on aquatic organisms. Med=medium

Major effects	Response variables	Laboratory/experimental setups	Experimental time frame†	Cost‡
Physiological and Metabolic	Growth rates	Aerated containers, Flow-through systems, Mesocosms	Med-Long	Low-Med
	Size at emergence	Aerated containers, Flow-through systems, Mesocosms	Med-Long	Low-Med
	Performance curves	Water baths	Short	Med-High
	Secondary productivity and assimilation	Mesocosms, Artificial streams, Natural streams	Long	High*
	Respiration	Respiration chambers	Short	Med
Phenological	Total development time	Aerated containers, Flow-through systems, Mesocosms	Long	Low-Med
	Voltinism and flexibility	Aerated containers, Flow-through systems, Mesocosms	Long	Low-Med
	Timing and length of emergence	Aerated containers, Flow-through systems, Mesocosms	Med-Long	Low-Med
Reproductive success and fitness	Fecundity	Water-filled containers, Aerated containers, Flow-through systems	Med	Low-Med
	Juvenile survival and recruitment	Water-filled containers, Aerated containers, Flow-through systems	Med	Low
	Rates of egg development and hatch success	Water-filled containers	Med	Low
Behavioural	Migration and movement	Thermal Gradient tanks	Short	Low-Med
	Drift	Thermal Gradient tanks, Artificial streams, Natural streams	Short-Med	Med-High*
Ecological	Density	Mesocosms, Artificial stream, Natural streams	Long	Low-High*
	Distribution patterns	Mesocosms, Artificial stream, Natural streams	Long	Low-High*
	Species composition	Mesocosms, Artificial stream, Natural streams	Long	Low-High*
	Species richness	Mesocosms, Artificial stream, Natural streams	Long	Low-High*

*High cost only where Mesocosms and Artificial streams are used; †Times frames are roughly estimated as: Short (hours, days), Med (days, weeks), Long (months, years) – variable dependent on organisms to be studied, acclimation time and desired duration; ‡Cost ratings (estimates) are: Low (R500-R5000), Med (R5000-R10000), High (>R10000) – costs are estimated under the assumption that major laboratory infrastructure exists (e.g. Constant Environment rooms)

4.3.2.3 Water baths

Water baths have built in thermostats that control water temperature with a high degree of precision and many new models are controlled via a digital interface or through computer based programmes which allow for full customisation of heating rates as well as temperature heating profiles and for durations to be programmed. Generally baths have operating temperatures from ambient to 95°C with a uniformity of ±0.1-0.2°C, while more advanced baths also have cooling capabilities with built-in temperature logging capabilities. Capacities of single baths can range from 1.5l to 43l, while some units even consist of two chambers which have independent controls for operating at different temperatures simultaneously. The interior of the baths are usually constructed from seamless sheets of stainless steel which are corrosion resistant and chemically inert and thus do not affect aquatic organisms. Many newer baths control temperature uniformity by circulation of water using built in pumps – which concurrently serve to aerate the water.

Water baths thus serve as advanced aerated containers which allow for temperatures a) to be increased and decreased at constant rates b) kept constant or c) programmed to fluctuate according to a user defined profile. Where water baths are placed in controlled temperature environments, the effects of ambient air temperatures are able to be controlled and where permitting, the photoperiod as well. Most experimental procedures requiring the use of water baths for performance curve studies or CTM experiments are short duration experiments, not necessitating the need for a standardised food source – unless however, food and temperature interactions are the focus of the investigation.

4.3.2.4 Flow-through systems

These are relatively small man-made channels, flumes or containers (constructed of a chemically inert substance) and of varying sizes (almost any dimensions), that receive controlled and constant in-flows of fresh water (normally dechlorinated mains drinking water that has been circulated in a reservoir for approximately 24 hours) that is then circulated and aerated by motorised paddle wheels or pumps (see for example Warren and Davis 1971, Oldmeadow et al. 2010, Ditsche-Kuru et al. 2012). Flow rates in the system are precisely regulated to mimic natural stream velocities at constant rates. Overflow pipes or overshot weirs allow excess water to drain out of the system, thus maintaining water depth and velocity whilst also allowing for the removal of unwanted nutrients and ensuring water remains chemically stable. Additional aeration by means of compressed air via airstones can be used to ensure oxygen content remains close to saturation. Importantly these systems are generally housed in controlled temperature environments, where full spectrum lighting is controlled through a time switch which allows for an adjustable or constant photoperiod. Where several controlled temperature environments are available to provide a range of constant temperature treatments (e.g. 10-25°C at 5°C intervals), a single setup can be positioned in each treatment. Alternatively where only a single controlled environment is available, a set of these flow-through systems can be used and the environment either adjusted weekly/monthly during the course of a year to reflect ambient natural stream temperatures (thereby providing seasonal variation in stream temperatures) or the environment can be set to the coldest experimental treatment temperature (i.e. the control).

In the latter scenario, flow-through systems can also be modified for use in precision water baths set at constant temperatures above the control environment, or alternatively simple calibrated aquarium heaters can be placed in the systems to obtain constant temperature treatments above the control environment. Several water baths can also be used to heat the water contained in the separate reservoirs which is subsequently pumped into each flow-through system. Some advanced controlled temperature environments allow for fluctuating thermal regimes based on a diel cycles. Where these environments are available they would allow for a more accurate simulation of natural conditions thereby providing useful additional insights.

Dependant on the length of the experiment to be conducted using such systems, the organisms involved and the variables being measured, a standardised food source could be added to the system on a regular basis such that it is either a controlled variable or non-limiting/constant (e.g. a set amount of fish flakes or leaves in the case where fish and a detritivorous insect are being studied). Additionally sterilised suitable substrate (cobbles, pebbles, gravel or sand) could also be added to provide a more natural habitat (fish) and to provide a natural surface for attachment (insects). In all cases where a food will be provided to organisms in these systems, it is advised that pilot studies be conducted beforehand to determine a) a suitable food source for the study organism, b) the rate at which food should be replaced before it becomes a limiting factor, and c) the effects the added food source will have on water quality/chemistry in the system.

4.3.2.5 Respiration chambers

Respiration chambers specially designed for aquatic organisms are widely available and come in a range of sizes (from 0.5ml to 30l) that are able to measure respiration in individual eggs, embryos and larvae (invertebrate and fish) to mature adult fish (e.g. www.qubitsystems.com). Chambers are constructed from chemically inert materials that do not act as oxygen sinks and provide air tight seals (e.g. borosilicate glass, perspex). Chambers are designed to allow for oxygen probes to be hermetically mounted or inserted (some needle like fibre optic oxygen probes) inside the chamber to continuously log or allow for manual measurements of oxygen concentration to be recorded at regular intervals. Respiration is then recorded as other environmental factors are adjusted (normally temperature and or photoperiod). Some advanced designs allow for respiration to be measured in swimming fish, by creating a flow-through system using a pump which is built into the design (Jones et al. 2007, Kieffer et al. 2009, Pettersson et al. 2010). For smaller and simpler systems, water within the respiration chambers are usually circulated by means of a magnetic stirrer, where a false bottom or piece of nylon mesh protects the study organism from the stirrer and provides an attachment surface (Verberk et al. 2013).

In a simple experiment to determine the sublethal effects of water temperature on respiration rates of aquatic insects, nymphs are collected, transported to the laboratory and housed in flow-through systems fed with dechlorinated mains drinking water that has been circulated in a reservoir for approximately 24 hours and treated to reflect the water chemistry and quality of the field site. The flow-through system should be maintained in a controlled environment set at either ambient stream temperatures to reflecting those of the study site or set at 10°C. Where possible a constant photoperiod of 12L: 12D should be

provided. Nymphs are then maintained in this system for at least 48 hours in order to acclimate them to ambient temperature, prior to conducting trials. A standardised food source, dependant on the study taxa, can be provided to ensure survival. Following initial acclimation, separate trials are performed whereby each nymph is placed in a sealed glass respiration chamber which is then immersed in a constant temperature water bath, set at trial temperature treatments of 5, 10 and 15°C. Approximately 20-30 nymphs are required to be measured separately at each temperature treatment. Upon immersing the chamber in the water bath the water inside the chamber is stirred using a magnetic stirrer and mesh is put in place to prevent the organism from being harmed by the stirrer. The top of the chamber is left open and the organism is left to acclimate to the trial temperature for 60min, after which the oxygen content of the water is measured using a handheld oxygen meter, before closing and sealing the lid and thereafter leaving the chamber immersed for at least a further 60min (60-140min). At the end of the trial period the chamber is opened and the oxygen content of the water in the chamber quickly measured. Each nymph is thereafter killed and weighed to determine mass. In this manner, the level to which nymphs deplete oxygen from their initial values can be determined for each temperature and the consumption for each nymph can be expressed as $\mu g\ O_2 h^{-1}$ g fresh weight^{-1}.

4.3.2.6 Mesocosms

Mesocosms are essentially water-filled containers, aerated containers, flow-through systems or sets of either of these, that are maintained outdoors under semi-natural conditions (Caquet et al. 2000). The term semi-natural is used because certain environmental variables can be controlled, while others cannot. For instance several mesocosms have comprised flow-through systems setup inside greenhouses (e.g. Harper and Peckarsky 2006, Clements et al. 2013) where air temperature (and water temperature if heaters are used) and humidity can be controlled, but photoperiod remains natural. Elaborate mesocosms generally tend to be on a larger scale (see Harper and Peckarsky 2006,Wesner 2010, Greig et al. 2012) than water-filled containers, aerated containers and flow-through systems housed in laboratories, however they can be used for the same experimental investigations (e.g. growth, development times, emergence timing and length of life cycle, voltinism flexibility) that the other setups allow for and also more depending on the size and nature of the mesocosm (e.g. secondary productivity, density). Water in these systems is generally fed directly from a municipal source or diverted from natural streams. When constructed with glass observation ports, mesocosms have also been used for studies of territorial and reproductive behaviour of salmonid fishes (e.g. Wesner 2010, Grieg et al. 2012).

In a simple experiment by Moore and Schindler (2010) to determine the timing of emergence of aquatic insects *in situ* but in the absence of substrate-disturbing salmon, a basic and small-scale mesocosm was used that consisted of plastic crates (68l) filled with substrate, populated with aquatic insects and fed with water from a nearby lake. Aquatic insects were collected with a Surber sampler from three nearby streams, prior to salmon entering the streams to spawn. Subsamples of each taxa were then placed in the mesocosms in order to monitor growth and timing of emergence in mesocosms compared to insects in the natural streams. Crates were fitted with netting to retain emergent adults. These crates were then left outside to track ambient temperatures and photoperiod and

were monitored every 2-4 days for signs of emergence (Moore and Schindler 2010). The effects of trout (present in natural streams vs excluded in mesocosms) could then be determined on emergence timing of these insects when growing under the same environmental conditions (temperature and photoperiod).

4.3.2.7 Thermal gradient tanks

Myrick et al. (2004) defines three major categories of thermal gradient tanks 1) rectangular chambers that produce a horizontal thermal gradient, 2) cylindrical chambers that produce a vertical thermal gradient and 3) electronic shuttleboxes that produce a horizontal gradient controlled by the movement of organisms. A fairly recent and more complicated design is also that of an annular chamber (see Myrick et al. 2004, and for modifications see Reiser et al. 2013). Dependent on the organisms to be studied, either horizontal, or vertical gradients may be preferential. For instance horizontal gradients, are able to provide uniform light intensity, shallow water depths (<3cm) to prevent the effects of thermal stratification (Myrick et al. 2004) and a uniform bottom/attachment surface onto which organisms can attach and move (e.g. aquatic insects) or walk on (e.g. newts). However the corners that are present in such setups can provide places of extra cover thus making them preferable refugia for certain organisms thus potentially biasing the results. Vertical gradients tanks on the other hand are well suited to studies on free swimming organisms (e.g. zooplankton, fish) and are able to investigate the effects of thermal stratification, deep waters, and also negligible currents. However, vertical gradient can indeed also suffer from interacting effects such as disproportionate light intensities and also water pressures (especially in larger systems) (Myrick et al. 2004). Similarly shuttleboxes, while they allow the organism to regulate the temperature in a horizontal gradient can incur problems with being shallow and with features that provide different amount of perceived cover to organisms (Myrick et al. 2004). All categories of thermal gradient tanks are constructed from chemically inert substances (usually perspex, glass or steel) and are housed in controlled temperature environments, with full spectrum lighting set on an adjustable photoperiod (usually 12L: 12D).

There are numerous ways in which thermal gradients in horizontal and cylindrical tanks can be obtained. A typical and fairly simple horizontal gradient tank is described by Ybarrondo (1995) in a thermal preference experiment for two species of water scavenger beetles (Coleoptera: Hydrophilidae). The design consisted of a shallow (ca. 3cm), rectangular plastic tank (11cmx1m) able to contain 7.5l of water (dechlorinated mains drinking water that has been circulated in a reservoir for approximately 24 hours). At one end of the tank two submersible 250W aquarium heaters were positioned behind a screen (a fine nylon mesh would suffice) which prevented the heaters coming into direct contact with the study organisms. At the other end of the tank also positioned behind a screen ice water was circulated through copper coils, thus acting as a cooling element. Both heating and cooling elements were attached to copper plates to act as conductors, which extended approximately one third of the length of the tank from either side. These conducting plates were buried under a gravel substrate which covered the bottom of the tank. Normoxic conditions and circulation of water were provided by means of several air bubbling fixtures buried in the gravel substrate along the length of the tank. This setup allowed for a stable linear thermal gradient of 10°C-50°C.

Using such a setup, one would collected study organisms from a field site, return them to the laboratory where they would be acclimated in a controlled environment set to match diurnal natural ambient stream temperatures at the time of collection for at least 48 hours. Oxygen levels and photoperiod would ideally similarly mimic natural diurnal cycles. Following acclimation, a subsample of organisms (approximately 10 individuals) would first be measured (to allow for thermal preference to be related back to size should difference be observed), prior to being introduced to the tank from approximately one third of a meter from either end at random. Introducing organisms in this manner prevents organisms from merely clinging to the substrate at the point of release, however it can also result in the inability to escape critical temperatures resulting in death (this is more prevalent in slow moving organisms, such as gastropods). Upon introduction of each organism into the tank, is to monitor position at 5 min intervals while the temperature at the location of each organism is recorded (using a small hypodermic thermocouple probe – so as not to disturb the organism). Observations would be recorded for 40min to an hour after introduction into the tank. Other methods exist for making these observations whereby the gradient tank could be demarcated or semi-partitioned (i.e. still allowing the study organisms access) at regular intervals before starting the experiment. In one approach digital thermometers are placed at these demarcations to provide passive, real-time measurements of water temperature at given areas of the tank. In the second approach water temperatures can be predetermined in each of the partitions (Hernández-Rodríguez et al. 2002, Marek and Gvoždík 2012). Observations are then similarly made at 5 min intervals but rather the number of organisms at each demarcated temperature, or in each partition of a particular temperature, is recorded (Marek and Gvoždík 2012). An alternative to 5 min observations is to introduce the organisms into the tank and to leave them for one hour, after which their position is recorded. Preferably replicate experiments should be carried out, involving 10 organisms in each of four replicate trials. Data collected using these methods provides an indication of temperatures completely avoided and those preferentially migrated to over time.

4.3.2.8 Artificial streams

These attempts to replicate natural stream conditions, where certain variables can still be controlled, represent some of the most expensive and elaborate experimental setups (Warren and Davis 1971). These systems are man-made, artificial stream channels that are dug adjacent to natural streams. They receive constant regulated in flows of water which is either directly diverted or pumped from the adjacent natural stream. During the diversion or pumping process, water provided to this artificial stream can be heated (to mimic climate change scenarios), chemically treated and even enriched in order to control for several environmental variables. The streams can be designed to provide combinations of habitat types (e.g. riffle- pool-runs), to have a constant known gradient and they can be lined such that the effects of natural/artificial groundwater inflows can either be included or excluded. Substrate type and size in artificial streams can be added directly from the natural stream to replicate conditions or selected for to provide insight into the interacting effects of substrate type. Aquatic invertebrates naturally colonise these systems from the adjacent natural stream, or through manual stocking and also through drift from upstream (this is applicable where in channel manipulations have taken place – see Hogg and Williams 1996).

Artificial streams are best used to investigate questions, amongst others, pertaining to the ecological effects of sublethal water temperatures on aquatic organisms (e.g. effects of global climate change temperature increase scenarios – Hogg and Williams 1996). These systems can be sampled regularly as would natural streams over long time frames to investigate questions related to ecological effects of temperature (e.g. density, distribution, species composition, species richness and also secondary productivity, growth rates, voltinism flexibility, fecundity emergence and timing at emergence). For this reason experimental procedures are not discussed here in detail for artificial or natural systems. Instead one is directed to texts such as Hauer and Lamberti (2006).

4.4 Conclusions

This chapter provides an overview of the methods for estimating lethal and sublethal thermal limits for aquatic organisms. The choice of field or experimental setup ultimately depends on the objectives of the study, the logistics and available finances. Even the simplest designs can yield valuable results, which when interpreted in the context of natural systems, provides useful information on the biotic responses of aquatic organisms to changes in water temperature. The extensive literature included directs the reader to further information should they require it.

Chapter 5. Estimation of upper thermal limits for aquatic macroinvertebrates

5.1 Introduction

Tolerance to changes in temperature has been documented for several aquatic organisms (for example Nebeker 1971a, b, Ward and Stanford 1982, Brittain 1991, Hogg et al. 1995, Huryn 1996, Wellborn and Robinson 1996, Dallas and Ketley 2011, Dallas and Rivers-Moore 2012). No studies have however compared thermal limits of taxa amongst different geographic regions or thermal limits of a single species among sites that have different thermal regimes, thereby investigating the extent to which thermal history of an organism affects its thermal limits. Further, no studies have compared the thermal limits of a single species at the same site over time, thereby determining the extent to which thermal tolerance limits are fixed or whether they vary seasonally. This has bearing on the setting of environmental water temperature guidelines, which ideally should include both *in situ* water temperature (to characterise the river's thermal signature) and experimental data such as upper thermal limits.

The aim of this chapter was therefore to:

- Determine the upper thermal limits of a number of taxa that occurred at sites across different geographical regions of South Africa.
- Determine the upper thermal limits of the mayfly, *Lestagella penicillata* (Family Teloganodidae, Ephemeroptera) and the stonefly, *Aphanicerca capensis* (Family Notonemouridae) from sites on different rivers in the Western Cape, South Africa. It should be noted that the genetics of *L. penicillata* is currently under review and it is in fact a species complex (Ross-Gillespie 2014).
- Determine the upper thermal limits of the amphipod, *Paramelita nigroculus* (Family Paramelitidae) and the mayfly, *Lestagella penicillata* (Family Teloganodidae, Ephemeroptera) from sites on Skeleton and Window Gorge streams in the Western Cape, South Africa, respectively.
- Determine the influence of acclimation temperature and rate of change on CT_{max} values for two species, the amphipod, *Paramelita nigroculus* and the mayfly, *Lestagella penicillata* from sites on Skeleton and Window Gorge streams in the Western Cape, South Africa.
- Determine the extent to which CT_{max} values vary amongst species within the families Notonemouridae, Teloganodidae and Leptophlebiidae from sites in the Western Cape, South Africa.

5.2 Methods

5.2.1 Description of study areas

Hourly water temperature data were collected using Hobo UTB1-001 TidbiTs V2 loggers (Onset Computer Corporation, 2008) installed at 18 sites in rivers across five regions of South Africa, including the Western Cape, Southern Cape, Eastern Cape, Mpumalanga and KwaZulu-Natal (Figure 5.1, note that for the Eastern Cape data were collected at three hourly intervals). A full year of temperature data was available for all sites, although the start and end date varied due to differences in the date of installation and in some instances water temperature data were obtained from related studies (Sterkspruit Site, Rivers-Moore and Karssing 2014). Sites spanned a range of ecoregions, geomorphological zones and stream orders (Table 5.1). All sites were characterised by a single thread, confined to moderately confined river channel with low sinuosity, with the exception of the site on the Lower Sabie River in Mpumalanga, which was multi-channelled and anastomosing.

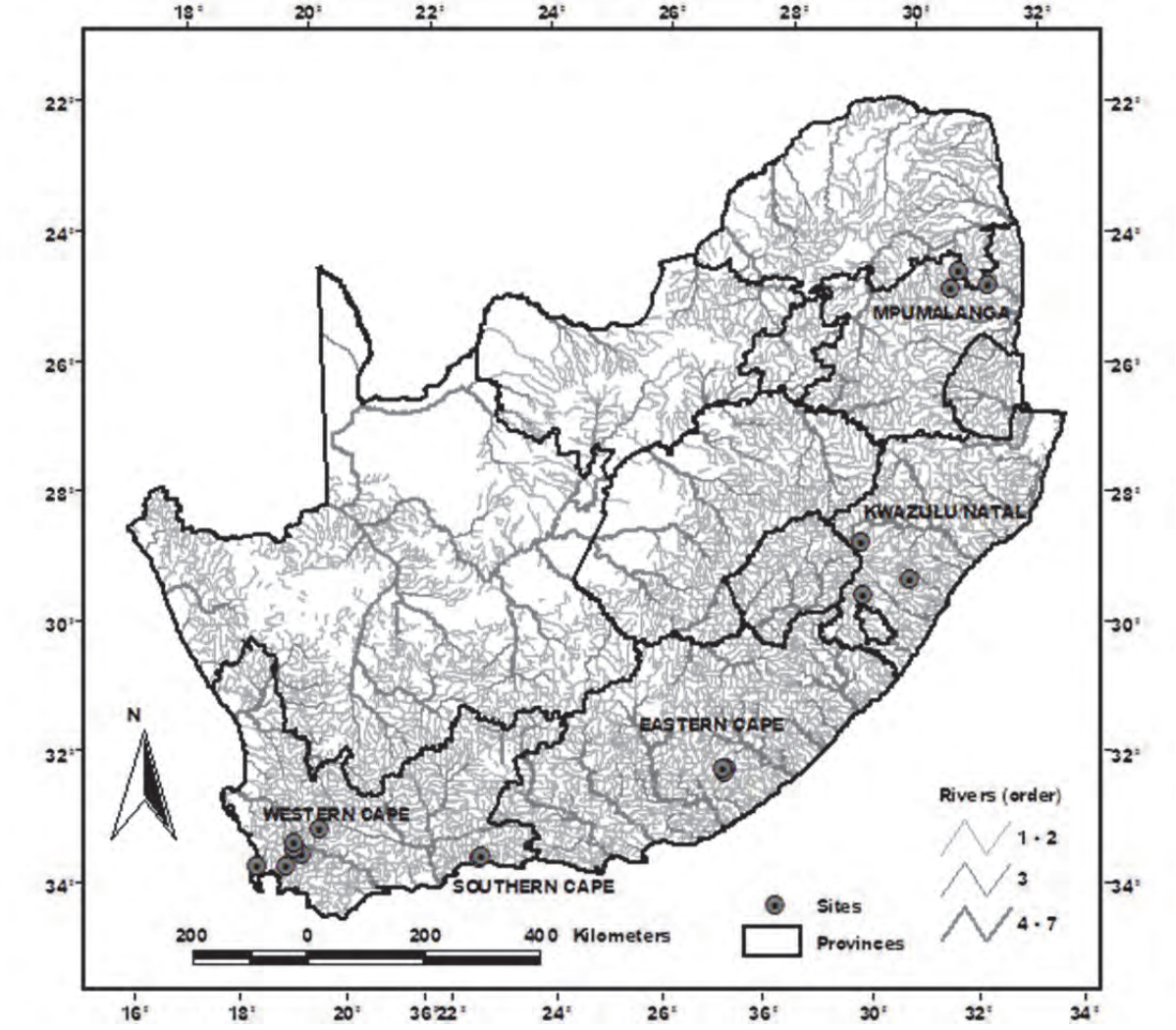


Figure 5.1. Study regions showing sites used for thermal logging and experiments

Table 5.1. Physical and geographical information, geomorphological characteristics and channel morphology characteristics pertaining to study rivers. Alt = altitude and WSW = water surface width.

Site	River	Latitude	Longitude	Alt (m)	Ecoregion Level I	Geomorph-ological Zone	Order	Channel type	Canopy Cover	Turbidity	WSW (m)
WESTERN CAPE											
WG	Window Gorge	-33.98394	18.43183	180	Southern Folded Mountains	Mountain stream	1	Mixed bedrock and alluvial: Boulder	Closed	Clear	1 to 2
SG	Skeleton Gorge	-33.98583	18.42917	164	Southern Folded Mountains	Mountain stream	1	Mixed bedrock and alluvial: Boulder	Closed	Clear	1 to 2
BE	Berg	-33.13090	18.86279	30	South Western Coastal Belt	Lowland River	4	Alluvial with dominant type: Sand, cobble	Open	Discoloured	20 to 50
EE	Eerste	-33.99377	18.97555	380	Southern Folded Mountains	Mountain Stream	1	Alluvial with dominant type: Boulder	Open	Clear	2 to 5
MO	Molenaars	-33.73139	19.11500	440	Western Folded Mountains	Transitional	2	Alluvial with dominant type: Boulder, cobble	Open	Clear	1 to 20
WI	Wit	-33.63709	19.10789	660	Western Folded Mountains	Transitional	1	Mixed bedrock and alluvial: Boulder	Open	Clear	2 to 5
RO	Rooielskloof	-33.46110	19.61786	481	Western Folded Mountains	Mountain stream	1	Mixed bedrock and alluvial: Cobble, boulder	Closed	Clear	2 to 5

Site	River	Latitude	Longitude	Alt (m)	Ecoregion Level I	Geomorph-ological Zone	Order	Channel type	Canopy Cover	Turbidity	WSW (m)
SOUTHERN CAPE											
KA	Kaaimans	-33.95230	22.53440	137	South Eastern Coastal Belt	Upper Foothill	2	Mixed bedrock and alluvial: Boulder	Open	Clear	2 to 5
TO	Touws	-33.94660	22.61260	154	South Eastern Coastal Belt	Upper Foothill	2	Mixed bedrock and alluvial: Boulder	Open	Clear	10 to 20
EASTERN CAPE											
CA	Cata	-32.57728	27.12375	890	South Eastern Uplands	Upper Foothill	1	Alluvial with dominant type: Boulder, cobble	Closed	Opaque	2 to 5
MU	Mnyameni	-32.61211	27.07438	950	South Eastern Uplands	Upper Foothill	1	Alluvial with dominant type: Boulder, cobble	Partially open	Opaque	2 to 5
ML	Mnyameni	-32.58762	27.04873	850	South Eastern Uplands	Lower Foothill	1	Alluvial with dominant type: Cobble, boulder	Open	Opaque	2 to 5
KWAZULU-NATAL											
CS	Cascades	-29.55984	30.31818	890	South Eastern Uplands	Upper Foothill	1	Mixed bedrock and alluvial: Cobble, sand	Open	Discoloured	1 to 2
MZ	Mzimkhulu	-29.83233	29.52150	1122	South Eastern Uplands	Lower Foothill	3	Mixed bedrock and alluvial: Cobble, boulder	Open	Discoloured	30 to 50
ST	Sterkspruit	-29.02887	29.42668	1250	Eastern Escarpment Mountains	Upper Foothill	1	Alluvial with dominant type: Boulder, cobble	Open	Discoloured	2 to 5
MPUMALANGA											
TT	Treur Tributary	-24.79297	30.89406	1500	Northern Escarpment Mountains	Mountain stream	1	Mixed bedrock and alluvial: Boulder	Open	Clear	5 to 10
SU	Klein Sabie	-25.06352	30.79092	1200	North Eastern Highlands	Upper Foothill	2	Alluvial with dominant type: Cobble	Open	Discoloured	2 to 5
SL	Sabie	-24.96972	31.40655	400	Lowveld	Lower Foothill	3	Mixed bedrock and alluvial: Gravel, sand	Open	Opaque	20 to 30

5.2.2 Collection of aquatic organisms

Organisms for regional comparisons were collected from sites between July 2013 and July 2014. Regions were sampled at different 2 week periods as logistically it was impossible to undertake simultaneous sampling and experimentation. Organisms for site comparison in the Western Cape were collected from sites in September and October 2013, while organisms for temporal analysis were collected from Skeleton Gorge and Window Gorge steams every two months from February 2013 to April 2014. Organisms for acclimation and rate of change experiments were collected in November 2013.

Sampling was undertaken using a sampling net of 950 mm diameter mesh, which ensured that only larger organisms were collected. Stones were removed and rinsed to dislodge organisms, which were caught in a net held downstream of the stones being rinsed. Hand-picking was also used if the number of organisms was inadequate or if the organisms were damaged during the collection process. Test organisms were transferred into a collecting bucket, which was kept cool using crushed ice packed around the bucket, and transported to a freshwater aquarium. Prior to tests, organisms were held at approximately 16^{o}C, which resembled water temperatures at capture, and maintained in aerated tubs filled with dechlorinated tap water until experiments commenced. Dechlorinated tap water was used for experiments and a photoperiod of 12:12 LD (light from 06h00 to 18h00) was maintained throughout the experimental period. Experiments commenced after approximately 24 hours and test organisms were not fed during the experiments.

5.2.3 Experimental procedure

Upper thermal limits of test organisms were determined using two methods, the critical thermal method (CTM) and the incipient lethal temperature (ILT) technique (see Dallas and Ketley, 2011 and Dallas and Rivers-Moore, 2012 for details of these methods). For CTM experiments, organisms were placed in an experimental chamber, which was immersed in a water bath in the temperature controlled aquarium. Each experiment was approximately 90 min in duration, which included a 30 min control phase, followed by a 60 min experimental phase during which the water temperature was raised at a constant rate of 0.34^{o}C per minute using a circulating heater (JulaboTM). The circulating pump ensured that the percentage saturation of dissolved oxygen remained above 70% during the experiments. Organisms were monitored throughout the experiment and as an organism exhibited behavioural signs of reaching the endpoint, CT_{max}, it was removed from the experimental chamber and placed in aerated tubs at the start (acclimation) temperature. The CT_{max} for each test organism was recorded and only those organisms that recovered were included in the results. Identifications were confirmed after the experiments were concluded.

For ILT experiments, organisms were placed in experimental chambers immersed in aquarium tanks, filled with dechlorinated freshwater, which was heated using water heaters to eight different temperatures (one control temperature at 16^{o}C and seven experimental temperatures: 18^{o}C, 21^{o}C, 24^{o}C, 27^{o}C, 30^{o}C, 33^{o}C and 36^{o}C). Standard deviation of water

temperature in each chamber was generally < 0.4^{o}C. Water temperature was recorded at one hour intervals throughout the experiment using Hobo TidbiTs v2 loggers (Onset Computer Corporation, 2008) placed in each of the tanks. Aquarium tanks were aerated using two air stones per tank or alternatively a pump to ensure that the percentage saturation of dissolved oxygen remained above 75%. Individuals of each taxon were placed in each experimental chamber, with a total of 30 individuals per experimental temperature. In some instances it was not possible to collect enough organisms in which case fewer individuals were used per experimental temperature.

Starting at the acclimation temperature, the temperature of holding tank was gradually increased by 2 to 4^{o}C/h, until the appropriate target temperature was reached. A control temperature, which approximated water temperatures at capture, was included to test potential mortality factors such as influences from handling, chambers, air, water or a lack of food. Chambers were checked for survival every 24h for four days (96h) subsequent to the target temperature being reached.

5.2.4 Statistical analysis

The Indicators of Thermal Alteration (ITA) method (Rivers-Moore et al. 2012, 2013a) was used to characterise and compare thermal signatures of each river. This method involves the conversion of sub-daily water temperature data for a full year to be converted to daily data (mean, minimum, maximum and range). From these data, 37 metrics are calculated to describe water temperatures with respect to magnitude of water temperatures, frequency, timing and duration of thermal events (Table 5.2). Data were normalised and agglomerative hierarchical cluster analysis (based on Euclidean distance, using a Group Average joining algorithm) and multidimensional scaling was then undertaken to determine groups (Primer, 2012). A subset of the metrics that were not highly correlated with one another were then normalised and analysed using principal component analysis (PCA) to determine which temperature metrics had the greatest influence on site groupings. Using the method of Rivers-Moore et al. (2012, 2013a), 7-day moving averages of mean, minimum and maximum temperatures, together with 95% confidence bands, were generated from water temperature data for each site.

For CT_{max} experiments, data were tested for normality (Kolmogorov-Sminorv test, Statistica Version 12 for Windows) and found to be largely non-normally distributed. The non-parametric Kruskal-Wallis one-way analysis of variance by ranks method was thus used to test the hypothesis that rankings are the same in different groups, with the significance level set at $p<0.05$. For ILT experiments, LT_{50} values (the temperature at which 50% of the sample survives in a specified time) and 95% confidence intervals were calculated using the Trimmed Spearman-Karber analysis (USEPA TSK Programme Version 1.5). This method is used extensively for estimating median lethal concentrations in toxicity bioassays and has been shown to be accurate, precise and robust and is easily computable (Hamilton et al. 1977). LT_{50}s were calculated for each time period (every 24 h) and plotted against treatment temperature and analysed using linear regression. The resulting regression formula was then used to determine the incipient lethal upper temperature (ILUT), which is the temperature survived by 50% of the population for 96 h.

To define a chronic stress threshold in this study, the approaches of Brungs and Jones (1977) and Armour (1991) were followed, whereby a Maximum Weekly Allowable Temperature (MWAT) threshold is calculated using experimental temperature data. Both methods use an optimal temperature (OT) and either the range of preferred temperatures from laboratory derived growth curves (Brungs and Jones 1977) or an Incipient Lethal Upper Temperature (ILUT). If OT is unknown, the midpoint of a range of measured water temperatures may be used (Brungs and Jones 1977). The MWAT represents that temperature that can be tolerated as long as the ILUT is not exceeded for sustained periods (Equation 1).

$$\mathrm{MWAT} = \mathrm{OT} + \frac{(\mathrm{ILUT} - \mathrm{OT})}{3} \qquad (1)$$

Where, OT is optimal temperature, and ILUT is the Incipient Lethal Upper Temperature (Armour, 1991). OT values were calculated for each taxon by using hourly water temperature data recorded at sites where each taxon was collected. These represent the median optimum temperature for the taxon. Where a family was represented by more than one genus, OTs was determined for each genus.

5.3 Results

5.3.1 Thermal signatures of rivers

The periods for which water temperature data were available varied from river to river, although a full year's data was used to calculate the ITAs (see Table 5.3 for details). The thermal regimes of the 18 rivers differed substantially from one another. Mean Annual Temperature (MAT) of river water varied from 12.1^{o}C (Upper Mnyameni River) to 21.8^{o}C (Lower Sabie River), whilst the coefficient of variation of MAT ranged from 10.61 (Upper Sabie River) to 32.68 (Wit River). Degree days ranged from 919 heat units for the Sterkspruit to 4310 heat units for the Lower Sabie, representing a four-fold difference in heat units over a one year period. The 7-day Moving Average was lowest for the Sterkspruit River (16.7^{o}C) and highest for the Lower Sabie River (28.0^{o}C) (Figure 5.2). As expected monthly averages, minimums and maximums were lowest in winter (June to August) and warmest in summer (December, January and February). Ranges in monthly temperatures were greatest in the Wit River in summer, compared to the Sterkspruit which was highest in early spring, and the Treur Tributary which was highest in winter.

Cluster and ordination using the full set of temperature metrics (including monthly values) for each site indicated that sites formed four groups (Figure 5.3) with warmer lowland sites forming one group (Berg and Lower Sabie), cool upland sites forming a second group (Cata and Upper Mnyameni and Sterkspruit), more thermally predictable sites forming a third group (Treur Tributary and Upper Sabie), while the remaining sites formed a fourth group (including those with greater thermal variability such as the Wit, Mzimkhulu and Molenaars). Examination of this data using PCA analysis showed that cumulative % variance explained by the first, second and third axes were 47.9%, 35.5% and 9.4% respectively with axis 1 linked to MAT, minimum and maximum temperature metrics, and axis 2 to predictability and thermal variation (CV of MAT and SD of MAT) (Figure 5.4).

Table 5.2. Temperature metrics for disaggregating thermal time series (from Rivers-Moore et al. 2013a)

Annual descriptive statistics		Mean + Std. Dev. of annual temperature
		Annual coefficient of Variability (% CV)
		Predictability (Colwell 1974)
		Mean annual range, mimima, maxima
		Degree days (annual)
Group 1	Monthly magnitudes	Jan-Dec mean, minimum and maximum temperatures
Group 2	Magnitude and duration of annual extreme water temperature conditions	7-day mean
		7, 30 and 90-day minima
		7, 30 and 90-day maxima
		Mean daily minimum
		Maximum daily range
Group 3	Frequency and duration (number of successive days of event above or below a threshold)	7-D mean threshold[1] count and duration
		7-D minimum threshold[2] count and duration
		7-D maximum threshold[3] count and duration
Group 4	Timing – Julian date of maximum and minimum metrics (thermal triggers)	Date of onset of longest exceedance of mean threshold
		Date of onset of longest exceedance of minimum threshold
		Date of onset of longest exceedance of maximum threshold

[1] 18°C for upper catchments sites and 25°C for lower catchment sites; [2] 12°C; [3] 96h ILUT as determined experimentally for organisms from each site was used for the maximum threshold

Table 5.3. Thermal metrics calculated for each river (Site codes are given in Table 5.1)

	WG	SG	BE	EE	MO	WI	RO	KA	TO	CA	MU	ML	CS	MZ	ST	TT	SU	SL
Water temperature data logging period	May 13-May 14	May 13-May 14	Mar 10-Feb 11	Jan 10-Dec 10	Jan 10-Dec 10	Jan 10-Dec 10	Jan 10-Dec 10	Oct 13-Sep 14	Jan 14-Oct 14	Aug 13-Jul 14	Aug 13-Jul 14	Aug 13-Jul 14	Nov 13-Oct 14	Nov 13-Oct 14	Oct 07-Sep 08	Jul 13-Jul 14	Jul 13-Jul 14	Jul 13-Jul 14
Mean Annual Temperature (MAT)	14.4	13.9	19.0	14.7	15.2	15.2	14.8	16.0	15.8	12.5	12.1	15.4	15.6	15.9	12.5	14.8	17.0	21.8
SD of MAT	3.21	2.89	4.92	3.83	4.65	4.97	3.48	4.19	4.30	3.58	3.66	4.08	3.37	4.77	3.07	2.10	1.80	3.59
CV% of MAT	22.26	20.83	25.85	26.08	30.51	32.68	23.55	26.22	27.12	28.77	30.25	26.46	21.64	30.02	24.54	14.17	10.61	16.48
Predictability	0.71	0.70	0.62	0.59	0.60	0.55	0.65	0.63	0.59	0.63	0.59	0.62	0.65	0.54	0.66	0.77	0.86	0.62
Mean of annual range	1.03	1.14	1.17	3.32	2.94	3.90	1.44	2.39	1.72	1.45	1.53	2.95	2.25	2.19	3.63	3.01	2.16	2.23
Mean of annual min	14.0	13.4	18.5	13.2	13.9	13.4	14.1	14.8	15.1	11.7	11.3	14.1	14.5	14.8	11.0	13.5	16.1	20.7
Mean of annual max	15.0	14.5	19.7	16.5	16.8	17.3	15.6	17.2	16.8	13.2	12.9	17.0	16.8	17.0	14.7	16.6	18.2	22.9
Degree days	1622	1420	3042	1704	1911	1895	1738	2010	1618	898	763	1982	2038	2154	919	1760	2557	4310
Mean_7*	20.1	19.6	27.9	22.0	23.2	24.4	20.3	24.1	23.9	18.7	18.4	22.3	20.8	22.6	16.7	18.0	19.6	28.0
Min_7*	9.1	9.4	10.7	7.3	7.5	7.0	7.4	7.6	8.3	6.0	5.1	7.0	7.4	4.2	4.5	9.0	11.8	12.7
Min_30^{+}	9.7	9.7	12.0	9.2	8.7	8.4	9.2	8.9	9.7	7.2	6.5	8.3	9.1	6.4	5.5	9.5	11.8	12.7
Min_90^{+}	10.4	10.2	12.8	9.7	9.2	8.9	9.9	9.9	10.9	7.2	6.5	8.3	10.2	8.2	6.7	9.5	11.8	12.7
Max_7*	20.7	20.5	29.3	24.3	25.4	28.6	21.1	26.2	25.0	19.6	19.1	24.2	21.9	23.8	21.0	20.9	21.4	29.7
Max_30^{+}	19.5	12.6	25.2	23.0	24.5	27.6	20.3	17.3	23.8	8.6	8.4	12.3	18.9	21.6	15.6	13.3	15.9	18.0
Max_90^{+}	19.8	18.9	27.5	22.7	24.3	25.6	20.3	23.1	22.0	18.1	17.7	22.1	20.8	21.5	21.0	18.3	20.0	26.6

	WG	SG	BE	EE	MO	WI	RO	KA	TO	CA	MU	ML	CS	MZ	ST	TT	SU	SL
Julian MWAT[@]	18	24	68	7	7	7	7	16	21	5	48	48	357	342	0	0	347	6
Julian min[@]	195	152	192	158	156	124	148	156	157	117	108	152	138	138	85	194	192	0
Julian max[@]	0	0	8	64	7	7	55	31	0	0	0	0	358	242	0	0	0	0
MWAT count & (duration)[#]	76 (51)	41 (15)	56 (49)	96 (80)	112 (83)	109 (84)	92 (79)	122 (58)	82 (73)	12 (6)	7 (4)	0 (0)	108 (103)	149 (73)	0 (0)	0 (0)	147 (107)	83 (32)
Min count & (duration)[#]	126 (81)	145 (132)	44 (29)	165 (58)	161 (118)	171 (101)	117 (58)	35 (29)	77 (67)	194 (186)	203 (187)	121 (111)	88 (61)	104 (82)	207 (184)	113 (44)	3 (3)	1 (1)
Max count & (duration)[#]	0 (0)	0 (0)	7 (7)	19 (9)	114 (82)	135 (94)	56 (19)	41 (24)	7 (5)	0 (0)	0 (0)	0 (0)	92 (79)	164 (88)	0 (0)	0 (0)	0 (0)	0 (0)

** 7-day moving averages of mean, minimum and maximum temperatures; [+] 30 and 90-day moving averages of mi and max temperatures; [@ #]Julian date on which longest period of successive days of temperature threshold exceedance (MWAT, min, max) begins; [#] Number of times within a year (and duration) that the MWAT, min/max temperature threshold was exceeded*

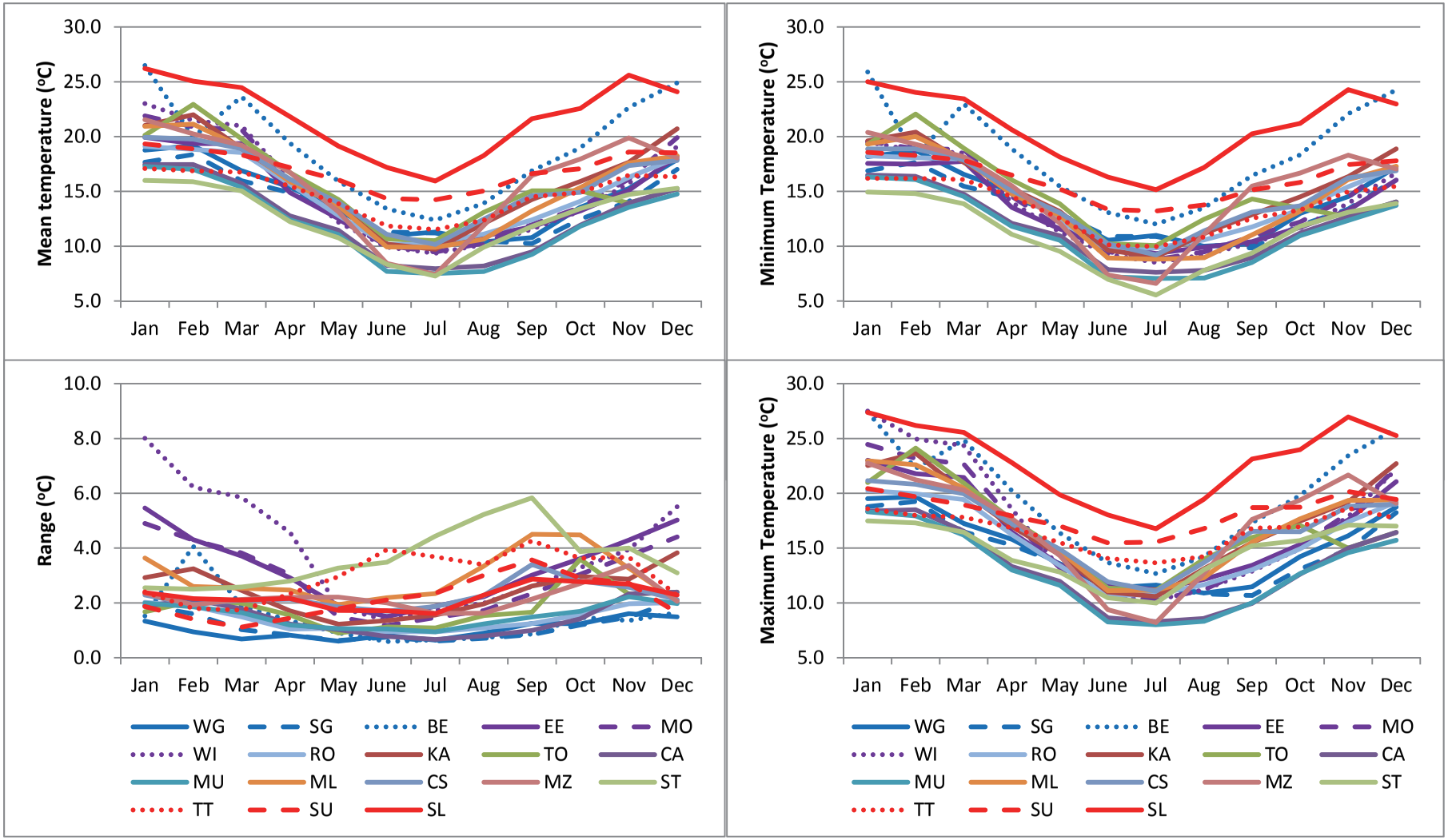


Figure 5.2. Monthly averages for mean, minimum and maximum temperatures (°C) as well as monthly temperature ranges for the study sites

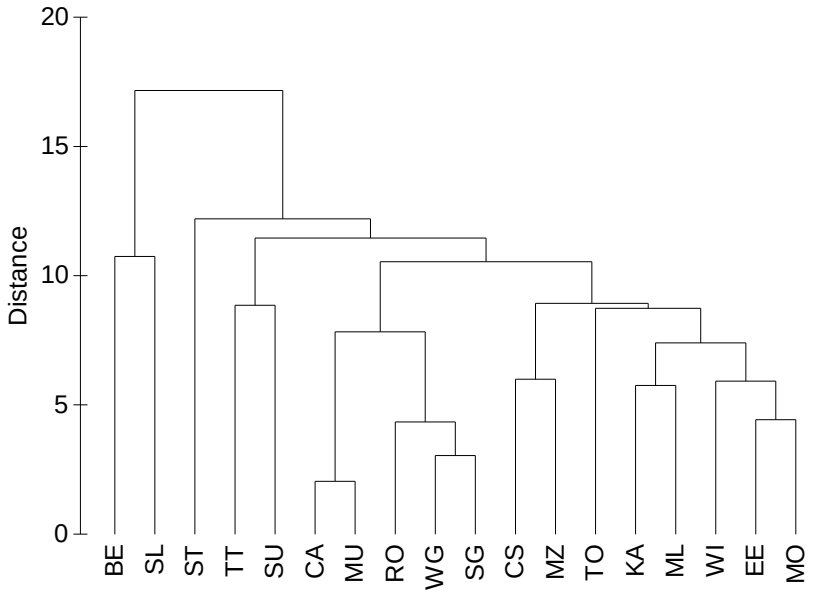


Figure 5.3. Cluster analysis of study sites based on variables derived from daily water temperature data using the Euclidean distance measure and group average relatedness.

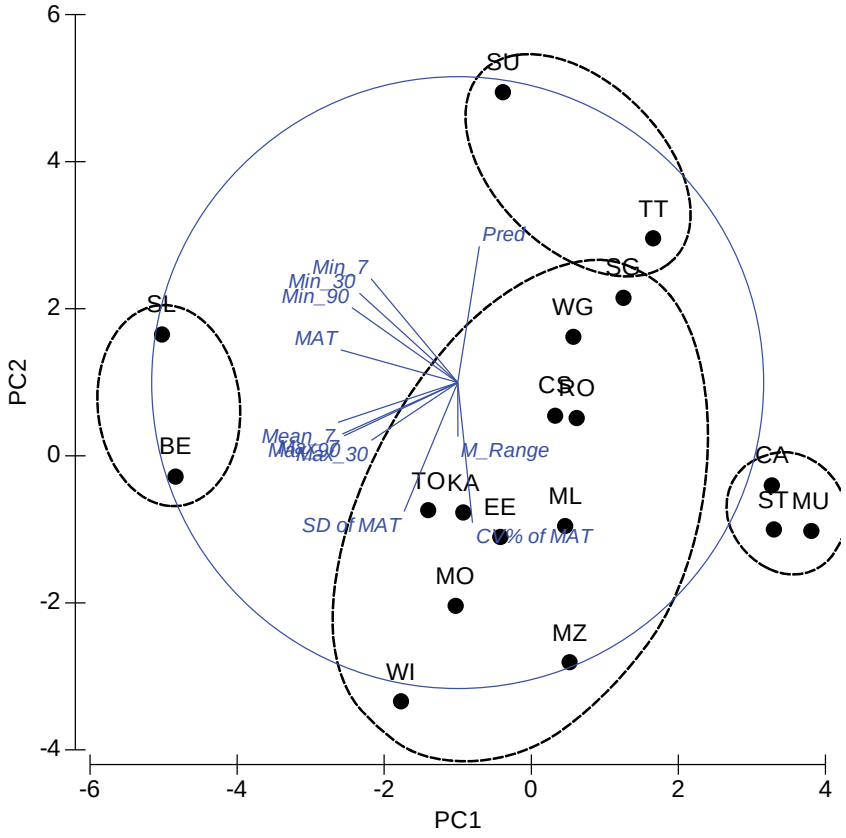


Figure 5.4. Principal Components Analysis of water temperature metrics. Vectors represent variables showing correlations (PC1 and PC2). The inner edge of the circle delineates a correlation of 1. Ellipses around sites (•) indicate the similarity between sites at a Euclidean distance of 3.9.

5.3.2 Comparison of upper thermal limits of aquatic macroinvertebrates amongst regions

5.3.2.1 Critical thermal maxima

Comparison of CT_{max} values amongst regions was undertaken for nine families of aquatic macroinvertebrates (Table 5.4). Some families were represented by a single genus, while for others the genera varied amongst sites. The number of regions compared for each family was dependent on availability of test organisms at the sites. CTM results for each family are discussed below and presented in figures 5.5 to 5.7.

Plecoptera: Notonemouridae and Perlidae

Analysis for Notonemouridae was restricted to autumn and winter periods throughout, even though CT_{max} values have been determined for a number of additional sites and periods in the WC. Comparison of CT_{max} values for *Afronemoura* from three regions revealed that there were significant differences amongst sites, specifically the Upper Mnyameni in the EC (median CT_{max} = 34.1°C) and the Treur Tributary (median CT_{max} = 29.1°C) in MPU, which were both significantly different from the other *Afronemoura* sites (Kruskal-Wallis test: H = 93.11711, $p < 0.05$, Figure 5.5). Differences were not significant between the two genera from Window Gorge in the WC, and CT_{max} values (30.7°C and 29.2°C for *Aphanicera* and *Aphanicercopsis* respectively) for this site were only significantly different from the two EC sites (Cata and Upper Mnyameni).

CT_{max} values for Perlidae varied significantly amongst the regions (EC, KZN and MPU) with median values ranging from 31.5°C for the Lower Sabie in MPU to 34.2°C and 35.5°C for the Lower Mnyameni in the EC (Kruskal-Wallis test: H = 69.46492, $p < 0.05$, Figure 5.5). Only the Upper and Lower Sabie sites varied significantly from one another within a region.

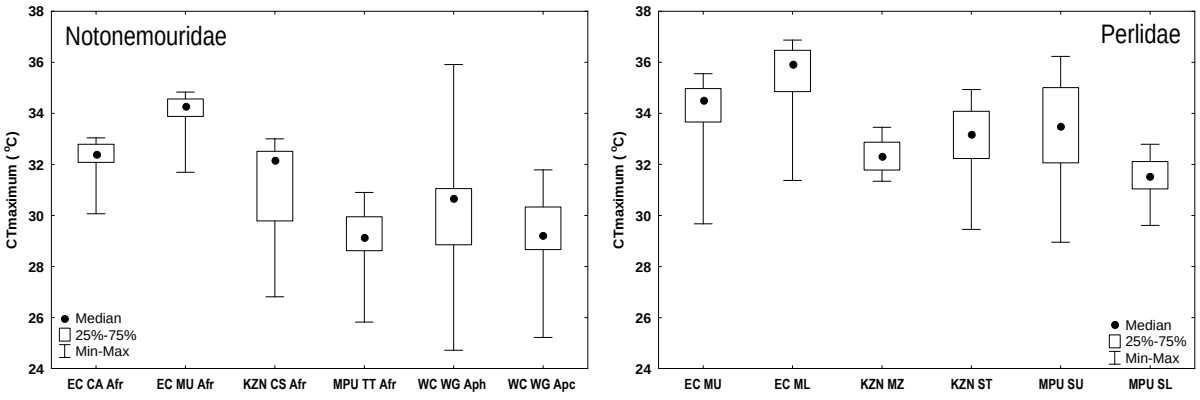


Figure 5.5. Median CT_{max}, 25th and 75th percentile and minimum and maximum values (°C) for Notonemouridae (Afr=*Afronemoura*, Aph=*Aphanicera* and Apc= *Aphanicercopsis*) and Perlidae (*Neoperla*) (EC=Eastern Cape, KZN=KwaZulu-Natal, MPU=Mpumalanga, WC=Western Cape; sites codes as per Table 5.1)

Table 5.4. Families (and genera) of aquatic macroinvertebrates used for CTM experiments. Those indicated with an asterisk were also used for estimating ILUT for regional comparisons. (Site codes are given in Table 5.1). Season is indicated (A = autumn, W = winter and SP = spring)

		Western Cape							Southern Cape		Eastern Cape			KwaZulu-Natal			Mpumalanga		
Family	**Genus**	**WG**	**SG**	**BE**	**EE**	**MO**	**WI**	**RO**	**KA**	**TO**	**CA**	**MU**	**ML**	**CS**	**MZ**	**ST**	**TT**	**SU**	**SL**
Notonemouridae	*Afronemoura*										A	A*		W			W		
	Aphanicerca	A			SP*		SP*	SP*											
	Aphanicercopsis	A																	
Perlidae	*Neoperla*											A	A		W*	W*		W*	W
Heptageniidae	*Afronurus*					A				SP		A*	A*		W*	W		W	W
Leptophlebiidae	*Adenophlebia*				A							A*	A*			W*			
	Aprionyx				SP														
	Castanophlebia	A*			SP*			W											
	Choroterpes								SP*	SP*									
	Euthraulus														W*	W*		W*	W
Teloganodidae	*Lestagella*	SP*			SP*	SP*	SP*	SP*	SP*	SP*									
Tricorythidae	*Tricorythis*			SP							A*	A*	A		W*	W*		W*	
Philopotamidae	*Chimarra*				A		SP		SP*	SP*		A			W*	W		W*	W
Blephariceridae	*Elporia*		W*		W											W	W		

Ephemeroptera: Heptageniidae, Leptophlebiidae, Teloganodidae and Tricorythidae

CT_{max} for Heptageniidae (Genus *Afronurus*) varied significantly amongst the sites (WC, SC, EC, KZN and MPU), with median values ranging from 32.7°C for the Molenaars in the WC to 36.9°C for Lower Mnyameni in the EC (Kruskal-Wallis test: H = 101.3592, $p < 0.05$, Figure 5.6).

CT_{max} for Leptophlebiidae varied significantly amongst the groups (group = region+site+genus; (WC, SC, EC, KZN and MPU), with median values ranging from 31.2°C for *Aprionyx* from the Eerste in the WC to 36.3°C for *Adenophlebia* from the Lower Mnyameni in the EC (Kruskal-Wallis test: H = 245.5240, $p < 0.05$, Figure 5.6). Comparison of group pairs indicated that, with the exception of *Chloroterpes* from the SC, values were not significantly different amongst sites or regions within genera, and that differences were largely due to differences amongst genera (Kruskal-Wallis test: H = 189.9142, $p < 0.05$, Figure 5.6). Comparing genera, regardless of region, revealed that *Aprionyx* had the lowest CT_{max} (31.2°C) with remaining genera increasing as follows: *Castanophlebia* (32.6°C), *Euthraulus* (33.0°C), *Chloroterpes* (34.0°C) and *Adenophlebia* (35.7°C).

Comparison of CT_{max} values for Teloganodidae was only possible for sites from the WC and SC, because while *Lestagella* occur in the EC, they were not present in March. CT_{max} values for *L. penicillata* varied significantly amongst sites (Kruskal-Wallis test: H = 103.2606, $p < 0.05$), with lowest values from the Molenaars and Eerste in the WC (29.1°C and 30.1°C respectively) and highest from the Wit in the WC and the Touws in the SC (34.7°C and 34.1°C respectively) (Figure 5.6). Comparison of site pairs indicated that differences were due to significantly lower CT_{max} values from the Eerste and Molenaars Rivers in comparison to the other sites.

CT_{max} for Tricorythidae varied significantly amongst the groups (WC, EC, KZN and MPU) four regions represented), with median values ranging from 29.1°C for *Tricorythus* from the Upper Sabie in MPU to 38.7°C from the Berg in the WC (Kruskal-Wallis test: H = 174.7258, $p < 0.05$, Figure 5.6). Comparison of site pairs showed that the Berg in the WC was significantly different from all other sites except for the Lower Mnyameni in the EC, while sites within the EC and sites within KZN were not significantly different to one another. The Upper Sabie was significantly different to all sites except the Sterkspruit in KZN.

Philopotamidae

CT_{max} varied significantly amongst the regions (EC, KZN and MPU) with median values ranging from to 29.4°C for the Upper Sabie in MPU sites to 34.5°C for the Touws in the SC sites (Kruskal-Wallis test: H = 93.93631, $p < 0.05$, Figure 5.7). Only the Upper and Lower Sabie varied significantly from one another within a region.

Blephariceridae

CT_{max} varied significantly amongst the summer (WC) and winter (KZN and MPU) rainfall regions with median values of 26.0°C and 26.5°C for two WC sites, and 29.0°C and 29.2°C for KZN and MPU respectively (Kruskal-Wallis test: H =46.87196, $p < 0.05$, Figure 5.7).

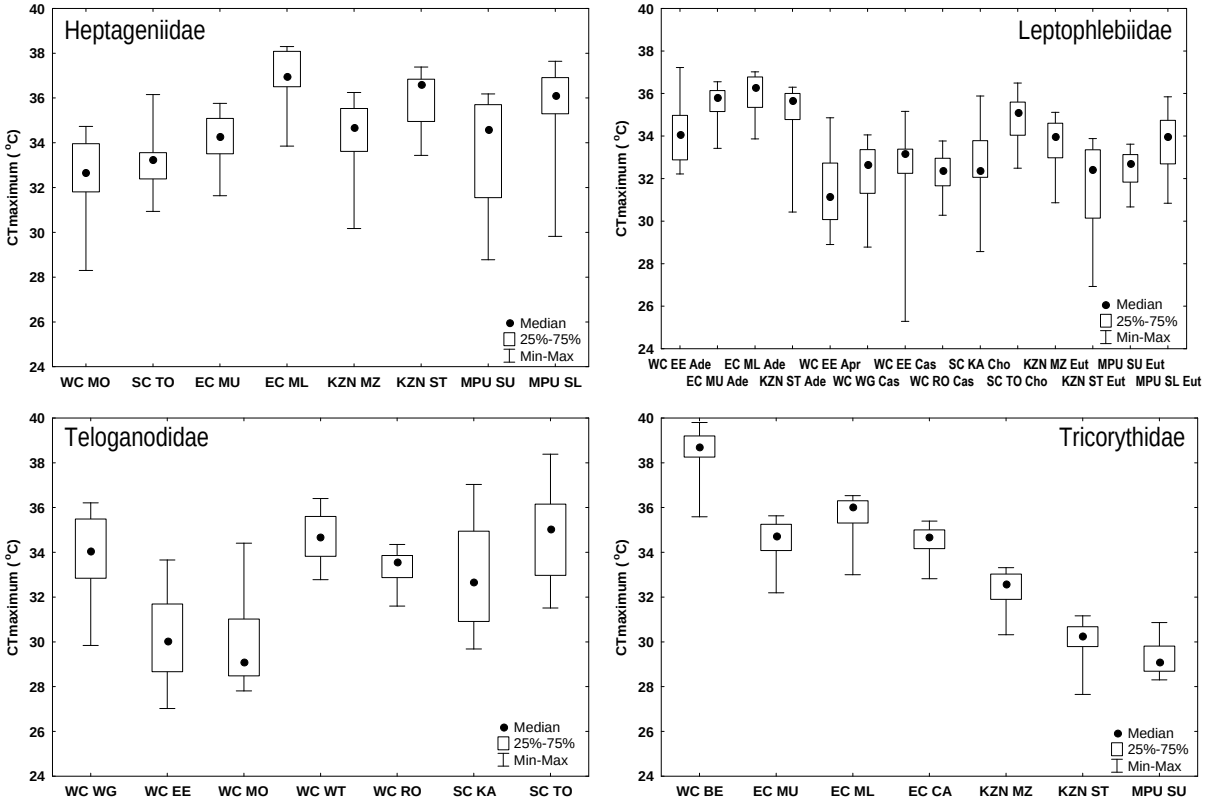


Figure 5.6. Median CT_{max}, 25th and 75th percentile and minimum and maximum values (^{o}C) for Heptageniidae (*Afronurus*), Leptophlebiidae (Ade=*Adenophlebia*, Apr=*Aprionyx*, Cas=*Castanophlebia*, Cho=*Chloroterpes*, Eut=*Euthraulus*), Teloganodidae (*Lestagella penicillata*) and Tricorythidae (*Tricorythus*) (WC=Western Cape, SC=Southern Cape, EC=Eastern Cape, KZN=KwaZulu-Natal, MPU=Mpumalanga; sites codes as per Table 5.1)

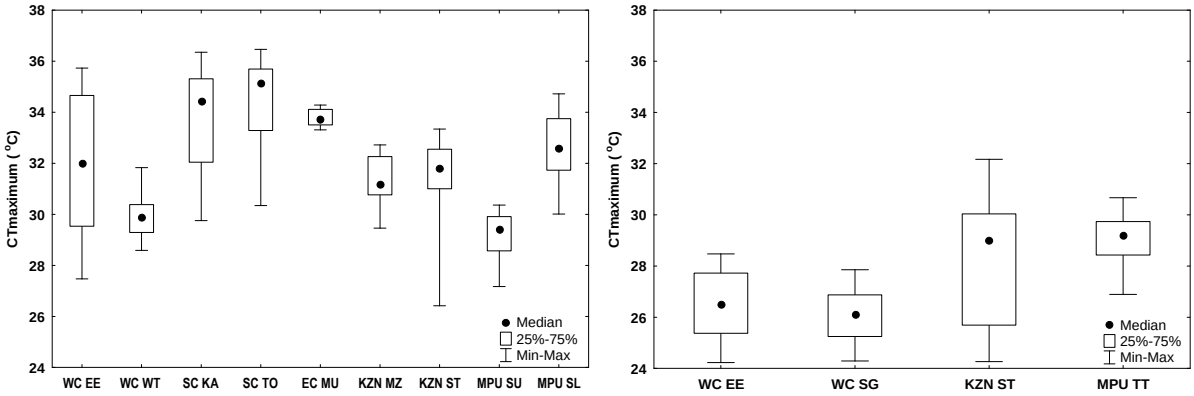


Figure 5.7. Median CT_{max}, 25th and 75th percentile and minimum and maximum values (^{o}C) for Philopotamidae (*Chimarra*) and Blephariceridae (*Elporia*). (WC=Western Cape, SC=Southern Cape, EC=Eastern Cape, KZN=KwaZulu-Natal, MPU=Mpumalanga; sites codes as per Table 5.1)

5.3.2.2 Incipient lethal upper temperature

96h ILUT estimated from regression equations generated from four day LT_{50} trials are summarised in Table 5.5 and discussed below.

Plecoptera: Notonemouridae and Perlidae

96h ILUT for the notonemourid, *Aphanicerca capensis*, were 21.7°C, 20.8°C and 21.9°C for the Eerste, Rooielskloof and Wit rivers in the WC respectively (all October) in comparison to 23.3°C for Skeleton Gorge in the WC, and 25.8°C for *Afronemoura amatolae* in the EC (March). 96h IULT for the perlid, *Neoperla*, ranged from 18.8°C for the Mzimkhulu in KZN to 21.9°C for the Upper Sabie in MPU.

Ephemeroptera: Heptageniidae, Leptophlebiidae, Teloganodidae and Tricorythidae

96h IULT for Heptageniidae (Genus *Afronurus*) was 22.2°C and 26.1°C for the Upper and Lower Mnyameni in the EC respectively, 23.3°C for the Mzimkhulu in KZN and 26.5°C for the Molenaars in the WC. 96h ILUT was determined for four genera of Leptophlebiidae, although only one of these was common to more than one region. Lowest values were for *Adenophlebia* and *Euthraulus* from the Sterkspruit in KZN (19.8°C and 20.9°C respectively), followed by *Castanophlebia calida* from the Eerste in the WC (21.7°C), *Euthraulus* from the Upper Sabie in MPU (24.9°C), *Adenophlebia* from the EC (25.1°C and 26.1°C for the two sites), with highest for *Chloroterpes* from the SC sites (27.4°C and 28.8°C). 96h ILUT were only determined for the WC and SC regions, with values for the teloganodid, *Lestagella penicillata*, ranging from 19.3°C for the Wit to 27.1°C for the Touws. 96h IULT for the tricorythid, *Tricorythus*, varied from 20.1°C for the Mzimkhulu in KZN to 25.2°C for the upper Sabie in MPU.

Philopotamidae

96h ILUT for the philopotamid, Chimarra, were relatively uniform across regions ranging from 24.8°C in the Touws (SC) to 26.0°C in the Molenaars (WC).

Blephariceridae

While LT_{50} experiments were undertaken for the Sterkspruit in KZN, high mortalities in the control prevented an estimate of ILUT. The 96h ILUT for the blephericerid, Elporia, was estimated at 21.6°C.

Table 5.5. 96h ILUTs estimated from 4 day LT_{50} experiments ordered by family. R^2 values are provided. (Site codes are given in Table 5.1)

Family	Genus species	Region	River	R^2	96h-ILUT
Notonemouridae#	*Aphanicerca*	WC	SG	0.985	**23.3**
Notonemouridae	*Aphanicerca*	WC	EE	0.907	**21.7**
Notonemouridae	*Aphanicerca*	WC	RO	0.969	**20.8**
Notonemouridae	*Aphanicerca*	WC	WI	0.935	**21.9**
Notonemouridae	*Afronemoura*	EC	MU	*	**25.8**
Perlidae	*Neoperla*	KZN	ST	0.523	**20.6**
Perlidae	*Neoperla*	KZN	MZ	0.887	**18.8**
Perlidae	*Neoperla*	MPU	SU	0.654	**21.9**
Heptageniidae#	*Afronurus*	WC	MO	0.984	**26.5**
Heptageniidae	*Afronurus*	EC	MU	0.977	**22.2**
Heptageniidae	*Afronurus*	EC	ML	0.993	**26.1**
Heptageniidae	*Afronurus*	KZN	MZ	0.595	**23.3**
Leptophlebiidae#	*Castanophlebia*	WC	WG	0.983	**26.3**
Leptophlebiidae	*Castanophlebia*	WC	EE	0.956	**21.7**
Leptophlebiidae	*Choroterpes*	SC	KA	0.999	**27.4**
Leptophlebiidae	*Choroterpes*	SC	TO	0.797	**28.8**
Leptophlebiidae	*Adenophlebia*	EC	MU	0.997	**26.1**
Leptophlebiidae	*Adenophlebia*	EC	ML	0.993	**25.1**
Leptophlebiidae	*Adenophlebia*	KZN	ST	0.976	**19.8**
Leptophlebiidae	*Euthraulus*	KZN	ST	0.982	**20.9**
Leptophlebiidae	*Euthraulus*	KZN	MZ	High mortality (21.7 for 24h)	
Leptophlebiidae	*Euthraulus*	MPU	SU	0.918	**24.9**
Teloganodidae	*Lestagella*	WC	WG	0.892	**23.3**
Teloganodidae	*Lestagella*	WC	EE	0.754	**23.1**
Teloganodidae	*Lestagella*	WC	MO	0.955	**20.1**
Teloganodidae	*Lestagella*	WC	WI	0.861	**19.3**
Teloganodidae	*Lestagella*	WC	RO	0.992	**19.9**
Teloganodidae	*Lestagella*	SC	KA	0.876	**23.6**
Teloganodidae	*Lestagella*	SC	TO	0.991	**27.1**
Tricorythidae	*Tricorythus*	EC	MU	0.975	**24.0**
Tricorythidae	*Tricorythus*	EC	CA	0.884	**22.1**
Tricorythidae	*Tricorythus*	KZN	ST	0.864	**21.6**
Tricorythidae	*Tricorythus*	KZN	MZ	0.924	**20.1**
Tricorythidae	*Tricorythus*	MPU	SU	0.833	**25.2**
Philopotamidae#	*Chimarra*	WC	MO	0.879	**26.0**
Philopotamidae	*Chimarra*	SC	KA	High mortality (25.5 for 24h)	
Philopotamidae	*Chimarra*	SC	TO	0.857	**24.8**
Philopotamidae	*Chimarra*	KZN	MZ	High mortality (24.2 for 24h)	
Philopotamidae	*Chimarra*	MPU	SU	0.676	**25.1**
Blephariceridae	*Elporia*	WC	SG	0.735	**21.6**

**regression based on 2 data points only due to low mortality for 24 & 48 hours; # from Dallas and Ketley (2011)*

5.3.3 Comparison of upper thermal limits of aquatic macroinvertebrates amongst rivers within a region – the Western Cape

5.3.3.1 Critical thermal maxima

CT_{max} values for *L. penicillata* varied significantly amongst sites (Kruskal-Wallis test: H =102.6968, $p < 0.05$), with lowest values from the Molenaars and Eerste Rivers (29.1°C and 30.1°C respectively) and highest from the Holsloot, Wit and Window Gorge Rivers (34.8°C, 34.7°C and 34.1°C respectively) (Figure 5.8). Comparison of site pairs indicated that the CT_{max} values were not significantly different amongst the following groups of rivers: the Eerste and Molenaars Rivers; the Holsloot, Window and Wit; and the Holsloot, Window and Rooielskloof Rivers. CT_{max} values for *A. capensis* varied significantly amongst sites (Kruskal-Wallis test: H = 34.13140, $p < 0.05$), with lowest values from the Eerste and Molenaars Rivers (28.1°C and 29.4°C respectively) and highest from the Wit and Holsloot Rivers (32.3°C and 31.9°C respectively). Comparison of site pairs indicated that the CT_{max} values were not significantly different amongst the following groups of rivers: the Eerste, Molenaars and Rooielskloof Rivers; and the Holsloot and Wit Rivers. Experiments were not undertaken on *A. capensis* from Window Gorge but previous research reports a CT_{max} of 30.7°C (Dallas and Ketley, 2011).

5.3.3.2 Incipient lethal upper temperature

96h ILUT were estimated from regression equations generated from four and ten day LT_{50} trials (Table 5.6). R^2 values were consistently higher for the four day trials, while estimated 96h ILUT were lower for estimates based on the four day trials compared to estimates based on the ten day trials, with the exception of notonemourids from the Molenaars River. 96h ILUT based on the four day trials ranged from 19.3°C for the *L. penicillata* from the Wit River to 23.3°C for Window Gorge. 96h ILUT based on the four day trials ranged from 20.8°C for the *A. capensis* from the Rooielskloof River to 23.2°C for Window Gorge.

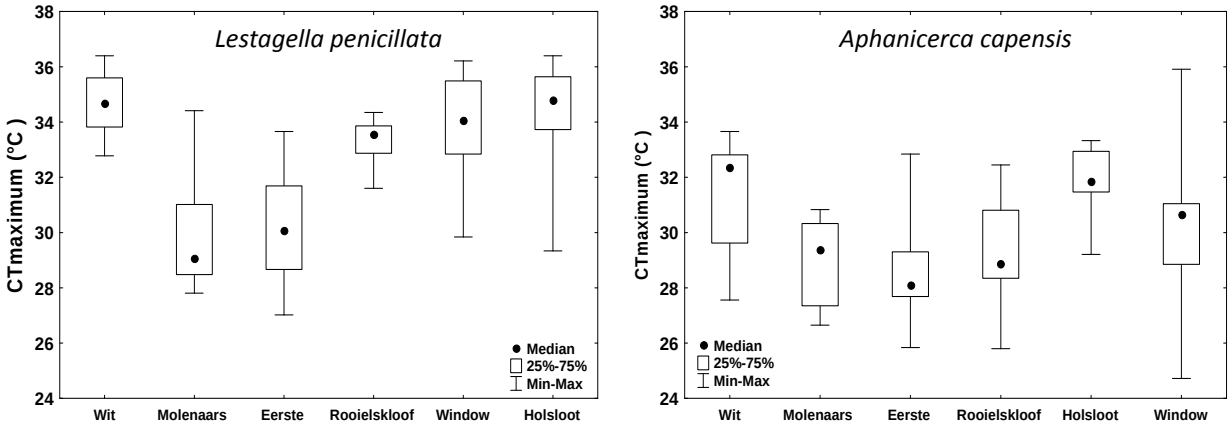


Figure 5.8. Median CT_{max}, 25th and 75th percentile and minimum and maximum values (°C) for *Lestagella penicillata* and *Aphanicerca capensis* and from six rivers

Table 5.6. 96h ILUTs estimated from 4 and 10 day LT_{50} experiments for *Lestagella penicillata* and *Aphanicerca capensis*. R^2 values are provided.

		4 Day Trial		10 Day Trial	
	River	**R^2**	**96h-ILUT**	**R^2**	**96h-ILUT**
Lestagella penicillata	Wit	0.861	**19.3**	0.499	**20.8**
	Molenaars	0.955	**20.1**	0.668	**22.4**
	Eerste	0.754	**23.1**	0.514	**24.1**
	Rooielskloof	0.992	**19.9**	High mortality	
	Window Gorge	0.892	**23.3**	0.764	**24.2**
	Holsloot	0.707	**22.3**	0.549	**23.1**
Aphanicerca capensis	Wit	0.935	**21.9**	0.602	**22.3**
	Eerste	0.907	**21.7**	0.742	**23.3**
	Rooielskloof	0.969	**20.8**	0.947	**21.0**
	Window Gorge+	0.916	**23.2**		
	Holsloot #	0.701	**22.4**	0.390	**23.1**

+ Data from Dallas and Ketley, 2011

5.3.4 Temporal variation in upper thermal limits of aquatic macroinvertebrates

5.3.4.1 Critical thermal maxima

CT_{max} values for *P. nigroculus* varied significantly amongst months (Kruskal-Wallis test: H = 169.5711, $p < 0.05$), with lowest values in October 2013 (24.7°C) and highest in February 2013 (29.0°C) (Figure 5.9). Comparison of pairs indicated that generally the CT_{max} values were significantly amongst seasons, where late summer (Feb) was different to winter (Jun and Aug), while differences between spring (Oct), early summer (Dec) and autumn (Apr) were less distinct and more variable. CT_{max} values for *L. penicillata* varied significantly amongst months (Kruskal-Wallis test: H = 101.3255, $p < 0.05$), with lowest values in April 2013 (30.2°C) and highest in April 2014 (36.9°C) (Figure 4). Comparison of pairs indicated that CT_{max} values were significantly different between summer (Dec) and winter (Aug), while differences between spring (Oct) and autumn (Apr) were less distinct and more variable, with interannual differences evident. Note that organisms were 1st and 2nd instars in February and thus could not be used in CTM experiments.

5.3.4.2 Incipient lethal upper temperature

96h ILUT were estimated from regression equations generated from four and ten day LT_{50} trials (Table 5.7). For *P. nigroculus*, 96h ILUT were generally higher for estimates based on the four day trials compared to estimates based on the ten day trials, and there was a seasonal trend, with lowest 96h ILUT in August and October (22.5°C and 22.2°C respectively) and highest in February and April (25.7°C and 25.5°C respectively) (Figure 5.10). A similar seasonal trend was evident for *L. penicillata*, with lowest 96h ILUT in August (21.9°C) and highest in December and April (26.5°C and 26.6°C respectively) (Figure 5.10).

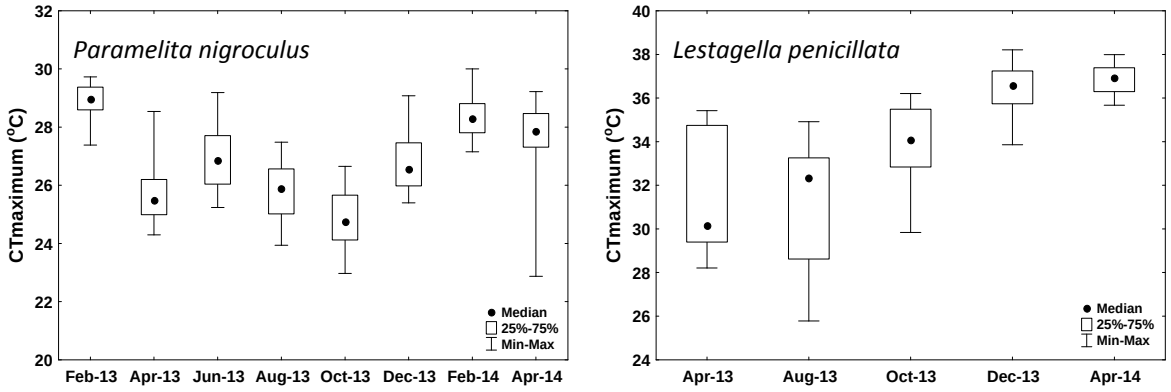


Figure 5.9. Median CT_{max}, 25th and 75th percentile and minimum and maximum values (^{o}C) for *Paramelita nigroculus* and *Lestagella penicillata* from Skeleton and Window Gorge respectively

Table 5.7. 96h ILUTs estimated from 4 and 10 day LT_{50} experiments for *Paramelita nigroculus* and *Lestagella penicillata*. R^2 values are provided.

		4 Day Trial		10 Day Trial	
	River	**R^2**	**96h-ILUT**	**R^2**	**96h-ILUT**
Paramelita nigroculus	Feb-13	0.899	**25.7**	0.920	**25.3**
	Apr-13	0.600	**25.5**	0.889	**25.1**
	Jun-13	0.977	**23.9**	0.995	**23.9**
	Aug-13	0.749	**22.5**	0.595	**23.2**
	Oct-13	0.981	**22.2**	0.982	**21.9**
	Dec-13	0.894	**22.6**	0.734	**23.6**
	Feb-14	0.913	**24.1**	0.983	**23.9**
	Apr-14	0.855	**24.9**	0.929	**24.8**
Lestagella penicillata	Feb-13	*			
	Apr-13	#	**26.1**		**26.3**
	Jun-13	+			
	Aug-13	0.700	**21.9**	0.525	**23.2**
	Oct-13	0.892	**23.3**	0.764	**24.2**
	Dec-13	0.995	**26.5**	0.970	**26.9**
	Feb-14	*			
	Apr-14	0.890	**26.6**	0.674	**27.4**

** Individuals too small to undertake experiments (first instars); # Low mortality at high temperatures prevented the calculation of ILUT and value given is 4 and 10 day LT_{50}; + organisms not collected due to experimental constraints*

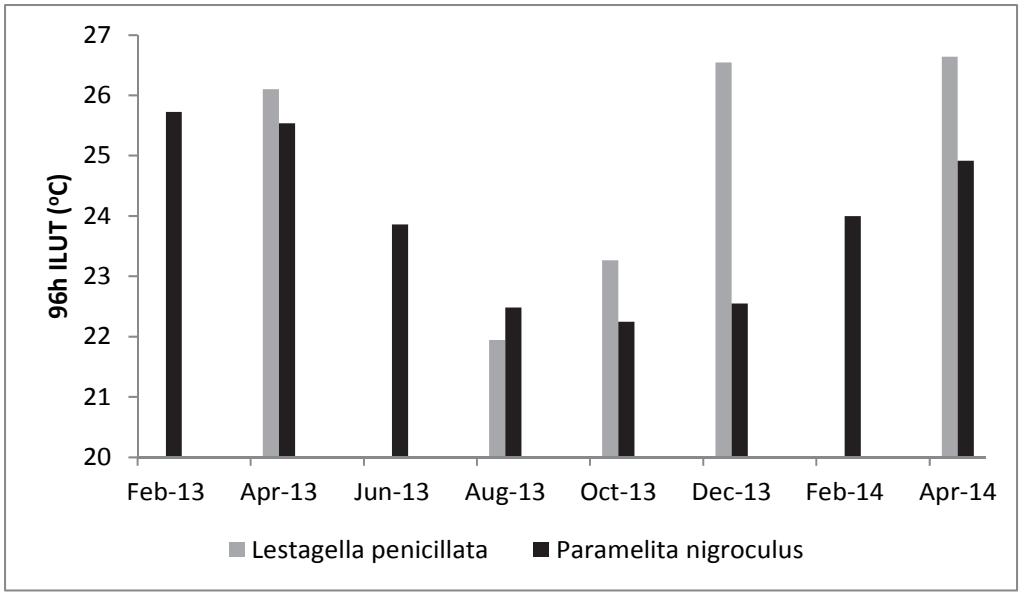


Figure 5.10. 96h ILUT estimates for *Paramelita nigroculus* and *Lestagella penicillata* showing seasonal variation in values

5.3.5 Effect of acclimation temperature and rate of change of temperature on upper thermal limits

5.3.5.1 Acclimation temperature

Organisms were collected from Window and Skeleton Gorge on the 3 November 2013 and were acclimated in aerated aquaria tanks at four different temperatures (10°C, 15°C, 18°C, 21°C) for 96 hours prior to commencement of the CTM experiments. A fifth temperature was included (24°C) for *L. penicillata.*

CT_{max} values for *P. nigroculus* varied significantly amongst acclimation temperatures (Kruskal-Wallis test: H = 54.34312, $p < 0.05$), with the lowest CT_{max} of 22.9°C at the acclimation temperature of 15°C and highest CT_{max} of 26.9°C at the acclimation temperature of 21°C (Figure 5.11) suggesting that CT_{max} increases as acclimation temperature increases. The same trend was however not evident for *L. penicillata* although Dallas and Rivers-Moore (2012) showed that two mayfly species, including *L. penicillata* and *C. calida*, had lower median CT_{max} when acclimated to 10°C in comparison to organisms acclimated to 17°C.

5.3.5.2 Rate of temperature change

Organisms were collected from Window and Skeleton Gorge on the 3 November 2013 and the effect of four rates of temperature change (0.17, 0.34, 0.68 and 1.03°C per minute) on CT_{max} were determined for two species *Paramelita nigroculus* and *Lestagella penicillata* (Table 5.8). CT_{max} values for *P. nigroculus* did not vary significantly amongst rates of temperature change which ranged from 24.1°C to 24.6°C. Mortality was higher at the faster rate of temperature change with ten individuals dying in comparison to one individual at slower rates. In contrast CT_{max} values for *L. penicillata* decreased significantly as rate of temperature change increased (Kruskal-Wallis test: H = 17.69866, $p < 0.05$), ranging from

34.1°C at the slowest rate (0.17°C per minute) to 32.1°C at the fastest rate (1.02°C per minute).

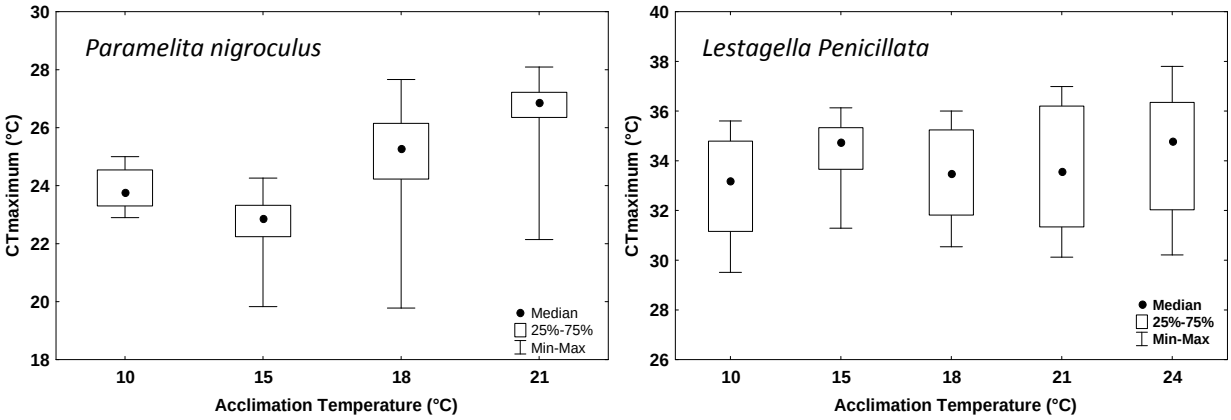


Figure 5.11. Median CT_{max}, 25th and 75th percentile and minimum and maximum values (°C) for *Paramelita nigroculus* and *Lestagella penicillata* at acclimated at different temperatures for 96h

Table 5.8. Median CT_{max} (°C) at four rate of temperature change. n = number of individuals; the number of fatalities are given in parenthesis

Rate of temperature change (°C/minute)	*Paramelita nigroculus*		*Lestagella penicillata*	
	n	**Median**	**n**	**Median**
0.17	30 (1)	24.1	30 (1)	34.1
0.34	29 (1)	24.4	29 (1)	35.1
0.68	29 (4)	24.3	27 (2)	34.4
1.02	22 (10)	24.6	28 (2)	32.1

5.3.6 Variation of upper thermal limits amongst genera or species within families

CT_{max} values were determined for a number of species in the following families: Notonemouridae, Leptophlebiidae and Teloganodidae (Table 5.9).

Notonemouridae

Median CT_{max} was significantly lower for *Aphanicercopsis tabularis* (29.2°C, n = 20, $p < 0.05$) compared to *Aphanicerca capensis* (30.7°C, n = 30) from Window Gorge and similarly median CT_{max} was significantly lower for *Aphanicerca capensis* (29.4°C, n = 10, $p < 0.05$) compared to *Desmonemoura pulchellum* (33.5°C, n = 11) from the Molenaars. No significant differences were observed between *Aphanicerca capensis* (31.9°C, n = 14) and *Desmonemoura pulchellum* (32.6°C, n = 11) from the Holsloot, although number of organisms are low.

Leptophlebiidae

Significant differences were observed amongst species within Leptophlebiidae, with median CT_{max} of 34.1°C (n = 15) for *Adenophlebia peringueyella*, 33.8°C (n = 23) for *Aprionx perterseni* and 33.0°C (n = 28) for *C. calida* from the Eerste, although this was attributed to differences between *A. peringueyella* and the other species, which were not different from one another. Median CT_{max} was significantly different for two species from the Sterkspruit (32.4°C for *Euthraulus* sp. and 35.7°C for *Adenophlebia* sp.)

Teloganodidae

Median CT_{max} was significantly lower for *Lithogloea harrisoni* (27.1°C, n = 30, $p < 0.01$) compared to *L. penicillata* (32.8°C, n = 30) from the Wit River, although no significant differences were observed between *L. penicillata* (31.6°C, n = 51) and *Ephemerellina barnardi* (32.3°C, n = 12) from the Rooielskloof river.

Table 5.9. Median CT_{max} (°C) for each species tested. n = number of individuals. Significant differences between species within families and sites are indicates with *

Family	River	*Genus species*	n	Median		
Notonemouridae	Window	*Aphanicercopsis tabularis*	20	29.2	*	
		Aphanicerca capensis	30	30.7		*
	Molenaars	*Aphanicerca capensis*	11	29.4	*	
		Desmonemoura pulchellum	11	33.5		*
	Holsloot	*Aphanicerca capensis*	14	31.9	*	
		Desmonemoura pulchellum	11	32.6	*	
Leptophlebiidae	Eerste	*Aprionx perterseni*	62	32.3	*	
		Adenophlebia peringueyella	15	34.1		*
		Castanophlebia calida	57	33.1	*	
	Sterkspruit	*Euthraulus sp.*	32	32.4	*	
		Adenophlebia sp.	29	35.7		*
Teloganodidae	Wit	*Lestagella penicillata*	30	32.8	*	
		Lithogloea harrisoni	30	27.1		*
	Rooielskloof	*Lestagella penicillata*	51	31.6	*	
		Ephemerellina barnardi	12	32.3	*	

5.3.7 MWAT Thresholds

Optimum temperatures calculated from hourly temperature data at sites where each taxon was present varied from 13.5^{o}C for the amphipod, *Paramelita nigroculus*, to 18.2^{o}C for the leptophlebid mayfly, *Euthraulus* sp. MWAT thresholds varied from 15.8^{o}C for the mayfly *Adenophlebia* in the Sterkspruit River to 20.9^{o}C for the mayfly *Lestagella penicillata* in the Touws River (Table 5.10). Using this information it is possible to generate a threshold temperature for each taxon. How these MWATs link to 7-day moving averages of mean, minimum and maximum temperatures is explored in chapter 8.

5.4 Summary and conclusions

Based on the results above the following conclusions may be drawn with respect to estimates of lethal limits for aquatic macroinvertebrate taxa studied.

- Thermal regimes differed substantially amongst rivers, ranging from cool headwater systems to warmer lowland ones. Predictability and variability account for additional differences. There was some grouping of sites based on region, although this was not consistent.
- CT_{max} values varied significantly amongst regions for all families examined. Differences were often evident amongst winter versus summer rainfall regions, although other aspects such as different genera amongst regions, and different sampling season also contributed to observed differences. 96h ILUT varied amongst regions for several taxa with the exception of philopotamids (WC, SC and MPU) and blephariceridis (only determined for WC). Differences were observed amongst winter and summer rainfall regions, although this was not consistent.
- Within the winter rainfall region of the WC, CT_{max} values varied significantly amongst sites, and 96h ILUT varied by approximately 2^{o}C amongst sites.
- CT_{max} values and 96h ILUT varied significantly amongst months with distinct differences between summer (highest) and winter (lowest), and less distinct and more variable differences in spring and autumn.
- The influence of acclimation temperature on thermal limits was unclear with amphipod exhibiting a trend of increasing CT_{max} as acclimation temperature increased, although this was not evident for mayflies.
- Similarly the effect of the rate of temperature change varied amongst taxa, with amphipods showing no response, while CT_{max} values for mayflies decreased as rate of temperature change increased.
- CT_{max} values varied significantly amongst genera within families for five of the seven comparisons undertaken.
- MWAT thresholds varied amongst taxa and rivers and ranged from 15.8^{o}C to 20.9^{o}C.

From these thermal experiments it is clear that both spatial and temporal variation in upper thermal limits is evident across a range of taxa. The extent to which this influences the generation of biological temperature criteria is explored in chapter 8.

Table 5.10. Maximum Weekly Allowable Temperature (MWAT) calculated for taxa where 96h ILUT were estimated. The optimum temperature (OT) calculated from hourly water temperature data is given. (Site codes are given in Table 5.1)

		MWAT (°C)													
		OT	**WG**	**SG**	**EE**	**MO**	**WI**	**RO**	**KA**	**TO**	**CA**	**MU**	**ML**	**MZ**	**ST**	**SU**
Paramelitidae	*Paramelita nigroculus*	13.5		17.0												
Notonemouridae	*Afronemoura amatolae*	14.8										18.5				
	Aphanicerca capensis	14.5		17.4	16.9		17.0	16.6								
Perlidae	*Neoperla sp.*	17.7												18.1	18.7	19.1
Heptageniidae	*Afronurus spp.*	16.9				20.1						18.7	20.0	19.0		
Leptophlebiidae	*Adenophlebia spp.*	13.8										17.6	17.3		15.8	
	Castanophlebia calida	14.3	18.3		16.8											
	Choroterpes sp.	15.3							19.3	19.8						
	Euthraulus sp.	18.2													19.1	20.4
Teloganodidae	*Lestagella penicillata*	17.8	20.1		19.6	18.6	18.3	18.5	19.7	20.9						
Tricorythidae	*Tricorythus sp.*	16.5									18.4	19.0		17.7	18.2	19.4
Philopotamidae	*Chimarra sp.*	16.4			19.6					19.2						19.3
Blephariceridae	*Elporia sp.*	14.4		16.8												

Chapter 6. Preliminary investigation of sublethal effects of temperature on aquatic macroinvertebrates

6.1 Introduction

The sublethal effects of temperature may be determined under natural conditions in the field, or under experimental conditions in the laboratory. Chapter 3 provided an overview of the approaches readily adopted to provide an estimate of sublethal effects of changes in temperature on aquatic organisms. This chapter provides an overview of the methodologies and experimental procedures explored during this project. The five categories into which sublethal effects of water temperature can broadly be divided are a) phenological effects, b) physiological and metabolic effects, c) effects on reproductive success and fitness, d) behavioural effects and e) broad scale ecological effects. Several of these were explored during this study and are discussed in detail below.

6.2 Life-histories (Phenological effects)

6.2.1 Introduction

Gathering, examining and understanding information on life-history patterns, through a combination of both field and laboratory work is of fundamental importance for virtually all ecological studies of freshwater invertebrates. Through the collection and assessment of this life-history data the potential effects of changes to hydrological and thermal regimes on aquatic insects commonly used in bioassessment methods (SASS) and used as bioindicators (EPT taxa) can be gauged. Life-history information gathered for the same species from multiple locations, where different environmental conditions prevail, help to determine the degree to which life-history-traits are moderated by these site specific environmental conditions versus being constrained by phylogenetic history.

6.2.2 Methods

Monthly sampling of invertebrates was carried out for the period April 2009-April 2010 in six rivers within the Western Cape. For each monthly sampling event, five replicate riffle sites were chosen and *in situ* measurements of flow and depth were taken. Sampling was performed by gently picking up, shaking and brushing the surfaces of cobbles within the riffle as well as stirring the sediment underneath and surrounding these cobbles, whilst an assistant held a standard square frame kick net (30cm x 30cm x 60cm), fitted with a 80µm mesh in place, no more than two meters downstream. Over allocated and timed periods of one minute per riffle, as many cobbles as possible within the separate riffles were sampled in this manner. After each replicate sample was taken, the collected invertebrates were emptied into separate 500ml plastic ball jars, preserved in 70% ethanol and then returned to the laboratory where they were stored at -20°C to optimize preservation until they could be processed (soft-bodied invertebrates gradually degrade in 70% alcohol). All target organisms collected each month were sorted, counted and hardened body parts for each

taxon were measured to obtain an indication of organism size. Field-collected data were analysed using size frequency histograms for assessing life-history patterns of the study taxa. Linear regression analyses and GLM techniques were then used to assess and interpret life-history data in relation to environmental data (flow, water temperature, physicochemical variables) and to determine tolerances and optima for growth. See Ross-Gillespie (2014) for more details.

6.2.3 Results

Voltinism was determined in each of the target taxa: *L. penicillata* and *Aphanicercella* spp. both exhibited a slow, seasonal univoltine cycle with a single cohort easily tracked throughout the year, while *C. ambulans* showed a non-seasonal or asynchronous multivoltine life cycle with multiple generations occurring simultaneously (Figure 6.1A). *C. ambulans* appeared to show a phenotypically plastic response to temperature, in that more generations (trivoltinism) were observed in warmer rivers, in comparison to univoltine populations observed to occur in colder rivers (see Ross-Gillespie 2014 for comparison of life histories between rivers). *C. ambulans* showed limited or no recruitment during periods of high flow (June-September), while individuals of the *Aphanicercella* spp. emerged as adults during high flow periods (Figure 6.1A). Thus for these two taxa the life cycle appeared to be timed such that larvae and nymphs avoided unfavourable high flow conditions in winter – either through undergoing a pupal stage in the case of the former or emerging as adults in the case of the latter (see Ross-Gillespie 2014 for further details).

The effect of physico-chemical variables on life-history patterns, however, remained somewhat unclear. Optimal thermal ranges for growth were established through the use of GLMs, and were found to be 13-21.5°C for *L. penicillata*, <11.5°C-14.5°C for *Aphanicercella* spp.*,*14.3°C->21.5°C for *C. ambulans*) (Figure 6.1B). More details regarding the GLMs, model parameterisation and results are given in Ross-Gillespie (2014).

6.2.4 Discussion

Ross-Gillespie (2014) found that life-history traits were indeed impacted and modulated by differences in the thermal and hydrological regimes among the sites, where the same species was concerned. This effect was more noticeable in *C. ambulans,* which exhibited less phylogenetic constraint and more flexibility in terms of its life-history. In comparison, *L. penicillata* and *Aphanicercella* spp. showed greater phylogenetic constraint and greater adaptation to site-specific conditions – congruent with molecular analyses (see Ross-Gillespie 2014) that showed higher genetic divergence among sites. Overall, the life-history responses of the target species assessed in Ross-Gillespie (2014) appeared to be finely tuned to the hydrological and thermal regimes of each river studied. This could have been as a result of site specific evolution and adaptation, perhaps showing similarities on a catchment scale. However, where the same species showed differences in life-history responses (number and duration of generations) amongst rivers, the data presented in Ross-Gillespie (2014) appeared to suggest that water temperature was the most likely factor for these differences. The hydrological regime, on the other hand, was found to be the major

driver in determining population size and mortality while possibly imposing a developmental time constraint for life-histories of the study taxa (especially *C. ambulans* and *Aphanicercella* spp.). Ross-Gillespie (2014) could however not discount the possibility that the putative effects of discharge on life-cycle and emergence might reflect synchronicity with availability of key basal resources, or the effects of seasonal conditions on adult fitness.

6.3 Growth rates/rearing (Physiological and metabolic effects)

6.3.1 Introduction

Knowledge of aquatic individuals' thermal tolerance limits, as well as an understanding of the sublethal effects of temperature on other phenological traits (e.g. emergence, growth, egg development and hatching, and fecundity), is of critical importance in determining not only an individual's life cycle but also the potential response of ecosystems to altered thermal regimes. Additionally, where such information can be coupled with fundamental life-history data in conjunction with long-term temperature data the ability to further model and forecast ecosystem responses (based on predicted future climate change scenarios) then becomes possible (see Ross-Gillespie 2014 and Rivers-Moore *et al.* 2013b for further discussion). Collecting such data from the field can be complicated and difficult to achieve and is thus better suited to being collected through rearing/mortality experiments conducted under controlled laboratory conditions. Ross-Gillespie (2014) reports on the results of laboratory rearing experiments that were conducted on two genetically distinct lineages of the larger putative species complex of *L. penicillata*, collected from the Window Gorge and Molenaars River sites. The major aim was to assess whether differences existed between the lethal and sublethal effects of temperature on these individuals specifically in terms of nymphal growth rates, upper Lethal Temperature (LT_{50}) mortality, and timing of emergence. Importantly, suspected differences in growth rates among *L. penicillata* populations (Molenaars River and Window Gorge) were experimentally evaluated before genetic analyses had been completed.

6.3.2 Methods

Using a novel, thermostatically controlled, flow-through system design, Ross-Gillespie (2014) assessed the sublethal effects of temperature on growth rates, body size, and the timing of emergence in conjunction with thermal tolerance limits (using a static LT_{50} experimental procedure) for a range of water temperature treatments within a CE room.

Early instar nymphs of the mayfly *Lestagella penicillata* were collected from two sites previously utilised (see Ross-Gillespie 2014), namely the Molenaars River and Window Gorge, in late September/early October 2011 and transferred to a constant environment room (with a 12 hour constant photoperiod provided by full spectrum fluorescent lighting) where they were acclimated to ambient stream temperatures (±10°C) for 3 days. After initial acclimation 60 nymphs were individually photographed under a dissecting microscope (Leica EZ D) fitted with a digital camera.

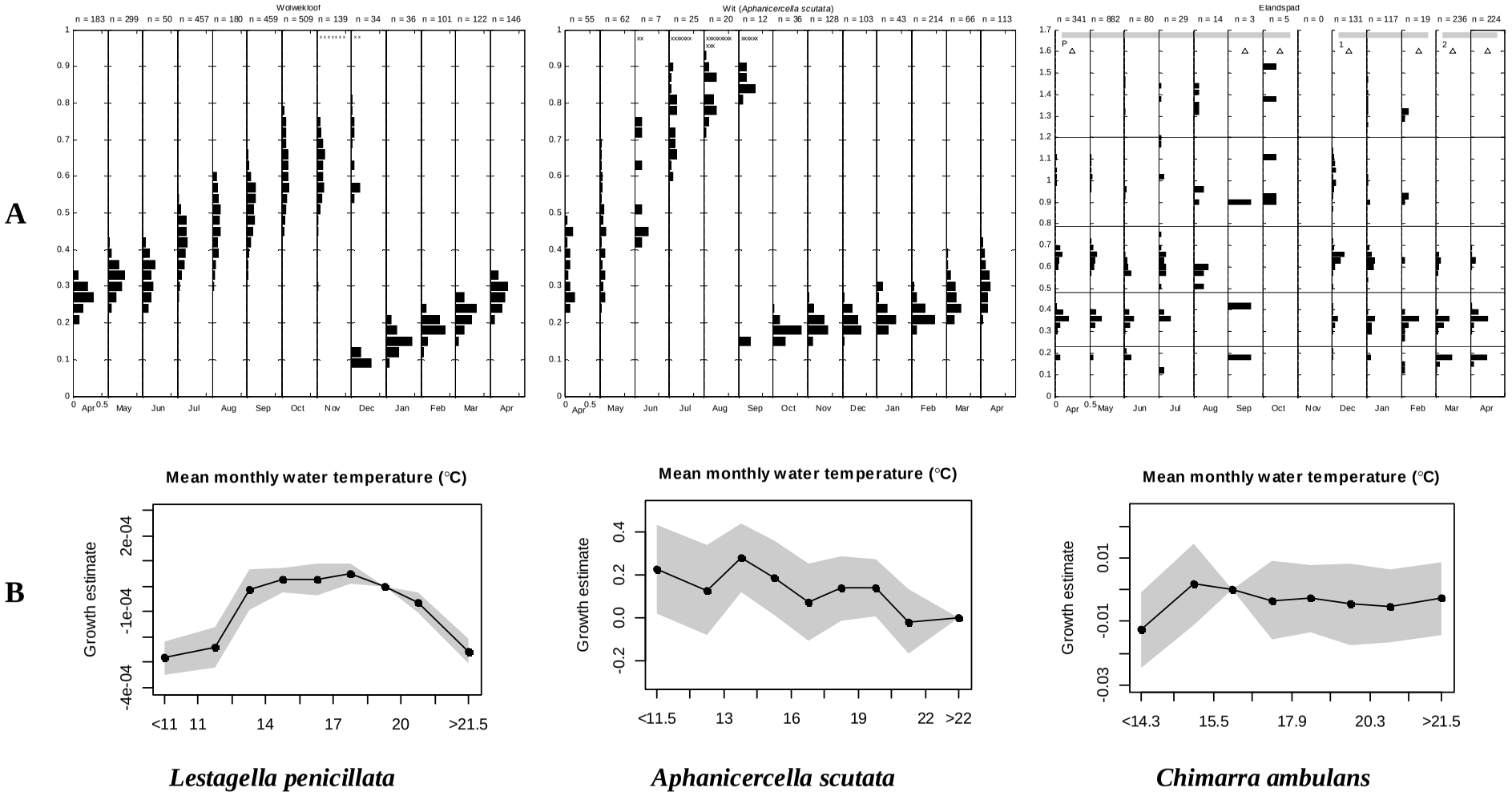


Figure 6.1 Examples of life-history plots (A) and the effect of factor levels of water temperature on the growth coefficient based on GLM modelling (B) for three species of aquatic insect. Life-history plots represent data from monthly samples collected from one of the six sample rivers in the Western Cape, South Africa (April 2009-April 2010). For the life-history plots the y axis represents a measure of size (mm) based on measurements of different hardened body parts for each species – Interocular Distance (IOD) for Lestagella penicillata, Head Capsule Width (HCW) for Aphanicercella scutata and Head Capsule Length (HCL) for Chimarra ambulans. Bar length in the life-history plots indicates relative numbers of individuals for a given size class in each month. Dashed horizontal lines indicate separation of larval instars (for Chimarra only). Grey bars with corresponding numbers 1, 2, or 3 indicate the number and estimated duration of multiple generations. P denotes a generation from the previous year. Triangles (Chimarra) represent months in which adults were present. Asterisks (Lestagella and Aphanicercella) represent the numbers of adults present in each month. Total number of individuals in the sample each month is given by the n values at the top of the graph. Life history data from all six rivers were used to inform the GLM plots. Grey shading in the GLM plots represents the 95% confidence interval.

For photographs, each individual nymph was carefully placed in a few drops of water on a microscope slide and a cover slip gently placed on top to standardise the measurement process by preventing distortion of the image. Several body size measurements (body length, head capsule width, thorax width and inter-ocular distance) as well instar number/age (number of antennal segments) could then be obtained from each captured digital image at a later stage using Leica software. This method was found not to harm the organisms and provided an accurate way of measuring several size parameters with minimal handling time.

After initial body measurements were recorded, individual nymphs were placed in small glass polytop vials, filled with dechlorinated mains drinking water that was circulated in a reservoir for approximately 24h prior to use, and two small algae covered pebbles for food and attachment (Figure 6.2). The lid of each vial was modified so that half of the lid was cut away allowing for through flow into the vial. A piece of mesh secured under the lid of each vial prevented the organisms from escaping. In the other half of the lid a small hole was punctured. Each vial was secured to a plastic automatic pipette end (which was inserted through the hole in the lid). Each pipette end was securely fitted to a series of plastic t-junctions linking aquarium tubing around the inside edge of each of eight 60L crates. The crates were positioned in the constant temperature room at 10°C where organisms were acclimated. Crates were half filled with dechlorinated mains drinking water that was circulated in a reservoir for approximately 24h prior to use and were each fitted with an aquarium pump (and attached filter canister) connected to the aquarium tubing. Six of the crates were fitted with calibrated aquarium heaters which heated water in the crates to a range of constant temperature treatments from 15-25°C (two crates served as the control at 10°C and did not require heating) (see Table 6.1). The aquarium tubing was held in place to the edge of the crates by S hooks which also served to suspend the tubing above the water level in each crate. The water in each crate was further aerated to saturation levels using airstones on a compressed air line. In total fifteen vials were placed in each of eight 60L plastic crates. The pumps provided flow of heated, aerated reservoir water (water heated, aerated and held in the crates) to each of the vials. The modified lid of each vial allowed for an overflow, such that water was continuously replaced, without the organism escaping.

Organisms were monitored daily for the presence of exuvia which indicated a moult. Where a moult was observed the individual was photographed using the method already described after which it was placed back in the system. Food in each vial was replaced every two weeks when a portion of the water in the crates was also replaced with fresh water. Organisms were monitored in this way until emergence or death. Growth rates were calculated from body size measurements and related to water temperature.

Table 6.1. Summary of water temperature data collected from experimental temperature chambers used in the growth and LT_{50} experiments. The total number of *L. penicillata* (n) from the Window Gorge and the Molenaars River study sites exposed at each temperature treatment are indicated. CON denotes control temperatures. Table from Ross-Gillespie (2014).

	Experimental temperature chambers							
	10#1^{CON}	**10#2^{CON}**	**15#1**	**15#2**	**20#1**	**20#2**	**25#1**	**25#2**
Mean (°C)	10.68	10.70	15.76	16.83	19.76	21.75	25.61	26.96
Maximum (°C)	11.32	11.47	16.25	19.03	20.46	22.71	25.87	28.19
Minimum (°C)	10.39	10.39	15.03	15.58	17.77	18.87	23.49	24.34
Std. deviation.	0.096	0.116	0.180	0.313	0.400	0.557	0.226	0.251
Window (n)	7	8	7	8	8	7	7	8
Molenaars (n)	8	7	8	7	8	7	7	8

6.3.3 Results

Ross-Gillespie (2014) found that the results of the experiment confirmed estimates of thermal optima for growth obtained from modelling procedures performed on life-history data. In addition the experiment allowed for life-history trait differences between two genetically distinct lineages to be evaluated and contrasted under common environmental conditions, providing insight into the degree to which genetic differences vs. phenotypic plasticity affect these traits (Ross-Gillespie 2014). In particular, individuals from both Window Gorge and the Molenaars River showed optimal growth rates converging at between 16-18°C (Figure 6.3) in accordance with GLM estimates for optimal growth of this species (Figure 6.1B), possibly suggesting that this is a common thermal optimum range for growth in the genus. Individuals from the Molenaars River, however, showed better growth than those from the Window Gorge individuals at all temperature treatments (Figure 6.3), likely owing to genetic differences in conjunction with adaptation to a warmer thermal regime that is experienced in the Molenaars River.

This conclusion was further supported by the observation that individuals from the Molenaars River showed higher upper LT_{50} limits than those from Window Gorge (Figures 6.4 and 6.5). Individuals of *L. penicillata* from the Molenaars River exhibited upper LT_{50} temperatures that were higher than those from the Window Gorge (23.2°C vs. 26.5°C) after the 168 h duration. The proportion of mortality after 168 h at each temperature treatment for *L. penicillata* from the Window Gorge and the Molenaars River sites are shown in Figures 6.4 and 6.5 respectively. *L. penicillata* from Window Gorge experienced 100% mortality at both the 25.6°C and the 26.9°C treatment after 168 h whereas mortality proportions of only 28.6% and 62.5% were recorded for individuals from the Molenaars River at the same temperature treatments after the same duration exposure. A maximum of ca. 68% mortality occurred in the Molenaars River population at 27.5°C.

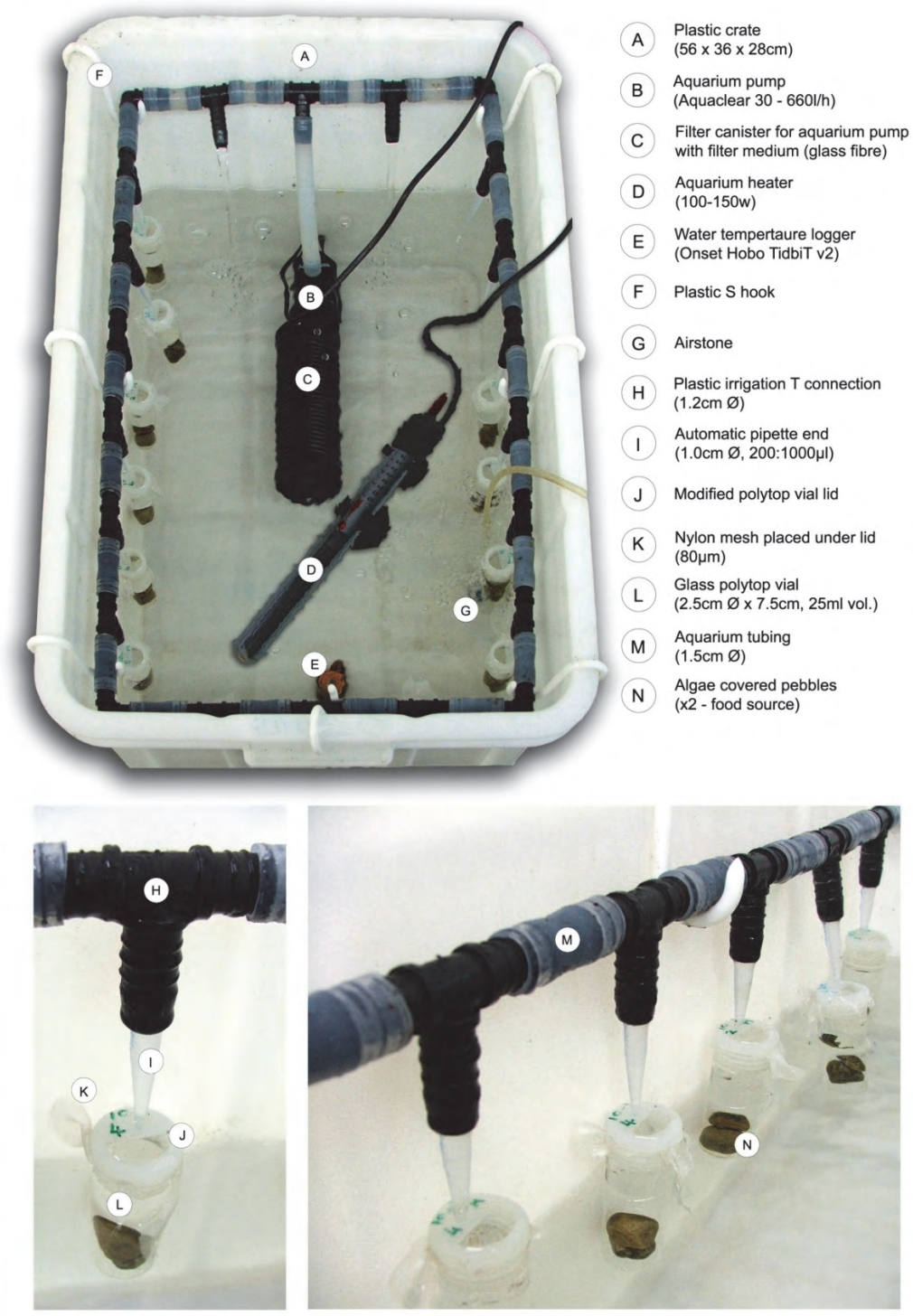


Figure 6.2. Design of temperature chambers and organism housings used in growth and LT_{50} experiments. Diameter is denoted by the symbol Ø. Figure from Ross-Gillespie (2014).

6.3.4 Discussion

The innovative flow-through system provided an effective environment for the rearing and monitoring of *L. penicillata.* Furthermore it served the dual purpose of providing an experimental environment for long-term LT_{50}s (lethal thermal tolerance experiments). Ross-Gillespie (2014) found that thermal optima for growth calculated during the rearing experiments were congruent with those calculated using modelling techniques based on the field-collected life history data. This highlights the importance of conducting laboratory experiments in conjunction with field studies. Further, it was found that the delayed timing of emergence of *L. penicillata* collected from both sites in laboratory experiments when compared to natural populations appeared to be an adaptive plastic response in the life-history trait of this species. Further differences in the timing of emergence were observed between individuals collected from the two sites; these too are also thought to be as a result of the different natural thermal regimes experienced at the two sites to which the species have become adapted. Notably, body size and the number of moults appeared to be regulated by the inter-moult duration, which generally decreased with increasing temperature. Differences under the same temperature treatments were again observed between individuals from the Molenaars River and Window Gorge, providing further evidence of genetic differentiation between the two lineages. More comprehensive results and discussions are given in Ross-Gillespie (2014).

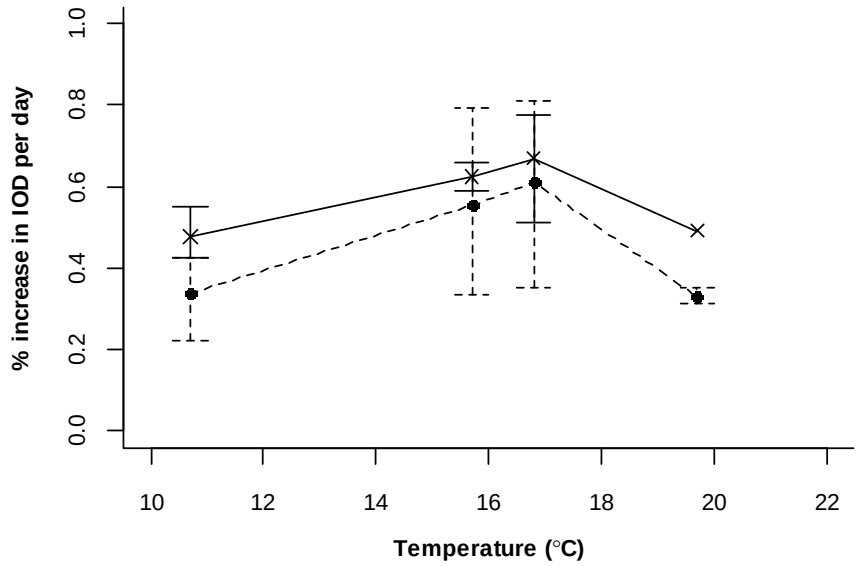


Figure 6.3. Average growth of *L. penicillata* (percentage increase in interocular distance – IOD – per day) from collected from the Window Gorge and the Molenaars River sites at different experimental temperature treatments. Dashed line with closed circles denotes Window Gorge, solid line with crosses denotes Molenaars River. Bars indicate range of growth values. Figure from Ross-Gillespie (2014).

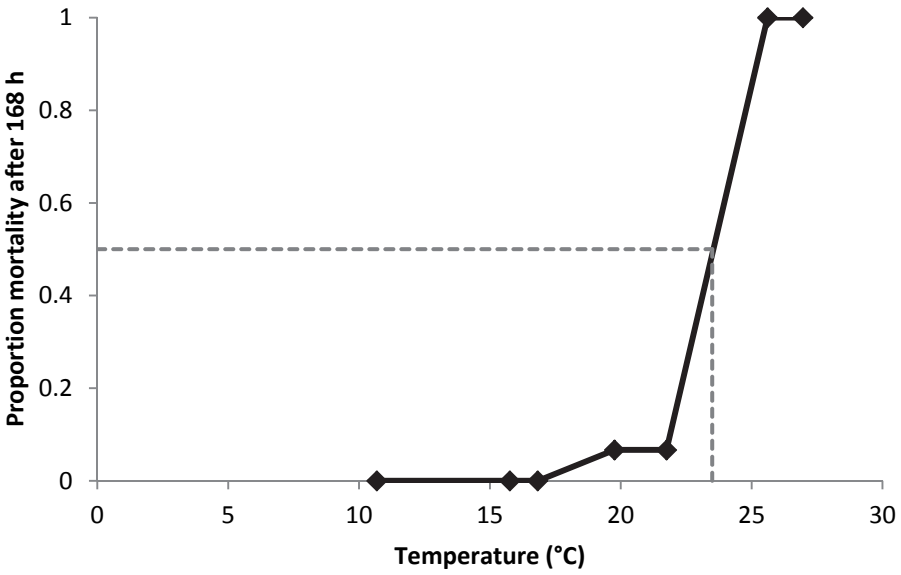


Figure 6.4. The proportion mortality at each temperature treatment of *L. penicillata* collected from Window Gorge calculated after 168 h exposure. The dashed grey line indicates 50% mortality. Figure from Ross-Gillespie (2014).

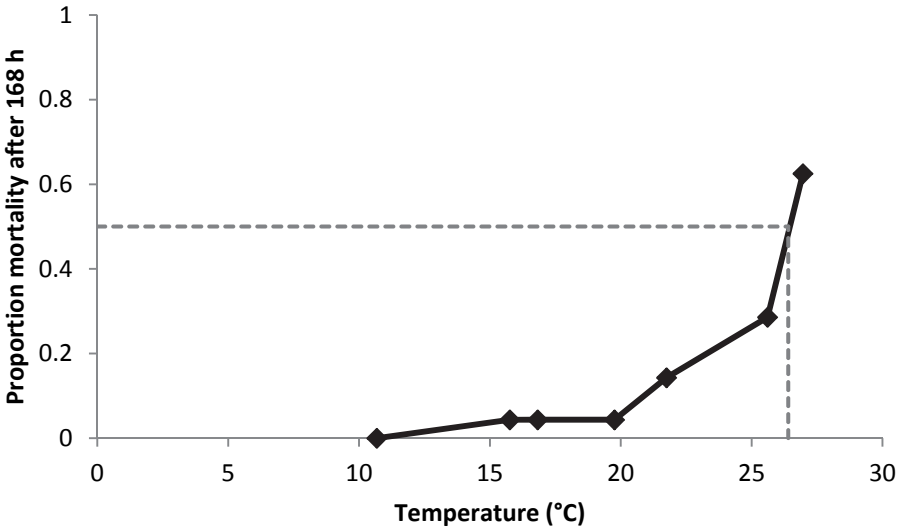


Figure 6.5. The proportion mortality at each temperature treatment of *L. penicillata* collected from the Molenaars River calculated after 168 h exposure. The dashed grey line indicates 50% mortality. Figure from Ross-Gillespie (2014).

6.4 Egg development (Effects of reproductive success and fitness)

6.4.1 Introduction

Data relating to the egg stage are often overlooked in life-history studies of aquatic insects, yet this stage comprises a crucial component of the life-history cycle and is suitable for experimental testing in laboratory conditions. An understanding of the egg stage, including egg development time and hatching success, is necessary for the correct interpretation of total life cycle duration, timing of emergence and hatching, population size and also environmental limits for successful development. Where such information can be collected through experimental testing in the laboratory, in conjunction with field experiments and life-history data, it can provide a wealth of fundamental information necessary for the establishment of conservation guidelines, while forming a platform for further studies on aquatic ecosystems. Currently in South Africa data relating to the egg stage of aquatic invertebrates represents a major knowledge gap for freshwater ecological research. To help address this gap, Ross-Gillespie (2014) conducted a study to investigate the sublethal effects of water temperature on the egg stage (specifically on egg development time, length of the hatching period and hatching success) of three target species representing the Ephemeroptera, Plecoptera and Trichoptera (EPT taxa). The three species were *Lestagella penicillata*, *Aphanicercella scutata* and *Chimarra ambulans*. Experiments were conducted under controlled environment conditions in the laboratory.

6.4.2 Methods

In his study, Ross-Gillespie (2014) collected eggs of each species, counted them and separated them into batches of approximately 100-200 eggs. Two to four replicate batches were then placed in water-filled petri dishes[2] (±25ml), which were floated in each of six water-filled glass aquarium tanks (Figure 6.6). Each tank was equipped with a calibrated aquarium heater and was set to a constant temperature in order to provide a range of temperatures from 5-30°C at 5°C intervals. The tanks were situated in a constant temperature environment set to 5°C. The environment was also equipped with full spectrum lighting on a time switch to allow for adjustable photoperiod, though photoperiod has been found to have little or no effect on egg development of aquatic insects (Brittain 1982).

The first tank in this controlled environment served as the control (which in this instance could be the temperature at which no development is expected) or the ambient natural temperature. The eggs in each dish were then checked daily for signs of development and hatching. Development of replicate egg batches at each temperatures treatment were

[2] Eggs can also be placed in water-filled beakers, which are subsequently placed in water-filled glass aquarium tanks or in separate water baths, such that the level of water is just below the lip of the beaker.

tracked through time at regular intervals (every 2-5 days) using digital photographs – taken with a dissecting or compound microscope fitted with a digital camera.

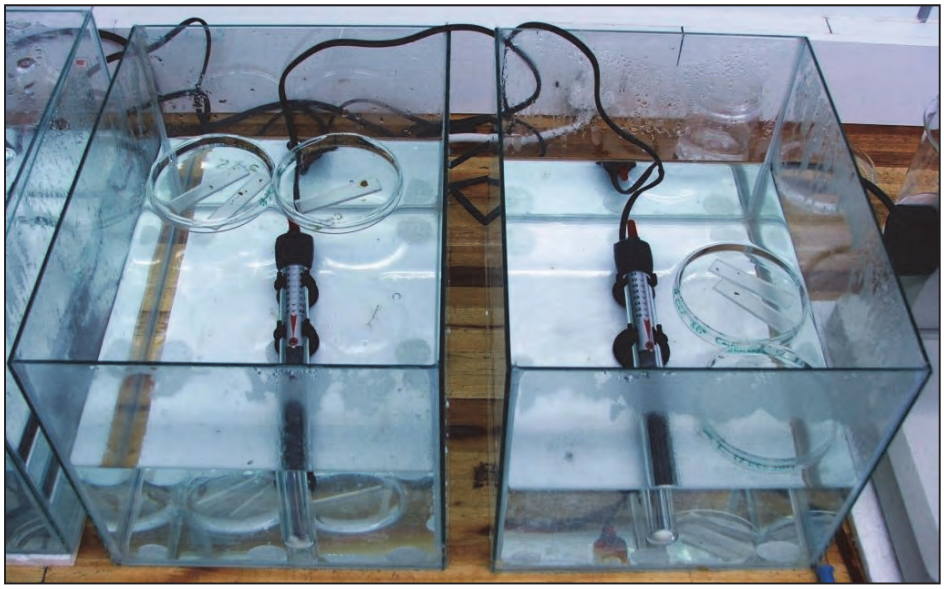


Figure 6.6. Aquarium tanks, heaters and petri dishes for egg hatching experiments

At the first signs of hatching, newly emerged hatchlings each day or time period were removed and counted. Removed hatchlings can be placed in separate water-filled containers – to monitor juvenile survival at different temperatures, or transferred directly to aerated/flow-through systems for use in growth experiments. Each day from the start of first hatching, the number of hatchlings and unhatched eggs were counted, in order that hatch duration and cumulative hatch percentage per day could be calculated. When no further hatching occurred, the number of unhatched eggs was recorded to provide an indication of the total hatch success at each temperature treatment. Egg development time (calculated as Degree Days – DD > 0°C) required for the mean[3] number of eggs to hatch at each temperature treatment was calculated and plotted to determine the relationship between development time and temperature for each species. Essentially the number of DD > 0°C is calculated as the temperature of the incubation treatment multiplied by incubation time in days until mean hatch is observed. In many species of aquatic insects the relationship between water temperature and the developmental time requirement to hatching (commonly measured in Degree Days (DD)) can be described by a power equation (Brittain 1982, Humpesch 1980a, b, 1984, Pritchard *et al.* 1996), as such, equations were then calculated that best represented the relationship between these values and the incubation temperature treatments.

Care should be taken to prevent fungal and bacterial build up at higher temperatures, as these can be detrimental to eggs, and prevent successful hatching. This can be managed through cleaning the tanks prior to the start of the experiment and if necessary, during the

[3] This refers to when 50% of the eggs that successfully hatched at a given temperature treatment had hatched.

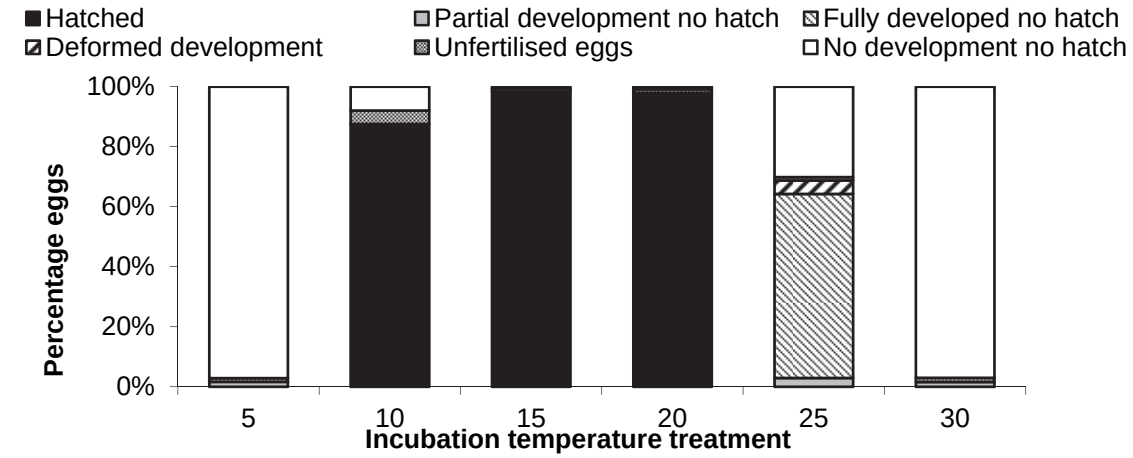
experiment at the first signs of fungal or bacterial build up. Anti-fungicidal products should only be used if pilot studies have been conducted to confirm that the products themselves don't affect hatch success rates or development (Ross-Gillespie 2014).

6.4.3 Results

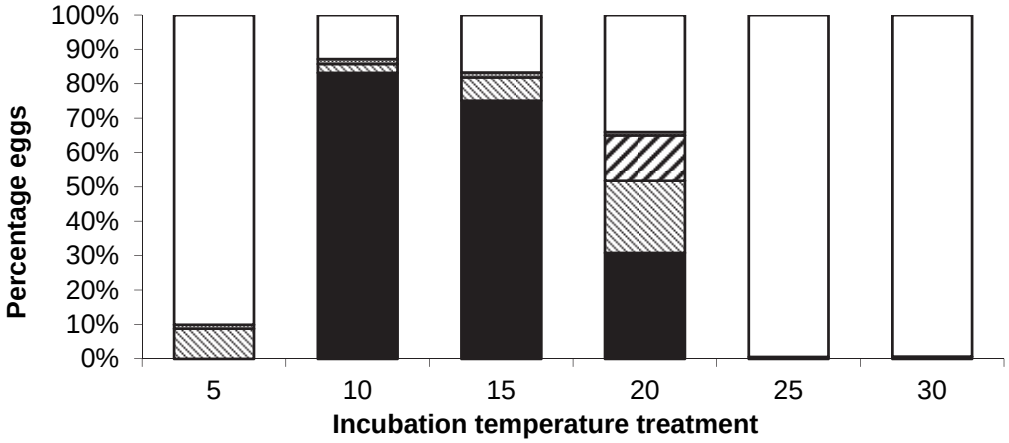
Ross-Gillespie (2014) found that water temperature affected the development of eggs of three genera quite differently. Experiments by Ross-Gillespie (2014) revealed that successful egg development and hatching occurred between 10-20°C for *L. penicillata*, with a high percentage hatch (80%) at 10, 15 and 20°C treatments (Figure 6.7). For *A. scutata* successful hatching occurred also between 10-20°C but with reduced hatching success (~30%) at 20°C compared to ~80% hatching success at 10 and 15°C treatments (Figure 6.7). For *C. ambulans*, successful hatching occurred at a wider range of temperatures from 10-25°C but with lower and more variable hatching success at all temperatures (average hatching success ranged from 5-20%) (Figure 6.7). The DD requirement to mean hatch for *L. penicillata* and *C. ambulans* decreased with increasing temperatures from 10 to 20°C (Figure 6.8). In the 25°C treatment, however, the DD requirement for eggs of *C. ambulans* started increasing (263.3 DD), being on average greater than that observed at in 20°C treatment (252.1 DD). For eggs of *A. scutata*, on the other hand, the DD requirement increased with increasing temperatures (Figure 6.8).

6.4.4 Discussion

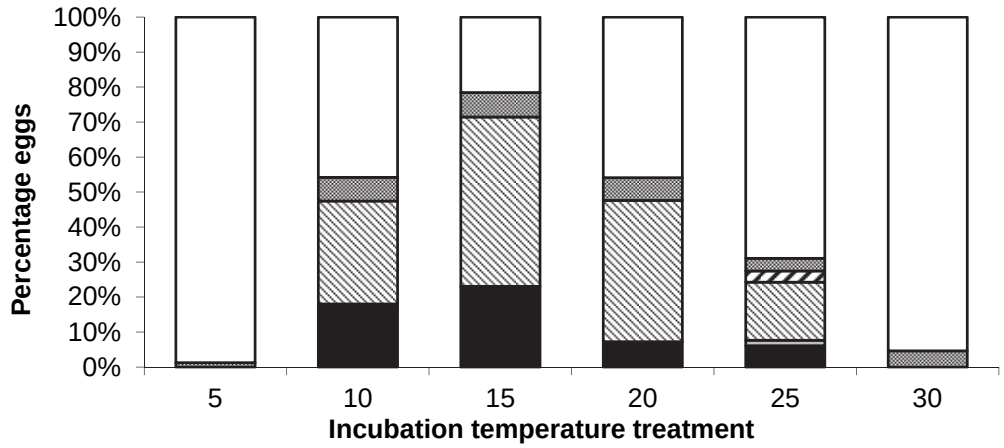
Overall, the data on the egg stage presented by Ross-Gillespie (2014) allowed for a more accurate interpretation of the life-history data presented for *L. penicillata*, *A. scutata* and *C. ambulans* from each study site (see Ross-Gillespie 2014). Lethal thermal limits for egg development along with the sublethal effects of temperature on hatch success provided valuable information that can be used to inform the establishment of thermal guidelines for the Ecological Reserve. Thermal reaction norms calculated for egg development in conjunction with morphological descriptions of the eggs provided useful insights into the evolutionary history, thermal origins/preferences as well as the oviposition behaviour of each species (see Ross-Gillespie 2014). Descriptions of first-instar nymphs were also used to confirm the presence of first-instar nymphs in samples collected from the field that informed life-history data.



A) Lestagella penicillata



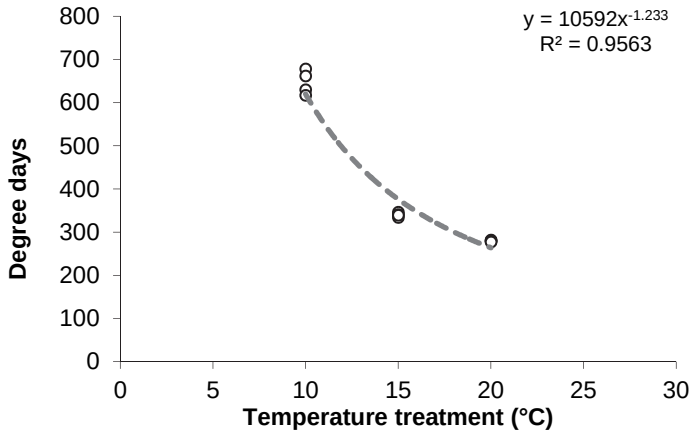
B) Aphanicercella scutata



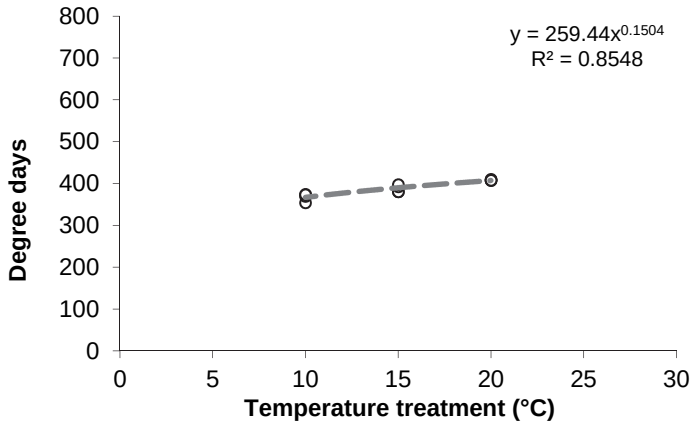
C) *Chimarra ambulans*

Figure 6.7. The percentage of the eggs of three species of South African aquatic insect categorized according to their final developmental outcome at several incubation temperatures (°C). Figure from Ross-Gillespie (2014)

A



B



C

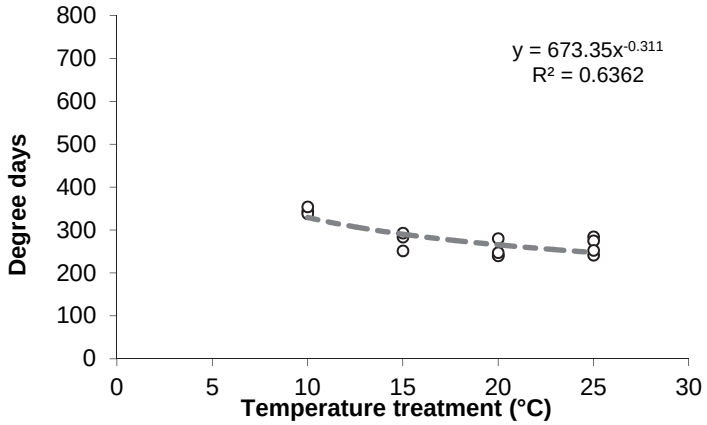


Figure 6.8. The relationship between degree days to mean hatch and temperature for eggs of three species of aquatic insect A) *Lestagella penicillata*, B) *Aphanicercella scutata* and C) *Chimarra ambulans* kept at varying incubation temperature treatments. Figure from Ross-Gillespie (2014)

6.5 Thermal preferences

6.5.1 Introduction

Thermal gradient tanks allow for the estimation of temperatures completely avoided and those preferentially migrated to over time. In this way it provides insight into the range of temperatures that an organism is able to utilise allowing for comparison with sublethal and lethal effects. The horizontal thermal gradient tank described below is a simple, portable design that allows for the estimation of thermal preferences of aquatic macroinvertebrates under experimental conditions.

6.5.2 Methods

The horizontal gradient tank comprised a shallow (ca. 10cm), u-shaped plastic tank (15cm width x3.0m length) which was filled with dechlorinated tap water (Figure 6.9). At one end of the tank a submersible 100W aquarium heater were positioned behind a screen of fine nylon mesh which prevented the heaters coming into direct contact with the organisms. At the other end of the tank, also positioned behind a screen, chilled water was circulated through copper coils, thus acting as a cooling element. Aeration and circulation of water was achieved by using a perforate tube running along the length of the tank, which allowed bubbling along the length of the tank. This setup allowed for a stable linear thermal gradient of 10°C to 25°C. Water temperature was recorded at one hour intervals throughout the experiment using Hobo TidbiTs v2 loggers (Onset Computer Corporation, 2008) placed at 10 cm intervals along the length of the tank. Alternatively digital thermometers could be placed at regular intervals to provide passive, real-time measurements of water temperature at given areas of the tank.

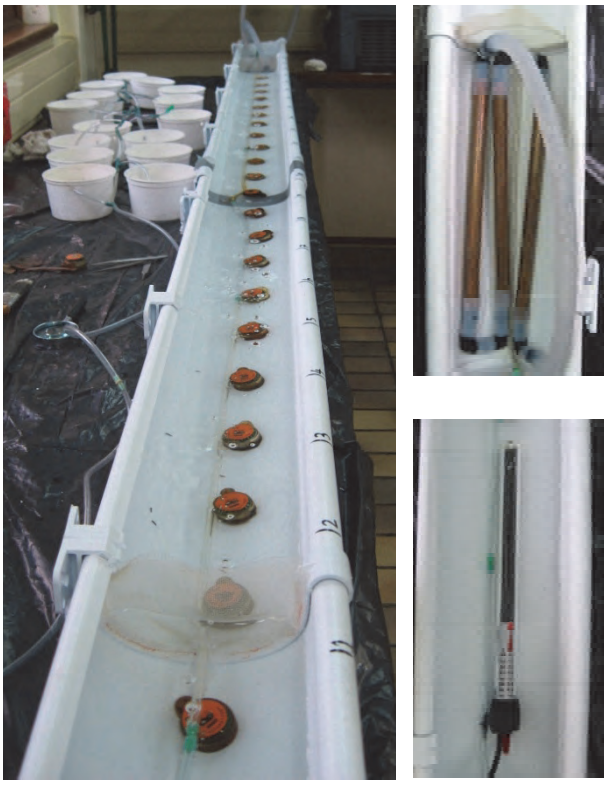


Figure 6.9. Thermal preference tank with water temperature loggers, copper cooling pipe and aquarium heater

Organisms for thermal preference experiments were collected from two rivers in the Southern Cape (Kaaimans and Homtini Rivers) in April 2014 using a using a sampling net of 950 mm diameter mesh. Test organisms were transferred into a collecting bucket, which was kept cool using crushed ice packed around the bucket, and transported to the aquatic

Ecology Laboratory of the Nelson Mandela Metropolitan University George Campus. Prior to tests, organisms were held at ≈ 16°C, which approximated water temperatures at capture, and maintained in aerated tubs filled with dechlorinated tap water until experiments commenced. Experiments commenced after 24 hours and test organisms were not fed during the experiments.

Organisms were added to the tank at a fixed position(s) and allowed to settle for one hour after which their position was noted and they were removed. Two sets of trials were undertaken, the first where organisms were added at a position where the temperature approximated the acclimation temperature (16.5°C); and the second where organisms were added at the two thermal extremes, i.e. 10.7°C and 24.7°C.

6.5.3 Results

Logged water temperatures in the thermal gradient tank were used to generate a "preference temperature" (T_p) for each organism following release and one hour of settling in the tank. This allowed for the calculation of mean T_p for eleven families, including Notonemouridae (Kaaimans: mix of *Aphanicerca capensis*, *Aphanicercella barnardi*, Homtini: *Aphanicerca capensis*), Baetidae (Kaaimans: Homtini), Heptageniidae (*Afronurus barnardi*), Leptophlebiidae (Kaaimans: *Chloroterpes* spp., Homtini: *Aprionyx* sp. and *Castanophlebia* spp.), Teloganodidae (*Lestagella penicillata*), Barbarochthonidae, Leptoceridae Philopotamidae (*Chimarra* spp.), Corydalidae, Dryopidae and Scirtidae (Table 6.1), although not all families were present in sufficient numbers at both the Kaaimans and Homtini Rivers.

Table 6.2. Mean T_p and number of organisms (N) temperatures recorded for each family in trials 1 and 2 from the Kaaimans and Homtini Rivers – indicates insufficient organisms

	Kaaimans		**Homtini**	
	Trial 1	**Trial 2**	**Trial 1**	**Trial 2**
Notonemouridae	18.4 (57)	18.4 (23)	20.0 (31)	18.2 (34)
Baetidae	16.9 (42)	-	18.5 (13)	19.1 (14)
Heptageniidae	17.8 (11)	-	17.3 (7)	-
Leptophlebiidae	16.7 (66)	17.7 (28)	18.3 (46)	16.4 (28)
Teloganodidae	19.2 (56)	19.6 (29)	17.9 (26)	18.2 (42)
Barbarochthonidae	17.3 (31)	-	17.6 (34)	-
Leptoceridae	20.2 (32)	17.4 (30)	16.5 (36)	20.0 (30)
Philopotamidae	18.0 (15)	17.9 (13)	19.7 (36)	19.5 (33)
Corydalidae	-	17.3 (30)	-	-
Dryopidae	-		19.0 (31)	17.8 (30)
Scirtidae	-		15.5 (12)	-

For the Kaaimans River, mean T_p differed significantly (Kruskal-Wallis test: $H = 28.4956$, $p < 0.05$) amongst "family x trial" groups, although differences were largely attributed to Baetidae and Leptophlebiidae for trial 1. Mean T_p ranged from 16.7°C for leptophlebiids to 20.2°C for leptocerids in trial 1 and 17.3°C for corydalids to 19.6°C for teloganodids in trial 2

(Table 6.2). Certain taxa such as leptophlebiids, barbarochthonids and philopotamids did not move substantially from where they were released.

For the Homtini River, mean T_p differed significantly (Kruskal-Wallis test: H = 26.8899, $p < 0.05$) amongst "family x trial" groups. Mean T_p ranged from 15.5°C for scirtids to 20.0°C for notonemourids in trial 1 and 16.4°C for leptophlebiids to 20.0°C for leptocerids in trial 2 (Table 6.2). Certain taxa such as barbarochthonids and philopotamids did not move substantially from where they were released. The overall mean T_p for each family was estimated by combining data from both rivers and trials. Mean T_p was significantly different amongst families (Kruskal-Wallis test: H = 30.5498, $p < 0.05$) although most differences were as a result of differences between Leptophlebiidae which has lower T_p compared to other Notonemouridae and Philopotamidae which had the higher T_p. Most taxa were recorded across the full range of temperatures in the thermal gradient tank suggesting that whilst some families may exhibited a preference the range of temperature (10°C to 25°C) was within their tolerance range.

6.5.4 Discussion

This preliminary exploration of using a thermal gradient tank to estimate thermal preferences of aquatic macroinvertebrates has provided some of the first data on thermal preferences for South African aquatic macroinvertebrate taxa. From a design perspective the tank generated a 14 degree gradient in water temperature which was considered suitable for exploring thermal preferences. The data obtained provide a first estimate of thermal preferences although further experiments are recommended. It has highlighted the variability in migration along the thermal gradient tank amongst different taxa, with some taxa appearing to be very mobile (e.g. baetid and teloganodid mayflies) whilst other are less mobile (e.g. barbarochthonid caddisflies, scirtid beetles and some leptophlebid mayflies). Specifically it is recommended that experiments should include a control tank, of similar design to the thermal tank, but in which no heating and cooling occurs. This would allow for the comparison of position of each taxon in the tank with the presence and absence of a thermal gradient. Further replicate trials should also be undertaken to increase the confidence in the thermal preferences.

Chapter 7. Development of a connectivity index to reflect resilience of rivers to climate change

7.1 Introduction

Globally, freshwater organisms are regarded as highly vulnerable to climate change as a consequence of rising water temperatures, alterations of hydrological regimes, compounding impacts from non-climatic stressors, and dispersal constraints imposed by the fragmentary nature of freshwater environments (Woodward et al. 2010, cited in Chessman 2012). Freshwater ecosystems will be profoundly affected by global climate change, especially those in mountainous areas, which are known to be particularly vulnerable to warming temperatures (Domisch et al. 2011).

These predicted global changes may reduce the resilience of current community states, largely driven by the impacts of extreme hydroclimatic events (Daufresne et al. 2007). Here, resilience refers to buoyancy, or the degree to which something bounces back to resume its original state after stretching, and we explicitly define resilience as a function of connectivity. Breaks in the river continuum differentially affect system resilience based on where they occur along the longitudinal axis. Disruptions can be a function of both physical barriers (dams and natural waterfalls) as well as abiotic factors (abstractions, changes in flow and temperature signatures affecting timing of life history cues and synchronies in the food web).

For reserve design to be resilient requires due consideration of connectivity (Dunlop and Brown 2008). It is increasingly being recognised in studies on species range shifts that species within and across different taxonomic groups will show differential resilience to water temperature changes, with some species 'winners' and others 'losers' (Jackson et al. 2007, Domisch et al. 2011, Eady et al. 2013). Levels of resilience will be promoted or compromised as a result of river connectivity allowing or preventing species from adapting to shifting environmental conditions. Such interactions have already been discussed in greater detail by Dallas and Rivers-Moore (2014) and a suite of tools to begin understanding potential current and future scenarios has been presented. Specific to this chapter is the use of various metrics to quantify river connectivity in terms of lateral, longitudinal and temporal components. The development of a connectivity index formed the basis of an MSc thesis (Ramulifho 2014, submitted) and has largely been achieved using rivers in KwaZulu-Natal as a study area and provides the basis for identifying areas of vulnerability of aquatic biota to climate change effects.

The development of a connectivity index, while useful in itself, is more importantly a means to a greater end, which is as a tool to make decisions about how to invest limited human and economic resources for maximum conservation benefit. It therefore has value beyond financial, i.e. its utility value. Utility may be defined on the basis of the degree of usefulness or profitableness of an entity or tool, and in decision theory, decisions are evaluated on the basis of the usefulness of their consequences. Typically, usefulness is measured on a numerical scale known as a utility scale such as from 0-1 (Jensen and Nielsen 2007). According to Kahneman (2011), expected utility theory is based on the premise that

individuals make choices on the basis of being risk-averse, so that the utility value of a choice may not directly relate to its monetary value. In other words, choices with a certain outcome will have a higher utility value than a gamble, even though the financial rewards may be the same. Importantly, the combination of cost and utility functions provides a useful approach for choosing the most suitable options for restoration or adaptation, particularly when applied within a Bayesian network model (see Stewart-Koster et al. 2010).

7.2 Methods and Results

To understand the utility value of a connectivity index requires first an understanding of how such an index was developed. For this project, the province of KwaZulu-Natal was selected as the study area. Weighted indices have been developed for all rivers of stream order 2 and above, based on disc-connectivity metrics linked to longitudinal position along the river axis, and lateral connectivity. Detail of the development of these indices is provided in Ramulifho (2014). Salient features from Ramulifho (2014) are however presented in this chapter, to illustrate the sequence of steps followed to use the final connectivity index in conjunction with existing conservation planning products (Rivers-Moore et al. 2011).

7.2.1 Development of a river connectivity index

In-channel barriers (weirs, waterfalls and impoundments) for all selected rivers within the study area were identified according to their position on the longitudinal axis (Figure 7.1). Weighted scores between 0 and 1 were assigned for each barrier, on the basis of the severity of the barrier. The impacts of impoundments on the natural pattern of turnover of downstream river biota have been shown to be considerable (for example, Rivers-Moore 2012; Figure 7.2), with impacts extending a considerable distance downstream.

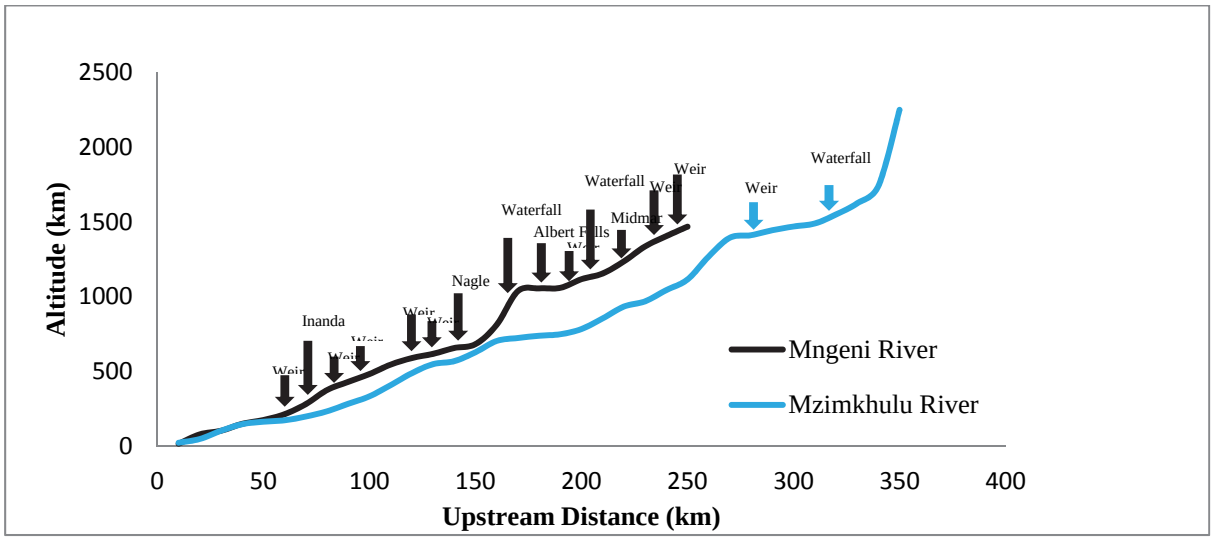


Figure 7.1 Sequence of upstream barriers (weirs, impoundments and waterfalls) on two rivers of KwaZulu-Natal – Mgeni River as a heavily impacted river system, and the Mzimkhulu River as a largely natural river system

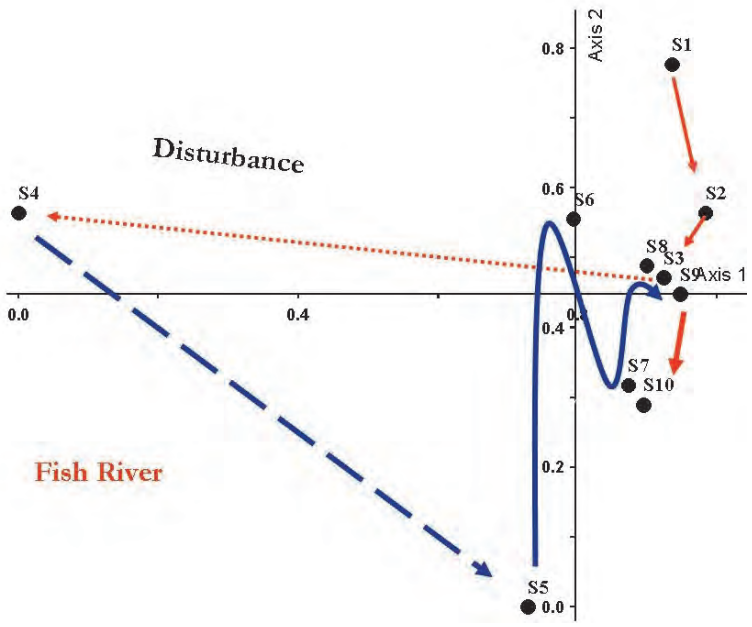


Figure 7.2 Bray-Curtis ordination of beta diversity differences for aquatic macroinvertebrate communities in the Great Fish River. Sites have been classified based on species relative abundance data. Lines indicate the impact of an impoundment on the downstream sequence of natural species turnover rates (from Rivers-Moore 2012)

This study recognises that connectivity is a function not only of longitudinal (in-stream barriers) and lateral (catchment transformation) connectivity, but also of temporal connectivity. This latter category is a function of changes in flow and water temperature regimes. The impacts of impoundments on downstream flows and simulated water temperatures were assessed by comparing data for pre- and post-impoundment periods, where good data were available for five sites within KwaZulu-Natal. Flow data were characterised in terms of metrics for magnitude, duration, frequency and timing of flow events (Richter et al. 1996), while water temperatures were defined in terms of monthly means and variation using simulated water temperature data based on daily air temperature and flow data. Results show distinct differences in flow and water temperatures for all five sites for pre- and post-impoundment periods (Figure 7.3).

The abovementioned analyses formed the justification for the use of a reset distance as a means of incorporating temporal connectivity into the longitudinal connectivity index. We calculated a cumulative disconnectivity score for all selected rivers in the study area, which was then reset according to a reset distance function based on mean daily flow volumes and using the relationship developed by Palmer and O'Keeffe (1989). Using this function, an accumulated disconnectivity score may be reset based on flow volumes, provided there is sufficient downstream distance between barriers for this to occur (Figure 7.4). This process is explained in greater detail in Ramulifho (2014).

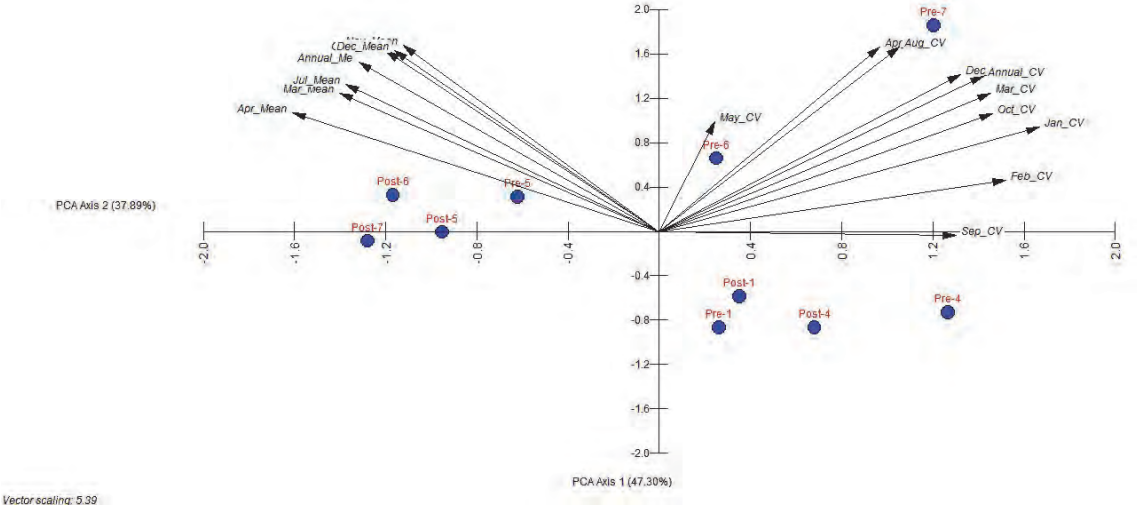


Figure 7.3 Principal Components Analysis biplot showing overall differences in monthly water temperatures at five sites where data have been compared for before and after impoundment

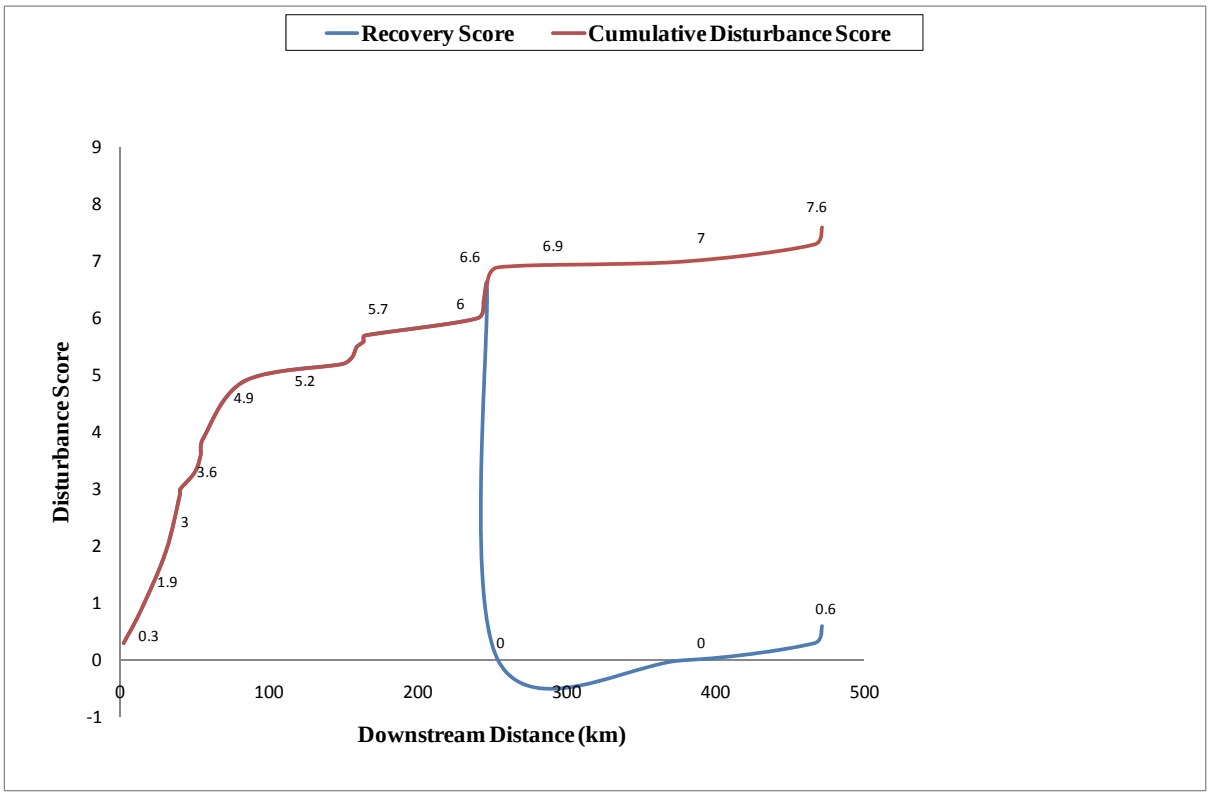


Figure 7.4 Cumulative disturbance score with and without reset function for the Thukela River

However, not only is the cumulative impact of downstream barriers of importance ranking rivers in terms of connectivity, but also the spatial pattern of barriers. Here, the association between points (degree of clustering versus regular spacing) is also useful to know in terms of clumping or dispersion at a range of scales. The co-ordinates of all in-stream barriers, and downstream distances between point pairs were calculated for 2 x *n* and *n* x *n* matrices for a highly impacted (Mgeni) and largely natural (Mzimkhulu) rivers. These matrices were used in Second Order Analyses, a suitable technique for assessing clustering of points in one or more dimensions (Fortin and Dale 2005, Rosenberg and Anderson 2011). Outputs are modified Ripley's K values that are a function of how many points fall within a series of different radius values for each point. Here, values of zero reflect random distributions, while values < 0 indicate clumping and values > 0 indicate regular spacing. Preliminary analyses indicate that this could be a useful technique for comparing connectivity patterns along river systems: The Mgeni River, which has at least 18 in-stream barriers along its downstream length of c. 250km, has Ripley's K values > 0 which indicates regular spacing of barriers with downstream distance (Figure 7.5) at large scales, but clumping at intermediate scales (2000-8000m radius). The plot for the Mzimkhulu River is very different, with only four barriers along the downstream axis of c. 350 km.

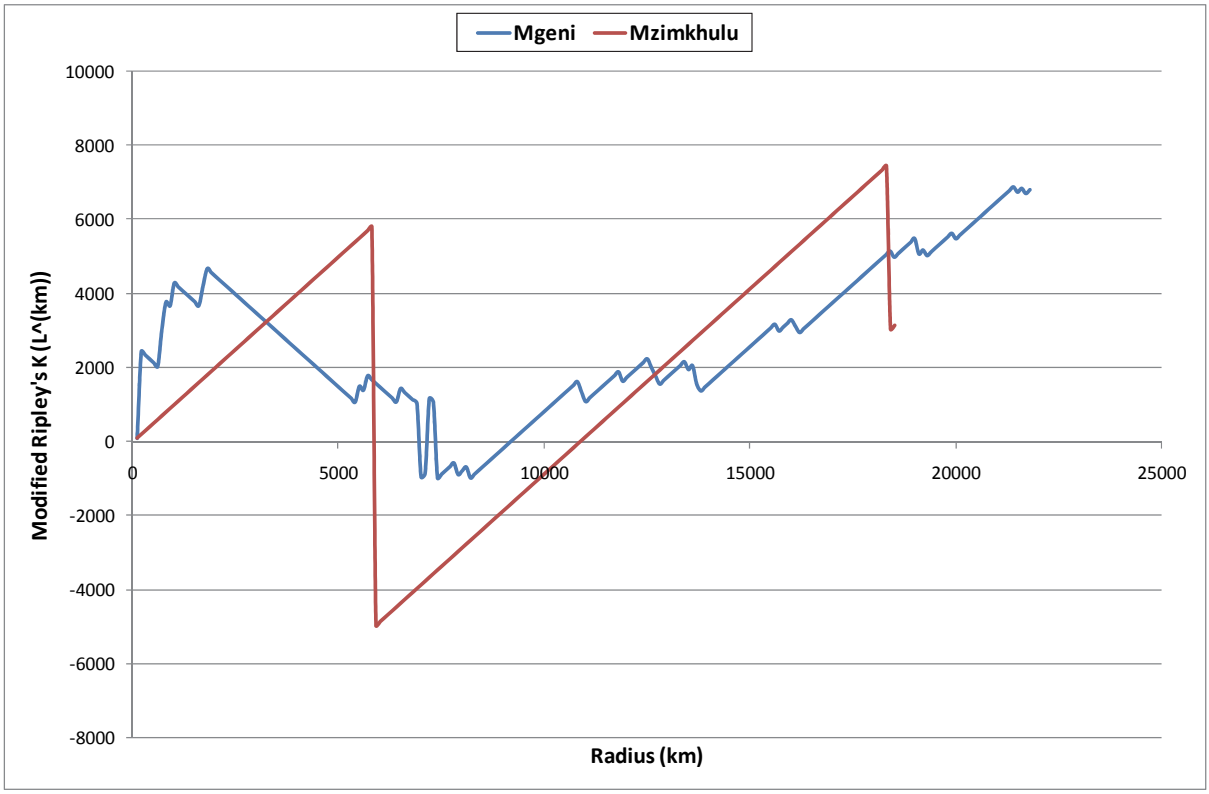


Figure 7.5 Results of Ripley's K analysis on one-dimensional data of barriers along the Mgeni and Mzimkhulu Rivers

In this study, lateral connectivity has been calculated as a combined function of land use fragmentation and density of small dams (ex-channel). While explained in greater detail in Ramulifho (2014), the basis for this index is that both farm dam density and land use change from natural have both been shown to impact on aquatic diversity patterns. A lateral and longitudinal disconnectivity, both standardised to 0-1 values, have been calculated at quaternary catchment scale (Figure 7.6).

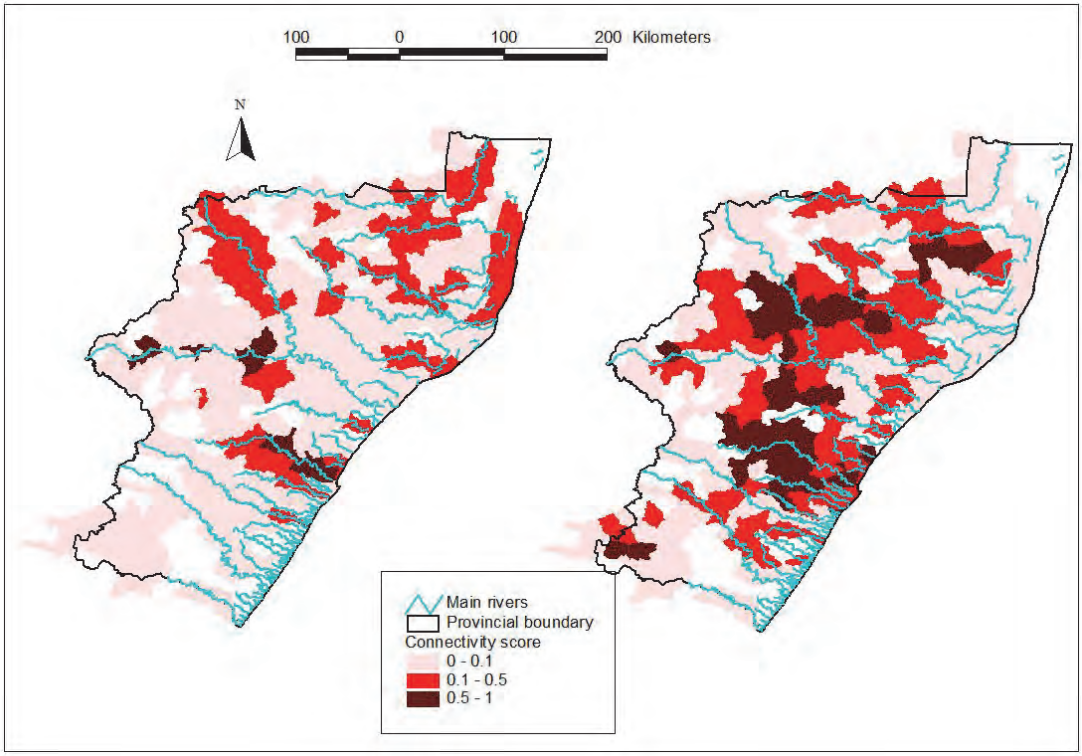


Figure 7.6 Standardised disconnectivity scores (0 = high connectivity and 1 = lowest connectivity across data range) for longitudinal (left) and lateral (right) components of an overall provincial disconnectivity index.

7.2.2 Utility value of the connectivity index

Disconnectivity scores for lateral and longitudinal (which includes reset distances as a means of including temporal disconnectivity) components were combined to provide a final catchment disconnectivity score layer where maximum values can be two. This layer was overlayed with the areas of conservation importance as developed for the provincial freshwater conservation plan by Rivers-Moore et al. (2011) (Figure 7.7). The combination of these data show areas of high conservation value relative to degree of connectivity, and when plotted together allow conservation planners to make choices between areas of high conservation value when resources are limited (Figure 7.8). In this example, conservation value may be assessed in terms of threat, and the choice made between conserving areas of high conservation value and high connectivity value (= low restoration costs) or high conservation value and low connectivity (= high threat but also high restoration cost).

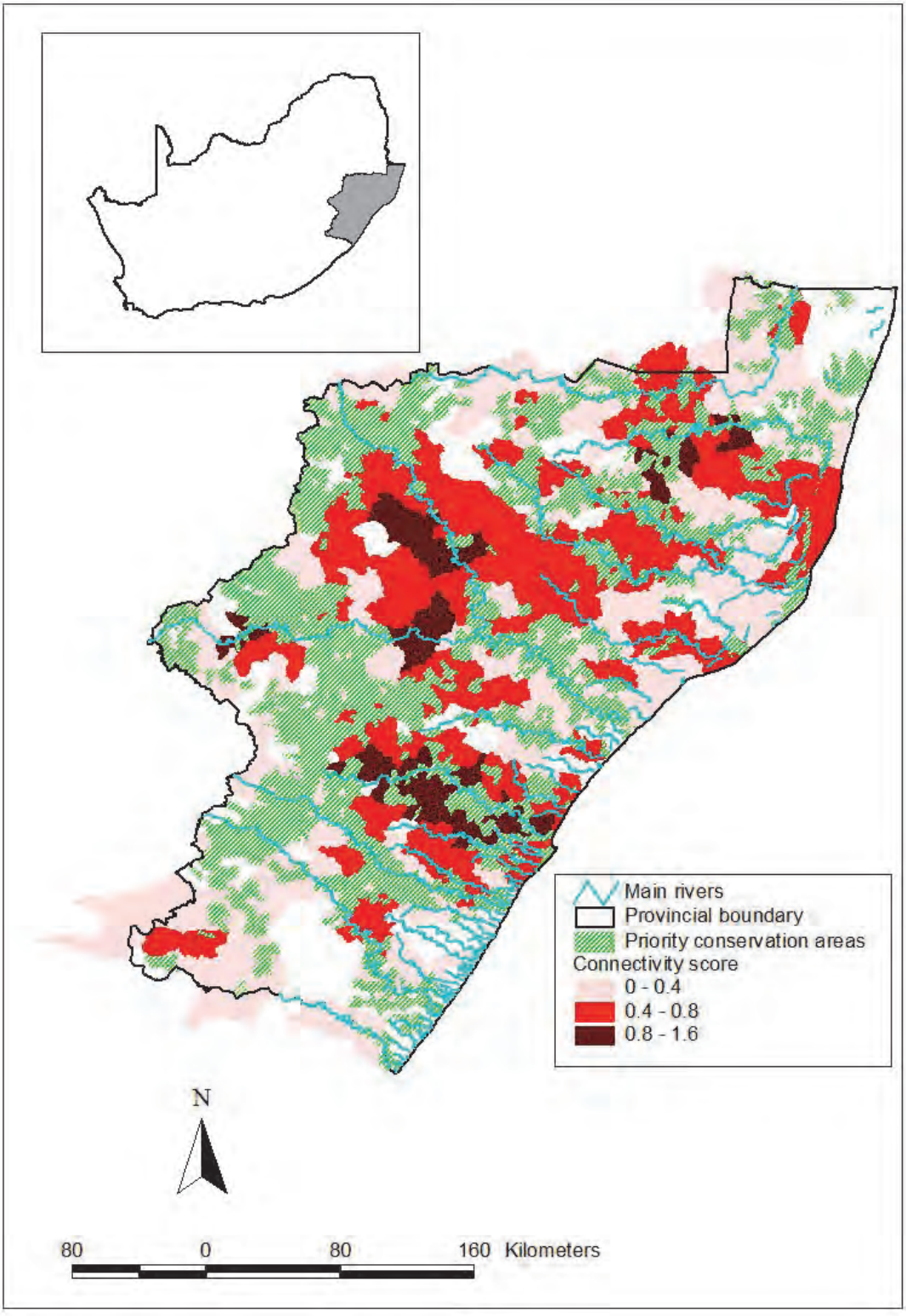


Figure 7.7 Combined connectivity score linked to areas of freshwater conservation importance for KwaZulu-Natal, as identified by Rivers-Moore et al. (2011)

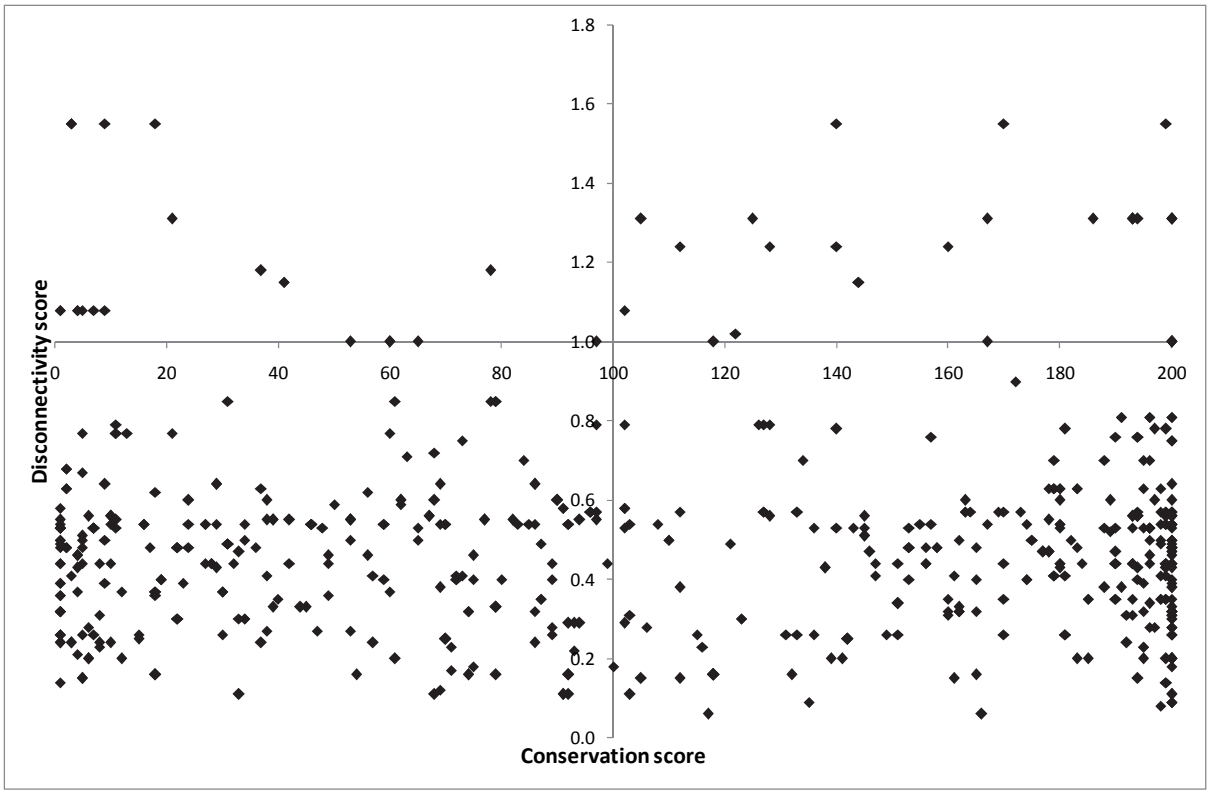


Figure 7.8 Scatterplot of conservation scores for planning units (sub-catchments) of the provincial freshwater conservation plan from Rivers-Moore et al. (2011) and their associated disconnectivity scores. The interaction between both variables allows for the comparison of conservation importance versus level of threat.

7.3 Discussion

This was previously defined as the degree to which a system is susceptible to, or unable to cope with, adverse effects of climate change, including climate variability and extremes. Vulnerability is a function of the character, magnitude, and rate of climate variation to which a system is exposed, its sensitivity, and its adaptive capacity (NAPA 2009), or alternatively, the product of sensitivity and exposure (Rieman and Isaak 2010 p24).

To identify areas on the longitudinal axis of rivers where biota are most vulnerable to climate change therefore requires consideration of the products of gradient, rate of thermal change, and level of disconnectivity. In theory, zones where these are highest will indicate zones of highest vulnerability, and consequently areas that require most urgent conservation intervention. Such areas facilitate identifying using a 'catchment acupuncture' approach to indicate hot-spots where risk (of an issue of concern – loss of species diversity or river function) is highest (Newson 2009).

Range shifts have been predicted as being the greatest impact of climate change on aquatic macroinvertebrates, and these have been predicted to contract for species that currently occur in streams with low annual mean air temperatures but expand for species that inhabit

rivers where air temperatures are higher (Domisch et al. 2011). Headwater streams and cold-water species are predicted to be the most sensitive river zones to climate change (Durance and Ormerod 2007, Buisson et al. 2008, Bush et al. 2012). Climate conditions will reorganise species composition and community structure along the river continuum, with expected reductions in population size of headwater species, eventually leading to a loss of genetic diversity (Domisch et al. 2011).

A shift in river species composition is likely to enhance the establishment of non-native macroinvertebrates in the lower reaches of the river continuum (Domisch et al. 2011). However, it will be difficult to fully categorise ecosystem-level responses by understanding responses of individual system components, because the synergistic effect of changed interactions between all components remains a large unknown. For example, Rivers-Moore and Karssing (2014) have shown that doubling of annual water temperatures results in reduced life cycle duration in tadpoles from two years to one and diminished condition, but that there remain a number of unknowns related to changes in predation and community structure, and food web asynchronies resulting from changes in life cycle timing which connect to periodicity and predictability (Colwell 1974). Such unknowns are in part due to trophic cascade effects and changes in behaviour of indigenous fish and invertebrates in response to establishment of new species in changing river communities (Townsend 2003, McDowall 2003). This is initially driven by selective removal of certain taxa, resulting in changes in community structure and functional feeding groups (Buria et al. 2003, Karssing et al. 2012). Conversely, small-mouth bass *Micropterus dolomieu* impact on aquatic community structure not through direct predation but through food web effects, due to the removal of indigenous fish that prey on aquatic macroinvertebrates (Lowe et al. 2008). Weyl et al. (2010) showed that impact of largemouth bass *M. salmoides* was biotope-specific, where impacts were apparent in marginal vegetation biotope rather than stones-in-current. Rivers-Moore et al. (2013c) was unable to draw conclusive impacts of trout on aquatic invert communities, but did show upstream/downstream differences where trout were absent and present, and in terms of community evenness and abundance indices.

To enhance the utility value of the connectivity index method currently being developed, and given all of these confounding effects, perhaps the most pragmatic approach is to recognise that the upper river zones will be the most vulnerable areas to climate change effects, and that this is largely a function of rate of thermal change in response to change in stream gradient. Certainly for the rivers of KwaZulu-Natal which are characterised by a sharp inflection point where lowland meets escarpment, the zone of greatest vulnerability will be in the upper river reaches where water temperatures change rapidly over a small downstream distance (Figure 7.9).

Ultimately, the utility of the connectivity index will be a function of the combination of vulnerability and connectivity as a measure of resilience (Equation 7.1). Trends in responses to climate change have been shown to apply at family-level (Bush et al. 2012, Chessman 2012), and two biological traits suitable for application at this level – thermophily (degree of preference for high versus low temperature) and rheophily (preference for flowing versus still water) – are useful measures to predict vulnerability to climate change, because rising air temperatures are expected to warm streams to the detriment of cold-adapted species with narrow thermal tolerances. The thermophily estimate for each family is the mean

instantaneous water temperature associated with samples in which that family was detected divided by the mean water temperature of all samples, ignoring samples for which temperature was not recorded. Similarly, rheophily is estimated as the mean hydraulic score of all hydraulically rated samples in which a family was detected, divided by the mean hydraulic score of all hydraulically rated samples in the total data set (Chessman 2009). Significant relationships have been found between the thermophily and rheophily of the families and the estimated strength and direction of their long-term trends, with families that favour colder waters and faster-flowing habitats more likely to have declined (Chessman 2009).

Resilience = vulnerability x connectivity [7.1]

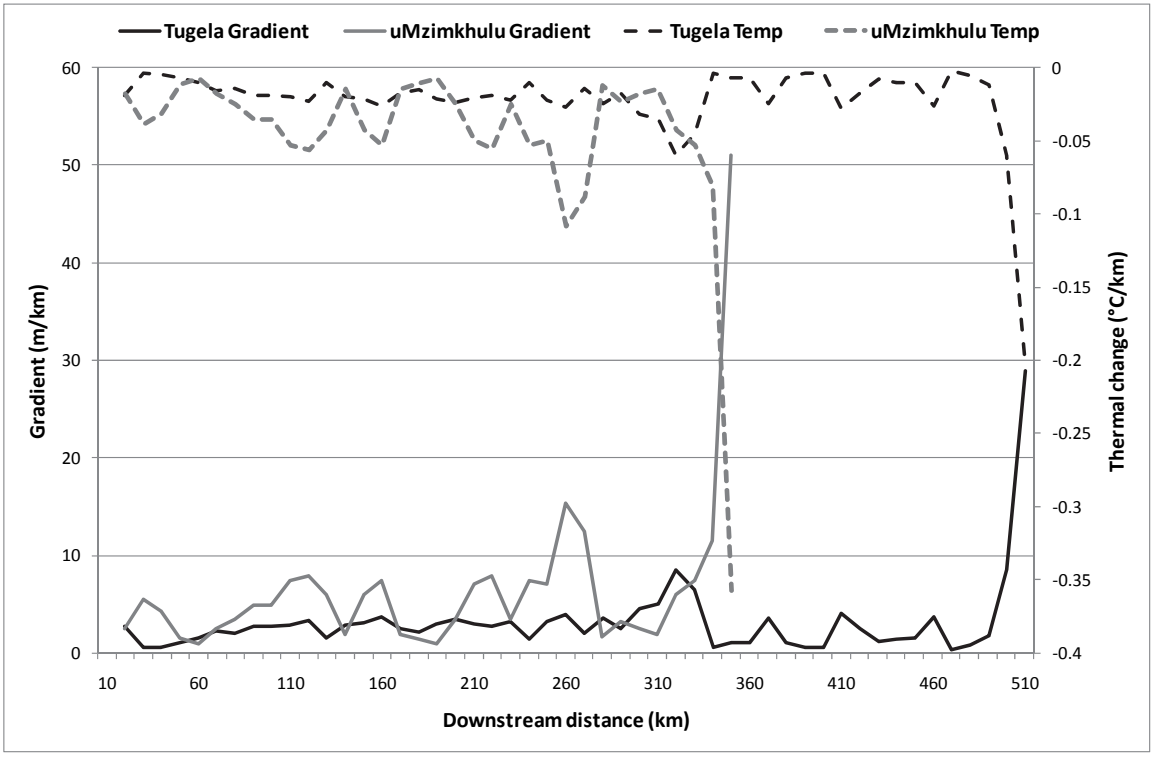


Figure 7.9. Hypothetical change of mean water temperatures as a function of stream gradient for the Mzimkhulu and Thukela Rivers, and based on an assumed lapse rate of 0.7°C per 100m change in altitude

Wilson et al. (2007) make the appeal that conservation planning consider not only land purchase and species richness, but also include consideration of threat to assist with where to allocate funding for conservation. Our connectivity index, when used as the basis for ranking catchments in terms of vulnerability and resilience, addresses this very issue through highlighting high threat catchments in terms of climate change.

Chapter 8. Development of biological temperature thresholds for setting thermal guidelines

8.1 Introduction

This project has explored several aspects related to thermal limits of aquatic macroinvertebrates, including estimates of lethal limits (CTM and ILUT), investigation of sublethal effects including physiological, phenological and developmental effects, as well as thermal preferences. These data allow for the generation of biological temperature thresholds that facilitate the establishment of environmental water temperature guidelines. These guidelines incorporate a statistical threshold (7-D moving average of mean, maximum and minimum daily temperatures) as well as a biological temperature threshold (Rivers-Moore et al. 2013a) based on experimental data.

Stream temperatures are affected by numerous regional, hydrological, structural and climatic factors (see Dallas 2008 for a review). Anthropogenic activities modify thermal regimes of rivers by altering hydrological factors such as rate of flow or discharge and water volume, and structural factors such as riparian vegetation cover, water depth and turbidity. Climatic factors that affect stream temperatures such as air temperature, precipitation and evaporation are predicted to change with global climate change, adding additional stress to systems already under pressure (Dallas and Rivers-Moore, 2014). Understanding the likely consequences of elevated water temperature on biota, through the application of biological thresholds, allows one to evaluate current thermal signatures and predict the potential effect of future increases in temperature. In this way it is possible to incorporate proactive management interventions to alleviate future environmental stress.

This chapter explores the process of utilising thermal tolerance data in the context of thermal signatures with the aim of developing environmental water temperature guidelines. A case study focusing on a single species evaluated in two regions, the Western and Southern Cape, has been selected to illustrate the process and highlight issues related to the establishment of water temperature guidelines. MWAT thresholds and thermographs were then derived for other regions in South Africa and the relationship between MWAT thresholds and temperature metrics determined.

8.2 Methods

8.2.1 Target river – Window Gorge

The river selected for the case study is Window Gorge, a small stream on the slopes of Table Mountain. Hourly water temperature data was collected for one year (May 2013 to May 2014) using Hobo UTB1-001 TidBit V2 loggers (Onset Computer Corporation, 2008) installed in the stream in the "least impacted" upper reaches of Window Gorge. This perennial, oligotrophic stream is in the winter rainfall region of the Western Cape. In extremely dry years, this stream ceases to flow and becomes a series of isolated pools joined by

subsurface flow. The underlying geology is quartzitic sandstone, subordinate shale and tillite; and dominant substrate is cobble/boulder.

8.2.2 Target species – Lestagella penicillata

The focus species is *Lestagella penicillata*, a mayfly in the family Teloganodidae. This species is considered to be of Gondwanan origin (Day 2005, Stevens 2009) with limited distribution and potentially unique thermal requirements. The life history of *L. penicillata* is that of a slow seasonal cycle, with a total development time of 12 to 13 months, making it univoltine (Ross-Gillespie 2014). Emergence of adults is from October to December, with recruitment occurring in from December to January (Ross-Gillespie 2014).

8.3 Results

8.3.1 Generation of a thermograph

The Indicators of Thermal Alteration (ITA) method (Rivers-Moore et al. 2012) was used to characterise the thermal signature of the stream. Sub-daily water temperature data for a full year was converted to daily data (mean, minimum, maximum and range). From these data, 37 metrics were calculated to describe water temperatures with respect to magnitude, frequency, timing and duration of thermal events (see Table 5.2 in chapter 5). Using the method of Rivers-Moore et al. (2013a), thermographs showing 7-D moving averages for mean, minimum and maximum temperatures, with a 95% confidence envelope, were then generated for Window Gorge (Figure 8.1).

8.3.2 Generation of biological temperature thresholds

The chronic stress threshold for *L. penicillata* was calculated using the approach of Brungs and Jones (1977) and Armour (1991), whereby a Maximum Weekly Allowable Temperature (MWAT) threshold is calculated using experimental temperature data. Both methods use an optimal temperature (OT) and either the range of preferred temperatures from laboratory derived growth curves (Brungs and Jones 1977) or an Incipient Lethal Upper Temperature (ILUT). If OT is unknown, the midpoint of a range of measured water temperatures may be used (Brungs and Jones 1977). The MWAT represents that temperature that can be tolerated as long as the ILUT is not exceeded for sustained periods (Equation 1).

$$\mathrm{MWAT} = \mathrm{OT} + \frac{(\mathrm{ILUT} - \mathrm{OT})}{3} \qquad (1),$$

where, OT is optimal temperature, and ILUT is the Incipient Lethal Upper Temperature (Armour, 1991).

The scientific rationale for using the MWAT as a temperature limit is based on data showing that moderate temperature fluctuations can be tolerated as long as the ILUT is not exceeded for long periods (Brungs and Jones 1977). The method assumes that optimum

temperatures are neither necessary nor realistic at all times to maintain viable aquatic populations (Rivers-Moore et al. 2013b). MWAT may be derived for different seasons and life stages (e.g. summer rearing or autumn/winter incubation). OT values were calculated for *L. penicillata* by using hourly water temperature data recorded at sites where this species was collected.

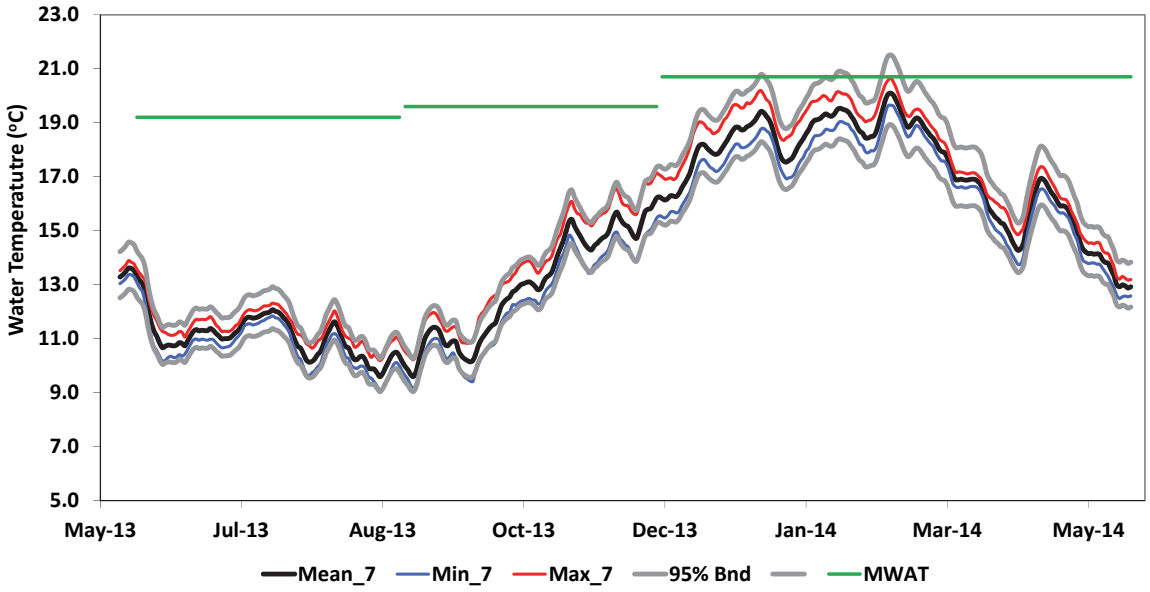


Figure 8.1. Thermograph showing 7-D moving averages for daily mean, minimum and maximum temperatures, with 95% confidence bands for Window Gorge. The MWAT threshold for *L. penicillata* is indicated (see below).

The 96h ILUTs for *L. penicillata*, calculated for four separate months (Aug-13, Oct-13, Dec-13 and Apr-14) were 21.9°C, 23.3°C, 26.5°C and 26.6°C respectively. The OT was calculated as 17.8°C, which resulted in MWAT thresholds of 19.2°C, 19.6°C, 20.7°C and 20.7°C for each of the months. The chronic stress threshold for each month was then taken to represent the season (June/July/August = winter; September/October/November = spring; December/January/February = summer and March/April/May = autumn) and was plotted on the thermograph to determine if and when the threshold was exceeded (Figure 8.1). From this it is evident that under the current water temperature profile, MWAT is not exceeded at any stage through the year, with the exception of the upper 95% band which is exceeded in mid-February. 7-D Mean and 7-D Maximum values remain below the MWAT threshold generated using the experimental temperature data. The MWAT threshold varied by approximately 1.5°C with the lowest MWAT in winter and the highest MWAT in summer.

8.3.3 Evaluating the effect of an increase in temperature on thermal stress

To determine the potential effect of an increase in water temperature on exceedance of MWAT thresholds, two scenarios were applied: a 2°C and a 4°C increase in both 7-D Mean and 7-D Maximum temperatures (Figure 8.2). These increments represent realistic global

climate change scenarios for this Mediterranean Climate Region, where climate change predictions are severe, with significant increases in water temperature and rainfall variability, and decreases in total runoff, forecast for the region. Results show that for the 2°C scenario, the 7-D Mean and 7-D Maximum exceeds the MWAT threshold for 51 and 77 days respectively, while the 4°C scenario results in exceedance of 110 and 146 days respectively. In both scenarios the exceedance period is over the warmer summer period through to early autumn (December to March) when flow volumes are lowest.

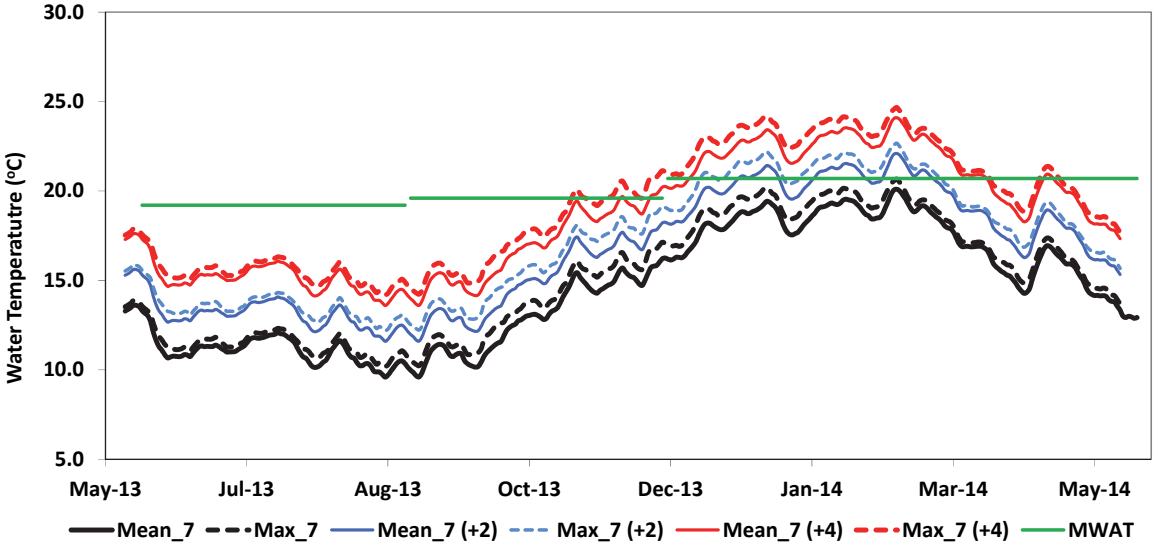


Figure 8.2. Thermograph showing 7-D moving averages for daily mean and maximum temperatures, with an increase of 2°C and 4°C to each. The MWAT threshold for *L. penicillata* is indicated.

Using this information, one is then able to confirm an upper thermal guideline based on the 7-D Mean value of 19.2°C in winter increasing by 1.5°C to 20.7°C in summer. Examination of the sublethal information for this species confirms an optimal thermal range for growth of 15°C to 18°C; and for egg development of 10°C to 20°C, with 80% hatching at these temperatures (Ross-Gillespie 2014), both of which tie in well with the proposed thermal guideline.

Examination of monthly mean, minimum and maximum water temperatures for this site reveal that the present thermal regime is highly suitable for recruitment, growth and emergence of this species. Adults utilised small pools gently fed by subsurface flows, for oviposition during a narrow time period in early summer when moderate temperatures (17 to 19°C) and low flow conditions prevail (Ross-Gillespie 2014). Experiments by Ross-Gillespie (2014) revealed that successful egg development and hatching occurred between 10 and 20°C for *L. penicillata*, with a high percentage hatch (80%) at 10, 15 and 20°C treatments Hatching at these temperatures can be expected to take between 15 and 20 days, with hatching taking place in January. Growth takes place from hatching to emergence with 15 instars recorded by the time they reach maturity and emergence in late spring (November) (Ross-Gillespie 2014).

8.3.4 Extrapolation of water temperature guidelines to other sites within the same region

Deriving water temperature guidelines on a site specific basis is time consuming and data heavy requiring water temperature and thermal tolerance data. The extent to which biological temperature criteria derived for a specific river reach can be extrapolated to other similar river reaches within the same region is unknown. MWAT thresholds for four additional sites (Eerste – EE, Molenaars – MO, Rooielskloof – RO and Wit – WI) in the same region, i.e. the Western Cape, together with their thermal signatures were therefore examined (Figure 8.3). MWAT thresholds for October (spring) were 19.6^{o}C, 18.6^{o}C, 18.5^{o}C and 18.3^{o}C for EE, MO, RO and WI respectively. If one assumes the same seasonal differences would hold for these sites as was observed for WG, then the summer MWAT would be approximately 0.75^{o}C higher, approximately winter 0.75^{o}C lower, and autumn the same as spring.

On this basis, 7-D Mean exceeded the MWAT threshold at EE briefly from late March to early February, and from mid-December to mid-March at both MO and WI. 7-D Mean did not exceed the MWAT threshold at RO. 7-D Maxima exceeded MWAT thresholds at all sites over the summer period with a longer duration observed for MO and WI (early December to late March) and a shorter duration at EE and RO (mid-December to mid-March) (Figure 8.3).

This reveals that a) the stress period is similar across rivers in the region, and b) the level of stress under current regimes varies in severity from 0^{o}C (WG) to 4^{o}C to 6^{o}C above 7-D Mean and 98 and 95 days (MO and WI respectively) (Figure 8.4). Linking this to the two scenarios whereby 7-D Mean and 7-D Maximum temperatures increased by 2^{o}C and a 4^{o}C, it is clear that substantial additional stress would be imposed upon all rivers, especially those that already have temperatures exceeding the MWAT thresholds.

8.3.5 Extrapolation of water temperature guidelines to other sites within a different region

The extent to which thermal regimes and MWAT thresholds and exceedance varied in a different region was further examined for two rivers in the Southern Cape region (Kaaimans – KA and Touws – TO) where *L. penicillata* occurs. MWAT thresholds for November (spring) were 19.7^{o}C and 20.9^{o}C for KA and TO respectively. If one assumes the same seasonal differences would hold for these sites as was observed for WG, then the summer MWAT would be approximately 0.75^{o}C higher, approximately winter 0.75^{o}C lower, and autumn the same as spring. On this basis, 7-D Mean exceeded the MWAT threshold for 75 and 70 days from mid-December to mid-March at KA and TO respectively. For both rivers maximum exceedance was approximately 3.5^{o}C (Figure 8.5). 7-D Maxima exceeded MWAT by up to 5.5^{o}C over the summer period for a longer duration (early December to late March). This confirms that the stress period is the same for both the Western and Southern Cape, both of which fall within the Mediterranean Climate Region, with rainfall predominantly in winter and warmer drier summers, although rainfall in the Southern Cape may also occur less frequently in other seasons.

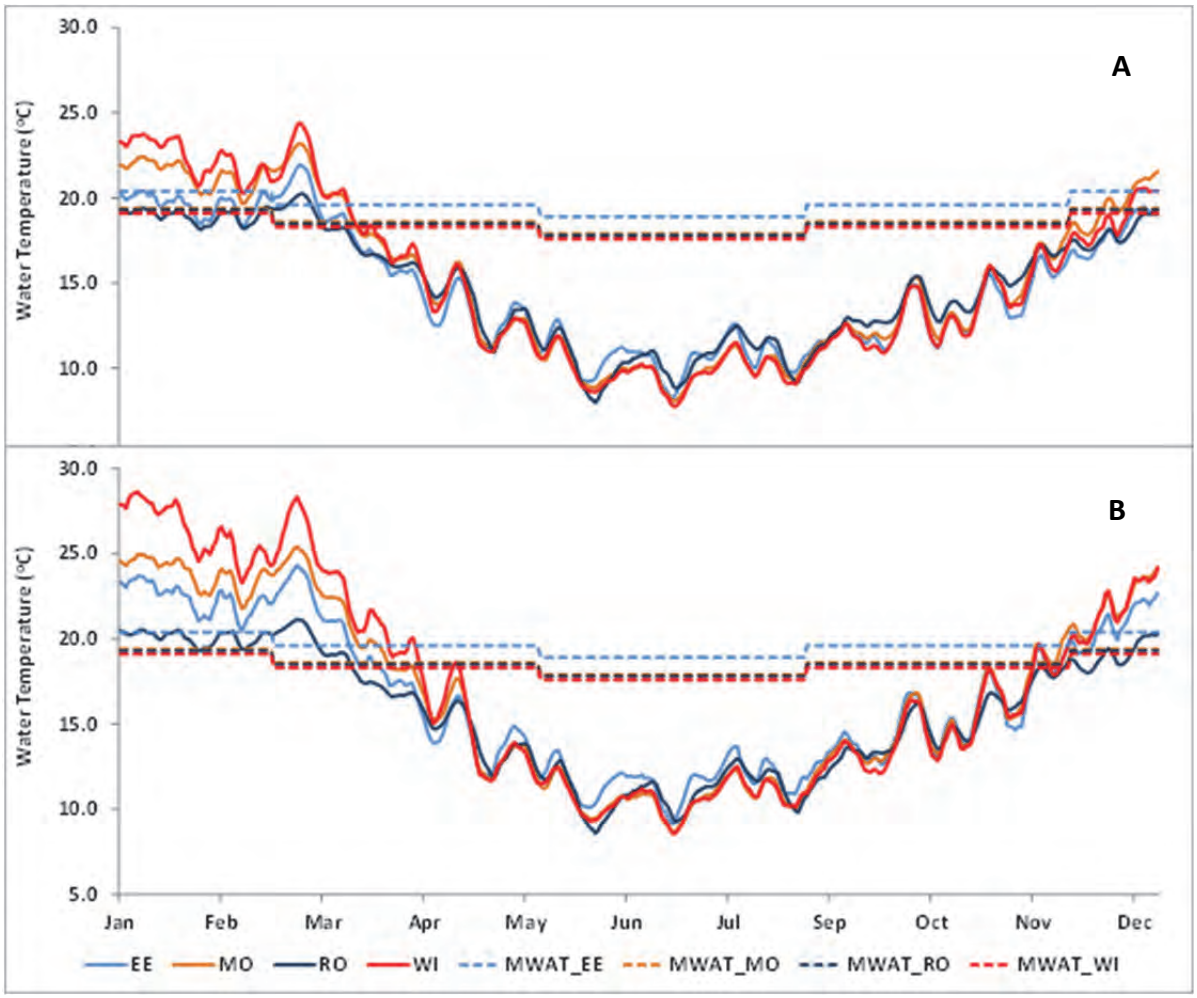


Figure 8.3. Thermograph showing 7-D moving averages for daily A) mean and B) maximum temperatures, for four sites (EE-Eerste, MO-Molenaars, RO-Rooielskloof and WI-Wit). The MWAT threshold for *L. penicillata* at each of the sites is indicated.

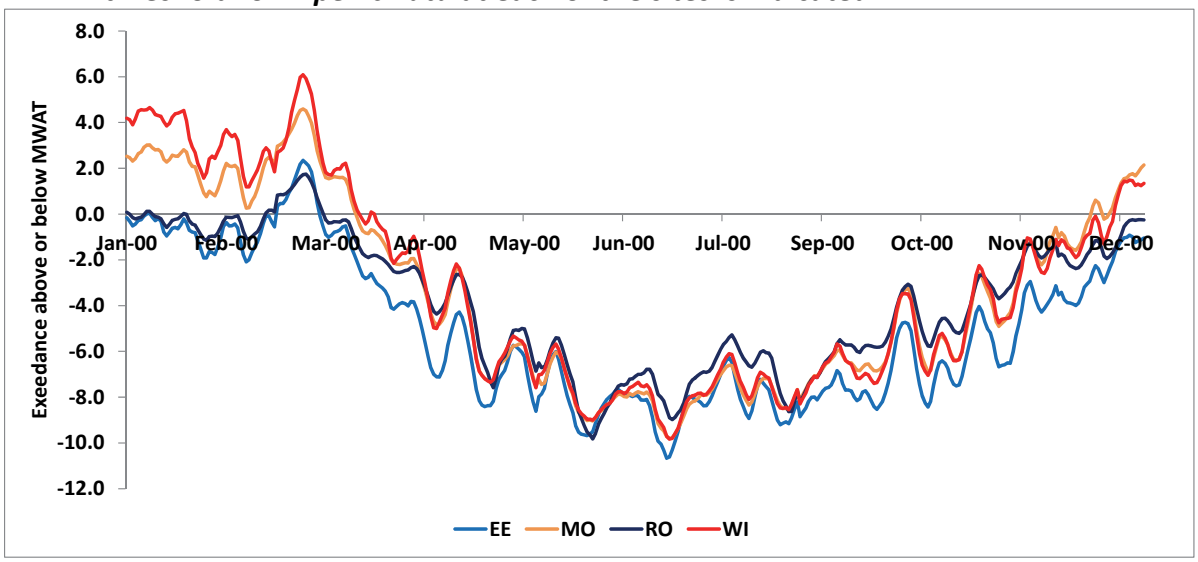


Figure 8.4. Exceedance of 7-D Mean temperatures above or below the MWAT threshold for *L. penicillata* for four sites (EE-Eerste, MO-Molenaars, RO-Rooielskloof and WI-Wit).

Figure 8.5. Thermograph showing 7-D moving averages for daily A) mean and B) maximum temperatures, for two sites in the Southern Cape (KA-Kaaimans, TO-Touws). The MWAT threshold for *L. penicillata* at each of the sites is indicated.

8.3.6 MWAT thresholds and thermographs for other regions in South Africa

The MWAT thresholds presented in the preceding sections are based on one species, *L. penicillata*, present at seven sites within the Western and Southern Cape. An average (± SD) MWAT threshold for *L. penicillata* of 19.4°C (± 0.95°C) at Western Cape sites was compared to MWAT thresholds for other taxa, in order to rank taxa in terms of thermal thresholds (Table 8.1). MWAT thresholds amongst taxa within the Western Cape, regardless of site or time of year (season), were lowest for Blephariceridae (16.8°C), Paramelitidae (17.0°C), Notonemouridae (17.0°C), higher for Leptophlebiidae (18.5°C), increasing for Teloganodidae (19.4°C), Philopotamidae (19.6°C) and highest for Heptageniidae (20.1°C). In comparison the combined MWAT thresholds for the Southern Cape were lowest for Philopotamidae (19.2°C), followed by Leptophlebiidae (19.6°C) and Teloganodidae (20.3°C) (Table 8.1). Taxa in Eastern Cape, KZN and MPU (at SU only) had MWAT thresholds < 19.4°C, < 19.0°C and <

20.4°C respectively. MWAT thresholds derived for each genus/species is also given separately for each site in the five regions (see Table 5.10 in Chapter 5).

Table 8.1. MWAT Thresholds for aquatic macroinvertebrate families given by region

	Western Cape	Southern Cape	Eastern Cape	KwaZulu-Natal	Mpumalanga
Paramelitidae	17.0				
Notonemouridae	17.0		18.5		
Perlidae				18.4	19.1
Heptageniidae	20.1		19.3	19.0	
Leptophlebiidae	17.5	19.6	17.5	17.3	20.4
Teloganodidae	19.4	20.3			
Tricorythidae			18.7	18.0	19.4
Philopotamidae	19.6	19.2			19.3
Blephariceridae	16.8				

In the Eastern Cape, using a MWAT threshold of 18.4°C (for *Tricorythus* sp.) derived for the Cata (CA), and average MWAT thresholds of 18.4°C and 18.6°C derived from four and two species for the Upper Mnyameni (MU) and Lower Mnyameni (ML) respectively, 7-D Mean exceeded the MWAT threshold for 7 at CA, 0 days at MU and 93 days at ML (late December to mid-March). For CA and ML maximum exceedance was approximately 0.3°C and 3.7°C respectively (Figure 8.6). 7-D Maxima exceeded MWAT by a maximum of 1.0°C, 0.7°C and 5.7°C for CA, MU and ML respectively over a longer period from early November to late March.

In KwaZulu-Natal, using an average MWAT threshold of 17.9°C and 18.3°C for the Sterkspruit (ST) and Mzimkhulu (MZ) respectively, 7-D Mean exceeded the MWAT threshold for 0 days at ST and 131 non-consecutive days from late October to mid-April. For MZ maximum exceedance was 4.3°C (mid-January and mid-February) (Figure 8.7). 7-D Maxima exceeded MWAT by a maximum of 1.0°C and 5.5°C for ST and MZ respectively over a longer period from mid-November to mid-March for ST and mid-September to mid-April for MZ.

In Mpumalanga, an average MWAT threshold of 19.6°C derived for the Upper Sabie (SU) and an estimated MWAT threshold of 22.7°C was derived for the Lower Sabie (SL) using the regression equation of Dallas and Ketley (2011), whereby median CT_{max} is used to derive an estimate of 96h ILUT, was applied. This 96h ILUT was then used to derive an MWAT threshold for taxa at this site, which was also corrected for season by adding 1.5°C. On this basis 7-D Mean exceeded the MWAT threshold for 0 days at TT, 1 day at SU and 173 non-consecutive days from mid-November to mid-February at SL. For SL maximum exceedance was approximately 5.3°C (Figure 8.8). 7-D Maxima exceeded MWAT non-consecutively by a maximum of 1.3°C, 1.8°C and 7.0°C for TT, SU and SL respectively over a longer period from mid-October to the end of March.

Figure 8.6. Thermograph showing 7-D moving averages for daily A) mean and B) maximum temperatures, for three sites in the Eastern Cape (CA, MU and ML). Average MWAT thresholds derived for three and four species respectively at MU and ML are indicated.

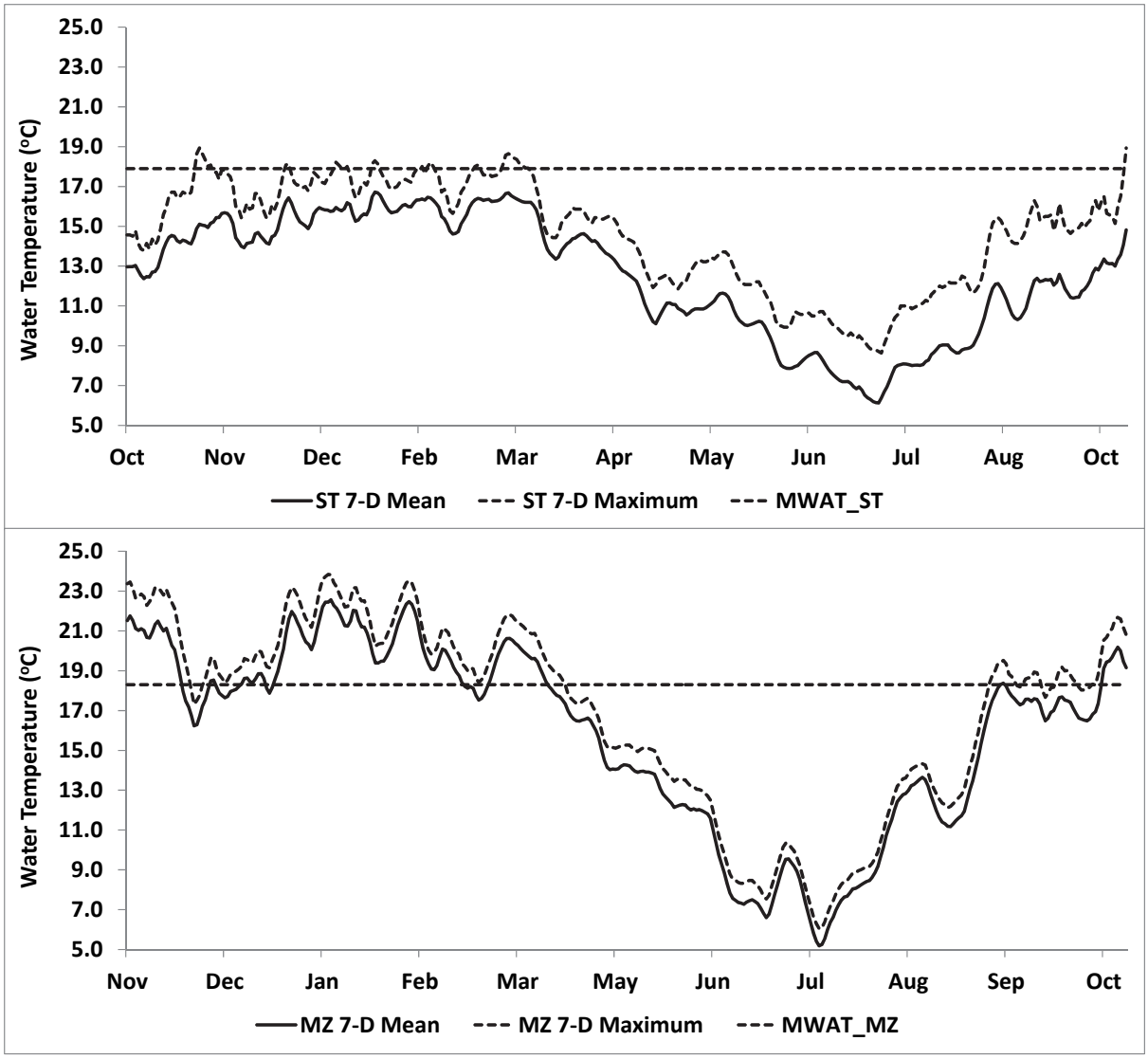


Figure 8.7. Thermograph showing 7-D moving averages for daily mean and maximum temperatures, for two sites in KwaZulu-Natal (ST and MZ). The average MWAT threshold derived for three and four species respectively at each of the sites is indicated.

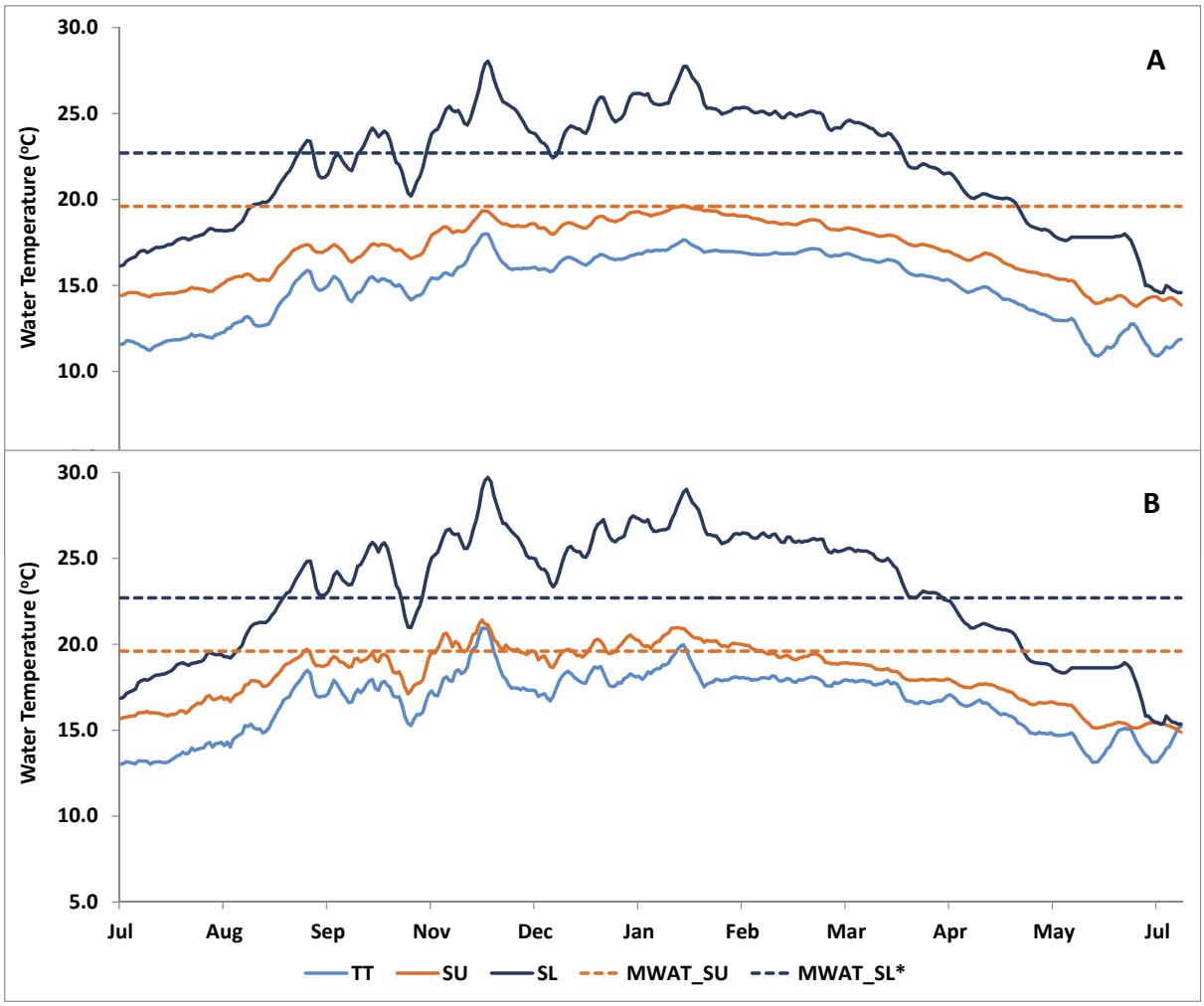


Figure 8.8. Thermograph showing 7-D moving averages for daily A) mean and B) maximum temperatures, for three sites in Mpumalanga (TT, SU and SL). The MWAT threshold for *Neoperla sp.* at each of the sites is indicated.

8.3.7 Exploring the relationship between MWAT thresholds and temperature metrics

When this study was first conceptualised it was postulated that a biological temperature threshold, derived through experimental work, could be related to one or several of the thermal metrics generated through the ITA method. In this way one could generate a thermal guideline using water temperature data for a site, without the need to undertake thermal experiments, which are time consuming and require suitable laboratory conditions and equipment. The extent to which these relationships were present was thus evaluated using MWAT thresholds calculated for taxa based on the 96h ILUT method. As no ILUTs were undertaken in the two rivers that had the highest MATs, namely the Lower Sabie and Berg Rivers, the regression equation of Dallas and Ketley (2011), whereby median CT_{max} is used to derive an estimate of 96h ILUT, was applied. This 96h ILUT was then used to derive MWAT thresholds for taxa at these sites. This analysis was undertaken for all individual MWAT

thresholds derived for all taxa on all rivers (n = 53; Figure 8.9A)) as well as average MWAT thresholds derived using all taxa from each site (n = 16; Figure 8.9B).

MWAT was significantly correlated (P < 0.05) with several metrics including MAT, degree days, 7-D mean, minimum and maximum threshold count and duration, monthly averages, minimums and maximums. Three of these have been shown graphically, MAT, 7-D moving average of mean and 7-D moving average of maximum (Figure 8.9). Of these MAT had the highest r^2 value with 62% and 56% of the variation in individual and averaged MWAT thresholds explained by variation in MAT.

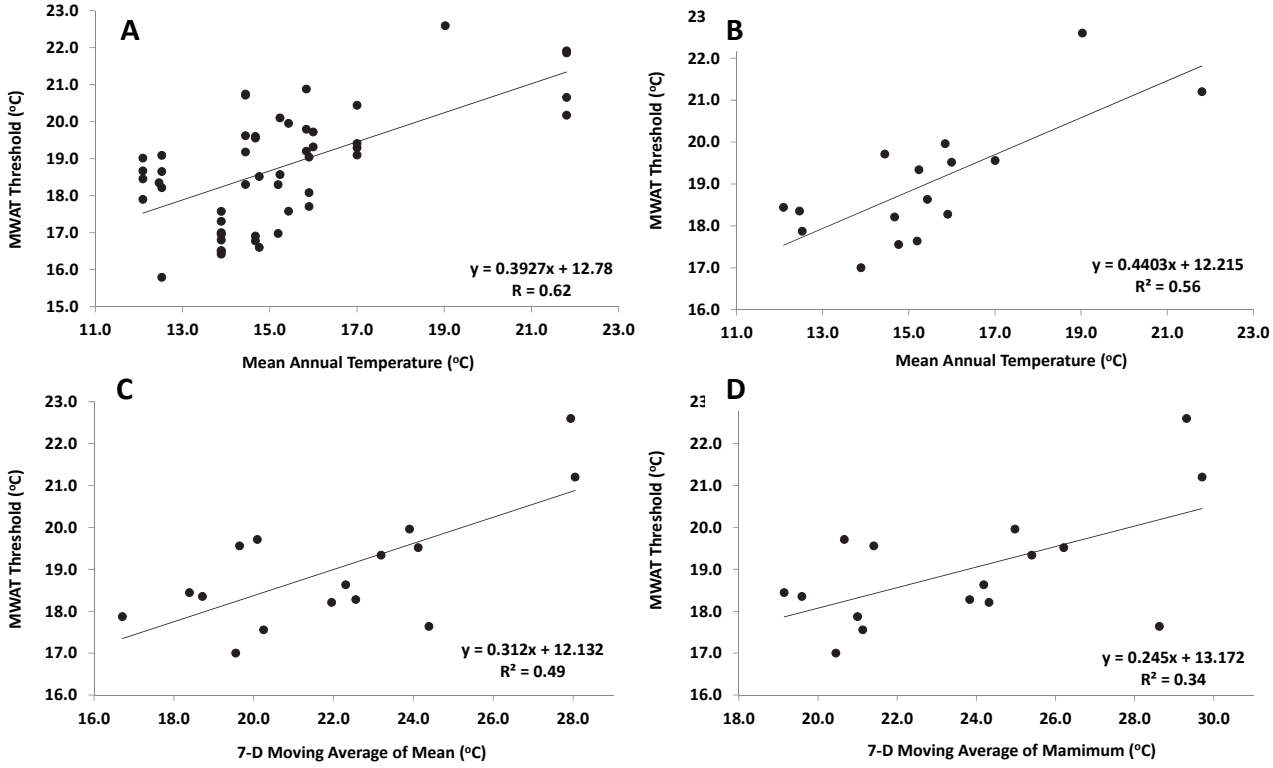


Figure 8.9. MWAT Thresholds plotted in relation to A and B) Mean Annual Temperature, and 7-D moving averages for daily C) mean and D) maximum temperatures. MWAT Thresholds are given for each individual taxon (A) and averaged for each site (B).

8.4 Discussion

Based on examination of MWAT thresholds and thermal signatures for 16 sites across five geographic regions in South Africa, it is clear that while the extent and duration of MWAT exceedance varied amongst sites, the period of exceedance was the same, i.e. over the summer period, regardless of region (and hence climatic zone). MWAT thresholds varied amongst taxa (15.8°C to 20.9°C) although the range of variation is substantially less than for CT_{max} and 96h ILUT, for the same taxa.

Some broad observations regarding MWAT thresholds were noted including:

- Sites within the same river reach and region vary in terms of the MWAT exceedance (Average ± Standard Deviation of 18.2^{o}C ± 1.2^{o}C all taxa excluding Blephariceridae which is a winter species; and 19.0^{o}C ± 0.7^{o}C for *L. penicillata*) and duration (0 to 98 days for 7-D Mean), but the extent of variation, i.e. 0.7^{o}C to 1.2^{o}C is unlikely to trigger a thermal response as both are still below 20^{o}C, which for *L. penicillata* has been shown to be suitable to egg and larval development (Ross-Gillespie 2014).
- Taxa from sites in upper catchments generally have lower MWAT thresholds than taxa from sites lower down in the catchment (e.g. Upper versus Lower Sabie).
- Taxa from a site below a dam (Lower Mnyameni) that released warmer surface water had higher MWAT thresholds than the site on the same river above the dam (Upper Mnyameni).
- In the absence of thermal data for a river, one can derive an MWAT threshold using the regression equation (y = 0.3927x + 12.78) relating MWAT to MAT. Given that this relationship only explains 62% of the variation, it provides an estimate of MWAT with a medium confidence level. Undertaking thermal experiments on a site specific basis would increase the confidence of this MWAT threshold, but logistical constraints may not always allow this.

The potential exists to use the correlations between MWAT and water temperature metrics as well as other spatial/meteorological variables (see Rivers-Moore et al. 2012) to model MWAT spatially. On this basis, a next step could be to produce spatial maps of MWAT exceedances for different genera or species and seasons for current and future scenarios (see Nelitz et al. 2007, Rivers-Moore et al. 2013b). This could be readily linked to global change scenarios using mean annual air temperatures, and the relationship between MWAT and air temperature, etc.

Chapter 9. Conclusions and recommendations

9.1 Conclusions

The establishment of thermal guidelines that adequately protect aquatic ecosystems and their biota is dependent on an understanding of a river's thermal signature and the vulnerability of its biota to changes in water temperature. This project has generated thermal signatures for a number of rivers covering five geographic regions and has experimentally determined the thermal thresholds of a range of aquatic macroinvertebrate taxa in these rivers. This information has enabled the evaluation of current thermal stress, together with scenarios of likely future stress given global climate change. Specifically it has focused on the effect of increases in water temperature relative to thermal thresholds.

The adaptability and vulnerability of riverine biota to global climate change is likely to vary amongst species and will in part depend on their biological traits. Those species with specialized habitat and/or microhabitat requirements; narrow environmental tolerances or thresholds that are likely to be exceeded at any stage in the life cycle; dependence on specific environmental triggers or cues; dependence on inter-specific interactions; and poor ability to disperse to or colonize a new or more suitable range; are likely to be more susceptible (Desta et al. 2012). An understanding of the life history, development, growth and thermal limits of key sensitive species is therefore critical if thermal guidelines are to be derived. The ability of organisms to adapt to a changing environment varies amongst taxa, with some organisms able to adjust their life-history pattern or behaviour to either cope with or avoid environmental change or stress. Phenotypic plasticity facilitates this process. Understanding how phenotypic plasticity or trait heritability affects an individual organism's or a population's ability to cope with different environmental conditions through the expression of variable life-history patterns is important for effective conservation, setting appropriate guidelines for environmental flows and water temperature and the Ecological Reserve. It informs policy and also allows for the prediction of impacts of global climate change and the ability of species to adapt to such changes.

This study has addressed the issue of vulnerability to climate change from two perspectives. The first, focused on the thermal sensitivity and tolerance of individual aquatic macroinvertebrate taxa to increased water temperature. This included estimates of both lethal and sublethal limits and effects. This allowed for the generation of biological temperature criteria based on exceedance of the MWAT thresholds relative to averaged water temperature data, specifically Mean Annual Temperature (MAT), and 7-D moving averages of mean and maximum temperatures. MWAT thresholds generated for 16 rivers across five geographically distinct regions allowed for an evaluation of the regional variation in thresholds as well as regional variation in thermal signatures. For all regions the period of maximum thermal stress is similar and occurred during the warmer months (September to April) although the extent and duration varied amongst regions, river zones and in some instances sites within a region. This is likely to be related to factors such as altitude, percentage of groundwater contribution and riparian vegetation, all of which have been shown to influence water temperature (see Dallas 2008 for a review). In the absence of

experimental data on thermal limits it is possible to utilise averaged water temperature data to set thermal guidelines on a river specific basis.

The second approach examined vulnerability from a regional basis by developing a connectivity index for rivers in one region, KwaZulu-Natal. Aspects such as gradient, rate of thermal change, and level of disconnectivity were used to identify where along the longitudinal axis of rivers biota are most vulnerable to climate change. Ultimately, the utility of the connectivity index will be a function of the combination of vulnerability and connectivity as a measure of resilience, where resilience = vulnerability x connectivity. Both of these aspects can inform management decisions on rivers or river reaches, and/or species likely to be vulnerable to global change scenarios.

Both these approaches provide essential knowledge for understanding the adaptability and vulnerability of riverine biota to climate change. Based on the results generated in this study the following recommendations may be made.

9.2 Recommendations

9.2.1 Establishment of long term water temperature monitoring sites

Strategic selection of long term monitoring sites for installation of water temperature loggers should receive the highest priority. To date water temperature monitoring has not been undertaken on a regular basis in South Africa, although the importance of monitoring water temperature in rivers was recognised as early as the late 1980s (pers. comm., N. Kleynhans, DWS). With global climate change exacerbating existing stresses on aquatic ecosystems, and given the expected increase in air temperature and change in precipitation across the country, it is no longer adequate to talk about monitoring water temperature. It is time for ACTION. Collection of water temperature data will facilitate the generation of thermal signatures for all rivers, from which thermal thresholds and guidelines may be established. Over time such data would allow for an estimation of long term trends associated with climate change, and enable comparisons to be made between actual thermal changes versus predicted thermal changes.

As a starting point it is recommended that the 40 DWA gauging stations for which a reliable flow record is available (based on previous research) be prioritised for installation of water temperature loggers. Given the importance of protected areas from the perspective of reference or natural rivers, it is further recommended that national (SANParks) and regional (e.g. CapeNature, Mpumalanga Tourism and Parks Agency, Eastern Cape Department of Economic Development, Ezemvelo KZN Wildlife) authorities be consulted. Including long term monitoring sites in such protected areas would greatly benefit the monitoring programme.

9.2.2 Generation of MWAT exceedances for South Africa

The potential exists to use the correlations between MWAT and water temperature metrics as well as other spatial variables to model MWAT spatially. On this basis, a next step would be to produce spatial maps of MWAT exceedances for different genera or species and seasons for current and future scenarios. This could be readily linked to global change scenarios using mean annual air temperatures, and the relationship between MWAT and air temperature, etc. The implementation of a water temperature monitoring programme as recommended above would greatly enhance the reliability of this spatial map. It would be further enhanced by the extension of the thermal experimental research to other regions, which would allow for the determination of MWAT values for more regions and taxa.

9.2.3 Further studies on biotic responses to water temperature (and flow)

The present study has provided some of the first information in South Africa on the responses of biota to water temperature and thermal stress. Since global climate change is likely to have "winners" (e.g. thermally sensitive taxa with limited geographic range) and "losers" (e.g. warm water pest species), further studies on additional species and regions would greatly advance our understanding of the biotic responses to elevated water temperature and allow for further predictions in terms of these "winners" and "losers". This could be done for aquatic invertebrates as well as other taxonomic groups including periphyton, fish and amphibians, in both riverine and wetland environments. Such studies could include aspects related to both the lethal and sublethal effects of water temperature, which would allow for enhanced understanding of, for example, life histories and environmental cues for critical activities such as emergence and spawning.

9.2.4 Understanding the relationship between groundwater and water temperature

The importance of groundwater in rivers and thermal buffering properties of groundwater is well recognised. However the potential consequences of groundwater abstraction on water temperatures in rivers that are largely groundwater dependent is not yet understood. A study focusing on this aspect would be beneficial and improve our understanding of the links between surface water, groundwater and water temperature.

9.3 New knowledge generated

This study has generated experimental data on thermal limits of aquatic macroinvertebrates, including both lethal limits and sublethal limits. This research has encompassed five geographic regions, 18 rivers and nine thermally sensitive taxa. These thermal limits have been used to generate biological temperature thresholds.

The project has developed a connectivity index for the rivers of Kwazulu-Natal, which may be used in subsequent validation of National Freshwater Ecosystem Priority Areas (NFEPAs) in South Africa. In addition, the protocol for which may be applied in other provinces.

9.4 Technology transfer

The research undertaken during the preceding (K5/1799) and current (K5/2182) WRC projects has seen the development of a protocol for establishing water temperature guidelines for incorporation into the ecological Reserve. This proposed method is based on sound science and encompasses both a statistical and biological threshold. The transfer of this knowledge to appropriate implementing bodies such as the Resource Directed Methods Directorate of the DWS is seen as key. Bridging the gap between scientific research and resource management, whilst challenging, is crucial.

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Appendix 1: Responses of rainfall and air temperature for the summer and winter rainfall regions of South Africa as predicted by global climate change models

Predicted change in climatic factors	
Summer rainfall region (central, north, east)	**Winter rainfall region (southwest)**
Rainfall	
Increase in mean annual precipitation (MAP) of 40 mm to 80 mm per decade in the east, particularly the mountainous areas. Northern and eastern regions likely to become wetter in summer and autumn, especially over regions of steep topography around the escarpment and Drakensberg.	Decrease in MAP of 20 mm to 40 mm per decade. Shorter winter rainfall season, weaker winter pressure gradients, more summer rainfall from January onwards, especially inland and towards the east.
Increase in year-to-year absolute variability of MAP in the east (from 30% up to double).	Decrease in year-to-year absolute variability of annual precipitation.
Wetting trend of varying intensity and distribution, particularly in the east and transitional region. Drying trend in the middle and towards the end of the wet season (i.e. January, April) in northern areas.	Drying trend in the west, mainly in the middle of the rainy season (July) and towards the end of the rainy season (October). Mountainous regions predicted to be relatively stable, while coastal regions likely to become drier.
Greater interannual variability, intensifying in autumn.	Greater interannual variability, more irregular rainfall events.
Increase in intensity of rainfall events.	Increase in the frequency of extreme events, including drought as a result of the predicted poleward retreat of rain-bearing frontal systems.
Air temperature	
Into the IF mean annual temperatures are projected to increase by 1.5-2.5°C along the coast and by 3.0-3.5°C in the far interior.	
Into the MDF mean annual temperatures are projected to increase by 3.0-5.0°C along the coast and by more than 6.0°C in the interior.	
Interannual variability (as standard deviation of annual mean) of temperature is projected to increase by ~10% over much of South Africa, with increases in excess of 30% in the north. Variability in mountainous areas in the south and west not projected to change (i.e. January, April).	
July (winter) minimum temperatures are projected to increase by a wider range from <2°C to >6°C, but with essentially a south to north gradient from the coast to the interior.	
January (summer) maximum temperature is projected to increase by 2-4°C.	January (summer) maximum temperature is projected to increase by 4-6°C.
In KwaZulu-Natal, mean daily air temperature is likely to increase by approximately 2.5°C.	Increase in days with hot, berg winds during December/January/February.

IF, intermediate future (2046-2065); MDF, more distant future (2081-2100).[5]
Note: model predictions are more in agreement for temperature than for rainfall.

Appendix 2: Global climate change drivers and ecological consequences of global climate change in freshwater ecosystems.

Ecological consequence	
Water quantity	Change in run-off patterns (flow variability, duration, timing)
	Increase in frequency and intensity of extreme events (droughts and floods)
	Change in groundwater recharge rate
Water quality	Increase in water temperature
	Increase in organic matter decomposition
	Decrease in the concentration of dissolved oxygen
	Changes in nutrient cycles (and carbon cycling) and loads
	Increase in algal growth and change in eutrophic condition*
	Increase in the incidence of cyanotoxins*
	Increase in sedimentation and turbidity
	Mobilisation of adsorbed pollutants such as metals and phosphorus from the riverbed
	Increase in transport of dissolved pollutants such as pesticides and pathogens
	Increased salinisation in semi-arid and arid areas (shallow groundwater and surface water)
Physical habitat	Change in channel geomorphology
	Decrease in longitudinal and lateral connectivity
	Change or reduction in aquatic habitat
Biological	Change in aquatic biodiversity
	Change in phenology and life-history patterns
	Change in communities
	Change in species distribution and range
	Extinction of vulnerable species
	Increase in the number and spread of invasive and pest species
	Increase in waterborne and vector-borne diseases

**Consequence is also biological*

Appendix 3: Bayesian Networks as a tool for coping with uncertainty and complexity

Bayesian Networks provide a method of representing relationships between variables, even if the relationships involve uncertainty, unpredictability or imprecision (Batchelor and Cain, 1999). They are also viewed as a strong tool for visualising complexity and engaging stakeholders (Zorilla et al. 2010). There is a rising interest in BNs as tools for water resource modelling (Kragt 2009, Uusitalo 2007, both citing a number of authors), even though their use in environmental sciences is still scarce (Aguilera et al. 2011). BNs are a useful addition to the toolkit of environmental scientists, because they allow assessment of relative changes in outcome probabilities linked to management actions, and because they offer a comprehensive way to portray complex system interaction (Kragt 2009). Furthermore, BNs are able to represent the catchment system as a whole. They are likely to become established as a standard method of analysis in problems dominated by uncertainty (Uusitalo 2007).

Advantages

- The simple graphical representation of BNs helps stakeholders understand and visualise complex problems (Kragt 2009). Additionally, and even before data are required, the process of constructing the model allows stakeholders to determine dependence (or independence) of relationships, and to see which variables are relevant to the problem (Aguilera et al. 2011). Potentially, complex situations with many variables can be modelled relatively quickly.

- As a control, the probabilistic presentation of knowledge prevents over-confidence in the strength of responses (Uusitalo 2007), i.e. model limitations are recognised explicitly.

- BNs can accommodate a variety of knowledge sources and data types (Kragt 2009), with incorporation of quantitative and qualitative data of a range of accuracies.

- The explicit recognition of uncertainty helps decision makers identify risks associated with different management actions (Kragt 2009, Aguilera et al. 2011).

- The process of BN construction helps represent current knowledge and identify knowledge gaps.

- Since BNs are a visual decision support tool, the transparent representation of causal relationships between system variables facilitates stakeholder buy-in.

- BNs are good at handling missing observations (Kragt 2009: Aguilera et al. 2011 citing various authors). Use of prior knowledge reflects the state of knowledge before the research was undertaken and as the study progresses these will be subsequently updated as knowledge expands (Uusitalo 2007). Even where data for prior probabilities are relatively poor, posterior probability distribution estimates are almost as accurate as when derived from extensive data (Goddard, N.D.), i.e. Bayesian networks are relatively "forgiving" of poor input data. Thus, this approach is free from the arguments of "too

little data" and BNs show good prediction accuracy even using small sample sizes (Uusitalo 2007).

- Structural and parameter learning, and new evidence can be incorporated (Kragt 2009, Uusitalo 2007). Various learning algorithms are used within the different software packages.

- Since numbers are attached to variables, it is possible to compute the probability of a particular hypothesis relatively quickly (Uusitalo 2007).

- Because of the natures of Baye's theorem, it is possible to calculate probability values of child nodes given the values of parent nodes (i.e. cause to effect) and vice versa (effect to cause) (Uusitalo 2007).

Limitations

A number of limitations in the use of BNs exist, although none of these should preclude using this approach in water resources management. Certain of these limitations are not problematic when BNs are used with other RSS tools, while the remaining limitations are merely constraints to be borne in mind when using BNs.

- Much data, especially in water management, is continuous (Aguilera et al. 2011), and BNs do not allow for the direct use of continuous data. Rather, there is generally a need to reduce the data into discrete states, which invariably results in data loss (Kragt 2009, Uusitalo 2007). States can be of different types: numerical values, intervals, probability distribution or categorical definition. It is also informed by the type of data available and level of model parsimony required (Kragt 2009).

- Spatial and temporal dynamics are not explicitly handled by BNs (Kragt 2009, Aguilera et al. 2011).

- No feedback loops (Kragt 2009, Uusitalo 2007)

- Developing a BN relevant to the problem at hand requires the model developer to have skills in stakeholder consultation and eliciting export knowledge, i.e. the model is only as good as the data-eliciting and stakeholder engagement processes (Kragt 2009, Uusitalo 2007).