Bioindicator monitoring and modelling for informing river health management

A thesis submitted in fulfilment of the requirements for the degree of
Doctor of Philosophy

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Abstract

Freshwaters are considered to be the most degraded ecosystems. In an attempt to monitor river health, macroinvertebrates and diatoms proved to be suitable for routine short- and long-term monitoring. However, making complex decisions for river health management based solely on the data generated by such monitoring efforts is challenging, mainly when data has limited predictive capacity. To overcome this, the thesis focuses on the integration of bioindicator monitoring and novel modelling techniques with the associated challenges to inform river health management. This thesis aimed firstly to document differences between two local catchments using ecological threshold models and then test the performance of two commonly used models. Results suggested three times higher total phosphorus (TP) thresholds in the Onkaparinga River catchment as compared to the River Torrens catchment. It was also found that thresholds for electrical conductivity (EC) specified by Hybrid Evolutionary Algorithms (HEA) exceeded those identified by Threshold Indicator Taxa Analysis (TITAN). Importantly, despite the observed differences in thresholds, results indicated that South Australian water quality guidelines for freshwater systems might be too high. Another study aimed to identify model-based bias in threshold identification, found that two commonly used methods, i.e. gradient forest (GF) and TITAN are robust in identifying change in species responses. Still, threshold identification differs depending on the analysis used and the nature of ecological data. Noting the differences in performance of the two methods on the same field and synthetic data, we recommend careful application of GF and TITAN, will improve their use for river health management. Another critical and neglected aspect of threshold identification is dependence on spatial scale. Data analysis from field monitoring of diatoms and water quality during autumn and spring for two years was further extended by merging with a broader data set from South Australian streams, to highlight spatial influences on thresholds. Consistent or lower thresholds were found across spatial scales when spatial resolution was increased from state to local scale. However, higher TP and EC thresholds were observed for the South East region than for the Adelaide and Mount Lofty Ranges. Thus we highlighted that thresholds derived at broad scales alone are unlikely to be appropriate for finer-scale assessment. Another study applied an integrated modelling approach using GF, Soil and Water Assessment

Tool (SWAT) and HEA to predict river health for future climate and land use. This study quantified population dynamics of sensitive taxa identified by GF under SWAT simulated future scenarios of deforestation, reforestation, climate change, and 10% increased urbanisation, as projected by local authorities over the next 30 years. HEA, used to predict future abundances, suggested a nonlinear response of species within commonly used Ephemeroptera, Plecoptera and Trichoptera grouping and we recommend to redirect focus of such studies from community to species-level. In reforestation and climate change scenarios, SWAT results also suggested a shift in the unusual permanent flowing stream of Sixth Creek towards intermittency under climate change and reforestation scenarios. Overall, outcomes of this research provide an improved understanding of local catchment processes in the context of global-scale challenges of climate and land use changes.

Publications arising from this thesis

Published

Sultana, J., Recknagel, F., Tibby, J. and Maxwell, S. (2019). Comparison of water quality thresholds for macroinvertebrates in two Mediterranean catchments quantified by the inferential techniques TITAN and HEA. *Ecological Indicators* 101 (867-877).

Sultana, J., Tibby, J., Recknagel, F., Maxwell, S. and Goonan, P. (2020). Comparison of two commonly used methods for identifying water quality thresholds in freshwater ecosystems using field and synthetic data, *Science of the Total Environment* 724, 137999: https://doi.org/10.1016/j.scitotenv.2020.137999

Sultana, J., Recknagel, F., Nguyen, H.H. and Tibby, J. (2019). Integrated approach for predicting impacts of future climate and land use changes on macroinvertebrates in a Mediterranean catchment using GF, SWAT and HEA models. *International Congress on Modelling and Simulation, 2019*
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In process

Sultana, J., Maxwell, S., Tibby, J. Evaluating spatial scale influences on threshold variations: Spatially-explicit responses of diatoms to water quality thresholds across multiple spatial scales (Intention to submit to Journal of Environmental Management)

National/International conference abstracts

Sultana, J., Tibby, J., Recknagel, F. and Maxwell, S. (2018). A rationale for threshold model selection and identification of ecological thresholds for macroinvertebrate assemblages: Comparison of two commonly used methods. *Australian Freshwater Sciences Society Conference 2018*, Adelaide, South Australia.

Sultana, J., Recknagel, F. (2018). Overall and site-specific response of the macroinvertebrate community of Swan Coastal Plain Wetlands (West Australia) to water quality gradients revealed by GF and HEA. *International Conference on Ecological Informatics 2018*, Jena, Germany

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Chapter 1: Introduction and Literature Review

1.1 General Introduction

Freshwater ecosystems support 6–10% of all species and are more at risk of ecological degradation than any other ecosystem on the planet (Dudgeon et al., 2006; Vörösmarty et al., 2010). The worldwide focus of river health management is the preservation of water quality and quantity. In an attempt to assess river health, enormous efforts are being taken to monitor different types of biological indicators and water quality stressors that produce complex data that as such have limited information to achieve good ecological outcomes (Goonan et al., 2012; Harris and Heathwaite, 2012). The analysis and synthesis of river data using novel modelling techniques promises to overcome this limitation and assist in informed decision making.

This chapter summarises the motivation for my research on the topic "Bioindicator monitoring and modelling for informing river health management". In Section 1.2.1, the rationale of bioindicator monitoring for river health assessment is reviewed. The chapter continues by reviewing the roles of traditional statistical tests and computational modelling in the field of ecology (Section 1.2.2) that leads to a discussion of recent developments in ecological threshold analysis by comparing benefits and limitations of models available for threshold identification and analysis. This section also addresses the potential of process-based and data-driven models in ecology, suggesting an integrated modelling approach for river health assessment. In Section 1.3, research gaps and challenges are highlighted. Section 1.4 defines the key research questions and significance of this research, and the final Section 1.5 outlines the thesis structure. The overall scheme of the thesis is given as Figure 1. The following section on literature review will elaborate on this scheme, but a more comprehensive assessment of the literature is provided in relevant chapters.

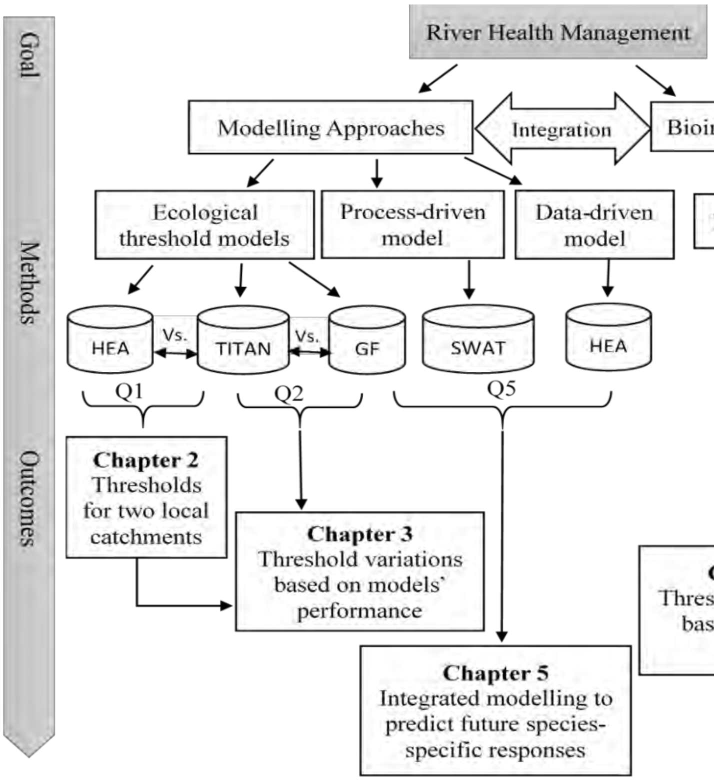

Figure 1: Overall scheme of thesis. This scheme is used in the following sections. HEA, TITAN, GF and SWAT are the modelling techniques presented later in Section 1.2.2. Q1, Q2, Q3, Q4 and Q5 refer to respective research questions mentioned in Section 1.4.

1.2 Integration of bioindicator monitoring and modelling approaches for river health management: a literature review

1.2.1 Bioindicator monitoring for river health assessment

The universal definition for the term 'river health' remained elusive as it implies human judgement, but refers to an overall integrity of river ecosystems (Davies et al., 2010; Karr, 1999; Norris and Hawkins, 2000). Different scientists defined it in slightly different ways. The term 'river health' was coined by Norris and Thoms (1999) with an emphasis on examining the combined effects of environmental factors that can affect aquatic species of a river ecosystem. Karr (1999) demonstrated that a healthy river is a living river, characterised by a minimum of human disturbance and a maximum of native biodiversity, itself essential to sustain the ecological functions of that ecosystem. Davies et al. (2010) proposed that a healthy river allows sustainable human interference while maintaining its essential character in terms of hydrological connectivity, resilience, stability, sediment transport, nutrient cycling and biodiversity. However, river ecosystem health is under enormous pressure in Australia and around the world due to numerous anthropogenic impacts including chemical pollution and physical habitat alterations owing to rapid urbanisation and unsustainable use of water in industry and agriculture (Bunn et al., 1999; Norris and Thoms, 1999; Pinto and Maheshwari, 2011; Vörösmarty et al., 2010).

The obvious effects of deteriorating water quality on aquatic organisms has led to the development of the concept of bioindication. Bioindication aims to use plants and animals as tools for the assessment of present, past or future environmental conditions (Markert et al., 2003). The idea of using organisms to investigate the potential adverse impacts of environmental contaminants is not a novel one. Kolkwitz and Marsson (1909) first proposed the use of biota for water quality monitoring. Since then, well-known methods were applied for mapping bioindicator data, depending on their tolerance or sensitivity to various levels of pollution (Džeroski et al., 2000). Jóźwiak and Jóźwiak (2014) affirmed that an ideal bioindicator should be of low cost, able to determine the source and levels of anthropogenic impacts, and can be used for both short-term and long-term observation. Although some bioindicators are considered to have more advantages than others, some studies argued about the optimal indicators to be used for assessment studies (Marzin et al., 2012). However, considerable efforts have been made to guide the appropriate choice of indicator and monitoring designs to achieve maximum outcomes (Downes et al., 2002; Strobl and Robillard, 2008). Since a change in focus of The European Water Directive Framework from use of single to several bioindicators for assessing river health (European Union, 2000), a number of studies have directed their focus to incorporate other bioindicators including macroinvertebrates and diatoms (Feio et al., 2007; Grenier et al., 2010; Hering et al., 2006; Juggins et al., 2016). The potential role of macroinvertebrates and diatoms as bioindicators, and their potential for river health assessment is discussed in the following section:

(i). Macroinvertebrates

Benthic stream macroinvertebrates have been extensively used as bioindicators of river health due to their trophic position, sensitivity to a range of environmental stressors, ubiquitous distribution and relatively long life cycle (Cairns and Pratt, 1993; Chessman, 1995). Short- and long-term monitoring programs for river health management regularly use macroinvertebrate analysis (Cortes et al., 2013; Smith et al., 1999). Macroinvertebrates are important for river management as they perform essential functions, including nutrient cycling, sediment processing, and transfer of energy within food webs (Covich et al., 1999). Researchers have demonstrated the potential of macroinvertebrate species as bioindicators at varying levels of pollution loads in streams and rivers around the world, e.g., development of AusRivAS (Australian River Assessment Scheme) and biotic index to assess river health of Australian rivers using macroinvertebrates (Chessman, 2003; Smith et al., 1999), determination of trophic conditions using composition of different macroinvertebrate species (oligochaete and mayfly) in the Great Lakes (Howmiller and Scott, 1977; Krieger et al., 2007), identification of ecological stress due to organic pollution in Ohio (Rae, 1989), heavy metal load due to mining activities in South American rivers (Smolders et al., 2003), land use and nutrient enrichment in mountain and lowland streams of Europe (Johnson et al., 2006), stream acidification in Germany (Braukmann, 2001) and human induced flow alterations on ecological responses in Australia (Leigh et al., 2012; Maxwell et al., 2010).

Due to the use of macroinvertebrates as bioindicators since early 1900s, the relationship between their biological characteristics and the environment is very well characterised (von der Ohe et al., 2009). For example, EPT--Ephemeroptera (mayflies), Plecoptera (stoneflies) and Trichoptera (caddisflies) are very sensitive to pollution; therefore their presence or absence in a freshwater ecosystem is a good indication of overall river health (Gerhardt et al., 2004). Conversely, Oligochaeta (worms) and chironomids (midges) are tolerant to high pollution levels and thus the dominance of these species indicate an impacted system (Azrina et al., 2006; Rae, 1989). Different studies have demonstrated the predictive potential of macroinvertebrates, including EPT taxa under individual future climate and land use change conditions (Chessman, 2009, 2012; Woznicki et al., 2016). Only a few studies explored the future prediction of macroinvertebrates under the combined impact of climate and land use change conditions, although exceptions include e.g., Guse et al. (2015) and Mantyka-Pringle et al. (2014).

In summary, stream macroinvertebrates served as potential candidates for bioindication. They gained worldwide importance due to their use in short and long-term assessments, and the ability to differentially respond to many sources of anthropogenic pollution. However, the use of macroinvertebrates as bioindicators for river health assessment also has some limitations. For example, quantitative sampling is not accessible due to their non-random distribution and, finding samples during some parts of the year is difficult because of their seasonal life cycles (Feld and Hering, 2007; Goethals, 2005). Besides, macroinvertebrates are considered more dependent on specific habitat characteristics than other bioindicators such as diatoms (Reid et al., 1995).

(ii). Diatoms

Diatoms are the most preferred phototrophic protists for use as bioindicators, as they are usually found in all water ecosystems, particularly in streams and rivers (Stevenson et al., 2010). Of the many potential bioindicators, diatoms are particularly useful because of their quick response to any physical, chemical or biological change, rapid growth owing to the short life cycle, and well-studied ecology and taxonomy (Reid et al., 1995; Stevenson et al., 2010). Additionally, it has been argued that diatoms are less dependent on habitat than macroinvertebrates (Reid et al., 1995; Stevenson et al., 2010), can be found in degraded habitat conditions where sensitive macroinvertebrates may not survive (Buffagni et al., 2004; Hering et al., 2006) and often exhibit stronger responses with less error (Johnson et al., 2006). Due to this advantageous nature, diatom-based models have been developed to infer past water quality parameters including salinity (Fritz et al., 1991; Gasse et al., 1997) total phosphorus (Reavie et al., 1995; Tibby, 2004) and pH (Battarbee, 1984; Enache and Prairie, 2002; Tibby et al., 2003). Studies using diatoms as stream bioindicators are mostly dominated by the development of diatom based indices (Chessman et al., 2007; Rimet, 2012). However, in the last decade literature has shown a slight shift in the use of diatoms to analyse ecological thresholds. A variety of techniques have been used worldwide to identify diatom thresholds including self-organising maps (Grenier et al., 2010), gradient forest (Tang et al., 2017) and threshold indicator taxa analysis (Baker and King, 2010; Tibby et al., 2019).

Comparative studies of diatoms and macroinvertebrates as indicators suggested either their combined use (De Jonge et al., 2008) or, in some case studies, diatoms outperformed macroinvertebrates as suitable bioindicators of water quality (Beyene, 2009; Newall et al., 2006). However, diatoms may be less predictable than macroinvertebrates and other bioindicators due to their short life cycles and rapid population fluctuations in natural conditions (Chessman et al., 1999) and less public appeal.

1.2.2 Modelling approaches for river health management

The effective management of river systems is governed by the continuous short and long term monitoring of their ecological status. The US Clean Water Act (CWA) developed in 1972 finds its parallels in the EU Water Frame Work Directive adopted in 2000, in terms of its long-term objective of protecting the biological integrity of water bodies. It has resulted in considerable progress towards implementing biological assessment in different monitoring programmes for aquatic ecosystem health (USEPA, 2016). Over 40 years since the establishment of the CWA, researchers and managers have progressed from traditional biological monitoring towards integration of modelling approaches, to redirect river management objectives.

(i) From conventional statistics to computational modelling

Computational bioindication in its present form has passed through a series of developmental phases. The origin of computational bioindication dates back to the use of conventional statistical techniques including multiple regression (Van Sickle et al., 2004), analysis of variance, discriminant function analysis and time series analysis. These analytical techniques are powerful tools to associate physical, biological and chemical data together, and to develop predictive models. However, the complexity of ecological systems that involves nonlinear relations among multiple variables limits their use (Hobbs and Hilborn, 2006).

More recently, with the advent of multivariate analysis, attempts were made to assess relationships between multiple environmental stressors and aquatic fauna (Griffith et al., 2002; Wright et al., 1984). During this era, scientists explored the potential of different ordination and clustering techniques to analyse community structure in relation to ecotoxicological and other stressors (Heino et al., 2003; Wijngaarden et al., 1995). Moss et al. (1987) used multivariate analytical techniques for predictive models e.g., River InVertebrate Prediction and Classification System (RIVPACS) and Australian River Assessment (AusRIVAS), became increasingly popular for freshwater bioassessment studies (Linke and Norris, 2003; Poquet et al., 2009). These models use macroinvertebrate data to evaluate the ecological status of rivers by comparing the taxa distribution and environmental variables at reference sites (i.e. minimally disturbed sites) to that of the observed ones (Clarke et al., 2003). Cluster analysis and discriminant function analysis are subsequently used to define and identify clusters that discriminate among different variables (Clarke et al., 2003). Based on the similar notion, benthic assessment of sediment (BEAST) was developed that replaced the use of presence/absence data of invertebrates with abundance (Reynoldson et al., 1997). Although RIVPACS and AusRIVAS have been predominantly used for over more than 15 years for river health assessment, these models face criticism due to their oversimplification and particularly the limitation of their dependence on having appropriate reference conditions. A number of predictive models (like AQUAFLORA, MoDi) have been used to evaluate the ecological quality of streams in recent years but the biggest challenge in their application is to find subsets of reference conditions that can be well classified by few environmental predictors (Feio et al., 2012; Feio et al., 2009).

With the advancement in computational modelling, there was a shift of ecological analysis to more sophisticated computing based on artificial intelligence (Recknagel et al., 1994), artificial neural networks (Edwards and Morse, 1995; Recknagel et al., 1997), Bayesian networks (Pollino et al., 2007) and genetic algorithms (Recknagel et al., 2002).

(ii) Ecological threshold models

The last two decades have observed a growing number of applications of threshold models in river health management. Ecological thresholds have become increasingly relevant for ecosystem managers by serving as guidelines for water resource management (Feng et al., 2019; Foley et al., 2015; Samhouri et al., 2010). The Institute of Scientific Information (ISI) Web of Science publication database results for the query 'threshold models and freshwater' show that the number of publications climbed sharply from five in 2002 to more than 45 at present (i.e. April, 2020) (Figure 2).

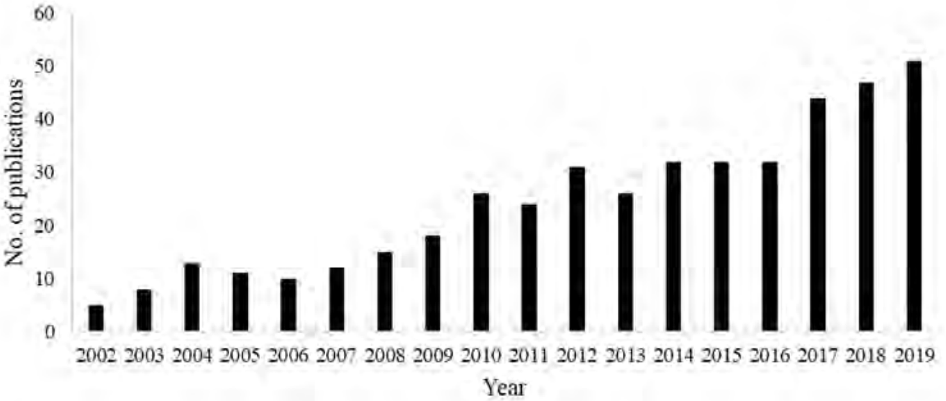

Figure 2: Total number of publications from Web of Science Publication database for the query 'threshold models and freshwater'. Total number of publications during 2002–2019 is 437; 5 in the year 2002 and 51 in the year 2019.

Worldwide, freshwater ecosystems are exposed to multiple stressors that adversely affect stream biota. Identifying thresholds beyond which ecosystem services may be diminished is often difficult due to the complexity of ecosystem dynamics that requires an understanding of the effects from multiple environmental stressors (Ormerod et al., 2010; Wagenhoff et al., 2012). Due to this complexity, freshwater managers are embracing sophisticated methods to inform management actions (King et al., 2011; Wagenhoff et al., 2017a), including threshold identification. A summary of threshold methods found in literature with their advantages and limitations is given in Table 1. Examples include breakpoint or piecewise regression (Toms and Lesperance, 2003), nonparametric change point analysis (Qian et al., 2003), cumulative frequency distribution curves (Utz et al., 2009), Significant Zero Crossings (SiZer) (Sonderegger et al., 2009), Hybrid Evolutionary Algorithm (HEA) (Recknagel et al., 2016), Threshold Indicator Taxa Analysis (TITAN) (Baker and King, 2010) and Gradient Forest (GF) (Ellis et al., 2012). These approaches differ by identifying either single or multiple thresholds along gradients, in the number of predictor variables that can be assessed simultaneously, and the ability to deal with multivariate or univariate response variables. Given that threshold identification models are based on different underlying statistical assumptions, each with their associated limitations and uncertainty, different models typically identify different thresholds (Dodds et al., 2010).

The recently developed methods of TITAN and GF are briefly discussed in the following section. A comparison of the two approaches with regards to data requirements, overcoming limitations of previous methods, use of multivariate, nonlinear responses of species abundance data, their suitability for management settings and support for setting criteria limits is discussed in Chapter 3.

Threshold Indicator Taxa Analysis

Threshold indicator taxa analysis (TITAN) (Baker and King, 2010) enables the identification of ecological community thresholds based on indicator species scores that integrate abundance, richness, direction and magnitude of individual taxon responses in the whole community across an environmental gradient (Baker and King, 2010). TITAN is an extension of change-point analysis (Qian et al., 2003) and indicator species analysis (Dufrêne and Legendre, 1997). It distinguishes taxa responding positively or negatively to the specific predictor variable in terms of z scores, and incorporates a bootstrap procedure to find taxon responses that are pure and reliable. Change points or thresholds are detected when there is a synchronous change in the abundance of a number taxa lying within a narrow range of the predictor variable (Baker and King, 2010).

TITAN has been applied to demonstrate stream assemblage thresholds in response to a variety of environmental gradients including anthropogenic pressures like the urbanisation and water quality of European and U.S rivers (Kail et al., 2012; King et al., 2016), Brazilian neotropical savanna streams (Firmiano et al., 2017), alpine rivers and lakes (Khamis et al., 2014) and salinisation of Canadian streams (Porter-Goff et al., 2013). In south-east Australia, TITAN in combination with an expert opinion, was used for discretisation of predictor variables in Bayesian networks (Lucena-Moya et al., 2015). In another study, TITAN was used to highlight the need for improving water quality guidelines in South Australia (Tibby et al., 2019).

While robust in identifying thresholds, TITAN finds its limitation where data sets dominated by abundant species occurring in all samples are not suitable for TITAN. It is due to the fact that it is based on indicator species analysis and this kind of data provides little information on which to base the analysis (King and Baker, 2014).

Gradient Forest

Gradient Forest (GF) (Ellis et al., 2012) is a novel machine learning approach based on regression trees. It is an extension of random forest (Breiman, 2001) which has been considered superior to other related methods (e.g. generalised linear or additive models, regression tree methods) in terms of prediction (Cutler et al., 2007; Prasad et al., 2006). Regression tree based approaches are well suited for handling complex ecological data with missing values, lack of balance and nonlinear relationships (De'Ath, 2002). GF consider responses from multiple species and incorporates a bootstrap procedure that calculates predictor importance and response curves along different gradients. Random forest is an ensemble of regression trees, whereas in GF separate random forests are grown for each species by partitioning the observations based on split functions. By aggregating information from recursive partitioning over all species, the method gives a function representing compositional change along environmental variables (Ellis et al., 2012).

GF has been applied to explore the significance of environmental variables that affect biodiversity composition in large marine regions of northeast Australia, the Gulf of Mexico, the Gulf of Maine (Roland Pitcher et al., 2012) and temperate Australian reefs (Thomson et al., 2014). GF also finds its utility in deriving ecological thresholds using different bioindicators in different regions of the world including nutrient and sediment gradients for limit setting in New Zealand freshwater systems (Wagenhoff et al., 2017a); community thresholds for benthic diatoms in Chinese streams (Tang et al., 2017); marine ecosystem thresholds for human-induced and environmental pressures in California (Samhouri et al., 2017) and ecological thresholds for fish and phytoplankton communities in French lakes (Roubeix et al., 2016; Roubeix et al., 2017).

GF was proved robust in identifying change in species' responses (Roland Pitcher et al., 2012) and also in identifying predictors' importance, however being an exploratory approach, it does not provide a measure of uncertainty around designated change points.

Table 1. Summary of ecological thresholds models in literature with advantages and limitations.

	Methods	**Description**	**Advantages**	**Limitations**	**Citation**
1	Breakpoint (or piecewise) regression	statistical determination of breakpoints for univariate response variable and single predictor	suitable for gradually changing distributions	considers univariate response variable and a single predictor, no guidance of optimum number of breakpoints, not suitable for stair-step or abupt change in responses	Toms and Lesperance, 2003
2	Nonparametric changepoint analysis	test for change in variance indicated by abrupt deviation from mean	works well in case when data on either side of mean significantly differ in mean	considers univariate response variable and single predictor, not suitable for data with wedge-shape distribution	Qian et al., 2003
3	SiZer	Signifiant Zero Crossings, model describes driver-response relationship using non-parametric weighted polynomial regression and derive thresholds based on derrivates of smoothed curve	multiple potential thresholds reflected by range of bandwidths	considers univariate response variable and single predictor, user-defined bandwidth can lead to false thresholds detection. Do not provide measures of uncertainty	Sonderegger et al., 2009
4	Cumulative frequency distribution curves	uses comparison of observed vs. expected cumulative frequency distribution curves to define lower and upper limit of specific taxon	allows comparison of rare and abundant taxa, identifies potential indicator taxa with relative sensitivities	require large sample size and broad range of environmental gradient values	Utz et al., 2009
5	TITAN	Threshold Indicator Taxa Analysis, a form of nonparametric changepoint analysis combined with indicator species analysis to identify change points in community structure	nonlinear responses at community level, identifies direction and magnitude of indicator species responses, provides measure of uncertainty	difficult to identify multiple abrupt change points, not suitable for data wih abundant species in all samples	Baker and King 2010
6	GF	based on random forest approach, identify points along the gradient where species abundance changes	nonlinear responses at community level, identifies most important stressors, assume Gaussian errors, uses dimensionless R^2 thus data from different surveys or regions can be combined	do not provide measures of uncertainty	Ellis et al., 2012

(iii) Process-driven models

Process-driven models are created by theoretical knowledge of ecological processes and offer a framework that can be used to predict species responses under altered environmental conditions. These models are advantageous in predicting the impacts of global change than the typical statistical, rule-based models that make use of previously collected data (Cuddington et al., 2013). Cuddington et al. (2013) argued that process-driven models are most appropriate and essential for most of the management challenges of ecological systems under global change. A review of process-based range modelling of many terrestrial species highlighted that process-based approaches yield better prediction as compared to species distribution models, because they model the ecological processes underlying distribution patterns (Evans et al., 2016). Numerous process-based models in hydrology utilise multiple distinct processes including land and atmospheric processes, vegetation, coupled surface and subsurface domains, solute transport and biogeochemistry (Gassman et al., 2007). These models vary in complexity and quantitative capacity to deal with the number of processes, which influences the suitability of any model for a specific purpose (Fatichi et al., 2016). A review of the hydrological process-driven model Soil and Water Assessment Tool (SWAT) is given in the section below. SWAT was selected because it is user-friendly, easily accessible, integrates a number of catchment processes (Arnold et al., 2012), can easily incorporate climate information and simulates river catchments of various sizes (Arnold et al., 2012; Francesconi et al., 2016).

Soil and Water Assessment Tool (SWAT)

SWAT is a semi-distributed, process-driven, open-access model that has demonstrated strength for assessing watershed resource pollution and land use change impacts on the water quality, for wide-ranging scales across the world (Gassman et al., 2007). Arnold et al. (1999) pioneered using SWAT to simulate river flow for the entire USA at almost 6000 gauging stations. Some of the other regional-scale applications of SWAT include: simulation of the twelve major rivers in India for quantification of climate change impacts on surface water parameters (Gosain et al., 2006), calculation of water resources at sub-basin level on a monthly time step for the entire African continent (Faramarzi et al., 2013; Schuol et al., 2008) and simulation of climate change impacts on hydrology of river basins from the glacial region of Northern Pakistan

(Anjum et al., 2019). A review on SWAT model applications in Australia discussed that SWAT applications are relatively limited as compared to other regions (Saha and Zeleke, 2015) but varied from simple streamflow simulations (Saha et al., 2014) to complex impacts of droughts, forests and different management practices on hydrology, water use efficiency and water pollution (Brown et al., 2015; Lee et al., 2012), In South Australia, SWAT predicted significant effects on eutrophication in catchment reservoirs and on water quality of the river catchment due to projected climate and land use changes (Nguyen et al., 2017; Shrestha et al., 2017).

SWAT is a modelling tool for river basins that includes mathematical descriptions of physical, hydrochemical and biogeochemical processes, and simulates catchment processes such as surface runoff and infiltration, percolation, evapotranspiration, nutrient and sediment transport (Neitsch et al., 2011). The major components of this model are hydrology, weather, temperature, soil properties, pesticides, nutrients, plant growth and land use management (Gassman et al., 2007). For SWAT, catchments are divided into multi sub-basins that are spatially linked to each other, and every sub-basin is further aligned into hydrological response units (HRUs). HRUs are the fundamental working units of SWAT that consist of unique but homogeneous land use attributes. Over the almost 40 years of modelling efforts using SWAT, different scientists evaluated the performance of the model applying different calibrations, sensitivity and uncertainty analysis approaches (Gassman et al., 2007). For the performance of SWAT, Shepherd et al. (1999) compared 14 models and concluded that SWAT outperformed all others for simulating phosphorous loss. Nasr et al. (2007) compared three catchment models and found that SWAT outperformed when calibrated for total phosphorus concentrations. Predicting flow, sediments and nutrient loads of the Sixth Creek catchment, South Australia, under the influence of land use changes by applying both SWAT and the Australian catchment model SOURCE, Nguyen et al. (2019) concluded that the depths of process representations in SWAT resulted in more credible scenario forecasts.

Although SWAT is considered as a promising tool for continuous simulations and predictions of water quality in a catchment, it has limitations for monthly or daily simulations based on extreme storm events (Borah and Bera, 2004). Indeed, Srivastava et al. (2006) observed more accurate results from an Artificial Neural Network (ANN) than SWAT for simulating flow in a small watershed in Pennsylvania.

(iv) Data-driven models

During the last two decades with advances in new tools and technology to enhance data acquisition, data-driven models also known as inferential modelling techniques, have been increasingly used for solving numerical prediction problems in hydrology (Remesan and Mathew, 2016). A number of interlinked areas that contribute to the data-driven modelling approach include computational intelligence, machine learning and pattern recognition (Solomatine et al., 2009). Computational intelligence broadly covers three broad areas, i.e. fuzzy systems, neural networks and evolutionary computing (Solomatine et al., 2009). For the scope of this project, I used the hybrid evolutionary algorithm (HEA) from evolutionary computation, to predict future abundance of macroinvertebrates under altered environmental conditions of climate change and land use change. I also used self-organising maps (SOMs) based on neural networks to determine the diatoms community structure. These two methods are reviewed in the following sections.

Hybrid Evolutionary Algorithm (HEA)

Evolutionary or Genetic Algorithms (EAs) form a well-known class of metaheuristics, also known as inferential or self-adaptive techniques. They are based on principles of biological evolution, such as reproduction, crossover and mutation and have been used for a variety of water resource-related problems (Maier et al., 2014). The Hybrid evolutionary algorithm (HEA) (Cao et al., 2013) has been applied for predictive modelling and early warning of algal blooms in lakes and rivers (Cao et al., 2013; Zhang et al., 2015), determining phytoplankton community dynamics of Lake Kinneret, (Israel) (Recknagel et al., 2013) and meta-analysis of cyanobacteria species across lakes with different climate and trophic states (Recknagel et al., 2019).

Due to the distinct representation of discovered models in the form of IF-THEN-ELSE rules, HEA models are easily understood even by non-technical users. Other advantages of HEA include the ability to incorporate qualitative attributes of the quantitative variables in the same rule set and the ability to make predictions based on data patterns without any prerequisite expert knowledge. However, HEA requires a large amount of input data to discern data patterns and hence may have limited applicability where there is limited field data available.

Artificial Neural Networks (ANNs)

Artificial Neural Networks (ANNs) are nonlinear mapping structures inspired by biological neural systems. ANNs are considered as highly flexible, powerful tools for ecological modelling, especially for data with unknown relationships (Basheer and Hajmeer, 2000). This machine learning approach can learn from data and identify correlations between input data. Learning in ANNs can be classified into supervised, i.e. based on previous knowledge vs. non-supervised learning, i.e. deriving information from data. Kohonen self-organising maps (SOMs) (Kohonen, 2001) belong to non-supervised learning that inherits all basic characteristics of ANNs.

SOMs are designed not only for determining data patterns, but also for data clustering, data mining and abstraction and information visualisation (Kohonen, 2001). SOMs have been used by biologists due to their clear visual representation and dealing with nonlinearities and also their ability to work with outliers and missing data (Lek and Guégan, 1999).

SOMs find a wide variety of applications and have been used to predict community-level responses including pattern recognition of benthic macroinvertebrates (Compin and Céréghino, 2007), predicting and understanding of causal relationships of algal populations (Recknagel et al., 1997), determining diatoms thresholds (Grenier et al., 2010) and investigating spatial variations in fish assemblages (Penczak et al., 2005). In recent era, combination of SOMs with other statistical techniques has been used for the analysis of ecological data (Zhang et al., 2008). (Coste et al., 2009) used combination of SOMs and multivariate techniques to update ecological profiles of key diatoms species, and highlighted a need to improve biological diatom index.

Despite the adaptive and self-organising capacity of ANNs that can handle complex nonlinear data, it faces criticism because of their incapability to represent models and therefore are categorised as black-box models.

1.3 Research gaps and challenges for modelling and river health management

Besides the limitations of specific modelling technique as discussed in the above sections, the practical applications of models present significant challenges to researchers and managers alike. These challenges and research needs for river health modelling are discussed in the following section:

(i) Robust data and models

Successful modelling relies on good monitoring of data and robust models. Models benefit from good quality monitoring data sets for calibration, model fitting and validation. Also, robust models in turn can test the hypothesis from monitoring data considering the goals of management programmes. With increasing threats to river ecosystems, informed management decisions need to be based on patterns and trends from the observed data to predict future trends. However, river health monitoring programmes may find it difficult to obtain consistent trends from the data, due to necessary changes of monitoring objectives from time to time (Goonan et al., 2012). Furthermore, to comprehend the complexity of ecosystems affected by multiple stressors may not be possible using a single model but may require an integrated modelling approach.

(ii) Appropriate model selection

To deal with complex and changing ecosystem dynamics to guide appropriate management actions, scientists and managers have applied more sophisticated models (Guntenspergen and Gross, 2014; Wagenhoff et al., 2017a). A wide variety of alternative models are available to solve a single problem, but the justification for appropriate model selection is given less attention (Kelly et al., 2013; Nguyen et al., 2019). Comparative studies on the performance of different models have shown varied results from different models. For instance, threshold identification varied markedly between piecewise quantile regression, nonparametric change point analysis and significant zero crossings (SiZer) based on sample size, data density and the pattern of linear change across an environmental gradient (Daily et al., 2012). Dodds et al. (2010) identified a three-fold difference in threshold values where different models were applied to a single response variable. For process-driven models, it was argued that being originating from theories of ecological processes, they offer more advantages in predicting global change impacts than rule-based or statistical models (Cuddington et al., 2013). However, comparison of two alternative process-driven models (SWAT and SOURCE) has shown that SOURCE produced robust estimates of streamflow but nutrient loads simulated by SWAT were more realistic (Nguyen et al., 2019). Greater choice of alternative models to solve a particular problem is associated with the challenge of appropriate model selection.

(iii) Appropriate spatial scales

Understanding the spatial distribution of organisms across multiple scales has become an important paradigm in aquatic ecology (Kuemmerlen et al., 2019; Schmidt et al., 2020; Schweiger et al., 2005). Riverine ecosystems are controlled by many linked factors acting at multiple spatial scales that determine the species composition, function and structure (Lowe et al., 2006).

Ecologists often measure scale in terms of spatial extent (i.e. the extent of study area) and its granularity (i.e. spatial resolution) (Alahuhta and Heino, 2013). There is extensive evidence that variations in both spatial extent and spatial grain influence alpha and beta diversity (Barton et al., 2013; Feld et al., 2009; Steinbauer et al., 2012). Successful modelling involves a series of compromises regarding the spatial extent and spatial resolution. Spatial scales used for modelling have been found not matching with management scales (Stevens et al., 2007). This mismatch between theory and practice needs to be overcome for successful management. For example, global circulation models are generally created to cover large geographical areas and downscaling is required to produce predictions for regional or local scales. Other examples are ecological threshold models that are mostly applied on broad spatial scales to cover a range of environmental gradients (e.g. King and Baker, 2010). However, the application of similar thresholds to manage a stream that is highly degraded or pristine, is still a problem. Thus, modelling for river health management needs to continue to consider appropriate spatial scales along with new modelling techniques.

(iv) Dealing with multiple stressors in a changing world

There is a growing need to evaluate the individual impacts of climate change and land use change on species diversity in riverine ecosystems (Chessman, 2009; Nukazawa et al., 2018; Woznicki et al., 2016). Some evidence also suggests that climate and land use change in combination will synergistically contribute to biodiversity loss at species, genetic or habitat level (Mantyka-Pringle et al., 2014; Nelson et al., 2009). However, there is a large uncertainty on which environmental stressors (such as altered flows due to climate or land use change, water temperature or nutrient enrichment) either alone or in combination will have the most substantial impact on shaping biodiversity in freshwater ecosystems. Identifying the combined effects of global changes, e.g. climate and land use change, is not a simple task. In this situation, process-based models with the potential to simulate beyond known conditions and to efficiently analyse multiple scenarios, are beneficial for managing ecological systems under changing global situations (Cuddington et al., 2013).

In conclusion, river ecosystems are affected by multiple stressors and understanding the individual as well combined effects of these stressors is another challenge for river health management (Ormerod et al., 2010; Wagenhoff et al., 2012). Thus models that take into account the effects of multiple stressors or provide measures of uncertainty for individual stressor-response relationships may benefit management decisions.

1.4 Research objectives and significance

This thesis focuses on improving understanding of, and decision support in, river health management by the integration of routine ecological monitoring (i.e. macroinvertebrates and diatoms) with different modelling approaches (see Figure 1). A variety of modelling approaches ranging from ecological threshold models (HEA, TITAN and GF), a process-driven model (SWAT) and a predictive model (HEA) were used to answer the following research questions:

1. Do differences between the River Torrens and Onkaparinga River result in the identification of different water quality thresholds for macroinvertebrates and how do thresholds identified by HEA compare to those identified by TITAN?

2. Is threshold identification affected by model-based bias, and how does threshold identification by TITAN and GF differ when applied to field and synthetic data?

3. Are there any stream-specific variations in diatom species distribution in relation to water quality drivers? And

4. Do diatom threshold responses vary among different hierarchical spatial scales and regions?

5. Is it possible to quantify species-specific macroinvertebrate responses to future climate and land use change scenarios? How might variations in future climate and land use changes affect species responses?

Research questions (1) and (3) were designed to improve the understanding of local scale water quality thresholds in the two catchments, and the stream-specific distribution of diatom indicator species from the two catchments. Evaluating the findings from these questions formed the basis for research question (2) that aimed to compare the performance of two ecological threshold methods using field and synthetic data. Research question (4) was designed to evaluate the spatial scale influences on threshold response variations needed to derive and apply meaningful thresholds for river health management.

Research question (5) aimed to develop and test the feasibility of an integrated modelling approach by combining some of the models used in answering the above questions in addition to process-driven model (SWAT), and quantify species-specific macroinvertebrate responses under climate and land use change scenarios.

1.5 Thesis Structure

This thesis consists of six chapters. The first chapter presented a general introduction, literature review of topics in subsequent chapters, research gaps, objectives, key research questions and significance. Chapter 2 and 3 are presented as published papers. Chapter 4 is presented in the form of a manuscript with the intention to submit to 'Journal of Environmental Management'. Chapter 5 is presented in the form of a manuscript, and is under review in 'Ecological Indicators'.

Chapter 2 presents a comparison of water quality thresholds from two local scale catchments i.e., the River Torrens and Onkaparinga River catchments in South Australia, using two different models, HEA and TITAN.

Chapter 3 evaluates the performance of two commonly used ecological threshold methods (GF and TITAN).

Chapter 4 extends the thresholds evaluation approach by integrating data generated from fieldwork with another broad-scale data set to investigate threshold response variations at multiple spatial scales.

Chapter 5 investigates how a combination of an ecological threshold model (GF), a process-driven model (SWAT) and a predictive model (HEA) can be used to predict species-specific responses, under changing scenarios of climate and land use.

The final Chapter 6 summarises key findings, limitations and recommendations for future research in the field.

Each chapter is followed by supporting information with additional results.

A further data analysis chapter based on fieldwork was written in publication style and given as Appendix B. Other information given as appendix A include: (1) a conference paper and two abstracts presented at international conferences.

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Comparison of water quality thresholds for macroinvertebrates in two Mediterranean catchments quantified by the inferential techniques TITAN and HEA

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ABSTRACT

Catchment managers face complex decisions to balance between competing targets such as water quantity and quality, land use and biodiversity. To balance these targets satisfactorily, decisions need to be grounded in reliable information. The present study aimed to identify potential water quality drivers that may impact the sustainability of the River Torrens and the Onkaparinga River catchments in South Australia by means of the Threshold Indicator Taxa Analysis (TITAN) and the Hybrid Evolutionary Algorithm (HEA). Fourteen years of data comprising abundance for 518 macroinvertebrate taxa and water quality variables were examined to determine possible stimulating and inhibiting effects of water quality conditions on macroinvertebrates at catchment and stream site level. When applied to the Torrens ($n = 262$) and Onkaparinga ($n = 227$), TITAN identified positive ($z+$) and negative ($z-$) responding species for both catchments and detected community change points in response to total nitrogen (Torrens: $z+ = 305\,\mu g\,L^{-1}$, $z- = 277\,\mu g\,L^{-1}$; Onkaparinga: $z+ = 420\,\mu g\,L^{-1}$, $z- = 397\,\mu g\,L^{-1}$), total phosphorus (Torrens: $z+$ and $z- = 22\,\mu g\,L^{-1}$; Onkaparinga: $z+ = 73\,\mu g\,L^{-1}$, $z- = 71\,\mu g\,L^{-1}$) and electrical conductivity (Torrens: $z+ = 931\,\mu S\,cm^{-1}$, $z- = 407\,\mu S\,cm^{-1}$; Onkaparinga: $z+ = 951\,\mu S\,cm^{-1}$, $z- = 428\,\mu S\,cm^{-1}$). These results suggested similar change points along electrical conductivity (EC) and total nitrogen (TN) gradients in both catchments, but three-times higher change points for total phosphorus (TP) in the Onkaparinga River catchment than in the Torrens catchment.

Since TITAN identified the species *Austrochiltonia australis* as responding positively to TP, TN and EC increases, and *Paramerina* spp. as responding negatively, these taxa were selected for modelling by HEA. In contrast to TITAN, HEA used averaged data from 37 stream sites in each of the two catchments. Resulting models for *A. australis* revealed much higher change points for TN, TP and EC in the Torrens catchment compared to the TITAN results. Models for *Paramerina* spp. suggested twice as high thresholds for TN and EC in Onkaparinga as compared to the Torrens. Whilst TN and TP thresholds specified by HEA fall within the range identified by TITAN, thresholds for EC exceeded significantly those identified by TITAN.

Overall, the study has shown that the nature of the data used in the two threshold approaches is likely to have resulted in differences in the derived thresholds. Importantly, despite the differences observed in thresholds based either on a catchment or the modelling technique, overall results indicate that South Australian water quality guidelines for freshwater systems are too high.

1. Introduction

Managing Mediterranean catchments with different land uses is a challenging task for river health managers and planners. Mediterranean stream ecosystems which already have to cope with intermittent flows, endure further pressure from urbanisation, physically altered habitats and flow regimes, as well as changing nutrient loads (Norris and Thoms, 1999).

In an attempt to assess impacts of deteriorating water quality and habitat conditions on aquatic organisms, the concept of ecological threshold analysis using bioindicators (Groffman et al., 2006) has been developed as a management tool. Ecological thresholds within

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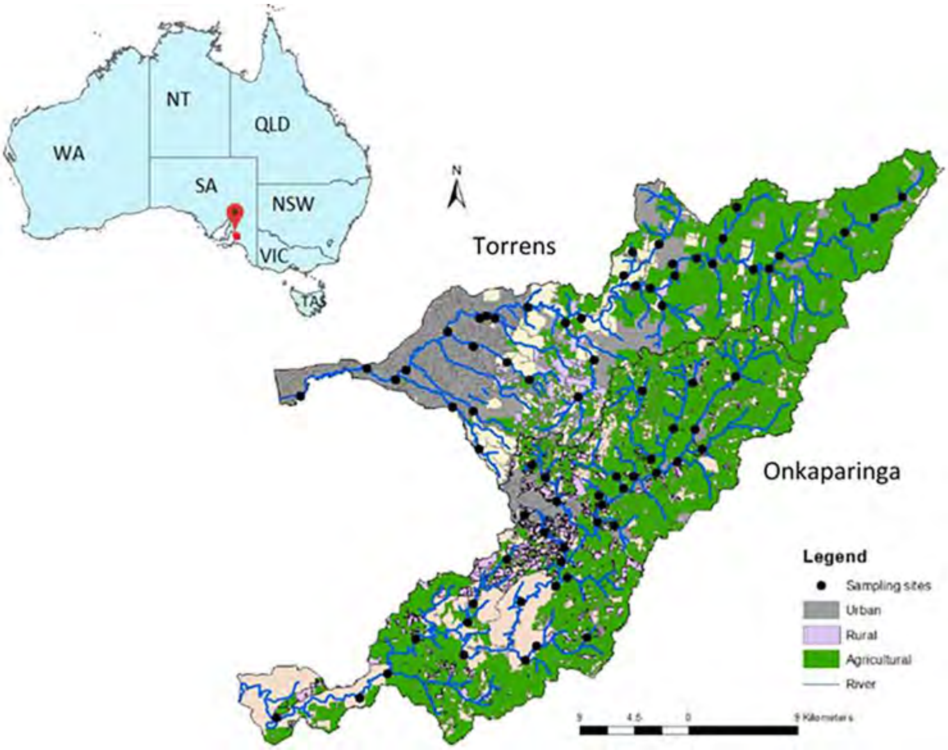

Fig. 1. Location and land use of the Torrens and Onkaparinga catchments.

catchments can assist managers in identifying hotspots of habitat degradation and preventing loss of biodiversity (King et al., 2016). Ecological thresholds are defined as tipping points along a continuum that show a shift to an imbalanced or an unacceptable state (Groffman et al., 2006). In contrast, Baker and King (2010) suggested the concept of community thresholds as change points where there is a synchronous change of indicator taxa along an environmental gradient (King and Baker, 2014). Martin et al. (2009) proposed the concept of "decision thresholds" that recommends management action when stressors exceed certain thresholds, while "utility thresholds" are based on management objectives, where small changes in an ecosystem produce positive outcomes for resource management (Samhouri et al., 2010).

Numerous methods for threshold quantification have been developed over the past decade (Baker and King, 2010; Dodds et al., 2010; Ellis et al., 2012; Recknagel et al., 2014; Sonderegger et al., 2009). There is a growing interest in applying threshold analysis for informing management regarding different environmental stressors such as water quality, urban land use, flow permanence, impervious cover, glacial cover and meltwater contribution that affect aquatic habitats (Firmiano et al., 2017; Hilderbrand et al., 2010; Kail et al., 2012; Khamis et al., 2014; King et al., 2016; Sundermann et al., 2015). Despite widespread use of different threshold models, implementation of environmental thresholds for management solutions remains a challenge. To aid in the progression from theory to application, reliance on a single method needs to be overcome by exploring the positives and negatives of various techniques.

Threshold indicator taxa analysis (TITAN) enables the identification of ecological community thresholds based on indicator species scores that integrate abundance, richness, direction and magnitude of individual taxon responses in the whole community across an environmental gradient (Baker and King, 2010). TITAN has been applied to demonstrate stream assemblage thresholds in response to a variety of environmental gradients including anthropogenic pressures like urbanization and water quality of European and U.S rivers (Kail et al., 2012; King et al., 2016), Brazilian neotropical savanna streams (Firmiano et al., 2017), alpine rivers and lakes (Khamis et al., 2014) and salinization of Canadian streams (Porter-Goff et al., 2013). In south-east Australia, TITAN in combination with an expert opinion was used for discretization of predictor variables in Bayesian networks (Lucena-Moya et al., 2015). The hybrid evolutionary algorithm (HEA) (Cao et al., 2013) has been designed to evolve either temporally- or spatially-explicit predictive models represented by IF-THEN-ELSE rules from sequential ecological data by combining genetic programming and differential evolution. IF-conditions of the rules display population, community or habitat thresholds discovered by HEA. HEA has been successfully applied for threshold analysis of plankton communities of Lake Kinneret (Israel), Lake Taihu (China), Lake Müggelsee (Germany), Lakes Lajes and Descoberto (Brazil) (Recknagel et al., 2016, 2014. 2013; Rocha et al., 2019) as well as for early warning of cyanobacteria blooms in Lake Wivenhoe (Australia) and Vaal Reservoir (South Africa) (Recknagel et al., 2017).

Ecological threshold analyses have been widely discussed in the literature, however, few applications at catchment scale have been reported. Distribution of macroinvertebrate assemblages is mainly affected by water quality at the catchment scale (Feld and Hering, 2007). Increased nutrient concentrations are of major concern in the Mediterranean catchments, and the management authorities are striving to revise corresponding water quality guidelines (ANZG, 2018). Thus, this study aimed to identify and compare total nitrogen (TN), total phosphorus (TP) and electrical conductivity (EC) thresholds for macroinvertebrates in two neighboring catchments that differ in land use and levels of human disturbance using two different models.

This study addresses the following research questions by applying a multi-model approach as suggested by Samhouri et al. (2017): (1) Do differences between the two catchments result in the identification of different water quality change points? (2) Do the change points derived by HEA correspond to the change points discovered by TITAN? (3) Do results of HEA complement the findings of TITAN?

2. Materials and methods

2.1. Study sites

The Torrens and Onkaparinga River catchments (Fig. 1) covers almost 1160 km^2 area including the city of Adelaide, its suburbs and surrounding rural-urban fringe which supports 75% of the South Australian population (Bourman et al., 2010). The catchments have multiple land uses including drinking water supply, irrigation for farms, vineyards, woodlands, horticulture, stock grazing, as well as recreation for local communities. Streamflow in these catchments is seasonal, i.e. high rainfall during winter maintains almost permanent flow during the cooler months but most creeks cease to flow or may be completely dry for several months in summer (SAEPA, 2012). The upper part of the Torrens catchment is characterised by grazing whilst the lower part runs through the city of Adelaide which is more likely to be affected by future urbanization impacts (Nguyen et al., 2018). Nutrient concentrations, algal blooms, pathogens and sediment loads in the Torrens catchment periodically diminish water quality below the acceptable standards for recreational use and for drinking water supply (Brookes, 2012; Gale et al., 2006). The Onkaparinga catchment is characterised by large areas of pasture, urban areas and intensive viticulture and horticulture in the western tributaries. Major water quality related issues in this catchment include high phosphorus and nitrogen concentrations, algal blooms, sediment loads and salinisation (Shrestha et al., 2016). The two catchments are also affected by water transfers from the River Murray (Tibby et al. 2010) that occurs in the upper reaches of the Torrens River, and in the mid to lower reaches in the Onkaparinga River.

2.2. Macroinvertebrate and water quality data

The macroinvertebrate data comprised the abundance of 518 taxa from 74 sites (sample size, $n = 489$) (Fig. 1), distributed across the two catchments from 1994 to 2007. The macroinvertebrate and water samples were collected using a variant of the AusRivAS method, under the Monitoring, Evaluation and Reporting Program (MERP) conducted by South Australian Environmental Protection Agency (SA-EPA). For macroinvertebrates, 10 m sweep samples were preserved in the field and a 10% subsample was sorted in the laboratory and identified to the lowest taxonomic resolution. Species names and abbreviations for all taxa are provided in the Appendix. Nutrients including total nitrogen (TN) and total phosphorus (TP) were analysed using standard American Public and Health Association methods in the laboratory (APHA, 2005). Within the scope of this study, threshold models for TN, TP and EC were tested to facilitate the process of improving water quality guidelines for South Australia. The annual sampling frequency varied among sites. Some sites were sampled once or twice, while others were sampled at regular intervals, and the remainder on several occasions but at irregular intervals. Thus, to overcome this data limitation, site-specific data was used for the HEA model, by averaging water quality data for each site (37 sites from each catchment) collected over the time period of 14 years irrespective of the temporal scale. For TITAN, this study utilised all data ($n = 227$ for Onkaparinga and $n = 262$ for Torrens) of macroinvertebrates with a taxa richness > 3.

The summary statistics of water quality and macroinvertebrate data collected by the South Australian monitoring programme are presented in Table 1. Twenty percent of the samples exceeded the limit values for nutrient concentrations $TN = 1000\ \mu gL^{-1}$ and $TP = 100\ \mu gL^{-1}$ as defined by the national water quality guidelines (ANZECC, 2000). For macroinvertebrate distributions, the mean Shannon-diversity index was significantly higher in the Torrens catchment than in the Onkaparinga catchment (means 2.14 and 1.83 respectively: $t = 2.072$, $p < 0.05$). However, there were no significant differences in mean abundance and richness ($t = -1.72$, $p > 0.05$ and $t = 1.17$, $p > 0.05$, respectively).

2.3. Threshold analysis by TITAN and HEA

This study applied the two inferential techniques Threshold Indicator Taxa Analysis (TITAN) and Hybrid Evolutionary Algorithm (HEA) to quantify TN, TP and EC thresholds of macroinvertebrates in the Onkaparinga and Torrens River catchments in South Australia, based on data monitored from 1994 to 2007. TITAN distinguishes between negative (z−) and positive (z+) taxon responses and tracks cumulative responses of the whole community. It allows the incorporation of multiple taxa and deals with non-linear responses of both species-rare and species-rich communities. Here we applied TITAN2, R-version 3.4.1 (Baker and King, 2010) with 500 bootstrap runs in order to estimate indicator reliability and purity, as well as uncertainty around the location of individual taxa and community change points.

The hybrid evolutionary algorithm has been designed to evolve either temporally- or spatially-explicit predictive models represented by IF-THEN-ELSE rules from sequential ecological data. It applies genetic programming (Koza, 1992) to evolve the optimum structure, and differential evolution (Storn and Price, 1997) to optimise the parameters of the "fittest" models. IF-conditions of the rules display population, community or habitat thresholds discovered by HEA. In this study, the C^{++} version of HEA (Cao et al., 2013) has been run on a Corvus super computer in a cyclic boot-strap scheme that randomly selected different data-subsets for training (75% of all data points) and testing (25% of all data points) for each of 80 generations of models. After 100 boot-strap runs, it determined the overall "fittest model" of all generations by cross-validation of the 37 stream sites for the two respective catchments. The best fit models with $r^2 > 0.5$ were selected for evaluation.

3. Results

3.1. Macroinvertebrate community change points identified by TITAN

TITAN identified positive (z+) and negative (z−) responding taxa change points for TN, TP and EC that are summarised as z scores in Table 2. Change points for significant indicator taxa with purity and reliability > 0.95 were considered. Community change points suggest that TN and TP thresholds for both catchments were below the national water quality guidelines ($TN = 1000\ \mu g\ L^{-1}$, $TP = 100\ \mu g\ L^{-1}$). Despite the fact that both catchments have similar mean and median TP levels, the Onkaparinga River catchment had a threefold higher change point ($TP = 71\ \mu g\ L^{-1}$) than Torrens ($TP = 22\ \mu g\ L^{-1}$). However, change points for TN and EC (both for sum z− and sum z+) have exhibited not big differences at catchment scale (Table 1).

Firstly, TITAN was used to determine the overall water quality thresholds for macroinvertebrate communities recorded from the two catchments. Secondly, the species *Austrochiltonia australis* and *Paramerina* spp., identified by TITAN as representative taxa that responded positively and negatively, respectively, to increasing water quality parameters were selected to quantify their thresholds using site-specific data by HEA. *A. australis* is a widespread amphipod in South Australia (King and Leys, 2011), which grazes on algae and thus is likely to increase in abundance with increased nutrient conditions. *Paramerina* spp. is a genus of predatory midges in the sub-family *Tanypodinae* that feeds on algal grazers, thus abundance may decrease at expense of increased competition for food resources among other taxa (Berg et al., 1995). Therefore, both species are well suited for testing HEA's capacity to model their population dynamics in response to site-specific water quality thresholds.

For all the water quality parameters considered, TITAN indicated higher number of indicator taxa for the Torrens compared to the Onkaparinga catchment. *A. australis* and *Paramerina* spp. showed a positive and negative response to most of the tested water quality variables, respectively. (Figs. 2–7a). We therefore selected these two taxa and plotted their TN, TP and EC ranges for both catchments in Figs. 2–7. Detailed results of taxon-

Table 1
Summary statistics of water quality parameters and macroinvertebrate assemblages from Torrens and Onkaparinga river catchments, collected over the time period (1994–2007).

	Torrens *(n = 262)*				Onkaparinga *(n = 227)*			
Variable	Minimum	Mean	Median	Maximum	Minimum	Mean	Median	Maximum
Water Quality								
EC ($\mu S\,cm^{-1}$)	139	1062	914	8010	151	1193	956	6030
Total-P (mg L^{-1})	0.003	0.088	0.057	0.870	0.003	0.089	0.063	0.560
Total-N (mg L^{-1})	0.000	0.527	0.521	4.450	0.120	0.541	0.480	5.640
Macroinvertebrate Assemblages								
Abundance	79	6942	4518	64,142	611	8302	5720	69,410
Richness	6	35	35	72	10	34	32	74
Shannon-diversity index	0.68	2.14	2.20	3.36	0.39	1.83	1.72	2.74

specific change points suggest that the representative positive taxon i.e. *A. australis* exhibited a low difference in change points between two catchments (TN = ~400, EC = ~600 $\mu S\,cm^{-1}$), however, on average higher change points were observed in Onkaparinga river catchment (for Torrens: TN = 360 $\mu g\,L^{-1}$, EC = 600 $\mu S\,cm^{-1}$; and Onkaparinga: TN = 420 $\mu g\,L^{-1}$, EC = 651 $\mu S\,cm^{-1}$) shown in Figs. 2c, 3c, 6c and 7c. *Paramerina* spp., a negative respondent with species-specific change point, occurred at approximately two times higher levels of TP in the Onkaparinga than in the Torrens River catchments (Torrens: TP = 40 $\mu g\,L^{-1}$; Onkaparinga: 90 $\mu g\,L^{-1}$) shown in Figs. 4 and 5. TN and EC change points at which *Paramerina* spp. showed a maximum change in response appeared to be lower for the Torrens (TN $\mu g\,L^{-1}$ = 500 $\mu g\,L^{-1}$, EC = 407 $\mu S\,cm^{-1}$) (Figs. 2b, 4b and 6b) than for the Onkaparinga data set (TN = 900 $\mu g\,L^{-1}$ and EC = 428 $\mu S\,cm^{-1}$) (Figs. 3b, 5b and 7b). Overall, similar trend was observed for the similar species identified by TITAN in both the catchments (Figs. 2–7).

3.2. *Species-specific thresholds identified by HEA*

In order to compare the species-specific TN, TP and EC thresholds for *A. australis* and *Paramerina* spp. identified by TITAN, we developed inferential models of the two species. Figs. 8 and 9 document the models that have revealed TN thresholds for *A. australis* and *Paramerina* spp. in the two catchments. Whilst similar concentrations of TN > = 600 and TN > = 700 $\mu g\,L^{-1}$ have been identified as change points indicating highest abundances of both *A. australis* and *Paramerina* spp. in the Onkaparinga River catchment (Figs. 8c and 9c), TN change points for the Torrens River catchment differed significantly with 1240 = < TN $\mu g\,L^{-1}$ < 1700 for *A. australis* and TN < = 300 $\mu g\,L^{-1}$ for *Paramerina* spp. (Figs. 8f and 9f). The models in Figs. 10 and 11 revealed the TP change points for *A. australis* and *Paramerina* spp. in the two catchments. The highest abundances of *A. australis* were determined by concentrations of TP > = 225 $\mu g\,L^{-1}$ at upstream sites of the Onkaparinga and TP > = 130 $\mu g\,L^{-1}$ at upstream sites of the Torrens catchment (Fig. 10b, e and c, f). However, high abundances of *A. australis* at downstream sites of both catchments were indicated by lower TP concentrations. High abundances of *Paramerina* spp. have been predicted in both catchments by similar concentrations of TP < 25 and TP < = 26.6 $\mu g\,L^{-1}$ (Fig. 11c and f). The Figs. 12 and 13 display the models that identified EC thresholds for *A. australis* and *Paramerina* spp. in the two catchments. As shown in Fig. 12b and c, highest abundances of *A. australis* occurred at EC > = 894 $\mu S\,cm^{-1}$ in the Onkaparinga, whilst *A. australis* tend to be most abundant in the Torrens at EC < 1517 $\mu S\,cm^{-1}$.

4. Discussion

We aimed to identify and compare the thresholds from two stream catchments beginning with community-level thresholds derived by TITAN. We also compared findings from TITAN with HEA to determine taxon-specific thresholds within the region.

When applied to all data of the Torrens and the Onkaparinga River catchments, TITAN results suggested similar community change points of TN and EC for sum (z+) taxa in both catchments, but three times higher change point of TP in the Onkaparinga compared to the Torrens. This may reflect the fact that Onkaparinga has a much higher observed TP concentrations in some streams than the Torrens, likely as a result of intensive viticulture and horticulture. The appearance of *A. australis* as a distinct sum (z+) taxa suggested its tolerance to increasing nutrient levels that are typical for grazers of algae such as of *A. australis* (King and Leys, 2011).

TITAN identified lower change points of TN, TP and EC for *Paramerina* spp. in the Torrens than the Onkaparinga River catchments. These findings imply that *Paramerina* spp. tolerates higher TN and TP levels in the Onkaparinga catchment before showing inhibitory effects compared to the Torrens. *Paramerina* spp. belongs to the subfamily *Tanypodinae* which has shown similar negative responses to water pollution in European rivers (Kail et al., 2012). Overall sensitivities of the two species corresponded well with the SIGNAL score (a scoring system for macroinvertebrates in Australian Rivers), where the

Table 2
Water quality thresholds in two catchments identified by TITAN. Sum (z−) and sum (z+) represent negative and positive responses. Values in parenthesis are the 5th, 50th and 95th percentiles of 500 bootstrap replicates.

	Torrens		Onkaparing	
Water Quality	sum (z−)	sum (z+)	sum (z−)	sum (z+)
Total-N ($\mu g\,L^{-1}$)	**277** (281, 359, 412)	**305** (294, 360, 559)	**397** (275, 392, 600)	**420** (388, 503, 672)
Total-P ($\mu g\,L^{-1}$)	**22** (18, 21, 24)	**22** (21, 27, 76)	**71** (29, 72, 75)	**73** (46, 57, 160)
EC ($\mu S\,cm^{-1}$)	**407** (394, 435, 576)	**931** (796, 931, 1203)	**428** (323, 430, 1090)	**951** (944, 1165, 1661)

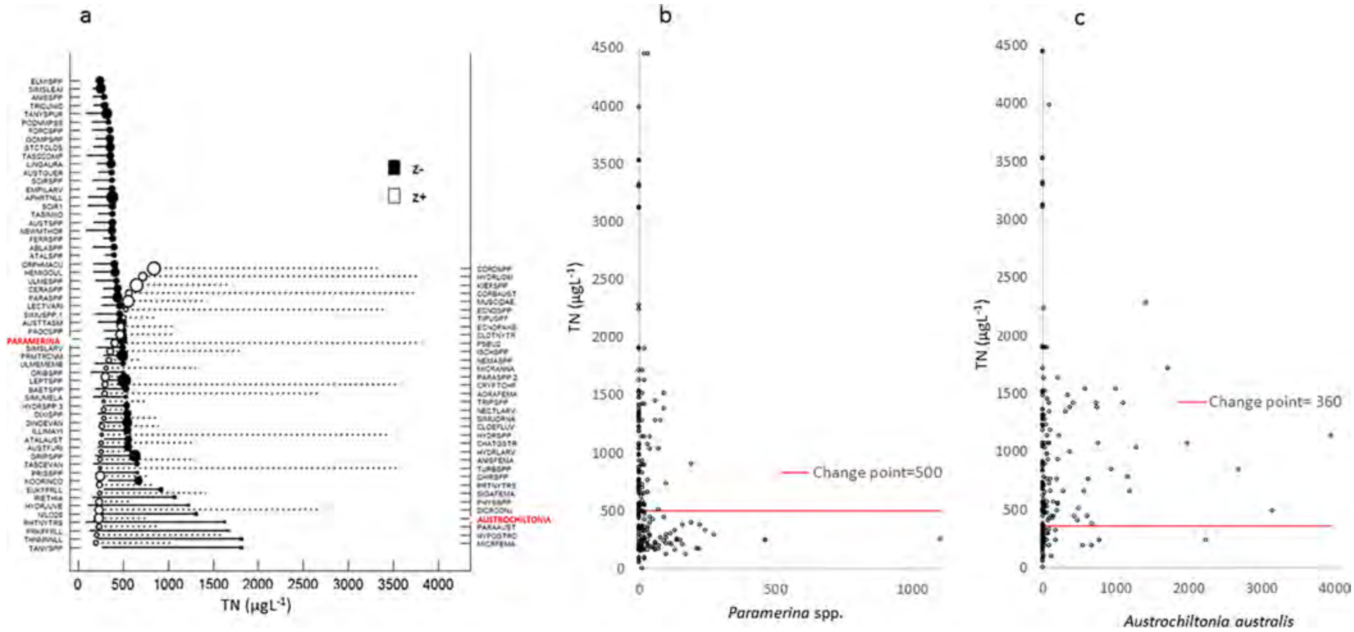

Fig. 2. TN thresholds discovered by TITAN for the Torrens catchment. (a) Thresholds for macroinvertebrates community (sample size, n = 262). (b) Change point for *Paramerina* spp. visualised by means of observed data, and (c) Change point for *A. australis* visualised by means of observed data.

subfamily *Tanypodinae* has a higher sensitivity grade (SIGNAL grade 4) compared to the sensitivity grade of 2 of family *Ceinidae*, to which *A. australis* belongs (Chessman, 2003).

When HEA was applied to data from the Torrens, results showed similar trends for *A. australis* in response to TN, TP and EC as TITAN, but revealed higher thresholds of each water quality parameter compared to TITAN. TN and EC thresholds for *Paramerina* spp. discovered by HEA were two-times higher in the Onkaparinga compared to those for the Torrens. When compared with results from TITAN, changing points of HEA for TP appeared to be in a similar range for both catchments. However, changing points of TP and EC suggested by HEA for *Paramerina* spp. for both catchments exceeded significantly those discovered by TITAN. Despite the differences of observed thresholds between the two catchments and the modelling techniques, overall results indicate that water quality guidelines for South Australian streams consider too high TP and TN concentrations. This study was not conducted at a state-wide level but comprised of data of some of the most degraded streams allowing to draw this conclusion. Results of this study will assist in defining management objectives, where decisions based on small changes in an ecosystem can produce substantial positive outcomes for resource management.

Differences in the results by TITAN and HEA may be explained by the frequency distributions of the water quality data that were used. The negatively skewed distributions show that frequencies of low values of TN, TP and EC used by TITAN were at least 10-times higher than those used by HEA Therefore, medium to high values of TN, TP and EC may have been underestimated by TITAN, and possibly explain significantly lower threshold values for TN, TP and EC derived by TITAN

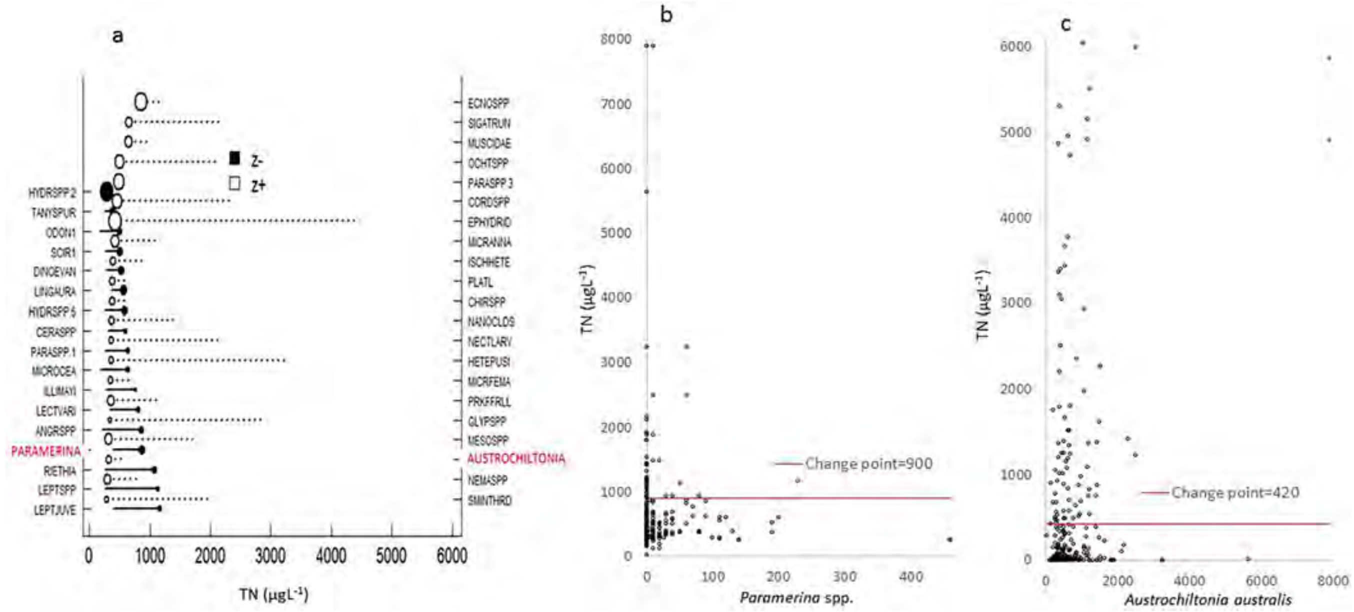

Fig. 3. TN thresholds discovered by TITAN for the Onkaparinga catchment. (a) Thresholds for macroinvertebrates community (sample size, n = 227). (b) Change point for *Paramerina* spp. visualised by means of observed data, and (c) Change point for *A. australis* visualised by means of observed data.

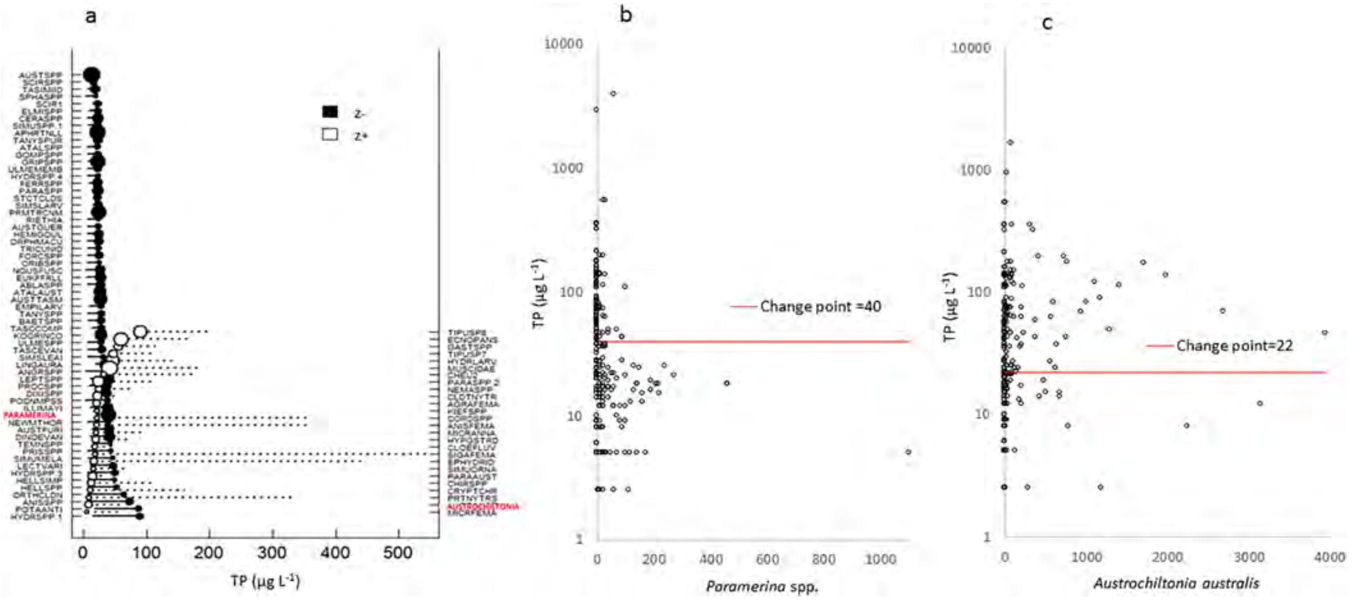

Fig. 4. TP thresholds discovered by TITAN for the Torrens catchment. (a) Thresholds for macroinvertebrates community (sample size, n = 262). (b) Change point for *Paramerina* spp. visualised by means of observed data, and (c) Change point for *A. australis* visualised by means of observed data.

compared to HEA. Moreover, TITAN used aggregate community metrics that may obscure the single species responses.

Due to the complex nature of ecological data sets, limitations associated with different models and underlying statistical assumptions, there is a need to complement community-related results with findings from other inferential methods based on single-species or via experimentation (e.g. King and Baker, 2014; Wagenhoff et al., 2017). Species-specific numerical thresholds identified by HEA, in general, were higher than community-related thresholds revealed by TITAN. These differences were not surprising since data extracted for species-specific analysis by HEA was just a small segment of the whole community and water quality data. Thus results from the two approaches are likely to benefit managers by providing information on thresholds both at the community level and at species-specific level. The study has also

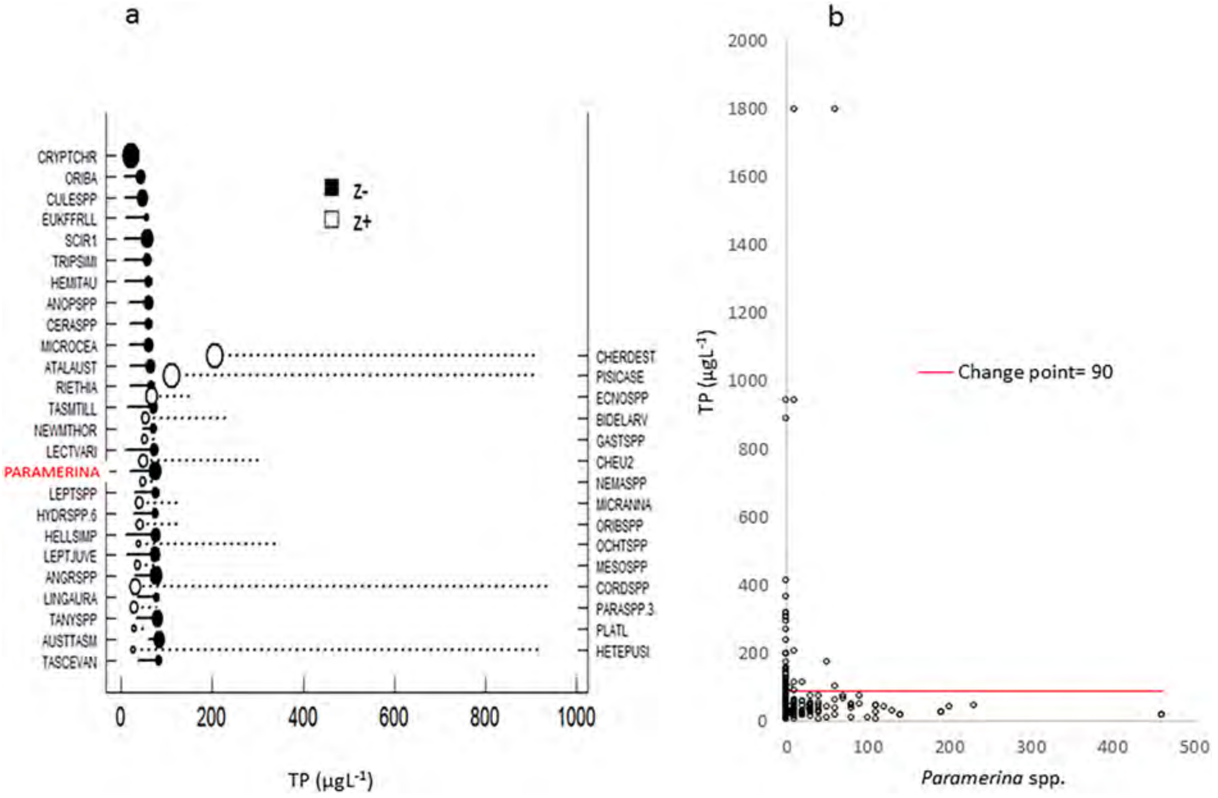

Fig. 5. TP thresholds discovered by TITAN for the Onkaparinga catchment. (a) Thresholds for macroinvertebrates community (sample size, n = 227). (b) Change point for *Paramerina* spp. visualised by means of observed data. Note: Since *A. australis* was not matching the criteria of pure and reliable taxa (i.e. > 0.95), only the change point for the sum (z−) taxa were considered.

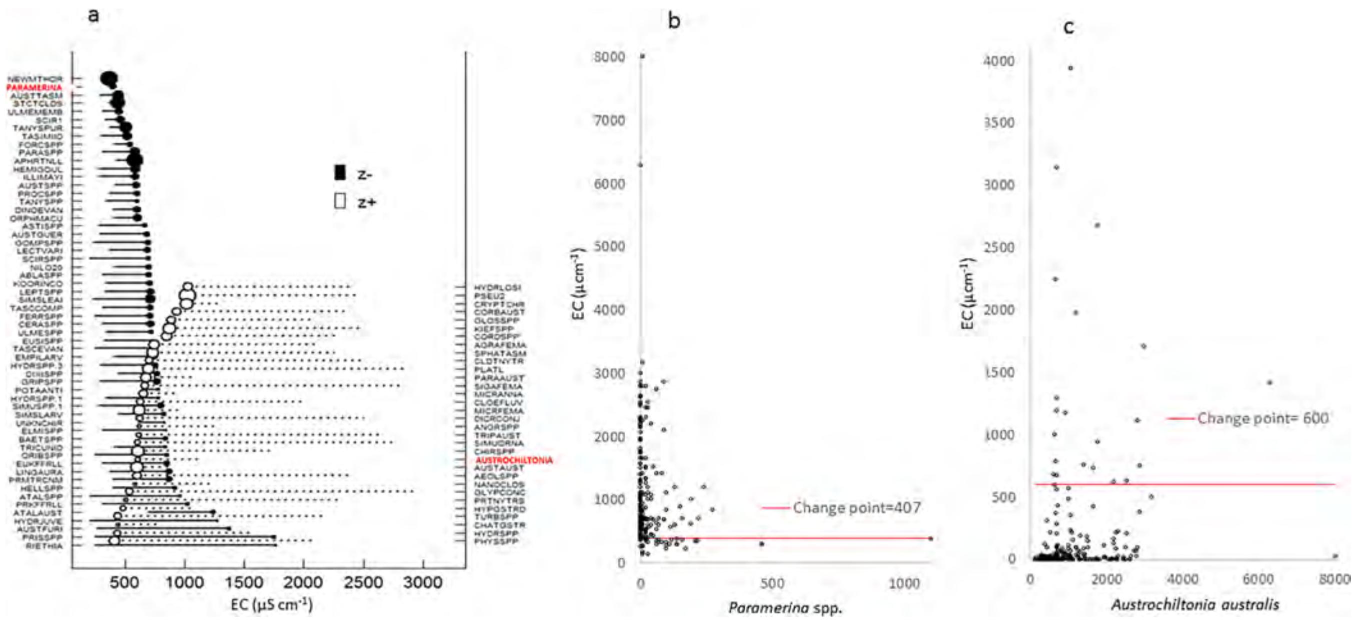

Fig. 6. EC thresholds discovered by TITAN for the Torrens catchment. (a) Thresholds for macroinvertebrates community (sample size, n = 262). (b) Change point for *Paramerina* spp. visualised by means of observed data, and (c) Change point for *A. australis* visualised by means of observed data.

demonstrated that *A. australis* and *Paramerina* spp. prove to be useful indicators of changes in nutrient concentrations in agricultural landscapes.

5. Conclusions

This study allows to draw following conclusions: (1) TITAN and HEA identified higher TP and EC changing points for the Onkaparinga River catchment reflecting its more intensive agricultural land uses compared to the Torrens River catchment. (2) Species-specific change points of TN, TP and EC for *A. australis* and *Paramerina* spp. identified by HEA were higher than community change points identified by TITAN. These differences may be due to the different quantities and distributions of data used by TITAN and HEA. However, this finding doesn't suggest that the two models require similar data distributions. (3) Overall results indicate that South Australian water quality

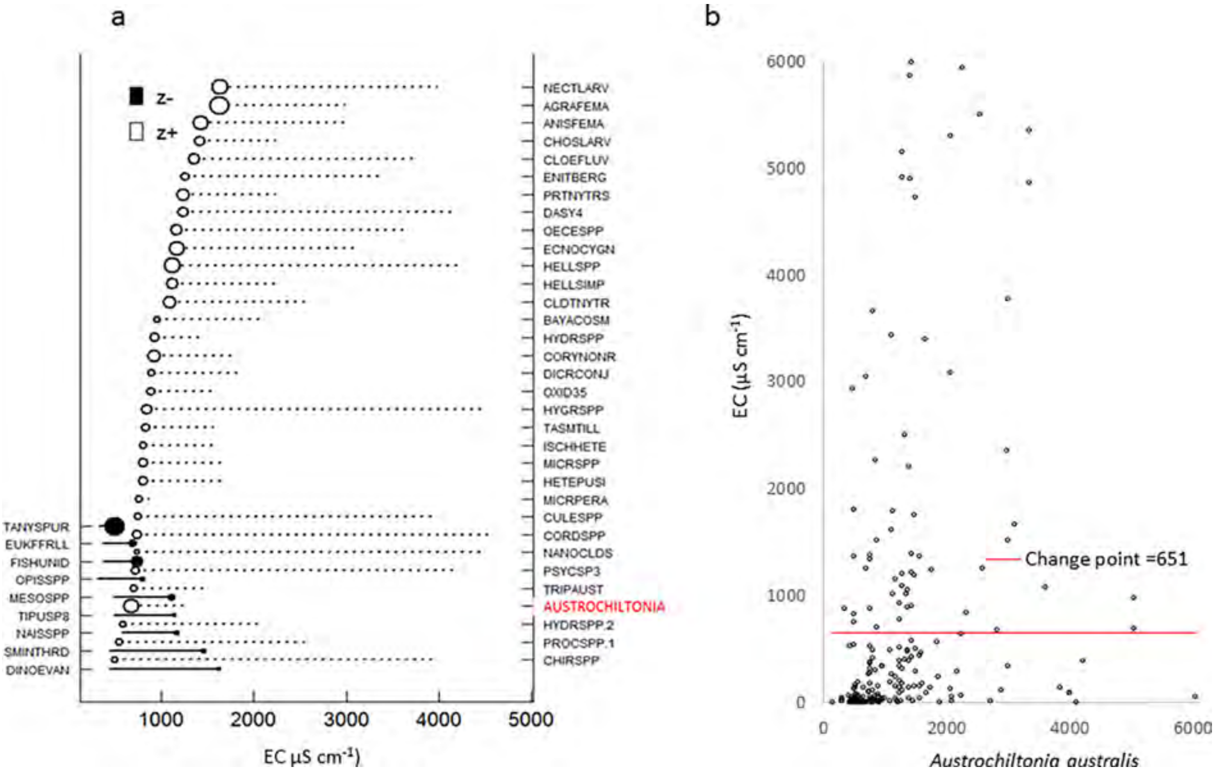

Fig. 7. EC thresholds discovered by TITAN for the Onkaparinga catchment. (a) Thresholds for macroinvertebrates community (sample size, n = 227). (b) Change point for *A. australis* visualised by means of observed data. Note: Since *Paramerina* spp. was not matching the criteria of pure and reliable taxa (i.e. > 0.95), only the change point for the sum (z−) taxa were considered.

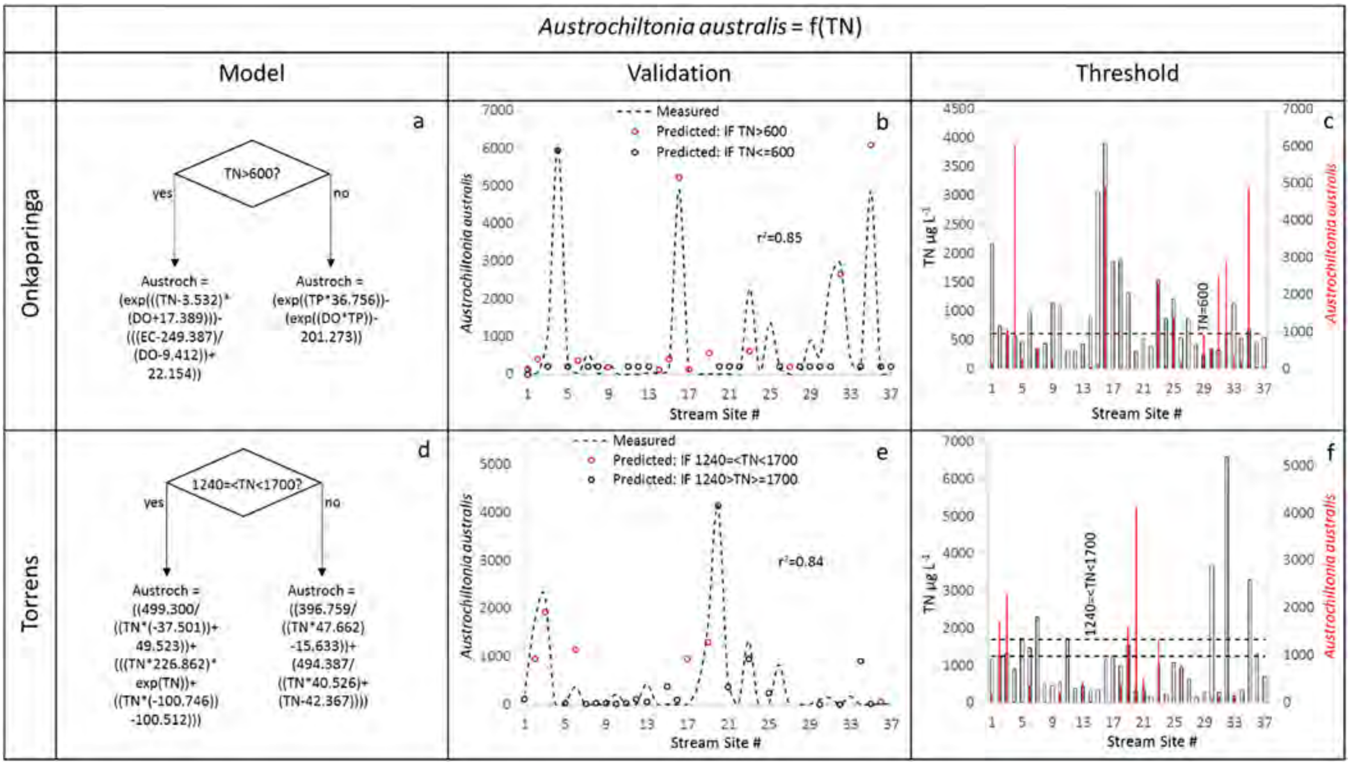

Fig. 8. HEA-based modelling of the abundance of *A. australis* in response to TN gradient along 37 stream sites of the Torrens and Onkaparinga catchments. (a) and (d): IF-THEN-ELSE models for *A. australis*. (b) and (e): Validation of the IF-THEN-ELSE models. (c) and (f): Visualisation of TN thresholds identified by the IF-THEN-ELSE models.

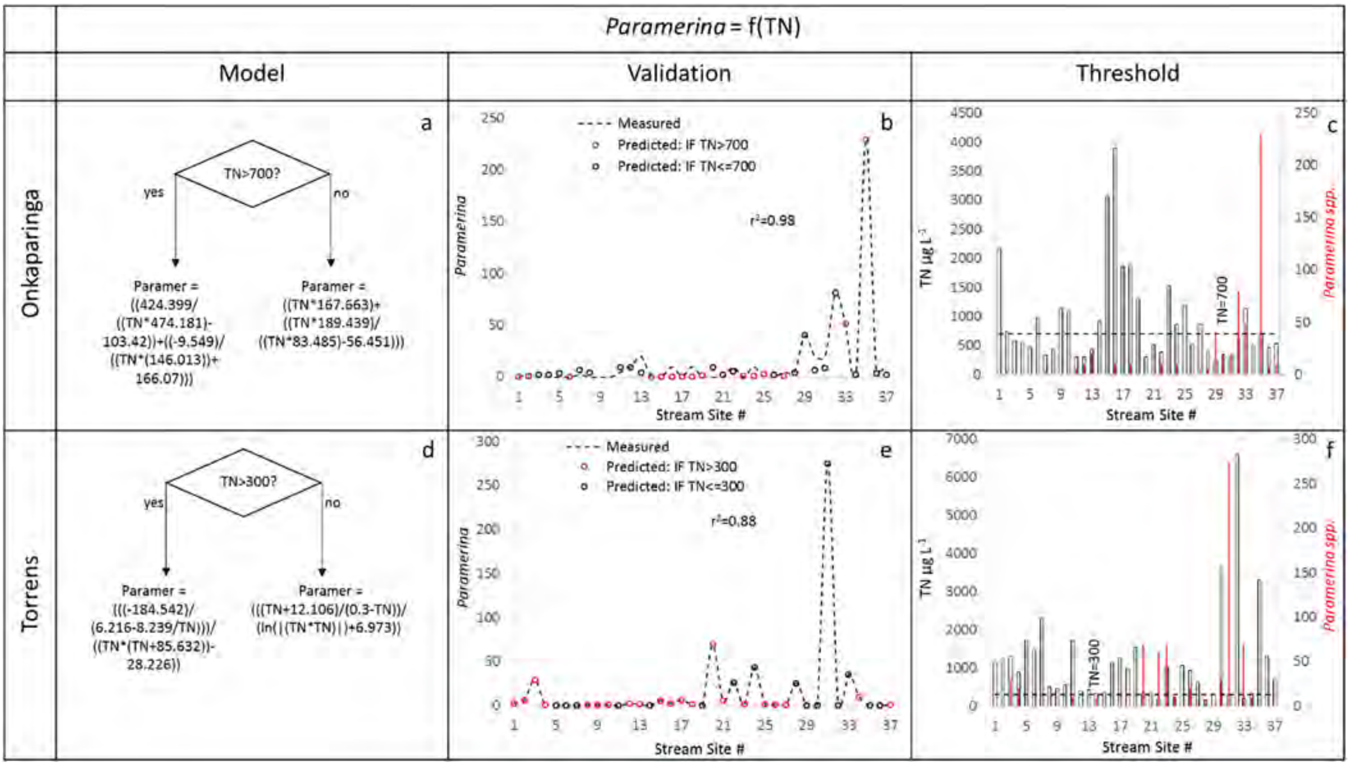

Fig. 9. HEA-based modelling of the abundance of *Paramerina* spp. in response to TN gradient along 37 stream sites of the Torrens and Onkaparinga catchments. (a) and (d): IF-THEN-ELSE models for *Paramerina* spp. (b) and (e): Validation of the IF-THEN-ELSE models. (c) and (f): Visualisation of EC thresholds identified by the IF-THEN-ELSE models.

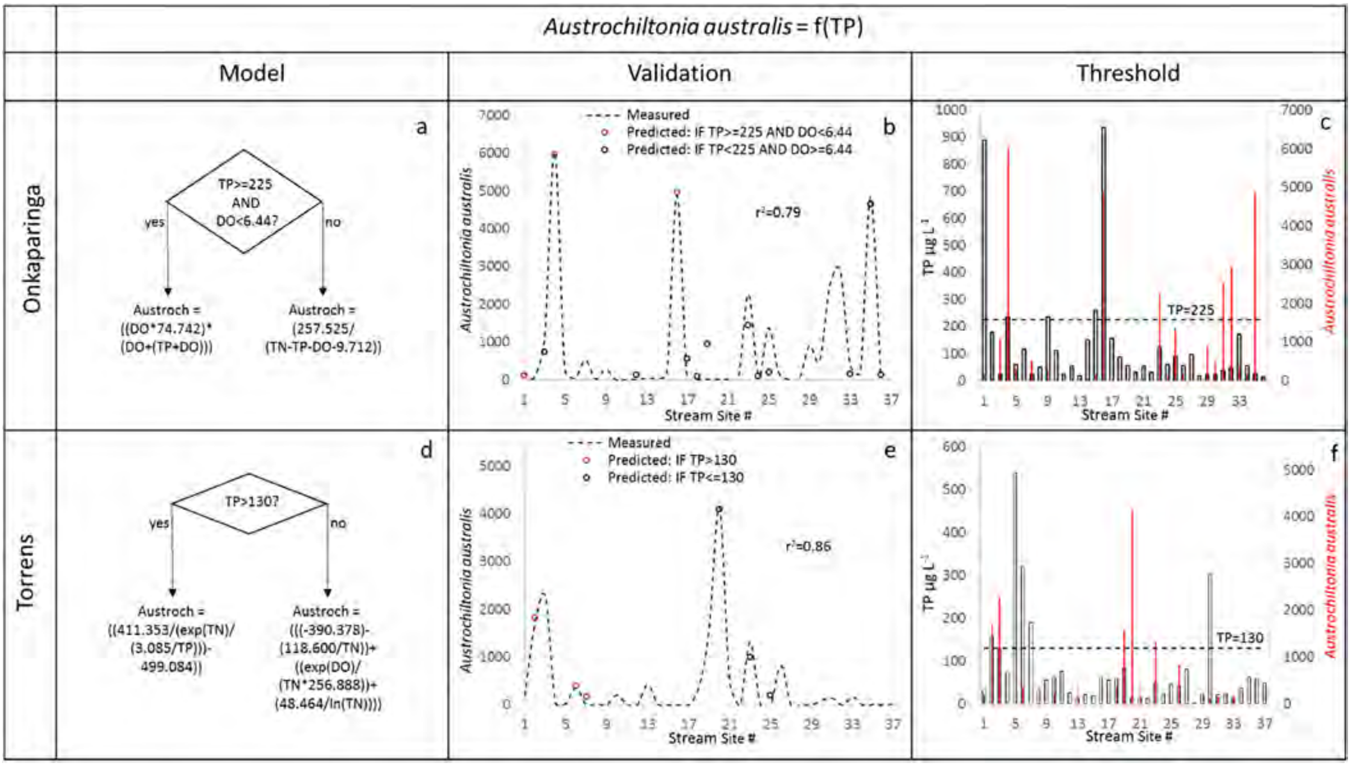

Fig. 10. HEA-based modelling of the abundance of *A. australis* in response to TP gradient along 37 stream sites of the Torrens and Onkaparinga catchments. (a) and (d): IF-THEN-ELSE models for *A. australis*. (b) and (e): Validation of the IF-THEN-ELSE models. (c) and (f): Visualisation of TP thresholds identified by the IF-THEN-ELSE models.

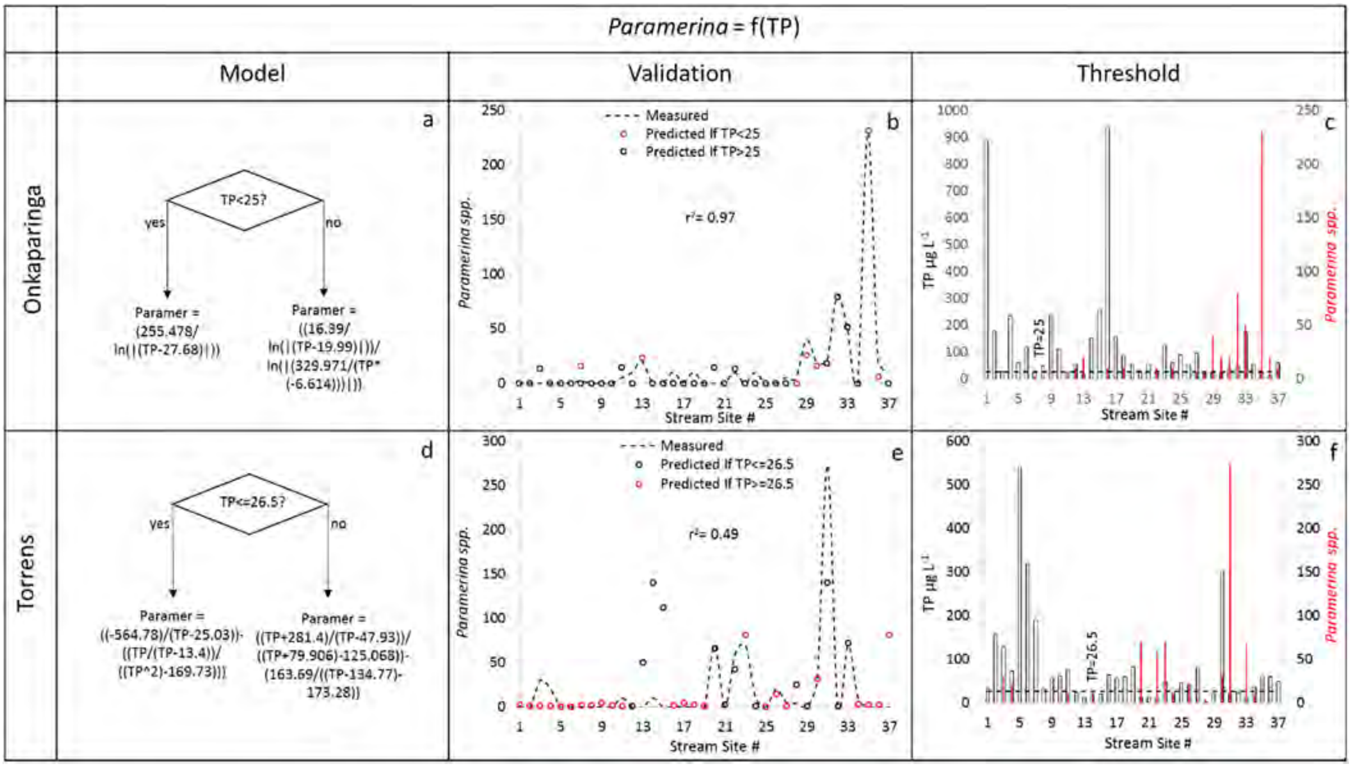

Fig. 11. HEA-based modelling of the abundance of *Paramerina* spp. in response to TP gradient along 37 stream sites of the Torrens and Onkaparinga catchments. (a) and (d): IF-THEN-ELSE models for *Paramerina* spp. (b) and (e): Validation of the IF-THEN-ELSE models. (c) and (f): Visualisation of TP thresholds identified by the IF-THEN-ELSE models.

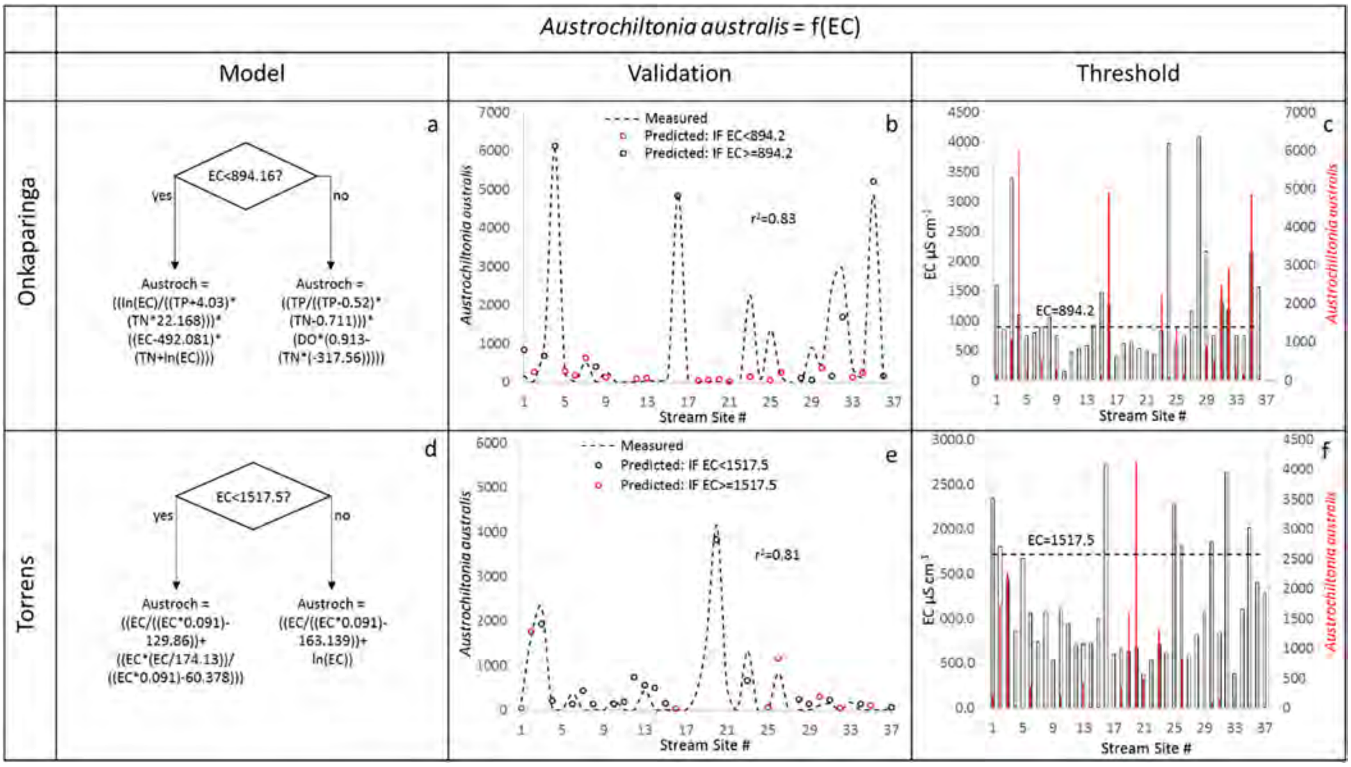

Fig. 12. HEA-based modelling of the abundance of *A. australis* in response to EC gradient along 37 stream sites of the Torrens and Onkaparinga catchments. (a) and (d): IF-THEN-ELSE models for *A. australis*. (b) and (e): Validation of the IF-THEN-ELSE models. (c) and (f): Visualisation of EC thresholds identified by the IF-THEN-ELSE models.

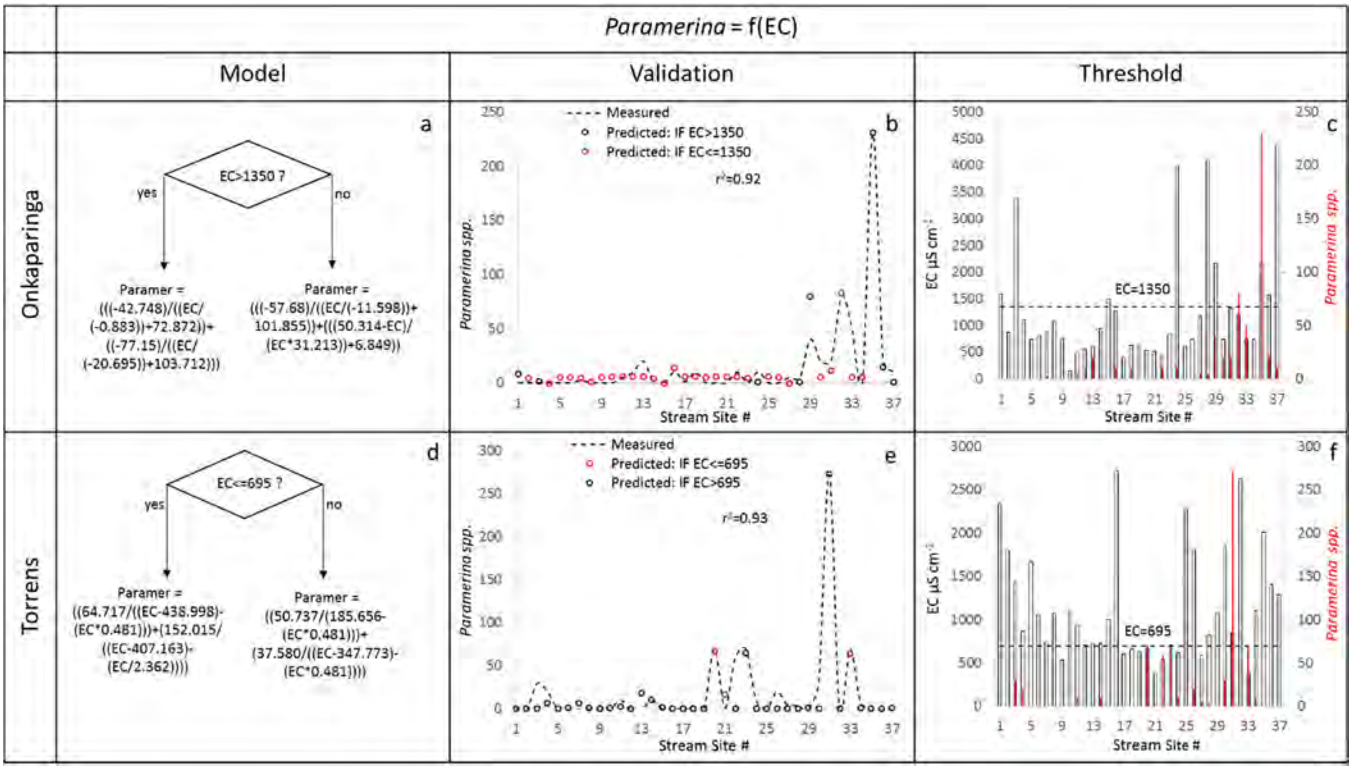

Fig. 13. HEA-based modelling of the abundance of *Paramerina* spp. in response to EC gradient along 37 stream sites of the Torrens and Onkaparinga catchments. (a) and (d): IF-THEN-ELSE models for *Paramerina* spp. (b) and (e): Validation of the IF-THEN-ELSE models. (c) and (f): Visualisation of EC thresholds identified by the IF-THEN-ELSE models.

guidelines for TN and TP concentrations in freshwater systems are too high.

Future research will aim at testing responses of macroinvertebrate taxa to changing flow regimes, land uses and climate. We have shown that TITAN and HEA may be applicable for this task by applying TITAN first to draw community level thresholds and HEA next for species-specific modelling across multiple years of monitoring. Since HEA is suitable for time-series modelling, it will allow to forecast macroinvertebrate population dynamics in response to scenarios of future land use and climate changes, and enable to estimate the timing when an ecological system is approaching a threshold.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2019.02.003.

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Supporting information (Chapter 2)

Appendix: List of macroinvertebrate species with abbreviations

Original name	Abbreviations	Original name	Abbreviations
Ablabesmyia spp.	ABLASPP	*Cladopelma spp.*	CLADSPP
Acarina spp.	ACARSPP	*Cladotanytarsus*	CLDTNYTR
Aedes spp.	AEDESPP	*Cloeon fluviatile*	CLOEFLUV
Aeolosomatidae spp.	AEOLSPP	*Cloeon spp.*	CLOESPP
Aeschnidae juveniles	AESCJUVE	*Coaustraliobates spp.*	COAUSPP
Aeshna brevistyla	AESHBREV	*Coenagrionidae spp.*	COENSPP
Agraptocorixa	AGRAFEMA	*Copelatus (Larva)*	COPELARV
Agraptocorixa eurynome	AGRAEURY	*Copelatus australiae (Adult)*	COPEADUL
Agraptocorixa parvipunctata	AGRAPARV	*Copelatus spp. (Adult)*	COPEADUL.1
Allodessus bistrigatus (Adult)	ALLOADUL	*Corbiculina australis*	CORBAUST
Alluauodomyia spp.	ALLUSPP	*Cordylophora spp.*	CORDSPP
Amphipoda spp.	AMPHSPP	*Corixidae spp.*	CORISPP
Angrobia spp.	ANGRSPP	*Corynoneura*	CORYNONR
Anisocentropus	ANSCNTRP	*Cricotopus*	CRICOTPS
Anisops hackeri	ANISHACK	*Cryptochironomus*	CRYPTCHR
Anisops hyperion	ANISHYPE	*Culex spp.*	CULESPP
Anisops spp.	ANISFEMA	*Culicidae spp.*	CULISPP
Anisops thienemanni	ANISTHIE	*Culicinae spp.*	CULISPP.1
Anisoptera spp.	ANISSPP	*Curculionidae spp.*	CURCSPP
Anopheles spp.	ANOPSPP	*Dasyhelea spp.*	DASYSPP
Antarctodrilus proboscidea	ANTAPROB	*Dero digitata*	DERODIGI
Antarctodrilus spp.	ANTASPP	*Dero spp.*	DEROSPP
Antiporus blakei (Adult)	ANTIADUL	*Dicrotendipes*	DCRTNDPS
Antiporus spp. (Larva)	ANTILARV	*Dicrotendipes conjunctus*	DICRCONJ
Antiporus spp. (females)	ANTIFEMA	*Dinotoperla evansi*	DINOEVAN
Aphroteniella	APHRTNLL	*Dixidae spp.*	DIXISPP
Apsectrotanypus	APSCTRTN	*Dolichopodidae*	DOLCHPDD
Archaeosynthemis macrostigma	ARCHMACR	*Dytiscidae spp.*	DYTISPP
Arrenuridae spp.	ARRESPP	*Ecnomus continentalis*	ECNOCONT
Arrenurus spp. (38)	ARRE38	*Ecnomus cygnitus*	ECNOCYGN
Astigmata spp.	ASTISPP	*Ecnomus pansus*	ECNOPANS
Atalophlebia australasica	ATALAUST	*Ecnomus spp.*	ECNOSPP
Atalophlebia australis	ATALAUST.1	*Ecnomus spp. EWS spp.1*	ECNO1
Atalophlebia spp.	ATALSPP	*Elmidae spp.*	ELMISPP
Atrichopogon spp.	ATRISPP	*Empididae spp. (Larvae)*	EMPILARV
Atrichopogon spp. 1 (NMVsp10)	ATRINMVS	*Enchytraeidae Group "gamma"*	ENCHGAMM
Atriplectides dubius	ATRIDUBI	*Enchytraeidae spp.*	ENCHSPP
Aturidae spp. (68)	ATUR68	*Enithares bergrothi*	ENITBERG
Aulonogyrus strigosus (Adult)	AULOADUL	*Enithares spp.*	ENITSPP
Aulonogyrus strigosus (Larva)	AULOLARV	*Enochrus (Adult)*	ENOCADUL
Austroaeschna parvistigma	AUSTPARV	*Entomobryidae*	ENTMBRYD
Austroaeschna spp.	AUSTSPP.4	*Ephydridae*	EPHYDRID
Austroaeschna unicornis	AUSTUNIC	*Ethochorema hesperium*	ETHOHESP
Austroagrion watsoni	AUSTWATS	*Eukiefferiella*	EUKFFRLL
Austrocerca tasmanica	AUSTTASM	*Eusiridae spp.*	EUSISPP
Austrochiltonia australis	AUSTAUST	*Eylais spp.*	EYLASPP
Austrogomphus australis	AUSTAUST.1	*Ferrissia petterdi*	FERRPETT
Austrogomphus guerini	AUSTGUER	*Ferrissia spp.*	FERRSPP
Austrogomphus spp.	AUSTSPP.3	*Ferrissia tasmanica*	FERRTASM
Austrolestes annulosus	AUSTANNU	*Forcipomyia spp.*	FORCSPP
Austrolestes cingulatis	AUSTCING	*Gastropoda spp.*	GASTSPP
Austrolestes leda	AUSTLEDA	*Glossiphoniidae spp.*	GLOSSPP
Austrolestes spp.	AUSTSPP.2	*Glyptophysa aliciae*	GLYPALIC
Austropeplea spp.	AUSTSPP	*Glyptophysa concinna*	GLYPCONC
Austropeplea tomentosa	AUSTTOME	*Glyptophysa spp.*	GLYPSPP
Austrosimulium furiosum	AUSTFURI	*Gomphidae spp.*	GOMPSPP
Austrosimulium spp.	AUSTSPP.1	*Gordiidae spp.*	GORDSPP
Baetidae spp.	BAETSPP	*Gripopterygidae spp.*	GRIPSPP
Bayardella cosmeta	BAYACOSM	*Gyrinidae spp.*	GYRISPP
Berosus queenslandicus	BEROQUEE	*Halacaridae spp.*	HALASPP
Berosus spp. (Larva)	BEROLARV	*Haloniscus spp.*	HALOSPP
Bidessinae (Larva)	BIDELARV	*Harnischia*	HARNISCH
Botryocladius grape	BOTRTH	*Harrissius*	HARRISSS
Branchiura sowerbi	BRANSOWE	*Hellyethira malleoforma*	HELLMALL
Bryophaenocladius	BRYPHNCL	*Hellyethira simplex*	HELLSIMP
Bryozoa spp.	BRYOSPP	*Hellyethira spp.*	HELLSPP
Cardiocladius	CARDCLDS	*Hellyethira spp.*	HELLMULT
Cecidomyidae spp.	CECISPP	*Helochares (Adult)*	HELOADUL
Ceratopogon spp.	CERASPP.1	*Hemianax papuensis*	HEMIPAPU
Ceratopogonidae Pupae	CERAPUPA	*Hemicordulia tau*	HEMITAU
Ceratopogonidae spp.	CERASPP	*Hemicorduliidae spp.*	HEMISPP
Ceratopogoninae spp.	CERASPP.2	*Hemigomphus gouldii*	HEMIGOUL
Chaetogaster	CHATGSTR	*Heterias pusilla*	HETEPUSI
Chaetogaster diaphanus	CHAEDIAP	*Hydra spp.*	HYDRSPP
Chaetogaster limnaei	CHAELIMN	*Hydracarina spp.*	HYDRSPP.6
Cherax destructor	CHERDEST	*Hydrachna spp.*	HYDRSPP.2
Cherax spp.	CHERSPP	*Hydraena*	HYDRAENA
Cheumatopsyche spp. 2	CHEU2	*Hydraenidae (Larva)*	HYDRLARV
Chironomidae	CHIRP	*Hydrobiidae spp.*	HYDRSPP.1
Chironominae spp.	CHIRSPP.2	*Hydrobiosidae spp.*	HYDRSPP.8
Chironomini spp.	CHIRSPP.1	*Hydrochus spp.*	HYDRSPP.7
Chironomus spp.	CHIRSPP	*Hydrodroma spp.*	HYDRSPP.4
Chostonectes gigas (Adult)	CHOSADUL.1	*Hydrodromidae spp.*	HYDRSPP.5
Chostonectes nebulosus (Adult)	CHOSADUL	*Hydrophilidae (larvae)*	HYDRLARV
Cladopelma spp.	CLADSPP	*Hydroptila calcara*	HYDRCALC
Cladotanytarsus	CLDTNYTR	*Hydroptila losida*	HYDRLOSI
Cloeon fluviatile	CLOEFLUV	*Hydroptila scamandra*	HYDRSCAM
Cloeon spp.	CLOESPP	*Hydroptila spp.*	HYDRSPP.9
Coaustraliobates spp.	COAUSPP	*Hydroptilidae juveniles*	HYDRJUVE
Coenagrionidae spp.	COENSPP	*Hydryphantidae spp.*	HYDRSPP.3

Hygrobatidae spp.	HYGRSPP	*Paratya australiensis*	PARAAUST
Hyphydrus elegans (Adult)	HYPHADUL	*Pentaneurini genus A*	PENTA
Hypogasturidae	HYPGSTRD	*Perthiidae spp.*	PERTSPP
Illiesoperla mayii	ILLIMAYI	*Phreodrilidae Group E*	PHREE
Insulodrilus lacustris	INSULACU	*Phreodrilidae spp.*	PHRESPP
Ischnura heterosticta	ISCHHETE	*Physa spp.*	PHYSSPP
Ischnura spp.	ISCHSPP	*Piona spp.*	PIONSPP
Isidorella hainesii	ISIDHAIN	*Pionidae spp.*	PIONSPP.1
Isidorella spp.	ISIDSPP	*Pisidium casertanum*	PISICASE
Isotomidae	ISOTOMID	*Planorbidae spp.*	PLANSPP
Johnstonianidae spp.	JOHNSPP	*Platynectes decempunctatus (Adult)*	PLATA
Kiefferulus martini	KIEFMART	*Platynectes decempunctatus (Larva)*	PLATL
Kiefferulus spp.	KIEFSPP	*Podonomopsis*	PODNMPSS
Koorrnonga inconspicua	KOORINCO	*Polypedilum nubifer*	POLYNUBI
Lancetes lanceolatus (Adult)	LANCADUL	*Polypedilum spp.*	POLYSPP
Larsia	LARSIA	*Potamopyrgus antipodarum*	POTAANTI
Lectrides varians	LECTVARI	*Potamothrix bavaricus*	POTABAVA
Lepidoptera spp.	LEPISPP	*Pristina aequiseta*	PRISAEQU
Leptocerid juveniles	LEPTJUVE	*Pristina longiseta*	PRISLONG
Leptophlebiidae spp.	LEPTSPP	*Pristina spp.*	PRISSPP
Leptorussa spp.	LEPTSPP.1	*Pristinella jenkinae*	PRISJENK
Limnesia spp.	LIMNSPP	*Pristinella spp.*	PRISSPP.1
Limnesiidae spp.	LIMNSPP.1	*Procladius spp.*	PROCSPP.1
Limnoxenus zealandicus (larvae)	LIMNLARV	*Procorticacarus spp.*	PROCSPP
Lingora aurata	LINGAURA	*Pseudosuccinea columella*	PSEUCOLU
Liodessus amabilis (Adult)	LIODADUL	*Psychodidae spp.*	PSYCSPP
Lumbriculus variegatus	LUMBVARI	*Ptiliidae spp.*	PTILSPP
Lymnaeidae spp.	LYMNSPP	*Pyralidae spp.*	PYRASPP
Macrogyrus spp. (Adult)	MACRADUL	*Rhantus suturalis (Adult)*	RHANADUL
Mesostigmata spp.	MESOSPP	*Rhantus suturalis (Larvae)*	RHANLARV
Mesovelia spp.	MESOSPP.1	*Rheotanytarsus*	RHTNYTRS
Micronecta annae	MICRANNA	*Richardsonianidae*	RCHRDSNN
Micronecta gracilis	MICRGRAC	*Riekoperla naso*	RIEKNASO
Micronecta robusta	MICRROBU	*Riethia*	RIETHIA
Micronecta spp. (female)	MICRFEMA	*Scatopsidae spp.*	SCATSPP
Microvelia oceanica	MICROCEA	*Sciomyzidae*	SCIOMYZD
Microvelia peramoena	MICRPERA	*Scirtidae spp.*	SCIRSPP
Microvelia spp.	MICRSPP	*Sclerocyphon spp.*	SCLESPP
Muscidae	MUSCIDAE	*Sigara spp. (Females)*	SIGAFEMA
Naididae spp.	NAIDSPP	*Sigara sublaevifrons*	SIGASUBL
Nais bretscheri	NAISBRET	*Sigara truncatipala*	SIGATRUN
Nais communis	NAISCOMM	*Simsonia leai*	SIMSLEAI
Nais elinguis	NAISELIN	*Simsonia spp. (Adult)*	SIMSADUL
Nais spp.	NAISSPP	*Simsonia spp. (Larva)*	SIMSLARV
Nais variabilis	NAISVARI	*Simuliidae spp.*	SIMUSPP.1
Nanocladius	NANOCLDS	*Simulium melatum*	SIMUMELA
Naucoris congrex	NAUCCONG	*Simulium ornatipes*	SIMUORNA
Necterosoma dispar (Adult)	NECTADUL.1	*Simulium spp.*	SIMUSPP
Necterosoma penicillatus (Adult)	NECTADUL	*Slavina spp.*	SLAVSPP
Necterosoma spp. (Adult)	NECTADUL.2	*Sminthuridae*	SMINTHRD
Necterosoma spp. (Larva)	NECTLARV	*Sphaeridae spp.*	SPHASPP
Nematoda spp.	NEMASPP	*Sphaerium tasmanicum*	SPHATASM
Nemertea spp.	NEMESPP	*Spirosperma spp.*	SPIRSPP
Newmanoperla thoreyi	NEWMTHOR	*Spongillidae*	SPONGLLD
Nilobezzia spp. (spp.20)	NILO20	*Staphylinidae spp.*	STAPSPP
Notalina spp.	NOTASPP	*Stempellina*	STEMPLLN
Notolina spira	NOTOSPIR	*Stenochironomus spp.*	STENSPP
Nousia fuscula	NOUSFUSC	*Sternopriscus multimaculatus (Adult)*	STERA
Ochthebius spp.	OCHTSPP	*Sternopriscus spp. (Females)*	STERFEMA
Odontomyia spp. (spp. 1)	ODON1	*Sternopriscus spp. (Larva)*	STERLARV
Oecetis spp.	OECESPP	*Stictocladious*	STCTCLDS
Offadens spp.	OFFASPP	*Stratiomyidae spp.*	STRASPP
Offadens spp. (Genus 1) (Baetis soror)	OFFASORO	*Stylaria lacustris*	STYLLACU
Oligochaeta spp.	OLIGSPP	*Synthemis eustralacta*	SYNTEUST
Onychiuridae spp.	ONYCSPP	*Talitridae spp.*	TALISPP
Opisthopora spp.	OPISSPP	*Tanypodinae spp.*	TANYSPP
Oribatida spp.	ORIBSPP	*Tanytarsini spp.*	TANYSPP.2
Orphninotrichia maculata	ORPHMACU	*Tanytarsus spp.*	TANYSPP.1
Orphninotrichia spp.	ORPHSPP	*Tanytarsus spp.*	TANYSPUR
Orthetrum caledonicum	ORTHCALE	*Taschorema complex*	TASCCOMP
Orthocladiinae	ORTHCLDN	*Taschorema evansi*	TASCEVAN
Oxidae spp. (spp. 35)	OXID35	*Tasimiidae*	TASIMIID
Parachironomus	PRCHRNMS	*Tasmanocoenis spp.*	TASMSPP
Paracnephia spp.	PARASPP.1	*Tasmanocoenis tillyardi*	TASMTILL
Paracymus (larvae)	PARALARV	*Temnocephala spp.*	TEMNSPP
Paracymus pygmaeus	PARAPYGM	*Thiara balonnensis*	THIABALO
Paraheptagyia	PARHPTGY	*Thienemanniella*	THNMNNLL
Parakiefferiella	PRKFFRLL	*Tipulidae spp.*	TIPUSPP
Paralimnophyes spp. (dark spp.)	PARASPP.2	*Triaenodes spp.*	TRIASPP
Paralimnophyes spp. (light spp.)	PARASPP.3	*Triplectides australicus*	TRIPAUST
Paramerina	PARAMERN	*Triplectides australis*	TRIPAUST.1
Parametriocnemus	PRMTRCNM	*Triplectides ciuskus*	TRIPCIUS
Paranacaena spp.	PARASPP	*Triplectides similis*	TRIPSIMI
Paratanytarsus	PRTNYTRS	*Triplectides spp.*	TRIPSPP
Paratya australiensis	PARAAUST	*Trombidioidea spp.*	TROMSPP
Pentaneurini genus A	PENTA	*Tubificidae Goup B*	TUBIB
Perthiidae spp.	PERTSPP	*Turbellaria spp.*	TURBSPP
Phreodrilidae Group E	PHREE	*Ulmerochorema membrum*	ULMEMEMB
Phreodrilidae spp.	PHRESPP	*Ulmerochorema spp.*	ULMESPP
Physa spp.	PHYSSPP	*Unionicolidae spp.*	UNIOSPP
Piona spp.	PIONSPP		

Chapter 3:

Comparison of two commonly used methods for identifying water quality thresholds in freshwater ecosystems using field and synthetic data

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Contribution to the Paper	Formal analysis, synthetic data simulations, results preparation, writing, original draft preparation. Acted as the first and corresponding author. I hereby certify that statement of the contribution is accurate.		
Overall percentage (%)	80%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Comparison of two commonly used methods for identifying water quality thresholds in freshwater ecosystems using field and synthetic data

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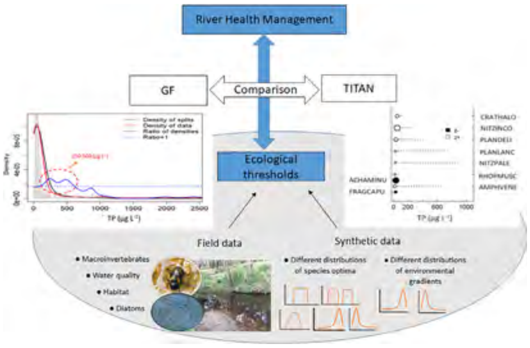
HIGHLIGHTS

- The performance of GF and TITAN were compared to identify true thresholds.
- Both models were robust in identifying change in species responses.
- TITAN identified realistic thresholds while GF overestimated at high end of gradient.
- Based on synthetic data GF outperformed when multiple change points were present.
- Distribution of species responses and evenness of data affect models' performance.

GRAPHICAL ABSTRACT



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ABSTRACT

Defining ecological thresholds has become increasingly relevant for water resource management. Despite the fact that there has been a rapid expansion in methods to evaluate ecological threshold responses to environmental stressors, evaluation of the relative benefits of various methods has received less attention. This study compares the performance of Gradient Forest (GF) and Threshold Indicator Taxa Analysis (TITAN) for identifying water quality thresholds in both field and synthetic data. Analysis of 14 years of macroinvertebrates data from the Mediterranean catchments of the Torrens and Onkaparinga Rivers, South-Australia, identified electrical conductivity (EC) and total phosphorus (TP) as the most important water quality variables affecting macroinvertebrates. Water quality thresholds for macroinvertebrates identified by both methods largely corresponded at low EC (GF: 400–900 μS cm^{-1} vs. TITAN: 407–951 μScm^{-1}), total phosphorus (TP) (GF: 0.02–0.18 mg L^{-1} vs. TITAN: 0.02–0.04 mg L^{-1}) and total nitrogen (TN) (GF: 0.2 mg L^{-1} vs. TITAN: 0.28–0.67 mg L^{-1}) concentrations. However, multiple GF-derived thresholds, particularly at high stressor concentrations, were representative of low data distribution, and thus need to be considered with caution. In another case study of South Australian diatom data, there were marked differences in GF and TITAN identified thresholds for EC (GF: 5000 μScm^{-1} vs. TITAN 1004–2440 μS cm^{-1}) and TP (GF: 250–500 μg L^{-1} vs. TITAN: 11–329 μg L^{-1}). These differences were due to the fact that while TITAN parsed species responses into negative and positive taxa, GF overestimated thresholds

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by aggregating the response of taxa that increase and decrease along environmental gradients. Given these findings, we also evaluated the methods' performance using different distributions of synthetic data i.e. with both skewed and uniform distribution of samples and species responses. Both methods identified similar change-points in the case of a uniform environmental gradient, except when species optima were simulated at centre of the gradient. Here GF detected the change-points but TITAN failed to do so. GF also outperformed TITAN when four simulated species change-points were present. Thus, the distribution of species responses and optima and the evenness of the environment gradient can affect the models' performance. This study has shown that both methods are robust in identifying change in species response but threshold identification differs depending both on the analysis used and the nature of ecological data. We recommend the careful application of GF and TITAN, noting these differences in performance, will improve their application for water resource management.

1. Introduction

Ecological thresholds have become increasingly relevant for ecosystem managers by serving as guidelines for water resource management (Feng et al. 2019; Foley et al., 2015; Samhouri et al., 2010). Worldwide, freshwater ecosystems are exposed to multiple stressors that adversely affect stream biota. Identifying thresholds beyond which species may be lost is often difficult due to the complexity of ecosystem dynamics and requires understanding of the effects from multiple environmental stressors (Ormerod et al., 2010; Wagenhoff et al., 2012). Due to this complexity, freshwater managers are embracing sophisticated methods to inform management actions (King et al., 2011; Wagenhoff et al., 2017) including threshold identification (Groffman et al., 2006).

The appropriate use of thresholds in management requires an adaptive approach for which different threshold types have been proposed (Huggett, 2005; Nichols et al., 2014). Different classes of thresholds that are relevant in decision making include ecological, utility and decision thresholds (Martin et al., 2009). Ecological thresholds can be viewed as components of ecological models (Martin et al., 2009), and different authors define them in different ways. Generally, ecological thresholds are viewed as tipping points along continuum beyond which a system is imbalanced or unacceptable (Groffman et al., 2006). In contrast, Baker and King (2010) suggested thresholds are change points where there is synchronous change of indicator taxa along an environmental gradient. In addition, Martin et al. (2009) proposed the concept of utility thresholds where small changes can have significant impact on management outcomes, whereas decision thresholds also called as management thresholds (Bennett and Balvanera, 2007) requires prompt management actions when stressor exceeds certain thresholds. The understanding of distinctions between different thresholds types and specific role of each in decision making is useful in conservation (Nichols et al., 2014). In South Australia, guidelines for freshwater quality were mostly viewed as decision thresholds, and recently, these guidelines have been scrutinised and are being revised in South Australia and at national level. The current values may be too high to ensure conservation of freshwater systems (Sultana et al., 2019; Tibby et al., 2019). Thus more work is needed to develop stringent water quality guidelines to ensure the protection of both degraded and pristine river ecosystems.

Methods for assessing ecological thresholds have rapidly progressed in the past two decades with many techniques identified. Current threshold identification models can be broadly classified as either inferential or exploratory. Exploratory methods quantify thresholds using nonlinear responses along environmental gradients, which can take the form of an abrupt but continuous change, subsidy-stress response, or a step function (Andersen et al., 2009; Ellis et al., 2012). Exploratory methods include significant zero crossings (SiZer) (Sonderegger et al., 2009) and gradient forest (Ellis et al., 2012). Gradient Forest is an extension of random forest (Breiman, 2001) which has been considered superior to other related methods (e.g. generalized linear or additive models, regression tree methods) in terms of prediction (Cutler et al., 2007; Prasad et al., 2006). Regression tree based approaches are well suited for handling complex ecological data with missing values, lack of balance and nonlinear relationships (De'Ath, 2002). In contrast to exploratory approaches, inferential methods statistically test and identify points of abrupt change. Most threshold methods use linear models to detect nonlinear responses along gradients. Examples of inferential methods include breakpoint or piecewise regression (Toms and Lesperance, 2003), nonparametric change point analysis (Qian et al., 2003), Bayesian change point analysis (Qian et al., 2003; Qian et al., 2004), Hybrid Evolutionary Algorithms (HEA) (Recknagel et al., 2016) and Threshold Indicator Taxa Analysis (TITAN) (Baker and King, 2010). TITAN is an extension of change-point analysis (Qian et al., 2003) and indicator species analysis (Dufrêne and Legendre, 1997). TITAN is considered preferable to other methods (e.g. non-parametric change point analysis) as it replaces aggregated community level data information with taxon-specific scores (Baker and King, 2010). In summary, threshold approaches differ by identifying either single or multiple thresholds along gradients, in the number of predictor variables that can be assessed simultaneously, and whether multivariate or univariate response variables are used. Given that threshold identification models are based on different underlying statistical assumptions, each with their associated limitations and uncertainty, different models typically identify different thresholds (Dodds et al., 2010; Moore and Palmer, 2005; Sultana et al., 2019). Understanding these differences is therefore of key importance to appropriate threshold identification.

A number of studies have argued that threshold model selection should be based on data distributions (Brenden et al., 2008) as well as knowledge of the ecological dynamics and potential threshold patterns (Qian and Cuffney, 2012). Only recently have some comparative studies been undertaken using different analytical techniques on the same data set (Brenden et al., 2008; Dodds et al., 2010). These have shown variation in thresholds estimates. For example, Dodds et al. (2010) identified a three-fold difference in threshold values where different methods were applied to a single response variable. Threshold identification can vary markedly between piecewise quantile regression, nonparametric change point analysis and significant zero crossings (SiZer) based on sample size, data density and the pattern of linear change across an environmental gradient (Daily et al., 2012). Spurious threshold detection can result from unrealistic statistical assumptions e.g. uniformly distributed samples across environmental gradient (Daily et al., 2012). Similarly, the specific shape and distribution of species' responses can affect model performance (García-Callejas and Araújo, 2016; Santika and Hutchinson, 2009). These features have been used to evaluate statistical models for predicting plant distributions (Austin et al., 2006), estuarine fish species distribution (França and Cabral, 2016; Large et al., 2015) and for ocean management (Hunsicker et al., 2016), but have received less attention for evaluating thresholds, particularly in freshwater systems.

Threshold comparisons based on field data can be confounded by sampling artefacts like variation in sampling effort, sampling frequency and scales, locations or timing (e.g. ecoregions and seasons, respectively). Generating synthetic data with known structure and patterns allows evaluation of the relative performance of threshold methods in a non-biased manner (Cuffney and Qian, 2013; Daily et al., 2012). Simulation methods have been used to generate synthetic data sets to evaluate the performance of newly developed techniques (Baker and King, 2010; Daily et al., 2012; Ellis et al., 2012), but have rarely been used to compare these techniques.

Gradient Forest (Ellis et al., 2012) and TITAN (Baker and King, 2010) use the multivariate, non-linear response of species abundance data for detecting ecological community thresholds. Both models consider the simultaneous response of multiple individual species (King and Baker, 2010) in contrast to SiZer, HEA and breakpoint regression. Despite numerous applications of these models in freshwater ecology (Berger et al., 2018; Costas et al., 2018; Monk et al., 2018; Tang et al., 2017) with both being used to identify thresholds and to support development of limit setting (Baker and King, 2010; Wagenhoff et al., 2017), to our knowledge their behaviour and relative performance have not been fully assessed. These two methods were selected because they have similar data requirements, overcome the limitations of previous methods, use the multivariate, non-linear response of species abundance data, are relatively easily applied in management settings and have similar aims in terms of supporting limit setting criteria.

Thus, this study applied GF and TITAN to both field and synthetic data to compare their performance. We focused this study on the following research questions: 1. Is threshold identification in field data affected by model-based bias? 2. Does threshold identification using TITAN and GF differ in synthetic data with different distributions? 3. How do different patterns of species responses affect TITAN and GF? 4. What are the requirements of ecological data for accurate identification of management thresholds? Answers to these questions provide a scientific rationale for facilitating the process of threshold model selection and accurate threshold identification. Accurate detection of thresholds enables policy makers and managers to more confidently set trigger values for management action, including the development of water quality guidelines.

2. Materials and methods

2.1. Data

2.1.1. Field data

For case study 1, we used macroinvertebrate species data collected by the South Australian Environment Protection Authority between 1994 and 2007. A total of 255 samples from 74 sites in the Torrens and Onkaparinga River catchments, South Australia, were collected. There were 518 macroinvertebrate taxa identified to the lowest taxonomic level (mostly species or genus). Water quality variables included total nitrogen (TN), total phosphorus (TP) and electrical conductivity (EC) (Table 1). Habitat characteristics were also measured (Table 1). For most sites, sampling frequency was not consistent. The two catchments have a broad land use gradient ranging from grazing and forest cover to intensive agriculture and high urban density. The sites also vary in topography (low elevation streams to mountains) and surface geology.

Table 1 summarizes the statistics of the variables from each site. Nutrient concentrations ranged from 0.003 mg TP L^{-1} to 1.8 mg TP L^{-1} and 0.06 mg TN L^{-1} to 7.9 mg TN L^{-1} with mean concentrations of 0.07 mg TP L^{-1} and 0.80 mg TN L^{-1}. Conductivity varied between 139 μS cm^{-1} and 5030 μS cm^{-1}, with a mean of 974 μS cm^{-1}. The most abundant and ubiquitous taxa in decreasing order of abundance were: *Oligochaeta* spp., *Hydrobiidae* spp., *Cricotopus* spp., *Austrochiltonia australis*, *Potamopyrgus antipodarum*, *Thienemanniella* spp., *Simulium ornatipes* and *Chironomus* spp. recorded with >40, 000 individuals recorded over 14 years. Names and abbreviations of all the taxa are given as supporting information (Appendix 2).

For case study 2, we used diatom data from a previous study designed to improve water quality guidelines (Tibby et al., 2019). This study used TITAN and showed that diatom communities had much lower thresholds for EC and TP than trigger values in South-Australian water quality guidelines. We used these diatom data to compare the performance of TITAN with GF. Data were monitored in South Australian streams during 2014–16. A total of 179 samples were

Table 1
Summary statistics of environmental variables for the observed data. Case study 1 is based on macroinvertebrate data from the Torrens and Onkaparinga River catchments (n = 255) and case study 2 is from South Australian diatom data (n = 179).

Predictors	Description/unit	Min	Max	Mean	Median
Case study 1					
Water quality variables					
Turbidity	Turbidity (NTU)	0.3	89	6.5	2.62
pH	pH units	6.5	9.07	7.8	7.75
Temperature	Temperature (°C)	4.7	24.1	14.1	14
DO	Dissolved oxygen (mg L^{-1})	2.2	14.8	9	9
EC	Conductivity (μS cm^{-1})	139	5030	974	708
TP	Total phosphorus (mg L^{-1})	0.003	1.8	0.07	0.03
TN	Total nitrogen (mg L^{-1})	0.06	7.9	0.80	0.49
Habitat characteristics					
Area_riffle	Area of site riffle (%)	5	95	44.05	40
Algae_r	% algae of riffle site	0	80	7.33	0
Detritus_r	% detritus of riffle site	0	100	25.82	10
Area_edge	Area of site edge (%)	5	95	53.84	50
Algae_e	% algae of edge site	0	90	6.65	0
Detritus_e	% detritus of edge site	0	95	27.14	20
% coarse sediments_r	% coarse sediments riffle site	0	100	53.0	60
% fine sediments_r	% fine sediments riffle site	0	60	14.4	10
% coarse sediments_e	% coarse sediments edge site	0	95	33.1	30
% fine sediments_e	% fine sediments edge site	0	95	32.2	30
Case study 2					
Conductivity	μS cm^{-1}	150	66,641	3797.0	1242
Total-P	Total phosphorus (μg L^{-1})	8	3817	110.0	41
Total-N	Total nitrogen (mg L^{-1})	0.12	6.96	1.4	1.003
NO_x	Nitrates (μg L-1)	1.5	5980	332.8	42.5
Dissolved oxygen	mg L^{-1}	2	17.6	8.2	6.9
pH	pH units	3.58	9.37	7.9	7.94

collected from the Adelaide and Mount Lofty Region, the south-east of South Australia and the Eyre Peninsula, covering broad environmental gradients. Data comprised of 275 diatom taxa identified to the lowest taxonomic resolution (mostly species). The most abundant taxa in decreasing order of abundance were: *Achanthidium minutissimum, Cocconeis placentula, Planothidium lanceolatum, Tabularia fasiculata, Planothidium delicatulum, Rhoicosphenia abbreviata, Nitzchia inconspicua* and *Rhopalodia musculus.* A summary of the water quality variables from this study is given in Table 1.

2.1.2. Synthetic data

We generated synthetic data by simulating different species distributions and different sample to environment distributions (SEDs) (Fig. 1). All synthetic data were generated with a sample size n = 625. Sample sizes of >600 occur in many monitoring data sets (Bryce et al., 2010; Daily et al., 2012). Gradient forest, which considers multiple stressors, requires a minimum of two gradients, thus two hypothetical environmental gradients X and Y were produced. Abundance data for 20 taxa were simulated.

To evaluate the presence of model-based bias, comparison of species responses along a uniform environmental gradient was conducted using the following experiments with different species optima:

1) species optima (i.e maximum species abundance) for all twenty species located in the middle of the gradient (species optimum = 120), with sharp change points at 80 and 160 (Fig. 1*a*).

2) four abrupt change points with ten species given an optimum = 120 and change points at 80 and 160, and other ten species with an optimum = 320 and change points at 280 and 360 (Fig. 1*b*).

3) different positions of species optima with change points in the form of skewed species response curves instead of abrupt change points (Fig. 1*c* and *d*) and species optima located in centre of the gradient (Fig. 1*e*).

We also modelled the effect of exponentially decreasing or increasing environmental parameters in the presence of uniform species' distributions (Fig. 1*f* and Fig. 1*j*).). Environmental data is often distributed in this way e.g. both our field case study data sets were mostly right skewed.

2.2. Methods

Gradient Forest (GF) (Ellis et al., 2012) was used to quantify the magnitude and shape of change in community response along multiple environmental gradients. GF is an extension of random forest method (Breiman, 2001) which has been used to assess predictor importance and change in abundance trends for univariate response variables (e.g. Lee et al., 2019). GF is a novel, machine learning approach that consider responses from multiple species. Random forest is an ensemble of regression trees, whereas in GF separate random forests are grown for each species by partitioning the observations based on split function. By aggregating information from recursive partitioning over all species, the method gives a function representing compositional change along environmental variables. It consists of two components. First, a regression tree consisting of 500 regression trees, based on split function and a bootstrap procedure that calculates predictor importance and response curves along different gradients. The importance of each variable in terms of the probability of representing a threshold, expressed by the coefficient of variation is then calculated for each predictor that is derived by maximizing the variance homogeneity for each subsequent split. Secondly, only information with a variance >0 is explained by the random forest models. As a result, the following three outputs are provided: 1) R^2 weighted importance for each predictor; 2) Species turn over functions based on predictor splits and data density; 3) Overall compositional turnover based on individual species and the change in cumulative response. In this study, we performed threshold analysis only on the three most important stressors with a weighted importance of $R^2 > 0.02$. This criterion is similar to other studies (Tang et al., 2017; Wagenhoff et al., 2017). GF analysis was carried out using the R package "gradientForest" (Ellis et al., 2012). To meet the statistical requirements, taxon abundances were log transformed ($x + \min[x, x > 0]$). However, predictor variables were not transformed as this is not a requirement of GF (Ellis et al., 2012).

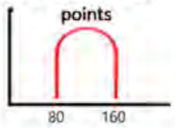
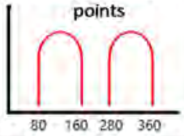
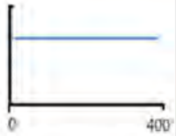
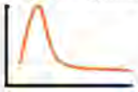
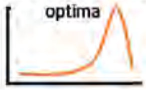
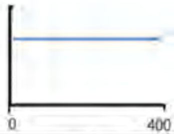
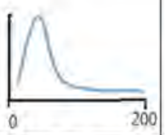
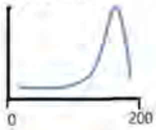
Synthetic Data Experiments	Environmental Gradient	Species Response	Location of results
1. Uniform environmental gradient			
a		two abrupt change points 	Figure 6
b		multiple change points 	Figure 6
c		right skewed species optima 	Figure 7
d		left skewed species optima 	Figure 7
e		species optima at centre 	Figure 7
2. Skewed environmental gradient			
f	right skewed 	evenly distributed 	Figure 8
j	left skewed 	evenly distributed 	Figure 8

Fig. 1. Summary of synthetic data experiments.

TITAN was applied to field and synthetic data using 500 bootstrap replicates in the R package TITAN 2 (Baker and King, 2010). Taxa with occurrences <3 were not considered following Baker and King's (2010) recommended minimum criterion for TITAN. TITAN distinguishes taxa responding positively or negatively to the specific predictor variable in terms of z scores. Change points or thresholds are detected when there is a synchronous change in the abundance of a

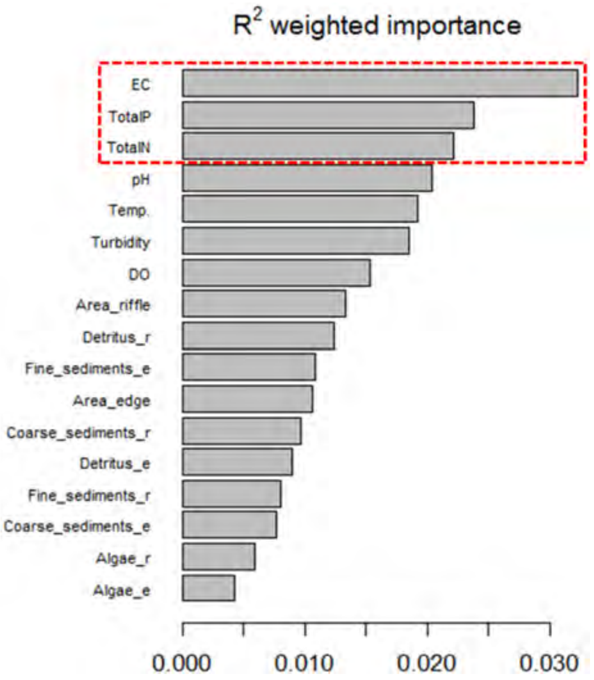


Fig. 2. Overall predictor importance for macroinvertebrate distribution calculated by gradient forest highlighting important variables under consideration for this study (Torrens and Onkaparinga River catchments, n = 255, time period: 1994–2007). Red dotted line denotes the most important predictors. Description of the variables are summarized in Table 1. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

number of taxa lying within a narrow range of the predictor variable. This method is based on IndVal (indicator values from species indicator analysis) (Dufrêne and Legendre, 1997) and incorporates a bootstrap procedure to find taxon responses that are pure and reliable. Nonmetric Multidimensional Scaling (NMDS) was used to assess the community composition based on environmental variables with the Bray-Curtis distance measure. All synthetic data were generated in R (version: 3.4.2) (R Develpment Core Team, 2018) and species abundance data were simulated using the R package coenocliner (Simpson, 2016).

3. Results

3.1. Analysis of observed data: case study 1

3.1.1. Importance of predictors

Gradient Forest identified EC, TP and TN as the most important stressors ($R^2 > 0.02$) in explaining macroinvertebrate distributions (Fig. 2). Additionally, NMDS did not show any separation of samples based on habitat characteristics and these were therefore less important (Supporting Information Appendix 1). As a result, we developed GF and TITAN-based thresholds for EC, TP and TN.

3.1.2. Thresholds of important predictors

EC, TP and TN thresholds for macroinvertebrate distributions are shown in Figs. 3, and 4. The GF split density plot (Fig. 3a), illustrates that data density is concentrated towards the low concentrations of EC gradient (red line). The data density standardized by split density (blue line) allowed assessment of compositional change across the gradient, with ratios above 1 indicating higher importance. EC thresholds can be seen at 400 μS cm^{-1}– 900 μS cm^{-1} and 2800 μS cm^{-1}. These results are further elaborated by plotting the abundance trends of most responsive species along EC, TP and TN gradient (given as supporting information Appendix 3 to 5). Along the EC gradient, multiple splits were observed in the range 400 μS cm^{-1}–900 μS cm^{-1}, indicating the most prominent changes in species' abundance were within this range. This threshold range was mostly due to changes in the abundance of *Aphroteniella* spp. (negative; −ve), *Orphninotrichia maculata* (−ve), *Astigmata* spp. (−ve), *Paramerina* spp. (−ve) *Newmanoperla thoreyi* (−ve) and *Austrochiltonia australis* (positive). *Aphroteniella* spp. declined markedly between 400 and 900 μS cm^{-1} whereas the other taxa showed a more gradual loss or gain (Fig. 3b). The locations

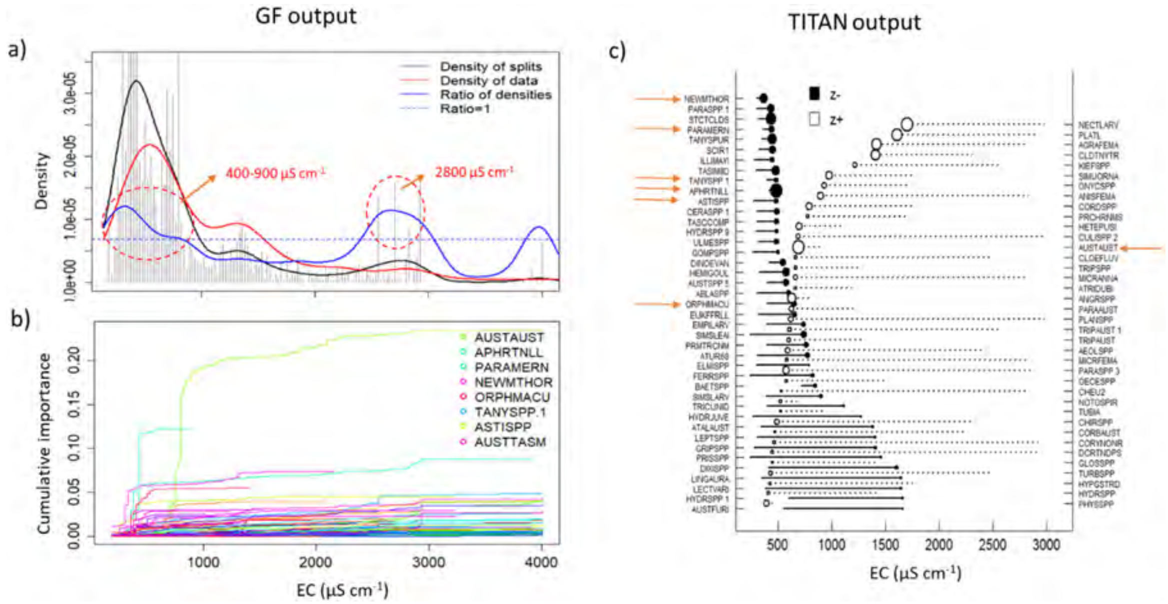


Fig. 3. Macroinvertebrate thresholds along the Electrical Conductivity (EC) gradient in the Torrens and Onkaparinga River catchments a). Split density plots from Gradient Forest (GF) output. Red dashed circles indicate the location of thresholds corresponding to significant change in taxon responses. b). Cumulative importance plots of all taxa, of which only eight most important are shown in legend; and c) significant indicator taxa change points based on TITAN output. Taxa and abbreviations shown as most responsive by GF are: AUSTAUST: *Austrochiltonia australis;* APHRTNLL*: Aphroteniella spp.;* PARAMERN: *Paramerina* spp.; NEWMTHOR*: Newmanoperla thoreyi;* ORPHMACU*: Orphninotrichia maculata; TANYSPP: tanytarsus* spp.; ASTISPP*: Astigmata* spp.; AUSTTASM*: Austrocerca tasmanica*. The cumulative importance plots of GF output refer to cumulative change in individual species abundance showing most responsive species and the cumulative change of whole community along the gradient. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

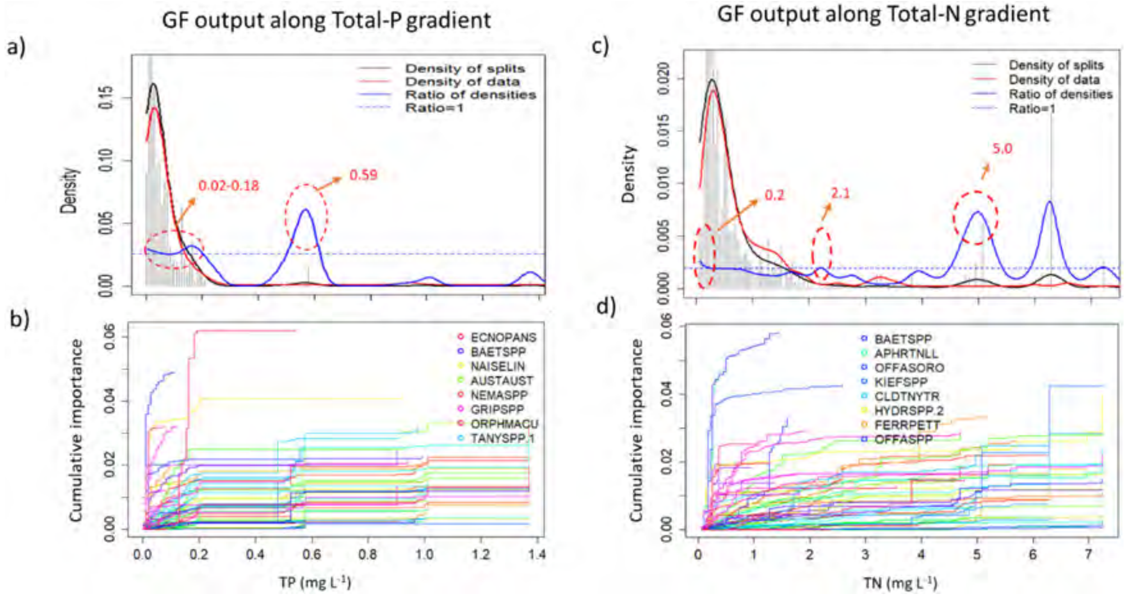


Fig. 4. Gradient forest (GF) based macroinvertebrate thresholds along the total phosphorus (TP) and total nitrogen (TN) gradient from the Torrens and Onkaparinga River catchments. a) Split density plots from Gradient Forest (GF) output along TP; b) Cumulative importance plots of all taxa along TP, of which only eight most important are shown in legend; c) Split density plots from GF along TN gradient; and d) Cumulative importance plots of all taxa along TN. Red dashed circles indicate the location of thresholds corresponding to significant change in taxon responses. Taxa and abbreviations shown as most responsive by GF are: ECNOPANS: *Ecnomus pansus; BAETSPP: Baetidae* spp.; NAISELIN: *Nais elinguis*; AUSTAUST: *Austrochiltonia australis*; NEMASPP: *Nematoda* spp.; GRIPSPP: Gripopterygidae spp.; ORPHMACU*: Orphninotrichia maculata; TANYSPP: Tanytarsus* spp.*; PARAMERN: Paramerina* spp.*;* BAETSPP: Baetidae spp.; APHRTNLL*: Aphroteniella spp.;* OFFASPP: *Offadens* spp.; KIEFSPP: *Kiefferulus* spp.; CLDTNYTR: Cladotanytarsus; FERRPETT: *Ferrissia petterdi.* (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

of individual change points for these species were similar to those identified by TITAN (Fig. 3d). However, the magnitude of response, as shown by the taxa with high z scores in TITAN, was different to the eight most responsive taxa from GF (Fig. 3c). Another change in species composition was also inferred by GF at 2800 μS cm^{-1}. This results from the cumulative response of increased abundance of species with broad environmental tolerances. As a result, and because of the small number of samples above this point, this "threshold" should be viewed with caution. TITAN identified change points for most of the positive and negative responding taxa within the EC range of 407 μS cm^{-1} to 951 μS cm^{-1} (Table 2), however the uncertainty band for positive responding taxa ranged up to 3000 μS cm^{-1} (Fig. 3c). The output from TITAN is shown in Fig. 3c as an example, while from here after these are given as supporting information. The suite of negative and positive responding taxa is similar to other studies with comparable salinity levels (e.g. (Botwe et al., 2015; Kefford et al., 2011).

For total phosphorus, gradient forest identified three main change points at 0.02 mg L^{-1}, 0.18 mg L^{-1} and at 0.59 mg L^{-1}. Macroinvertebrate assemblages changed most dramatically at 0.02 mg L^{-1} – 0.18 mg L^{-1}, mostly due to decreased abundances of a number of taxa (Fig. 4 a and b). Among the most responsive taxa (based on the highest cumulative importance identified by GF), *Austrochiltonia australis* showed a constant increase in abundance along the TP gradient (Fig. 4b). The change in species composition observed at 0.59 mg L^{-1} was mostly due to the cumulative response of a number of taxa. This threshold may need consideration depending on the management objective (i.e. utility threshold) because *Ecnomus pansus* was eliminated above 0.59 mg L^{-1} (Fig. 4b). However the very low data density at this point indicates that this threshold is based on a few samples and thus should be viewed with caution (Fig. 4a and b). The TITAN-based change points are concentrated at lower TP, where the maximum change in response for both negative and positive responding taxa was between 0.02 and 0.04 mg TP L^{-1} (Table 2).

Table 2
TITAN based water quality thresholds for macroinvertebrates and diatoms data. Sum (z-) and sum (z+) represent negative and positive responses. Values in parenthesis are the 5th and 95th percentiles of 500 bootstrap replicates.

Water quality variables	Sum (z-)	Sum (z+)
Macroinvertebrates		
EC (μScm^{-1})	407 (396, 448)	951 (695, 1750)
TP (mgL^{-1})	0.02 (0.008, 0.026)	0.04 (0.02, 0.05)
TN (mgL^{-1})	0.28 (0.24, 0.30)	0.59 (0.52, 0.67)
Diatoms		
EC (μScm^{-1})	1004 (681, 1214)	2440 (1525, 5129)
TP (mgL^{-1})	0.02 (0.01, 0.03)	0.17 (0.04, 0.32)

GF identified change points for total nitrogen at 0.2 mg L^{-1}, 2.1 mg L^{-1}, 5.0 mg L^{-1} and 6.2 mg L^{-1} (Fig. 4c). The macroinvertebrate community showed a response at TN concentrations below 0.2 mg L^{-1}, however, some taxa including *Aphroteniella* spp., *Offadens* spp. and *Baetidae* spp. were completely eliminated between 1.5 mg L^{-1} to 2.5 mg L^{-1} TN (Fig. 4c and d). Two potential thresholds were observed at TN concentrations of 5 mg L^{-1} and 6.2 mg L^{-1}. Some taxa (e.g. *Ferrissia petterdi)* appeared intolerant to TN concentrations above 5 mg L^{-1}, however data density at this point was very low (Fig. 4c). Another change point at 6.2 mg L^{-1} was driven by the positive response of a single taxon where there was very low data density (Fig. 4c and d). TITAN indicated change points for TN at lower concentrations than GF where the maximum change occurred for both sum (z-) and sum (z+) taxa within the range from 0.28 mg L^{-1} to 0.67 mg L^{-1} respectively (Table 2). TITAN output with change points showing magnitude and direction of z (+) and z (−) along TP and TN gradients are given as supporting information (Appendix 6 and 7).

3.2. Analysis of field data: case study 2

3.2.1. Importance of predictors

GF identified electrical conductivity and TP as the most important variables ($R^2 > 0.02$) driving the response of the diatom community (figure given as Supporting information Appendix 8). These findings

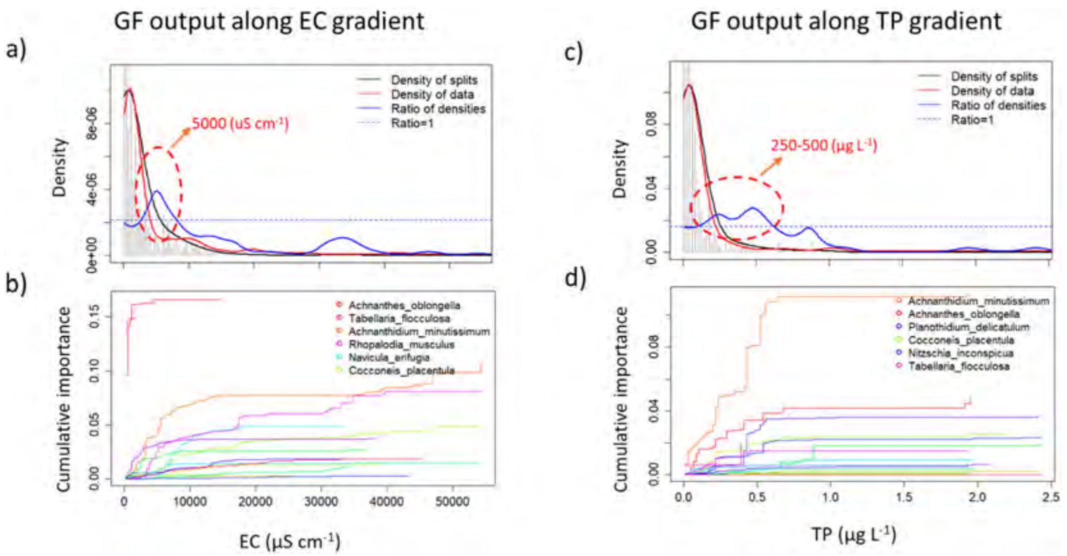


Fig. 5. Gradient forest (GF) based diatoms thresholds along the electrical conductivity (EC) and total phosphorus (TP) gradient from South Australian rivers. a) Split density plots from GF output along EC gradient. b). Cumulative importance plots of all taxa along EC, of which only eight most important are shown in legend; c) Split density plots from GF along TP gradient; and d) Cumulative importance plots of all taxa along TP. Red dashed circles indicate the location of thresholds corresponding to significant change in taxon responses. Most responsive taxa in GF species plot are: *Achanthes oblongella, Tabellaria flocculosa, Achanthidium minutissimum, Rhopalodia musculus, Navicula erifuga, Planothidium delicatulum, Nitzschia inconspicua, Rhopalodia musculus* and *Cocconeis placentula*. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

were consistent with the canonical correspondence analysis (CCA) results from previous study (Tibby et al., 2019).

3.2.2. Thresholds of important predictors

In the diatom data, both methods identified variation in overall thresholds detection. The split density plots generated by GF showed a right skewed distribution of samples on the EC and TP gradients (Fig. 5a and c), which is typical of many field data sets (Fletcher et al., 2005; Welsh et al., 1996). The EC threshold identified by GF was 5000 µS cm^{-1} which is considerably higher than the TITAN identified change points ranging from 276 µS cm^{-1} to 1314 µS cm^{-1} in the previous study (Tibby et al., 2019). Notably, *Tabellaria flocculosa* and *Achanthes oblongella* were eliminated completely well below 5000 µS cm^{-1} (Fig. 5b) and is reflected in the TITAN response z (−ve) for these taxa (Table 2, detailed TITAN plots are given as supporting information, Appendix 9 and 10). A similar phenomenon of higher TP thresholds identified by GF was observed with peaks at 250 µg L^{-1} to 500 µg L^{-1} (Fig. 5c) while TITAN-identified change points were at 18 µg L^{-1} to 330 µg L^{-1} (Table 2). TITAN thresholds were consistent with previous macroinvertebrate thresholds from same area which suggested much lower thresholds than South Australia's water quality guidelines (Sultana et al., 2019).

3.3. Analysis of synthetic data

3.3.1. Case 1: uniform sample to environment distribution (SED)

Thresholds for the simulated abrupt change points were identified using both methods (Fig. 6). GF and TITAN accurately detected the species change points along the uniformly distributed synthetic gradient (Fig. 6). When applied to multiple abrupt change points, GF accurately identified all four simulated change points with three identified in the density plot ($x = 80$, 280 and 360) (Fig. 6e) and all four identified in the cumulative importance plots (supporting information, Appendix 11). TITAN detected change points at $x = 160$ and 280 (Fig. 6f). However, the other two change points could only be identified with other peaks in summed z scores indicating points of maximum change in community structure (Fig. 6 f).

For skewed species response curves that mimic "real world" data (Fig. 7), GF accurately detected change points at key inflections in the species response curves for all the three experiments (Fig. 7b, e and h). TITAN produced similar results in these experiments (Fig. 7f). When species optima were located at the centre of the gradient, GF identified thresholds at 80 and 320 which represented inflection points in the response curve on either side of the optimum (200). By contrast, TITAN failed to identify change points as either z (+) or z (−) species in this experiment (Fig. 7h and i).

3.3.2. Case 2: skewed sample to environment distributions (SEDs)

Data sets with skewed sample distribution, but with uniform species responses, were used for testing the potential bias in GF and TITAN threshold identification. In this experiment no thresholds should be identified since the species' distributions are uniform. While thresholds were identified, both techniques also provided warnings that indicated caution in interpreting thresholds. However, the identified thresholds were substantially different between the methods. In both cases with skewed SEDs, GF output identified prominent threshold peaks at approximately 150 and 50 where there was also very low data density (Fig. 8b and e). For exponentially decreasing data along gradient X, GF identified one to two times higher thresholds at around 100 or 150 compared to the TITAN 35 to 48 (Fig. 8b and c). GF showed a similar trend of identifying thresholds at around 50 or 100 compared to TITAN change points at around 135, at the higher part of the gradient (Fig. 8e and f). These results indicate that considerable caution is needed when applying either method to detect thresholds in a setting with skewed environmental gradients.

4. Discussion and recommendations

This study compared the advantages and disadvantages of two well established methods for identifying thresholds in species responses along environmental gradients. We first sought to determine whether threshold identification in field data was affected by model-based biases, secondly to determine using synthetic data whether TITAN and GF identified different thresholds and thirdly to examine how different data distributions of species response affected the thresholds identified by GF and TITAN. Evaluation of alternative threshold detection methods is needed to find the model that is optimal for both the type of data and suitability for meeting management objectives (Burnham and Anderson, 2003; Samhouri et al., 2017).

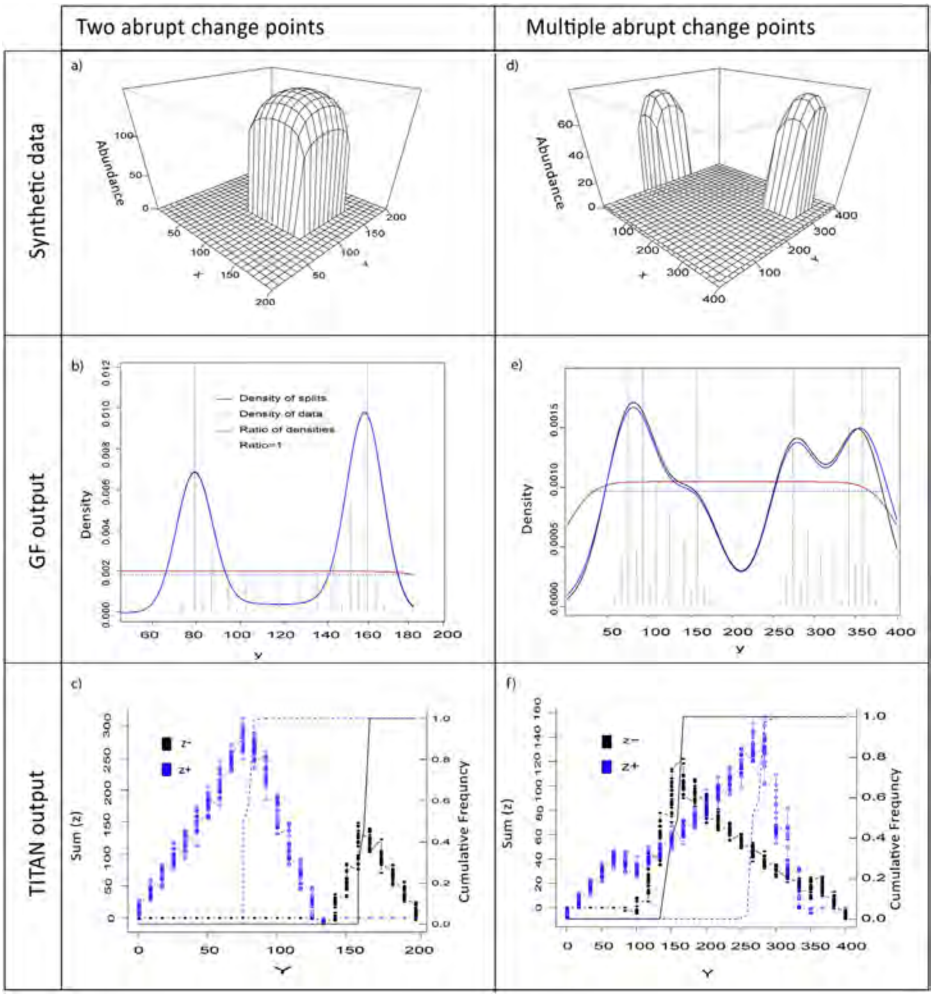


Fig. 6. Comparison of threshold locations identified by Gradient Forest (GF) and TITAN for abrupt species change points in species responses along a uniformly distributed environmental gradient. Abundance data for 20 species were simulated along hypothetical gradients X and Y. a) simulated change points at 80 and 160, d) simulated change points at 80, 160, 280 and 360. The TITAN output displays the sum (z) plots, where peaks in summed z scores of increasing (z+) and decreasing (z-) taxa indicate points of maximum change in community structure. Solid and dashed lines indicate cumulative frequency distributions (CFDs) of sum (z-) and sum (z+) respectively, whereby vertical CFDs (instead of sloping or stair-step) represented narrow uncertainty of change points (c and f).

GF ranks the relative importance of each biological stressor which TITAN does not. For TITAN other multivariate techniques are needed to "shortlist" the most important variables affecting biological assemblages, prior to threshold analysis (King and Baker, 2014). Gradient Forest analysis indicates that water quality variables i.e. EC, TP and TN are most important drivers affecting the macroinvertebrate community composition in the Torrens and Onkaparinga River catchments (Fig. 2) with habitat characteristics less important. Similarly, EC and TP were the most important factors affecting diatom communities in South Australian streams. This information allows freshwater managers to establish decision thresholds based on selection of priority managed stressors, which may account for the present condition of the resource. Therefore, we recommend use of GF as a first step to shortlist the most important driving factors.

In field-based studies, the identification of thresholds depends on the method and needs careful interpretation. In case study 1, with macroinvertebrate data from two catchments, Gradient Forest and TITAN identified similar thresholds at low concentrations of EC, TP and TN. These thresholds are lower than the national water quality guidelines for South Australia (ANZECC, 2000) (Table 3), which have been recently suggested to be too high (Sultana et al., 2019; Tibby et al., 2019). Our analysis supports these suggestions. However, at high stressor concentrations, multiple thresholds were inferred by GF. These thresholds appear to be spurious as they are related to a small number of outliers with very high stressor concentrations (Figs. 3a and 4c). This finding contrasts to the argument that regression tree based techniques such as GF are robust to outliers (De'Ath, 2002; Khoshgoftaar et al., 2007). More samples are required to characterize this part of gradient. In addition, the individual GF species response outputs indicate that the change points at higher EC and TP concentrations mostly relate to increases in the abundance of tolerant species rather than distinct species "loss" or "gain" (Figs. 3 and 4). This conclusion is supported by TITAN analysis that indicated no sharp synchronous change in positive-responding indicator taxa in relation to EC (Fig. 3c). A similar pattern is observed in other studies where positive-responding taxa tend to increase gradually along gradients and thus are not considered well-organised communities for thresholds consideration (King et al., 2011). Comparison of diatom and macroinvertebrate thresholds using TITAN suggest similar findings for TP thresholds i.e. 0.02 mg L^{-1}. However, diatoms appeared more tolerant to salinity with higher thresholds identified by TITAN (i.e. 1004 µS cm^{-1}) as compared to macroinvertebrate thresholds (407 µS cm^{-1}) (Table 2). In case study 2, GF overestimated thresholds as compared to TITAN, possibly due to the fact that threshold peaks at higher concentration was an aggregate response of significant positive and negative responding diatom species (Fig. 5). Thus we recommend exploration of the

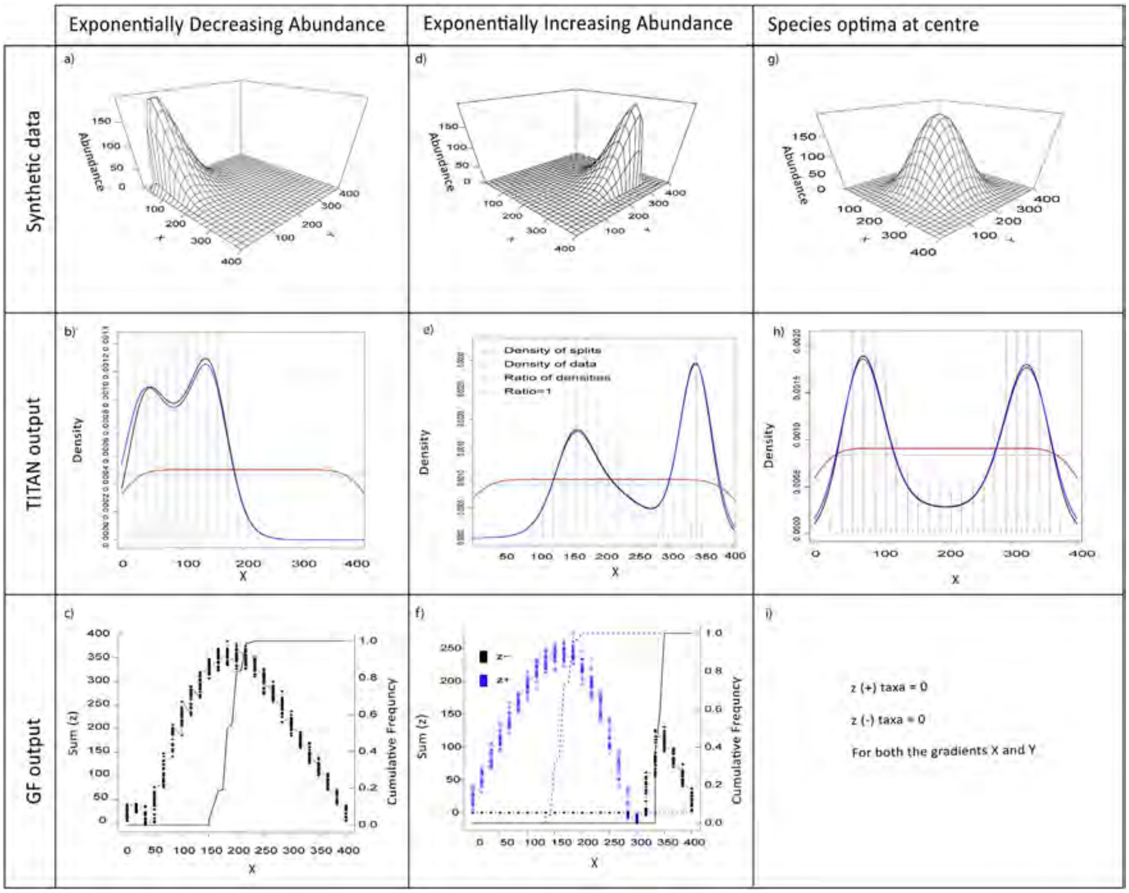


Fig. 7. Comparison of threshold locations identified by Gradient Forest (GF) and TITAN across a uniformly distributed environmental gradient with different species response curves. Abundance data for 20 species were simulated for each case.

individual species response to avoid this effect or a combination of these two methods for optimal threshold detection.

The comparison of GF and TITAN using synthetic data experiments provides a means to develop a rationale for appropriate model selection. A multiple model approach with qualitative comparison of model outputs has been suggested as an analytical framework for defining ecological thresholds (Samhouri et al., 2017). Similar change points were detected by the two models in the presence of uniform sample to environment distributions (SEDs) (Fig. 6). However, GF performed better when there were four simulated change points. Experiments with multiple change points can be used to screen the suitability of models to detect non-linear changes in stressor-response relationship. However, in the case of skewed distributions of environmental data, both models identified different species change points where none existed (Fig. 8 b and e). Daily et al. (2012) also warned of using non-parametric change point analysis when interpreting thresholds from non-uniform distributed data. Thus we recommend screening of data structure prior to analysis to avoid such an occurrence. Since field data are mostly skewed (Fletcher et al., 2005), caution is needed in interpreting thresholds from non-uniform environmental distributions, based solely on one model. In terms of limitations of both methods, a complementary approach has been recommended, whereby approaches with different underlying statistical assumptions are utilised (Ellis et al., 2012; King and Baker, 2014). This study has shown that GF and TITAN are complementary in defining thresholds and can be used in combination to detect thresholds.

The pattern of species responses along the gradient is another decisive factor that affects the location of thresholds (Bestelmeyer et al., 2011). Synthetic data experiments with different positions of species optima helped to distinguish between the models' performance. Both models identified similar change points when species had abrupt change points on uniform SEDs. The general understanding of the abrupt changes has been well studied and applied theoretically to test the threshold models along uniform distributed environmental gradients (Baker and King, 2010; Bestelmeyer et al., 2011; Francesco Ficetola and Denoël, 2009). Both models identified similar thresholds in case of skewed species responses, however TITAN failed to detect a threshold in the least likely case when species optima was in centre (Fig. 7). Other comparative studies confirmed that models' performance varied while testing shape of species response curves (i.e. abrupt, uniform, skewed and linear) and found that generalized additive modelling outperformed among other regression based methods (Francesco Ficetola and Denoël, 2009). Brenden et al. (2008) also found that shape of disturbance-response data is important in identifying true thresholds. Thus pattern of species responses need consideration while applying threshold models.

Both GF and TITAN take into account the non-linear, multivariate responses of taxa. But while TITAN parses species into those which respond negatively and positively and provides retrospective detection with clear visual interpretation, GF utilizes standardisation of data density which makes it well-suited for multiple threshold detection. However, without distinguishing positive and negative responding taxa, the aggregate response (i.e. threshold) can be overestimated for taxa which decline. To avoid overestimation of thresholds, we recommend either outlier deletion or using TITAN in conjunction with GF. TITAN is advantageous in providing a clearer indication of the magnitude and direction of response for the indicator taxa with associated uncertainty. By contrast GF requires specialised knowledge to interpret individual

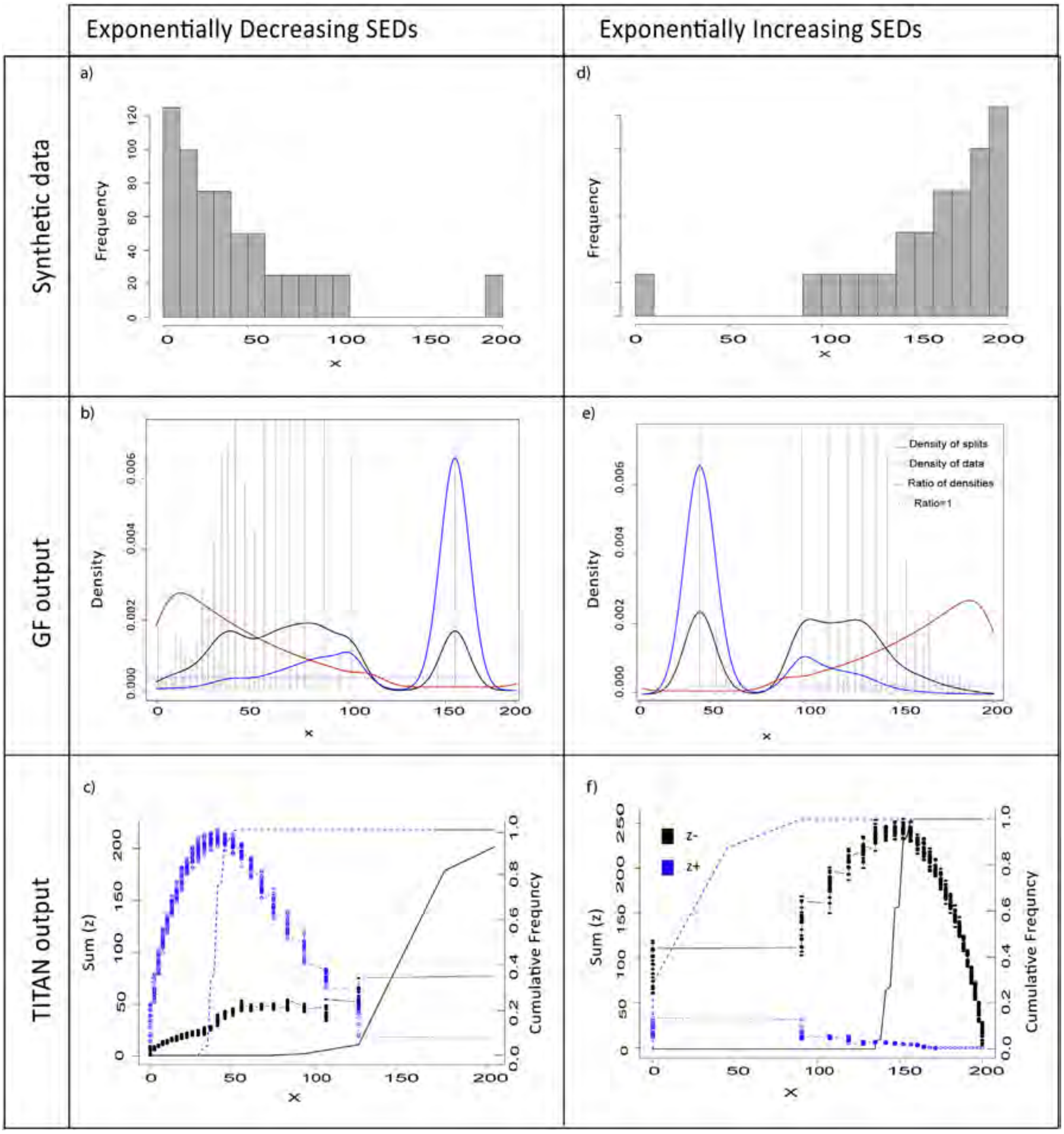


Fig. 8. Comparison of threshold locations identified by Gradient Forest (GF) and TITAN for a) exponentially decreasing sample-environment distributions (SEDs) and b) exponentially increasing SEDs.

species responses. As both models identified similar taxa as responsive indicators to the tested gradients, use of two models in combination further explains the magnitude and response of targeted taxa. Lastly, when there are no sharp thresholds and the TITAN uncertainty band is spread over all or much of the gradient, it is recommended that the data are split and rerun analysis to look for multiple secondary or tertiary thresholds (King et al., 2016). GF can serve that purpose by identifying multiple change points along gradients.

Table 3
Comparison of GF and TITAN identified thresholds for macroinvertebrates at low concentrations and for diatoms with ANZECC (2000) water quality guidelines.

	Macroinvertebrates		Diatoms		
Water quality variables	TITAN	GF	TITAN	GF	ANZECC (2000)
TP (mg L^{-1})	0.02	0.02	0.02	0.25	0.1
TN (mg L^{-1})	0.2	0.2	_	_	1
EC (μScm^{-1})	407	400	1004	5000	100–5000

5. Conclusions

Due to the complex nature of ecosystems, identification of environmental thresholds based solely on one model can lead to spurious outcomes. In conclusion, Gradient Forest and TITAN have strengths and weaknesses, but are generally robust in detecting changes in species responses and in identifying thresholds, despite some differences. Thresholds must be interpreted in the context of data structure and individual species' responses. In terms of management, any guideline or threshold value cannot be considered as an absolute compliance level but is used as a target. We recommend a combination of these methods to identify different signalling thresholds to be considered as management thresholds. This study has shown that a combination of the threshold models i.e. GF and TITAN allows a more comprehensive identification and description of ecological thresholds. The following conclusions can be drawn:

(1) The identification of true thresholds is dependent on the nature of sample to environment distribution and the pattern of individual species response curves.
(2) Both models identified the major thresholds in macroinvertebrate data at almost identical low concentrations of the most significant stressors (EC, TP, TN). However, in another case study of diatoms data, GF sometimes overestimates thresholds by aggregating the response of positive and negative responding taxa.
(3) Multiple thresholds identified by GF at high stressor concentrations need careful interpretation to avoid drawing incorrect conclusions based on outliers. However, carefully vetted multiple thresholds may provide valuable information about species preferences for higher stressor concentrations or management targets.
(4) Both the models identified similar thresholds when the sample to environment distribution was uniform in synthetic data. TITAN provides valuable additional information about the direction and magnitude indicator taxon responses and the associated uncertainty in location of thresholds.

CRediT authorship contribution statement

Jawairia Sultana: Conceptualization, Formal analysis, Writing - original draft.**John Tibby:**Conceptualization, Resources, Supervision, Writing - review & editing.**Friedrich Recknagel:**Supervision, Writing - review & editing.**Sally Maxwell:**Writing - review & editing.**Peter Goonan:**Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.137999.

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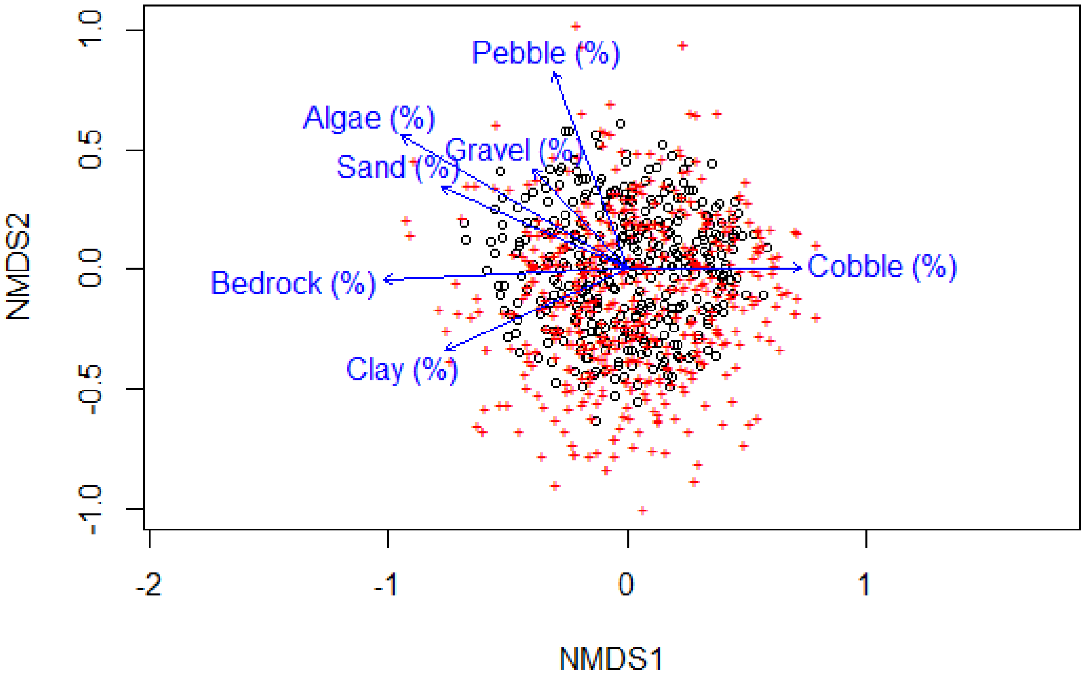
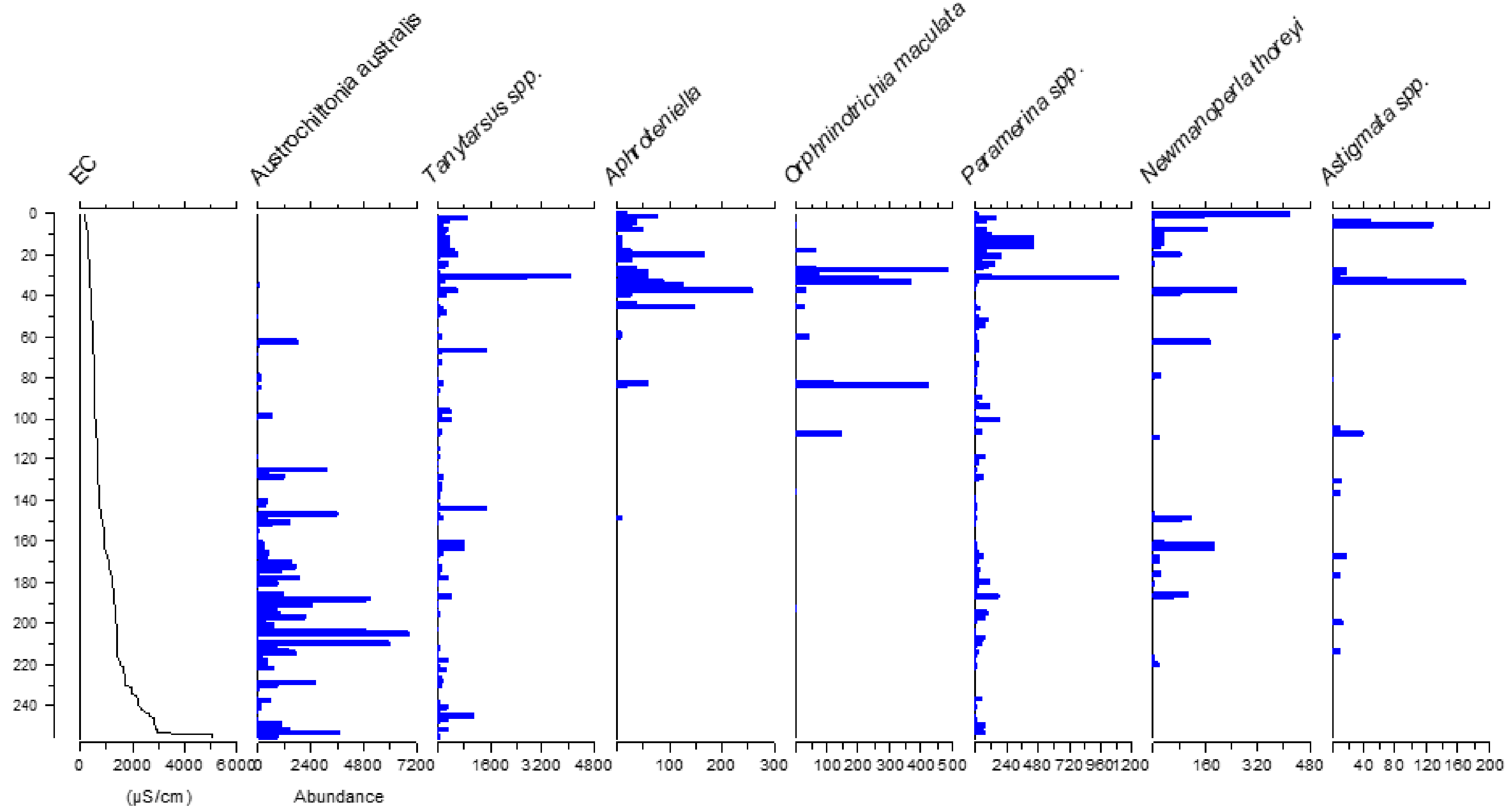
Supporting Information (Chapter 3)

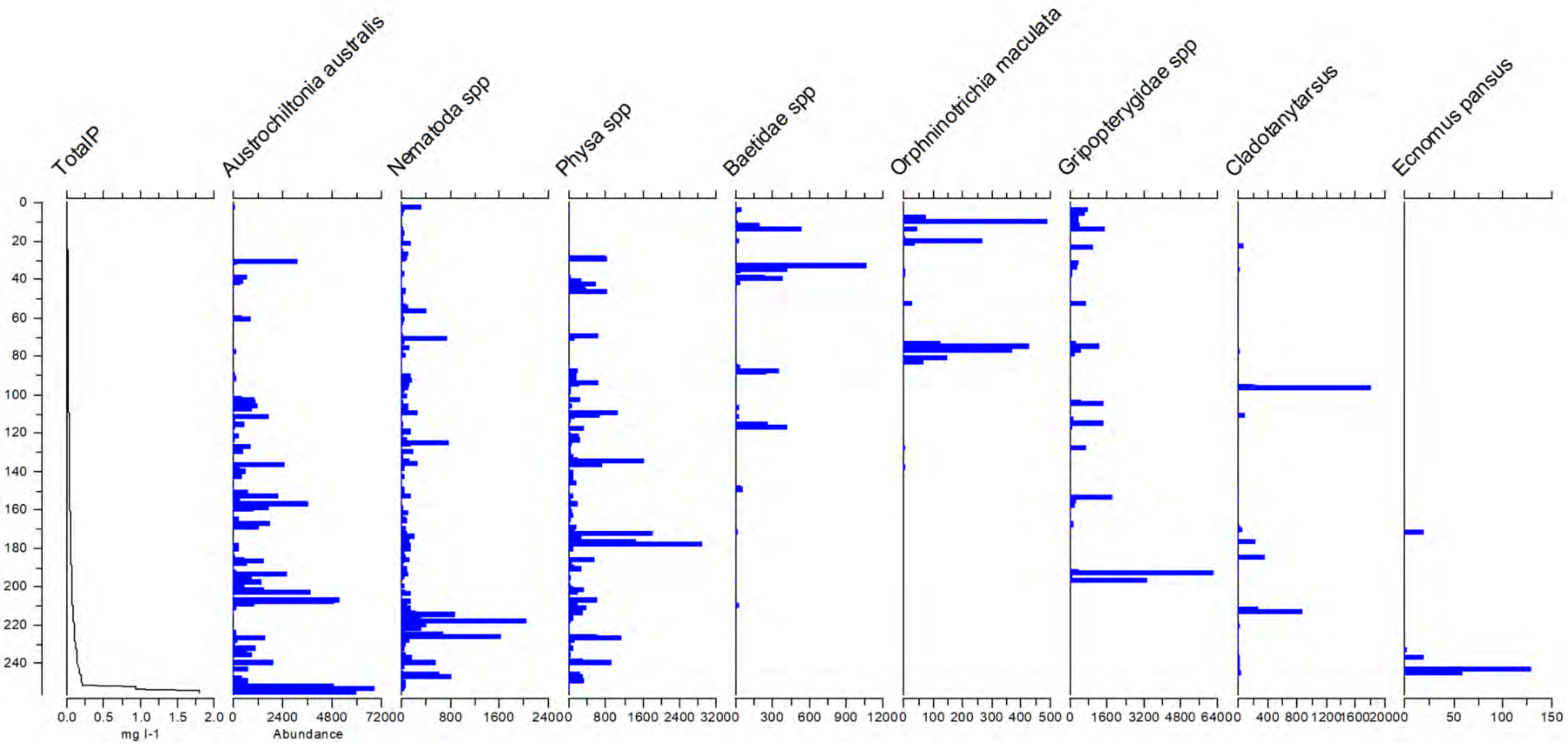


Appendix 1: Nonmetric Multidimensional Scaling plot for separation of samples based on habitat characteristics

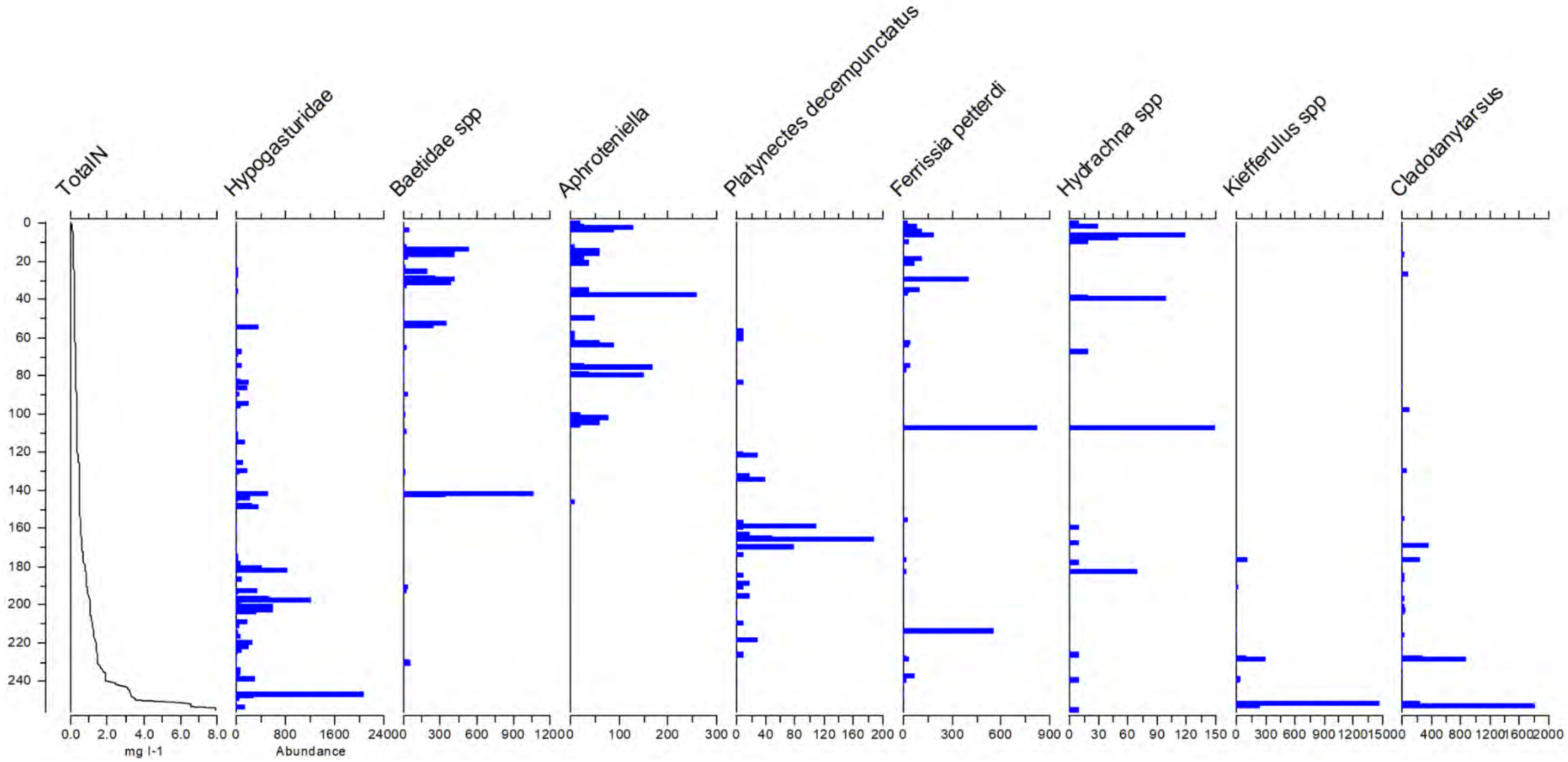
Appendix 2: List of macroinvertebrate species with abbreviations (please see page 44)



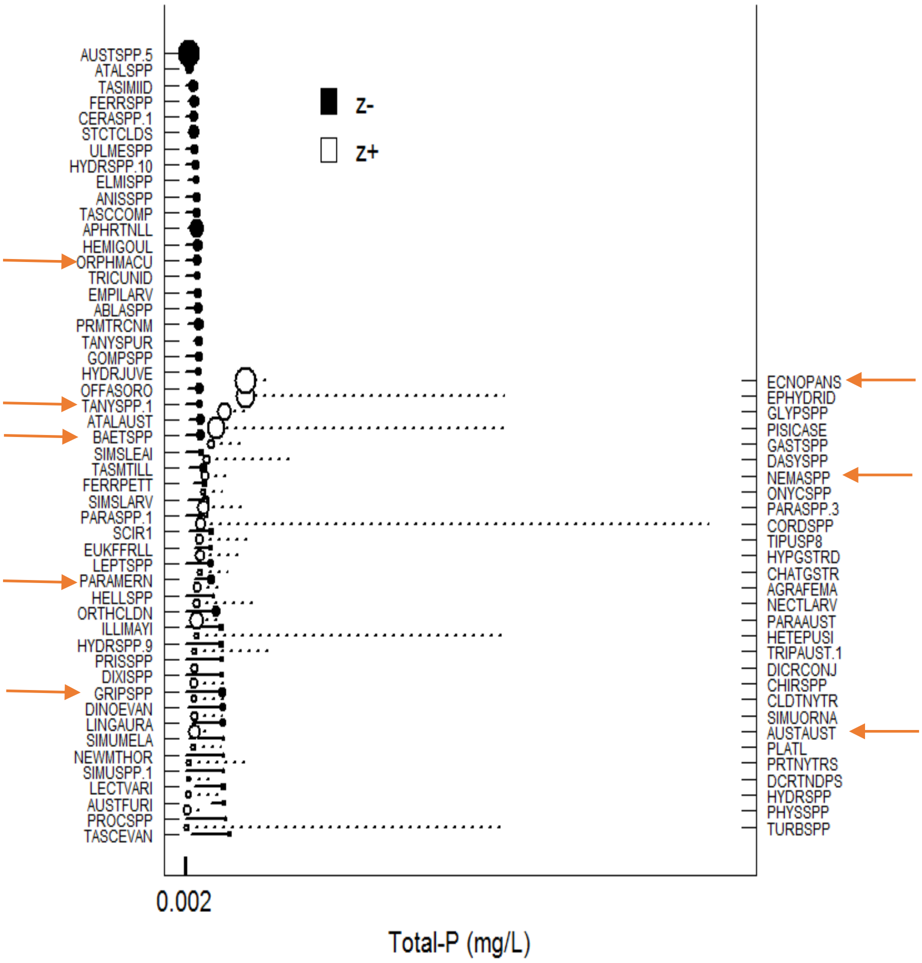
Appendix 3: Abundance trends of eight most responsive taxa identified by GF and TITAN along an increasing electrical conductivity (EC) gradient.



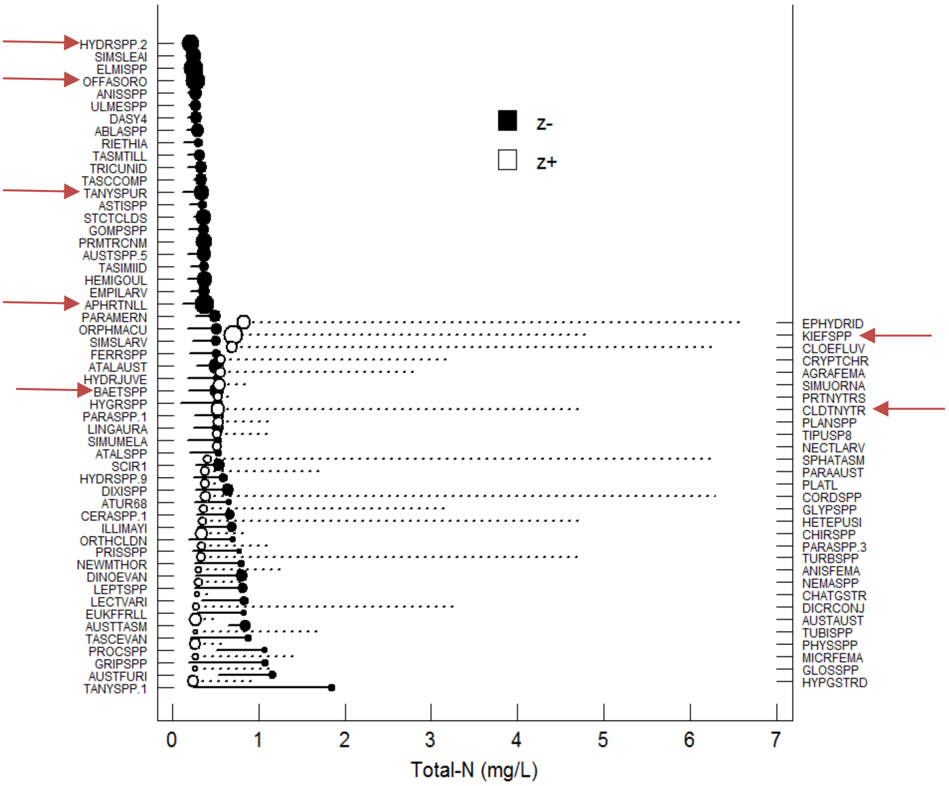
Appendix 4: Abundance trends of eight most responsive taxa identified by GF and TITAN along an increasing total phosphorus gradient.



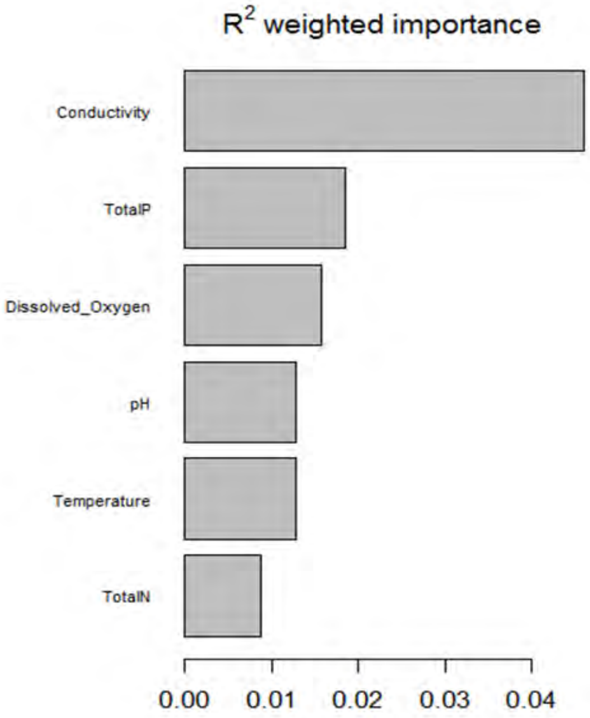
Appendix 5: Abundance trends of eight most responsive taxa identified by GF and TITAN along an increasing total nitrogen gradient.



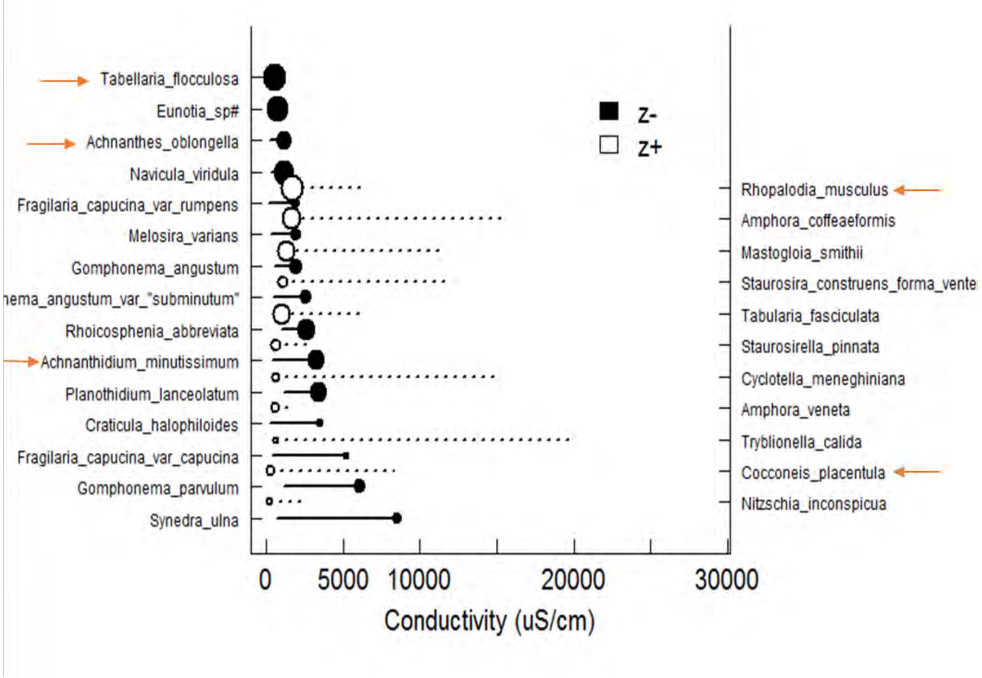
Appendix 6: Macroinvertebrate thresholds along the total phosphorus gradient showing significant indicator taxa change points based on TITAN output. Red arrows show similar species identified by gradient forest.



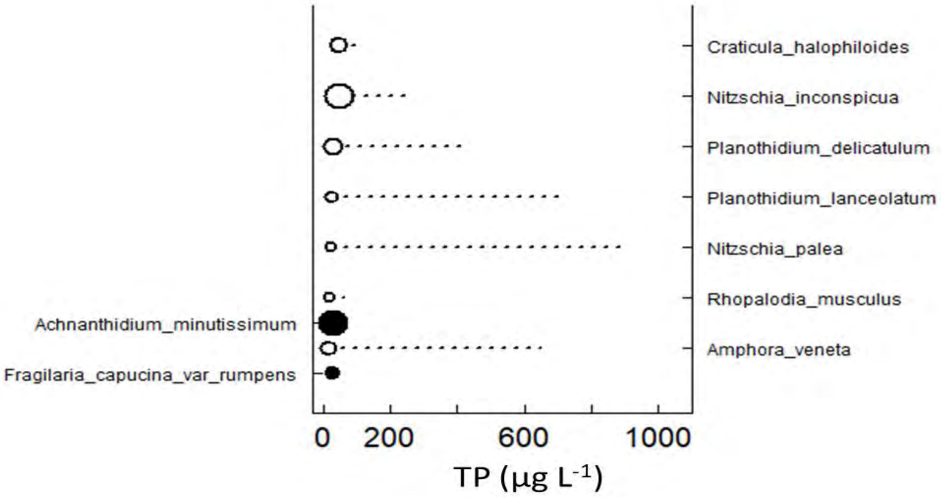
Appendix 7: Macroinvertebrate thresholds along the total nitrogen gradient showing significant indicator taxa change points based on TITAN output. Red arrows show similar species identified by gradient forest.



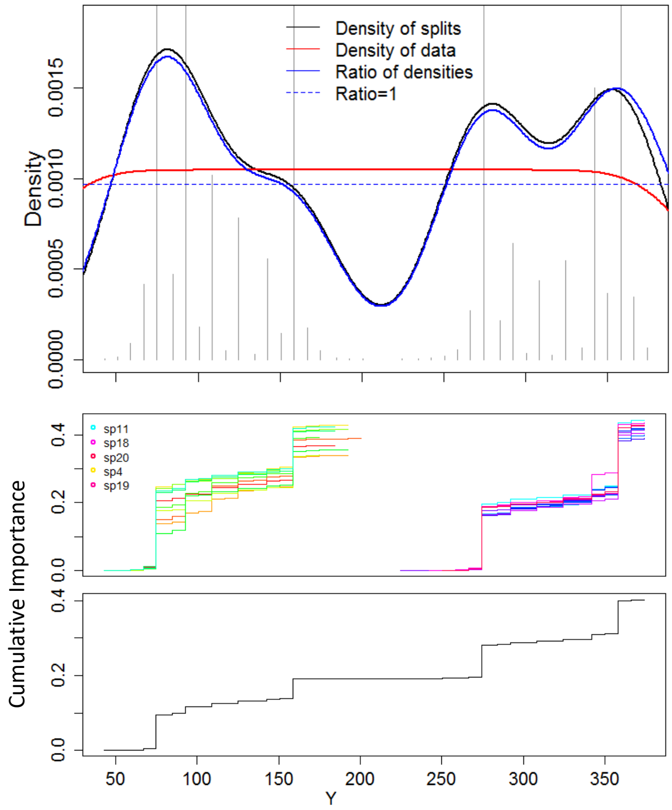
Appendix 8: Overall predictors' importance for diatoms distribution from South Australian streams (n=179) calculated by gradient forest highlighting important variables under consideration for this study.



Appendix 9: Diatom thresholds along the electrical conductivity gradient showing significant indicator taxa change points based on TITAN output. Red arrows show similar species identified by gradient forest.



Appendix 10: Diatom thresholds along the total phosphorus gradient showing significant indicator taxa change points based on TITAN output.



Appendix 11: Gradient forest identified four abrupt change points.

Chapter 4:

Evaluating spatial scale influences on threshold variations: Spatially-explicit responses of diatoms to water quality thresholds across multiple spatial scales

Statement of Authorship

Title of Paper	Evaluating spatial scale influences on threshold variations: Spatially-explicit responses of diatoms to water quality thresholds across multiple spatial scales
Publication Status	☐ Published ☐ Accepted for Publication ☐ Submitted for Publication ☑ Unpublished and Unsubmitted work written in manuscript style
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Principal Author

Name of Principal Author (Candidate)	Jawairia Sultana		
Contribution to the Paper	Local scale field work and diatoms identification, formal analysis, results preparation, writing, original draft preparation. I hereby certify that statement of the contribution is accurate.		
Overall percentage (%)	80%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	28-05-2020

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Sally Maxwell		
Contribution to the Paper	Conceptualisation, supervision, critical review and editing I hereby certify that statement of the contribution is accurate.		
Signature		Date	29/05/2020

Name of Co-Author	John Tibby		
Contribution to the Paper	Supervision, resources, provision of data, critical review and editing I hereby certify that statement of the contribution is accurate.		
Signature		Date	28/05/2020

Abstract

Ecological thresholds have been defined as tipping points beyond which ecosystem function is imbalanced. Identifying these thresholds is vital for conservation and restoration of freshwater ecosystems and for deriving management guidelines. However, different spatial and temporal scales complicate the identification and application of ecological thresholds for management. This study aimed at identifying key water quality variables and their thresholds affecting diatom community structures at multiple spatial scales in South Australian streams. Ecological thresholds were determined at four hierarchical spatial scales, i.e. state, regional, subregional and local. The congruence of thresholds was assessed among spatial scales and between different regions. Analysis of diatom and water quality data collected during autumn and spring from 2015–2019 revealed electrical conductivity (EC) and total phosphorus (TP) as the most important water quality variables affecting diatom assemblages, irrespective of spatial scale. Ecological thresholds were derived by threshold indicator taxa analysis (TITAN). There were either lower or consistent thresholds with increasing spatial resolution but differences between regions. For EC, similar thresholds were observed at the state scale (271 µS/cm) and within the three subregions (269–271 µS/cm). The thresholds for TP differed considerably across spatial scales with the regional scale change point (15 µg/L) around half that observed at the state scale (29 µg/L), but was similar at the subregional and local scale (12 µg/L). In contrast, the south east (SE) region exhibited higher thresholds for TP and EC (TP = 37 µg/L and EC= 1180 µS/cm, than the Adelaide and Mount Lofty Ranges (AMLR). Higher diatom thresholds for the SE compared to the AMLR and state scale thresholds, corresponding to the highest TP and EC concentrations being recorded in this region. Similar suite of threshold indicator species was observed among nested hierarchical spatial scales, but with considerable variations between regions. Overall, local scale thresholds in this study were lower than the state scale thresholds, possibly due to factors like differential land uses contributing towards site heterogeneity. Given the differences of thresholds observed between regions and across spatial scales, thresholds derived at broad scales alone are unlikely to be appropriate for finer-scale assessment. We recommend ecological thresholds be derived at the finest scale for which there is an appropriate amount of data. It may require adjustments to the scale at which data is collected to inform appropriate management guidelines.

Key words: site-specific thresholds, indicator species, threshold indicator taxa analysis (TITAN), local scale thresholds, water quality

4.1 Introduction

Ecological thresholds are defined as points of change along a continuum beyond which there is an abrupt change in ecosystem function (Groffman et al., 2006) or where there is a synchronous change in indicator taxa (King and Baker, 2014). Understanding an ecosystems' threshold response to change in stressors is fundamental to their effective management (Feng et al., 2019; Foley et al., 2015; Samhouri et al., 2010). Thresholds are controlled by many factors operating at different temporal and spatial scales (Groffman et al., 2006). A wide variety of techniques has emerged to quantify ecological thresholds in the last two decades (Baker and King, 2010; Ellis et al., 2012; Sonderegger et al., 2009), and since then there has been an explosion of application of these techniques with much success in freshwater management (King et al., 2011; Recknagel et al., 2016; Wagenhoff et al., 2017b). Studies on thresholds analysis ranges from development of new methods (Baker and King, 2010; Ellis et al., 2012; Sonderegger et al., 2009), comparison of methods (Daily et al., 2012; Sultana et al., 2020a) and application of new methods for freshwater management (Baker and King, 2010; Wagenhoff et al., 2017a). However, there has been less research on scale dependence in deriving and applying these thresholds.

Ecological thresholds have been most often analysed at broad spatial scales to cover the wide range of environmental gradients [e.g. (King and Baker, 2010; Tang et al., 2017; Taylor et al., 2018)]. For example, the Murray Darling Basin Plan in south-eastern Australia uses the same water quality thresholds across the entire basin despite significant regional differences in climate, rainfall, soil types and other variables (Basin Plan, 2012). Similarly, using a single threshold to manage streams that are highly degraded vs. pristine, is problematic (Olson and Hawkins, 2013; Soranno et al., 2008). In this context, Liang et al., (2020) introduced the concept of 'ecological fallacy' which defines that individual-level relationships can be different from group-level relationships and vice-versa. Comparative study of site-specific and ecoregional water quality thresholds highlighted that site-specific thresholds cannot be deduced from a broader spatial extent of ecoregions (Liang et al., 2020). Olson and Hawkins (2013) proposed that site-specific criteria can provide better scientifically defensible and ecologically meaningful water quality thresholds than regional. In South Australia, diatom thresholds have been derived to improve the water quality guidelines and a need for specific regional thresholds was identified (Tibby et al., 2019). The importance of potential differences in freshwater quality thresholds at different spatial scales is also emphasised in the recent updates on freshwater quality guidelines in Australia that have been established on more site-specific criteria (revised in 2018 and accessible online at www.waterquality.gov.au/guidelines). However, deriving site-specific thresholds is complex and may require extensive sampling on large temporal scales (Van Dam et al., 2019), which can be costly and difficult to manage. One approach to this trade-off between cost and utility is to find the optimal spatial scale by understanding the variation in thresholds at multiple spatial scales.

Understanding the spatial distribution of organisms across multiple scales has become an important paradigm in both terrestrial and aquatic ecology (Kuemmerlen et al., 2019; Schmidt et al., 2020; Schweiger et al., 2005). The concept of nested hierarchical spatial scales, which defines that small scale systems are determined by the larger-scale systems of which they are part (Frissell et al., 1986), is not a novel one. It finds its origin in landscape ecology (Delcourt et al., 1982). To date, several studies considered the effect of landscape heterogeneity on water quality and species composition at multiple spatial scales (Poole, 2002; Shen et al., 2015; Zhou et al., 2012). A study examining the effects of different spatial scales indicated that the role of spatial extent was unpredictable and subregions differed in heterogeneity of lake macrophytes composition (Alahuhta and Heino, 2013). Similarly, riverine ecosystems are controlled by different linked factors operating at multiple spatial scales that determine the species composition, function and structure (Lowe et al., 2006; Vannote et al., 1980). Some studies have highlighted the significance of variables acting at catchment scale, such as river bedform (Townsend et al., 2003) and basin geology (Allan and Johnson, 1997), whereas others have addressed reach (Richards et al., 1997) or microhabitat (Brosse et al., 2003) scale variables. Another study on multiple spatial scales highlighted that indicators of biodiversity generally differ between ecosystem types ranging from patch to global scale (Feld et al., 2009); and from site to catchment scale (Feld and Hering, 2007) due to impacts of different environmental gradients. A review about linking scales in stream ecology suggested that better understanding of ecological scales is needed where localised actions can lead to large-scale benefits and thus efficient management strategies (Lowe et al., 2006).

A wide variety of factors is responsible for shaping diatom community structure and responses and these vary among different regions worldwide and in Australia. A review on environmental and spatial control of diatoms in freshwaters highlighted regional stratification of diatoms and suggested considering spatial factors in bioassessment programs (Soininen, 2007). Another study found that regional scale diatom community structure was mainly affected by factors (e.g. agricultural, water quality and alkalinity gradients) that are different to watershed scales (e.g. local factor of acid mine drainage) (Smucker et al., 2011). A study in south-east Australia suggested that geographic position plays an important role in diatoms composition at subregional level (Philibert et al., 2006). Contrary to this, another broad-scale study from south-east Australian rivers indicated there was no significant relationship between geographical positions on diatoms species index (Chessman et al., 2007). By contrast, a diatom species index study at finer scale was strongly correlated with catchment urbanisation in Melbourne suburbs

(Chessman et al., 2007; Sonneman et al., 2001). Other studies have highlighted significant differences in diatom assemblages due to variations in water quality factors, particularly pH and salinity (Negus et al., 2019; Tsoi et al., 2017). While multiple ecological stressors affect diatoms community structure in different regions, their impacts are usually studied within the set objectives of regional domains, and variation among or within different spatial scales has been generally ignored.

This study aimed to highlight threshold variations at multiple spatial scales by evaluating change in diatom community responses within, and among, regions. The specific research questions were: 1. What are the most important water quality variables affecting diatom assemblages at different spatial scales? 2. Is there any variation in water quality thresholds among different spatial scales and regions of South Australia? 3. Is there any distinct variability in threshold indicator species among different spatial scales and regions responsible for different thresholds? 4. What are the appropriate scales to derive water quality thresholds? Answer to these questions can help to articulate spatial scale influences on threshold variations needed to derive suitable thresholds for different locations.

4.2 Material and Methods

4.2.1 Data Set and Study Area

For the assessment of local scale thresholds, sampling was extended, from previous monitoring at broad state scale, for another two years for four selected streams. For evaluating threshold variations within regions, we increased spatial resolution from state to regional, subregional and local stream-level. However, to cover data limitations associated with the highest spatial resolution at local scale, sampling was extended. Sampling protocol was consistent with the previous study design using rope as an artificial substrate to sample diatoms in both autumn and spring of 2017–2019. Diatoms and water quality data were collected from four perennial flowing streams with similar geographical positions. The four streams are tributaries of the River Torrens and Onkaparinga River catchments in WMLR (Figure 1b). First Creek and Sixth Creek were nearly pristine in a forested catchment; however, Aldgate and Cox Creek have agricultural land cover sites with nutrient enrichment [(Shrestha et al., 2017) and (Appendix B)]. For diatom sampling, lengths of identical polypropylene rope (100–200 cm) with 5cm frayed ends were deployed at each site for a period of over six weeks in both seasons. Ropes were collected after six weeks and the frayed ends were cut and preserved to process in the laboratory. Rope samples were treated with 25% hydrogen peroxide to remove organic material and then washed with distilled water in the laboratory. The diatom slurry was mounted on slides using Naphrax mounting medium. A total of 300 diatom valves were counted on each slide using a microscope (Carl Zeiss Axio Scope.A1), at a magnification of 1000X. Diatoms were identified to the lowest taxonomic level using reference keys (Krammer, 1986, 1988, 1991; Sonneman et al., 2000).

Physicochemical parameters i.e. pH, electrical conductivity, dissolved oxygen and water temperature were measured at each site where ropes were deployed using a multi-sensor data logger (YSI 6820). For nutrients, i.e. total phosphorus (TP), total nitrogen (TN) and nitrates, water samples were collected from same sites and further analysed in the laboratory using standard American Public and Health Association methods (APHA, 2005a).

For the assessment of regional variation in diatom thresholds, diatoms and water quality data was used from a previous study designed to evaluate diatom thresholds in South Australian streams, providing a broad spatial coverage and range of environmental gradients (Tibby et al., 2019). Samples were collected in autumn and spring during the years 2015–2016. The spatial scales used for regional comparisons in this study comprise three Natural Resource Management (NRM) regions of South Australia: The Adelaide and Mount Lofty Ranges (AMLR), South East (SE) and Eyre Peninsula (EP) (Figure 1). These NRM regions have diverse environments and have different water resource management plans. AMLR comprises of over 5,500 km^2 of surface water catchments and over 10,000 km of waterways. The largest river catchments in this region are Torrens, Onkaparinga, Gawler, South Para, North Para and Inman. Surface water streams in this region are perennial to intermittent mainly following seasonal flows in response to rainfall. The Western Mount Lofty Region (WMLR) is wetter than Eastern Mount Lofty Region (EMLR). The South East Natural Resource Management Region, hereafter termed South East (SE), covers an area of 28, 000 km^2 and has been categorised as a biodiversity transition zone due to variable climates ranging from temperate in southeastern to arid in central parts (Chowdhury et al., 2015). Average rainfall varies considerably from approximately 850 mm in the south to 450 mm in the north (www.natural resources.sa.gov.au). The Eyre Peninsula region covers a significant area of South Australia (55,000 km^2). Mean annual rainfall varies from 250 mm in the north and north-west to more than 500 mm in the south. The relative isolation of EP allows high endemic species biodiversity to develop (www.natural resources.sa.gov.au).

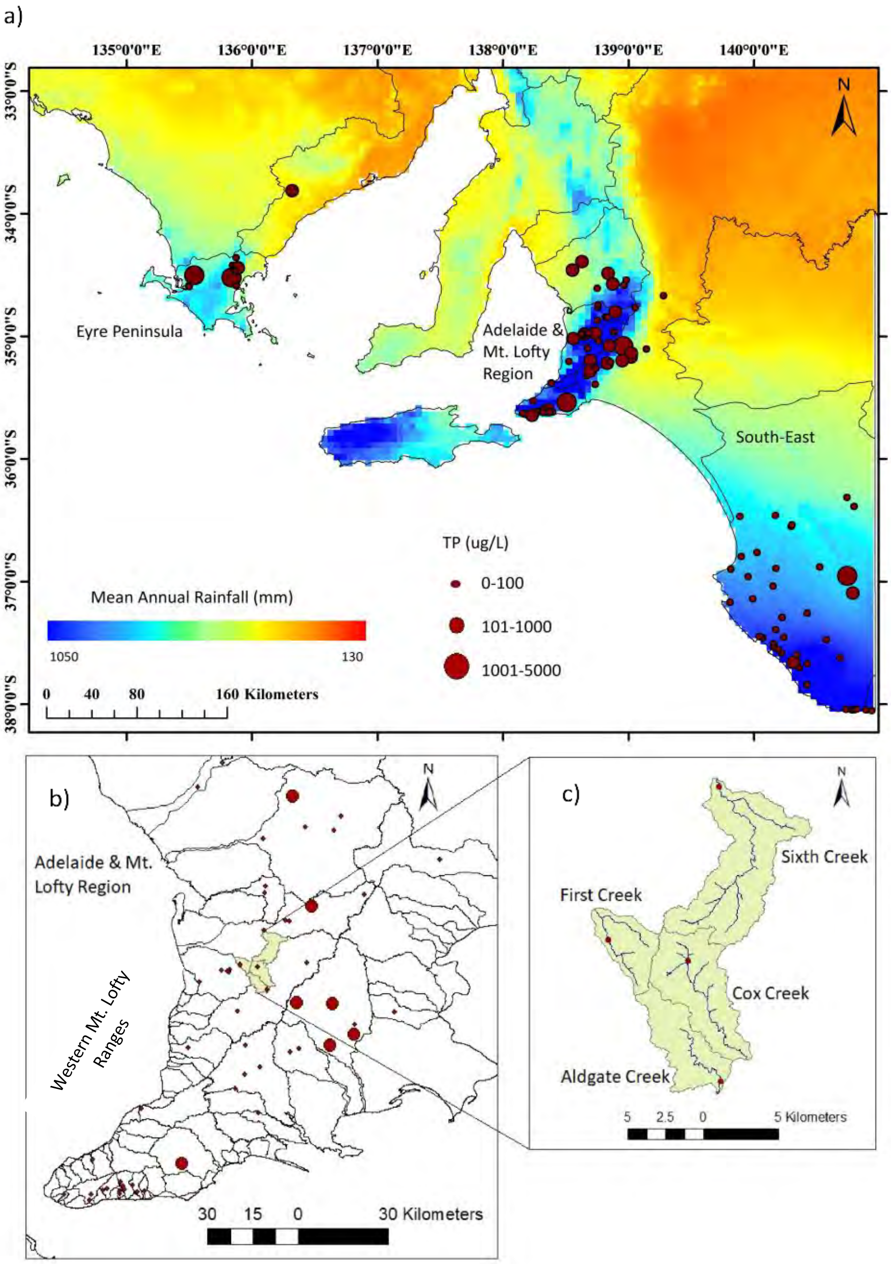


Figure 1: Sampling locations of diatom and water quality monitoring in South Australian streams with measured total phosphorus (TP) and rainfall variability: a) across South Australian streams; b). across Adelaide and Mt. Lofty Ranges region and; c) across four sites within the western Mt. Lofty Ranges region (WMLR).

4.2.2 Data Analysis

Gradient Forest (GF) and Nonmetric Multidimensional Scaling (NMDS) were used to detect the most important water quality variables affecting diatom assemblages. Gradient Forest (Ellis et al., 2012) is an extension of random forest method (Breiman, 2001) which has been used to assess predictor importance and change in abundance trends for univariate response variables (e.g. Lee et al., 2019). GF is a machine learning approach that consider responses from multiple species across multiple stressors. Random forest is an ensemble of regression trees, whereas in GF separate random forests are grown for each species by partitioning the observations based on split function. By aggregating information from regression trees based on split function and a bootstrap procedure, it calculates the predictor importance. GF was implemented using R package "gradientForest". To meet the statistical requirements, diatom relative abundance data were arcsine square root transformed. NMDS was used to assess the community composition based on environmental variables using Bray-Curtis distance measure in PRIMER (v6.01).

Threshold Indicator Taxa Analysis (TITAN) was then used for thresholds analysis of the identified most important stressors within and between regions of South Australia. We also identified the most abundant threshold indicator taxa using TITAN. TITAN distinguishes taxa responding positively or negatively to the specific predictor variable in terms of z scores (Baker and King, 2010). Change points or thresholds, used interchangeably throughout, are detected when there is a synchronous change in the abundance of a number of taxa lying within a narrow range of the predictor variable. This method is based on IndVal (indicator values from species indicator analysis) (Dufrêne and Legendre, 1997) and incorporates a bootstrap procedure to find taxon responses that are pure and reliable. TITAN was applied using 10,000 bootstrap replicates in the R package "TITAN2" (Baker and King, 2010; R Development Core Team, 2018). Taxa with occurrences <3 were not considered following Baker and King's (2010) recommended minimum criterion for TITAN.

4.3 Results

Summary statistics from the data collected from four streams at the local scale and the broader data set categorised into different regions and subregions are shown in Table 1. In this study, EC and TP concentrations varied markedly among different spatial scales and between regions. The highest mean EC and TP concentrations were found in the South East region, however when spatial resolution increased from state to local scale, mean EC and TP concentrations showed relatively low nutrient enrichment at studied local scale streams (Table 1). Due to the low number of samples from the Eyre Peninsula and the presence of large outliers, Eyre Peninsula data were not considered for further regional comparisons.

Table 1: Summary statistics of water quality variables collected from four streams at local scale in comparison to broad scale data at state scale, regional scales (Adelaide and Mount Lofty Ranges: AMLR; and South East:SE) and subregional (Western Mount Lofty Ranges:WMLR).

	***State scale (n= 178)**				**Regional scale: AMLR (n=101)**				**Subregional scale: WMLR (n=81)**				**Local scale: (n=28)**				**Regional scale: SE (n= 60)**			
Water quality variables	**Min**	**Max**	**Mean**	**Median**	**Min**	**Max**	**Mean**	**Median**	**Min**	**Max**	**Mean**	**Median**	**Min**	**Max**	**Mean**	**Median**	**Min**	**Max**	**Mean**	**Median**
TotalN (mg/L)	0.12	6.96	1.39	1.003	0	3.7	1.0	0.8	0.0	3.7	1.0	0.7	0.1	5.5	1.0	1	0.154	6.96	2.0	1.49
TotalP (µg/L)	8	3817	110	41	8	1010	99.9	49	8	1010	100.7	47	0.005	326	69.5	32	10	3817	137.7	27.5
Temperature (°C)	7.8	27.7	14.46	12.7	7.8	21.7	13.2	12.2	7.8	21.7	12.9	12.1	9.5	21.7	14.2	13	8.85	27.7	15.9	14.42
Conductivity (µS/cm)	150	66641	3797	1242	150	11913	1719.1	750.5	150	11913	1485.3	569.5	150.0	753	434.8	435	361	20590	3056.3	1436
Dissolved Oxygen (mg/L)	2	17.6	8.16	8.3	2.04	10.87	7.6	8.165	2.04	10.87	8.0	8.56	2.0	11.0	7.4	8	2.39	17.55	9.1	9.25
pH	3.58	9.37	7.94	7.94	6.85	8.67	7.8	7.77	6.99	8.67	7.8	7.775	6.7	8.4	7.8	8	7.13	9.37	8.3	8.26

*state scale data source: Tibby et al., 2019

3.1 Identification of important water quality stressors

EC and TP were identified as the most important water quality variables (R^2>0.02) in driving the response of diatom assemblages in South Australian streams (Figure 3). These findings were consistent with the canonical correspondence analysis from the previous study (Tibby et al., 2019). Comparison of local scale data to the broad scale identified the same variables as most important. However, slight variation in relative importance of EC and TP (R^2 >= 0.04) was observed at local scale (Figure given as supporting information, Appendix 1). NMDS clustering also identified conductivity and TP as most important factors affecting diatom assemblages in the study area (Figure given as supporting information, Appendix 2).

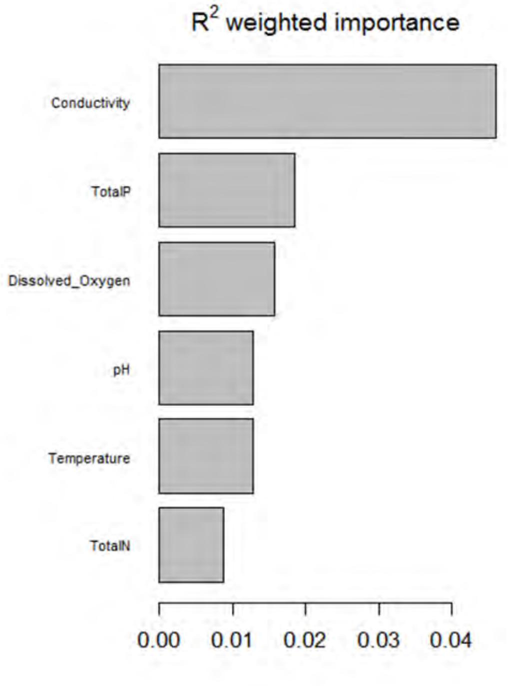


Figure 3: Gradient forest results showing the relative importance of water quality variables affecting the response of diatoms community in South Australian streams at state scale.

4.3.1 Threshold variations within and between regions

A variation in thresholds was observed across the spatial scales. Threshold variations among different spatial scales within the regions indicated either lower or consistent change points when the spatial scale was increased from state to local-scale. For example, EC exhibited similar sum z- change points at the broader state scale (change point= 271 µS/cm) and within Adelaide and Mt. Lofty region (change point= 269 µS/cm for AMLR; change point= 271 µS/cm for WMLR) (Table 2). However, no change points were observed at the local scale. In contrast, sum z- change points for TP at state scale (change point= 29 µg/L) were twice those at regional scale (AMLR) (change point= 15 µg/L), remained same at subregional scale and varied slightly at local scale (change point = 12 µg/L) (Table 2). Change points for sum z (+) taxa vary between scales both for EC and TP, indicating the tolerant behaviour of these species (Table 2). However, when regions were compared, the South East region had the highest change points (TP change point= 37 µg/L and EC= 1180 µS/cm, for sum z (-) taxa). Overall, a relatively broad range of change points was observed for sum z (+) as compared to sum z (-) taxa, indicating a gradual increase of tolerant taxa along the EC and TP gradient (Table 2).

Table 2: TP and EC thresholds among hierarchical spatial scales and between regions of South Australia. Sum (z-) and sum (z+) represent negative and positive responses. Values in parenthesis are 5th and 95th percentiles of 10,000 bootstrap replicates. AMLR = Adelaide and Mount Lofty Ranges region. WMLR = western Mount Lofty Ranges

Sampling scale	**TP (µg/L)**		**EC (µS/cm)**	
Within region	**f sum z-**	**f sum z+**	**f sum z-**	**f sum z+**
State scale*	29 (12,31)	175 (43.5, 271)	276 (219, 1176)	1314 (1287, 6660)
Regional scale: AMLR	15 (11, 27)	131 (66, 189.5)	269 (219, 543)	1341 (1046, 2366)
Subregional scale: WMLR	15 (11, 26)	123.5 (92, 189.5)	276 (219, 477)	2864 (764, 2864)
Local scale: 4 streams from WMLR	12 (12, 22.5)	38.5 (14.5, 43.5)	_	_
Between regions				
Adelaide and Mt. Lofty Region (AMLR)	15 (11, 27)	131 (66, 189.5)	269 (219, 543)	1341 (1046, 2366)
South-East (SE)	37 (27, 50.5)	42 (36, 247.5)	1179.5 (1113, 1544.5)	4968 (1205, 7025.5)

4.3.2 Variations of threshold indicator species within and among regions

Analysis of the five most abundant species at different spatial resolutions within the region has shown a similar suite of species (Figure 4). At state scale, the five most abundant taxa were *Achnanthidium minutissimum* (16%), *Cocconeis placentula* (14%), *Planothidium lanceolatum* (5%), *Tabularia fasciculata* (5%) and *Planothidium delicatulum* (5%) (Tibby et al., 2019). At regional and subregional scales (i.e. AMLR and WMLR), *Rhoicosphenia abbreviata* was more abundant than *Tabularia fasciculata*. In AMLR, the most abundant taxa were *Cocconeis placentula* (14%), *Achnanthidium minutissimum* (12%), *Planothidium lanceolatum* (8%), *Planothidium delicatum* (7%) *and Rhoicosphenia abbreviata* (7%) which were similar to observations in WMLR i.e. *Cocconeis placentula* (15%), *Achnanthidium* *minutissimum* (13%), *Planothidium lanceolatum* (9%), *Rhoicosphenia abbreviata* (7%) and *Planothidium delicatulum* (5%). At the local scale, *Achanthidium oblongella* (16%) was more abundant than *Planothidium delicatum (<5%)*, while the remaining four most abundant species were similar i.e. *Achnanthidium minutissimum* (21%), *Cocconeis placentula* (13%), *Planothidium lanceolatum (7%) and Rhoicosphenia abbreviata (5%).*

However, in the South East, the most abundant species were somewhat different i.e. *Achnanthidium minutissimum (28%), Cocconeis placentula* (13%), *Fragilaria capucina* (4%), *Tabularia fasciculata* (4%) and *Staurosirella pinnata* (3%) (Figure 4).

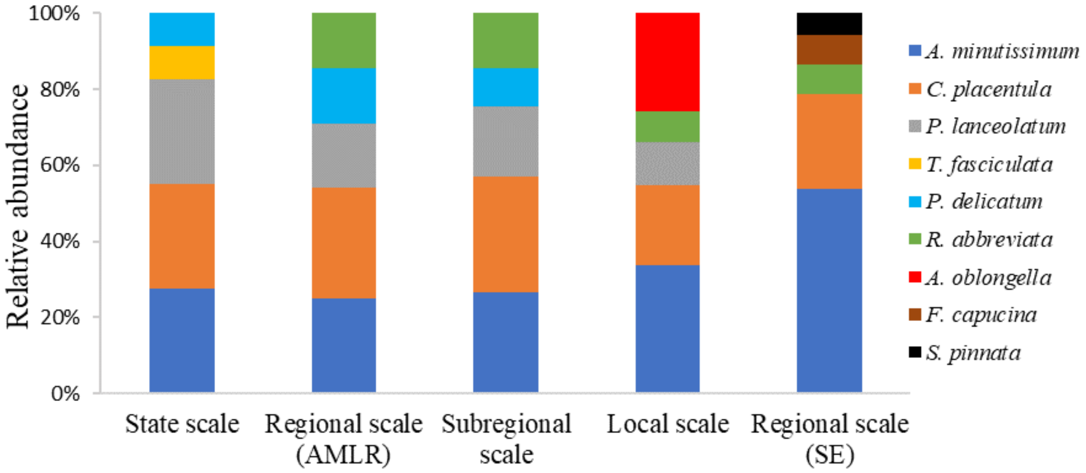
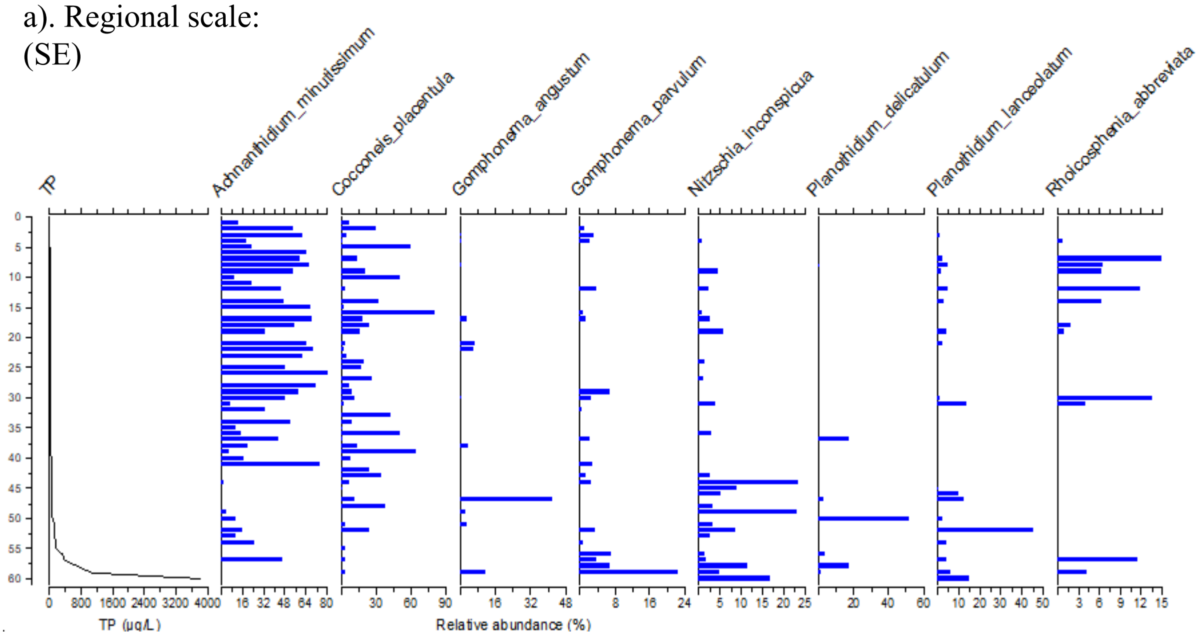


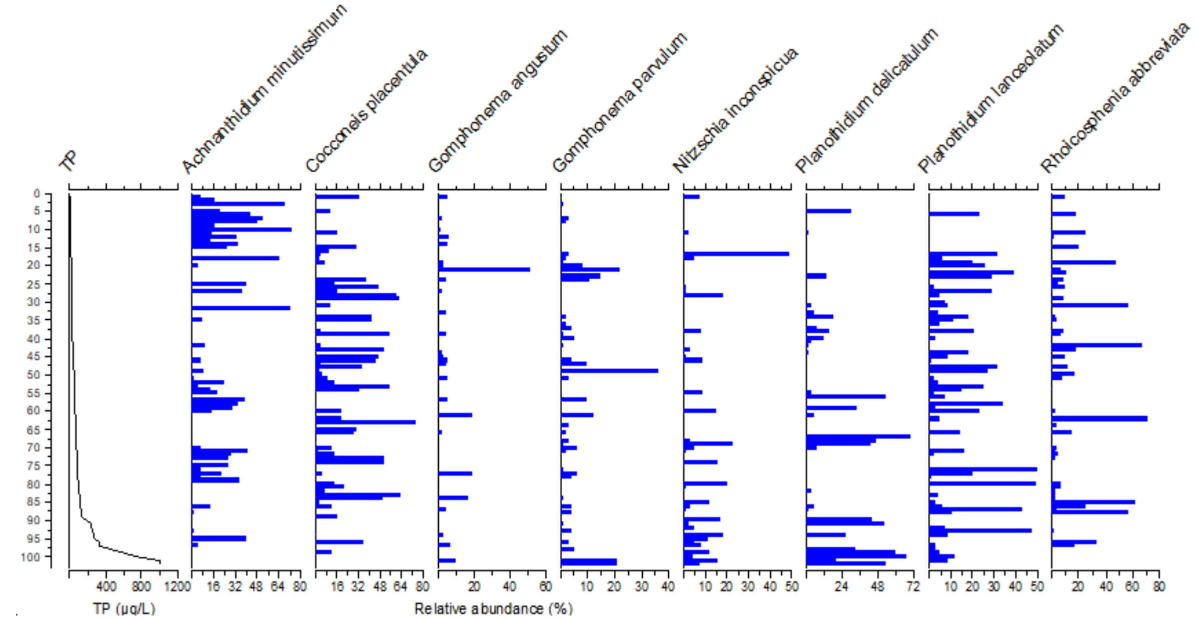
Figure 4: Relative abundance of five most abundant species observed across different spatial scales (i.e. state, regional, subregional and local) and between regions (i.e. Adelaide and Mount Lofty Ranges: AMLR and South East: SE).

Among all spatial scales and regions, *A.minutissimum* decreased while *N. inconspicua* increased with increasing TP concentration (Figure 5). These findings were in concordance with TITAN identified z (-) and z (+) taxa (given as supporting information, Appendix 3). A similar trend of these two species was observed along the EC gradient (Figure given as supporting information, Appendix 4). Other indicator taxa with high abundance like *Cocconeis placentula*, *Gomphonema parvulum, Planothidium lanceolatum* and *Rhoicosphenia abbreviata* were ubiquitous at almost all spatial scales. However, *Gomphonema angustum*, and *Planothidium delicatum*, were found at a only few sites in the SE region as compared to AMLR (Figure 5).

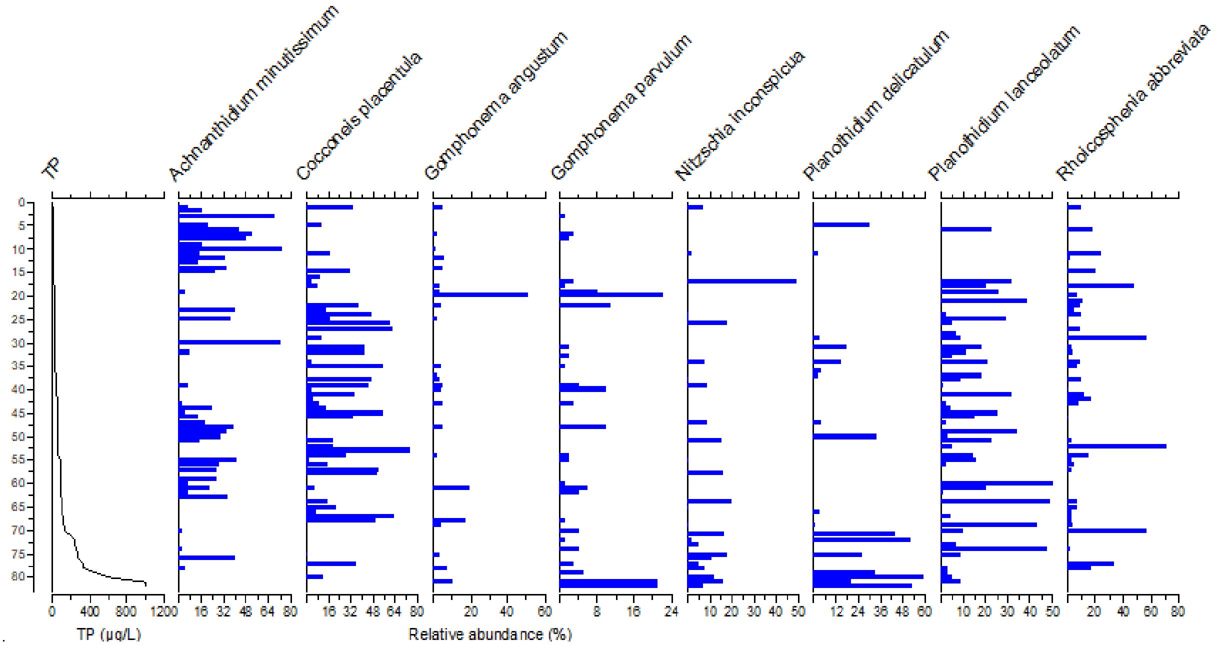
a). Regional scale:
(SE)



b). Regional scale:
(AMLR)



c). Subregional scale:
(WMLR)



d). Local scale

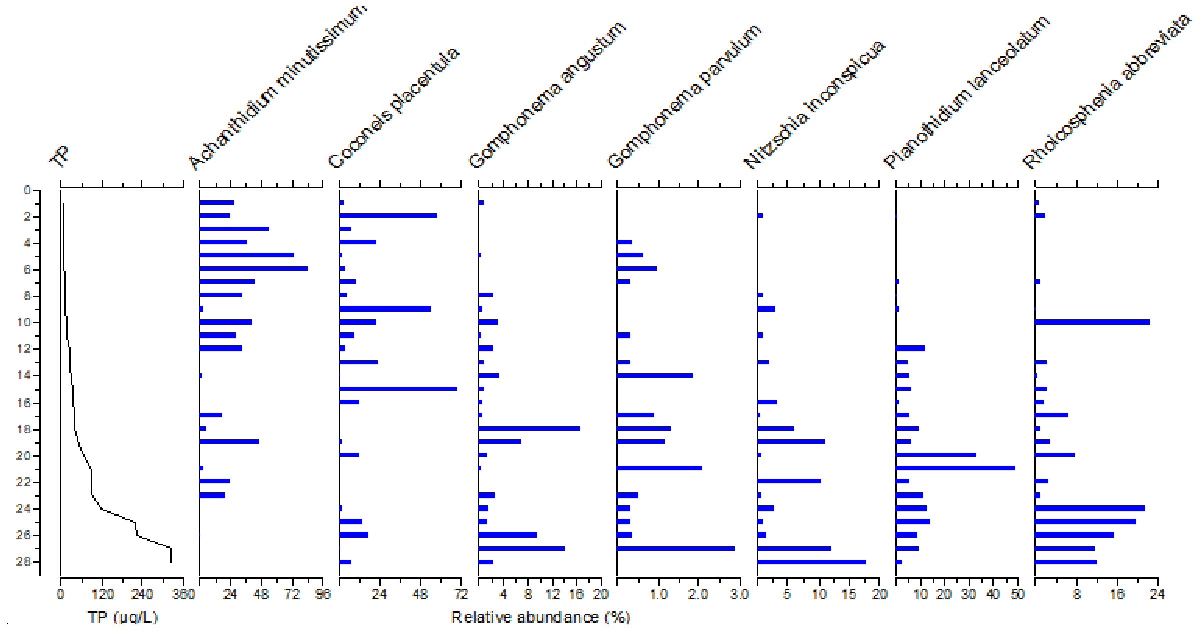


Figure 5: Abundance trends of most abundant threshold indicator species along total-phosphorus (TP) gradient among spatial scales and between regions of South Australian streams; a) regional scale of South East region; b) regional scale of Adelaide & Mt. Lofty Region (AMLR); c) subregional scale within AMLR region i.e Western Mt. Lofty Region (WMLR) and; d) local scale, i.e. four streams within the WMLR.

4.4 Discussion

Ecological thresholds have been widely used to develop water quality guidelines in different contexts worldwide. Identification of appropriate spatial scales to apply thresholds is vital to establishing and applying meaningful water quality guidelines. This study aimed to quantify water quality thresholds using diatoms at multiple spatial scales by increasing the spatial resolution from state to local scale. We also compared thresholds between two regions and evaluated the spatial variation in water quality variables and threshold indicator taxa. EC and TP were identified as the most important water quality stressors affecting diatom assemblages of South Australian streams at all spatial scales, irrespective of regional differences. Thus, threshold variations for EC and TP evaluated in this study can help to understand the contribution of differences at multiple spatial scales to overall thresholds. Salinity and TP have been recognised as important drivers affecting diatom assemblages worldwide and in Australia (Haynes et al., 2011; Pan et al., 1996; Reid et al., 1995; Tibby, 2004). Due to this, many studies focus on the use of diatoms as indicators of salinity and TP for managing river health (Taylor et al., 2014; Tibby et al., 2019).

We identified varying thresholds among different spatial scales and between regions of South Australian streams. Threshold variations among different spatial scales revealed overlapping change points, with either similar or lower thresholds at local scale than state scale. For EC, similar thresholds were observed when spatial resolution was increased from state to subregional scale. Thresholds were not found at the local scale. It may be because these particular streams may not have reached the threshold levels for EC, or more samples are required to fully understand EC thresholds at this level. The streams studied at the local scale had an EC of 150–753 μS/cm, compared to the overall EC range of 150–66641 μS/cm, further suggesting this may be the case. The TP thresholds observed at the state scale were almost two times higher than the local scale. However, consistent TP thresholds were observed at regional (AMLR) and subregional (WMLR) scales. These findings correspond to comparatively low nutrient enrichment observed at local scale. TP thresholds for sum z (-) taxa identified among different spatial scales are comparable to another study from streams of US with low TP nutrient enrichment, where TP limits of 18 μg/L to 48 μg/L were found using TITAN (Taylor et al., 2014). In another study on broad geographical area, Taylor et al. (2018) found a change in diatom assemblages at >25 μg/L.

The overlapping relationship of thresholds found in our study regions can be related to the concept of hierarchical landscapes proposed by Frissell et al. (1986), where spatial scales are interlinked from large to small spatial scales. Other factors driving consistent thresholds among spatial scales include similar climate and rainfall patterns. However, different thresholds observed at the local scale can be due to heterogeneity of site-specific factors which do not exist at the regional scale (Liang et al., 2020). Differential land use patterns can be the major factor affecting site specific heterogeneity in these regions. In this study, the South East (SE) region which had higher mean TP and EC concentrations, had correspondingly higher thresholds as compared to the AMLR region and South Australia as a whole (state scale). In another study (Olson and Hawkins, 2013) with an aim to develop site-specific criteria also found that site-specific thresholds for TP were higher than specific ecoregion across streams of western USA. In the context of our work, the default national water quality guideline values of 100–5000 µS/cm for EC and 100 µg/L for TP (ANZECC, 2000) were not appropriate for all spatial scales. Similarly, The Murray Darling Basin Plan, a world leading environmental management program (Hart, 2016), which governs the most extensive river system in Australia, sets out water quality thresholds for various types of systems including rivers, lakes and marine environments. The Plan aims to restore ecological health of the highly degraded Murray-Darling River system. But the lack of local-scale thresholds, such as those developed in this study, is likely to be an obstacle to full environmental protection. Thresholds for streams and rivers follow national default guidelines for TP but with amendments for EC (Basin Plan, 2012). Our findings suggest that these values are still too high for protection of sensitive (z-) taxa and are very unlikely to be appropriate for all sites within the basin. This study indicates that state scale thresholds are likely to mask more local scale variation and may therefore not be appropriate for application at finer spatial scales.

Over the past two decades, there is a growing awareness of the need for more local scale water quality guidelines. The United States Environmental Protection Authority (USEPA, 1994) considered the applicability of site-specific guidelines across large spatial scales, i.e. state, region or catchment in case where generic guidelines are inappropriate for water resource management (USEPA, 1994). In Canada, Australia and New Zealand, the use of site-specific guidelines is recommended over national generic guidelines (ANZG, 2018; CCME, 2003). Van Dam et al. (2014) illustrated different approaches to derive site-specific guidelines. Recently revised water quality guidelines in Australia and New Zealand highlighted the need to derive more local scale thresholds based on local conditions (ANZG, 2018). However, there has been insufficient guidance in deriving these guidelines. In light of this, guidance for deriving site-specific guidelines was reviewed, and a conceptual framework was proposed for selecting different approaches in deriving site-specific guidelines (Van Dam et al., 2019). A distinction was made between site-adapted thresholds (where an existing water quality criteria is modified) vs. site-specific thresholds (thresholds derived on basis of site-specific data), and terms like national, regional, catchment, subcatchment, local thresholds based on spatial scales were proposed (Van Dam et al., 2019). This evolution of water quality guidelines from national to site-specific highlights the importance of different types of water quality thresholds, i.e. national, regional, catchment or local, based on spatial factors.

Distribution of threshold indicator taxa among different spatial scales follow a similar pattern as the thresholds, showing an overlap within regions. Similar suites of indicator species were generally observed at regional, subregional and local scale. However, a distinct suite of threshold indicator species found in South East (SE) were driving higher thresholds in this region. Among the threshold indicator species, *A. minutissimum* responded negatively while *N.inconspicua* responded positively, in relation to TP and EC at different spatial scales in all regions. *A. minutissimum* and *N. inconspicua* have been found in streams with TP concentrations within the range of 20 µg/L to 200 µg/L and 50 µg/L to 1000 µg/L, respectively (Sonneman et al., 2000). Distinct threshold indicator taxa observed between regions can be related to the thresholds of habitat abundance concept, which affects organism's ability to move and locate suitable habitats for establishment (Groffman et al., 2006). However, in this study, two species found common have been identified as good indicators for salinity and TP enrichment in another study on state scale for South Australian streams (Tibby et al., 2019), which confirmed their usefulness as indicators, irrespective of spatial scale.

This study suggests that broad-scale thresholds are unlikely to be applicable at local scales. However, with the limitation of this study based on local scale sampling from one region, we recommend a hierarchical monitoring design by replicating each spatial scale in other regions. We also recommend derivation of local scale water quality thresholds based on local conditions, and their use at broader spatial scale as long this provides an appropriate level of protection. For deriving local scale water quality thresholds, statistical change points using methods like TITAN can be used (Sultana et al., 2019) in combination with other lines of evidence to provide appropriate thresholds for management.

4.5 Conclusions

This study highlighted the need to investigate water quality thresholds at multiple spatial scales. It has provided insights for identification and setting of meaningful thresholds at a level required for management. Revised freshwater quality guidelines in Australia and New Zealand, and around the world highlight the need to derive site-specific thresholds, and studies like this can help eliminate spatial dependence in deriving and applying these thresholds. It can be concluded that:

- TP and EC were major water quality drivers affecting diatom responses at multiple spatial scales.
- State scale thresholds are often not appropriate at the local scale. Overall, local scale thresholds were lower than the state scale thresholds. However, higher TP and EC thresholds were observed for the South East region compared to the AMLR due to the higher TP and EC concentrations in SE region streams.
- An overlapping trend in thresholds was found among hierarchical spatial scales whereby thresholds increased as the spatial scale increased. Thus we recommend to derive thresholds at a local scale and extend their applicability to broader scale as far it provides an appropriate level of protection.
- The threshold indicator taxa were considerably different between regions. However, *Nitzschia inconspicua* responded positively while *Achanthidium minutissimum* responded negatively in relation to TP and EC at all the spatial scales and in both regions. Thus, these taxa can be used by managers to assess salinity and nutrient enrichment of South Australian streams, irrespective of spatial scales.

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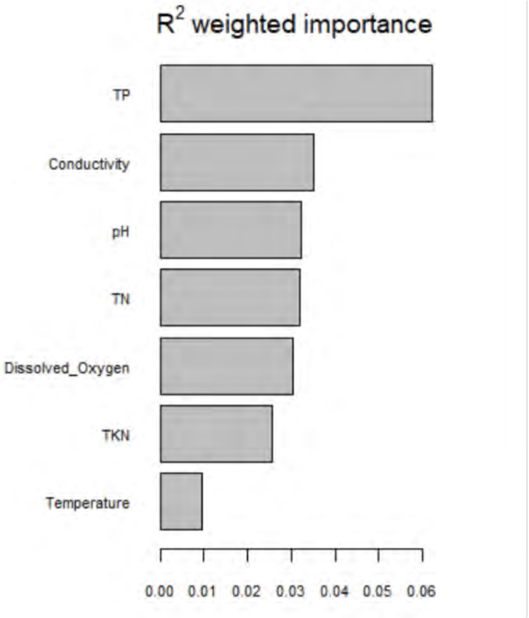
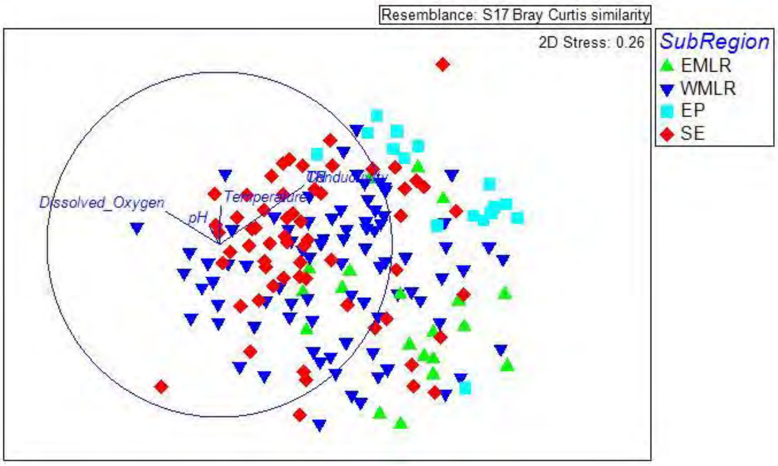
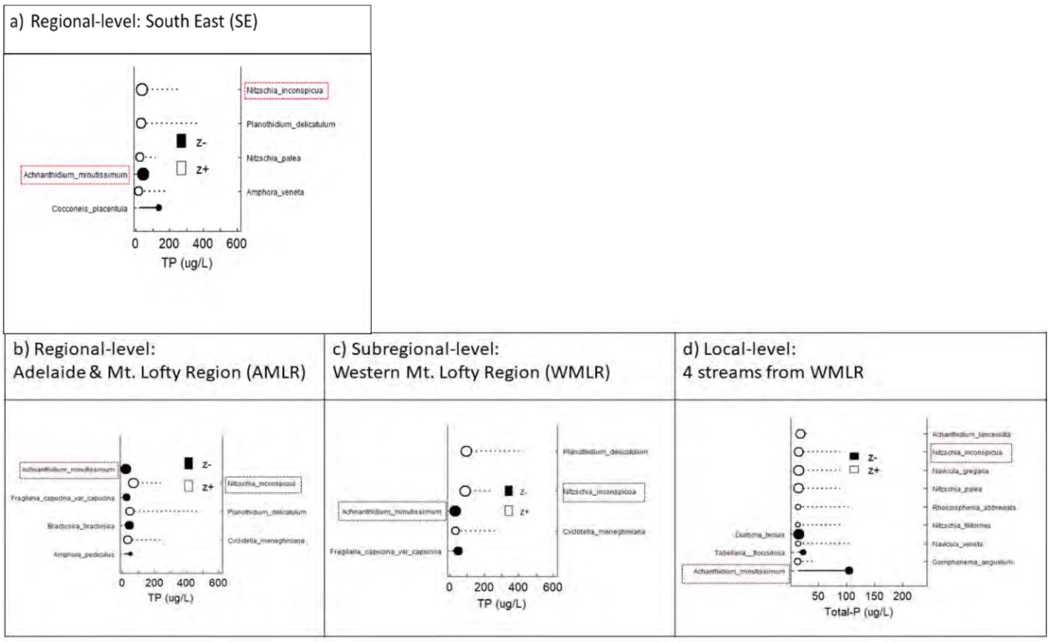
Supporting Information (Chapter 4)



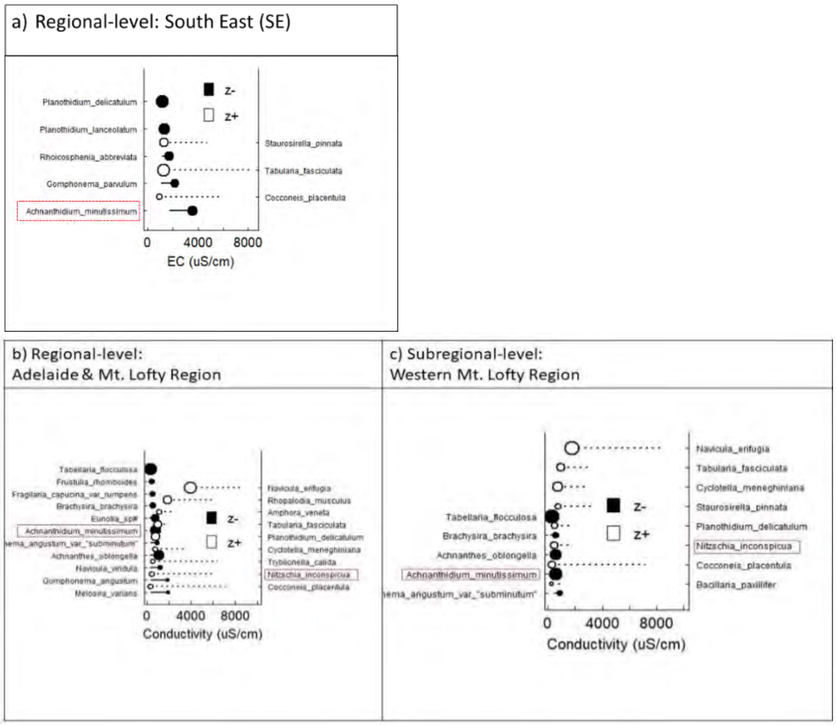
Appendix 1: Gradient forest results showing relative importance of water quality variables affecting response of diatoms community in South Australian streams at local scale.



Appendix 2: NMDS of diatoms community from South Australian streams including Eastern Mount Lofty Region (EMLR) and Western Mt. Lofty Region (WMLR), Eyre Peninsula (EP) and South East (SE). Relative abundance was arcsine square root transformed.

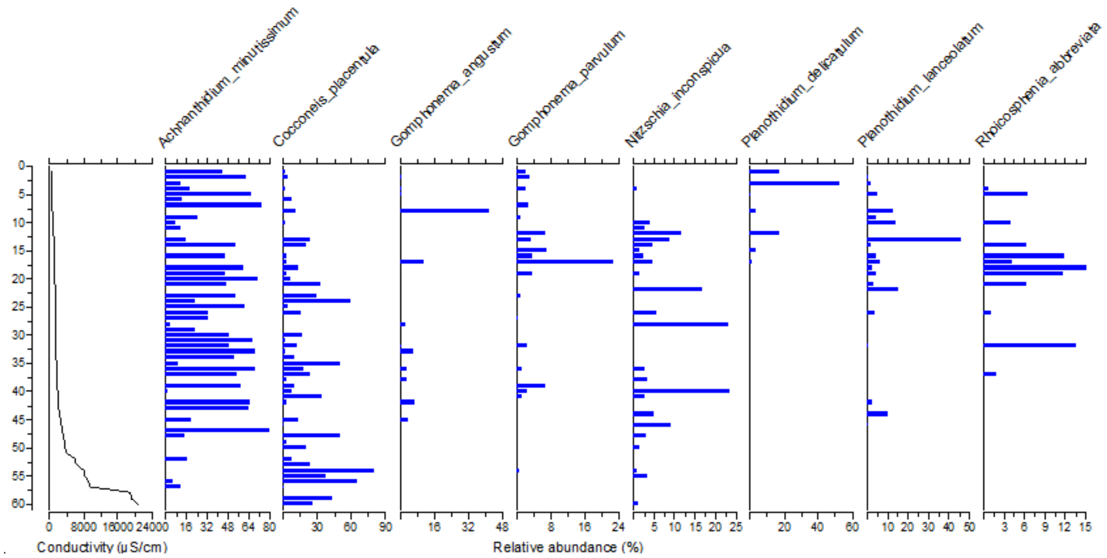


Appendix 3a: Significant indicator taxa change points based on TITAN output along total phosphorus (TP) gradient.

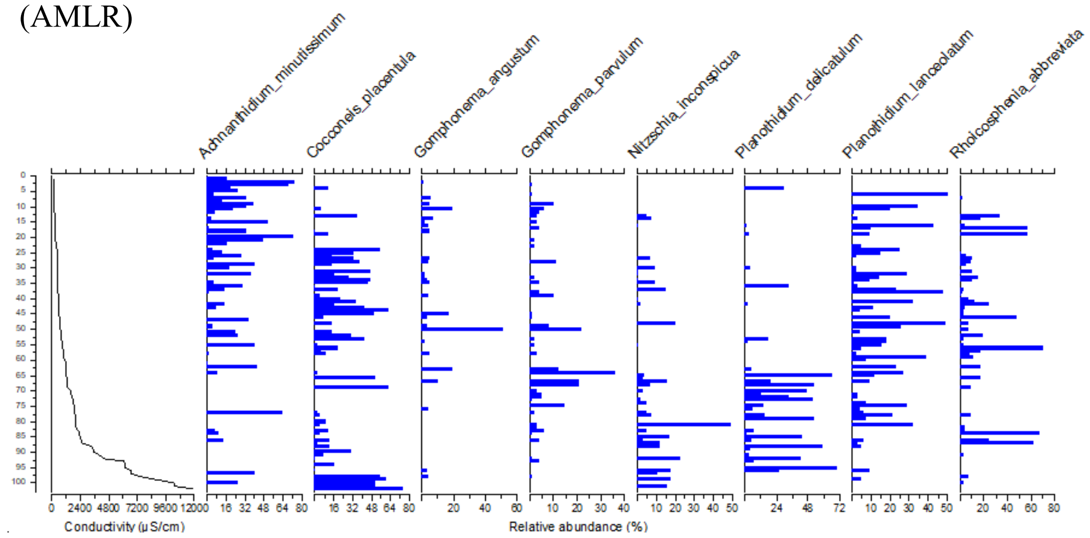


Appendix 3b: Significant indicator taxa change points based on TITAN output along electrical conductivity (EC) gradient.

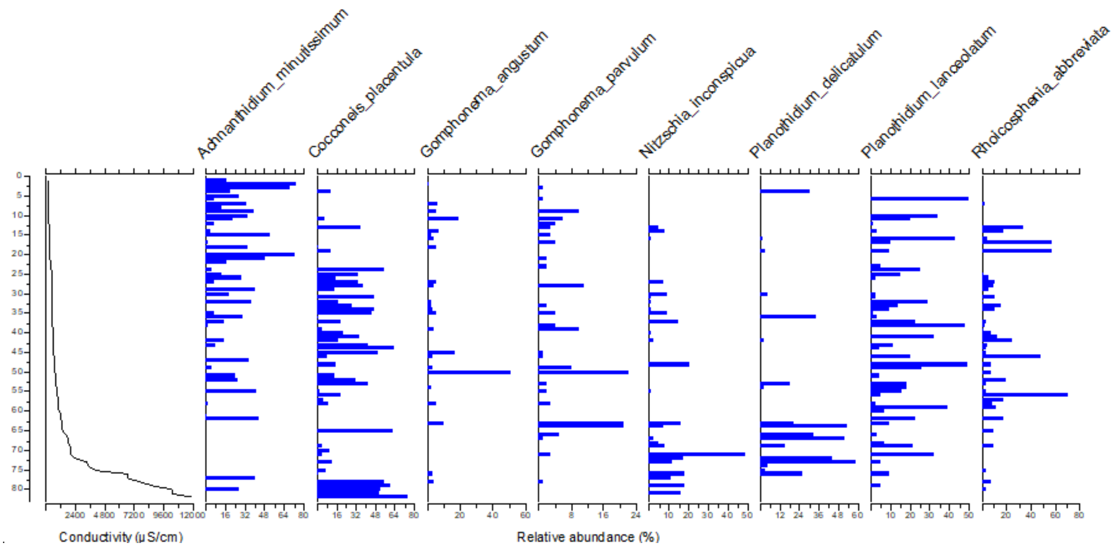
a). Regional scale: (SE)



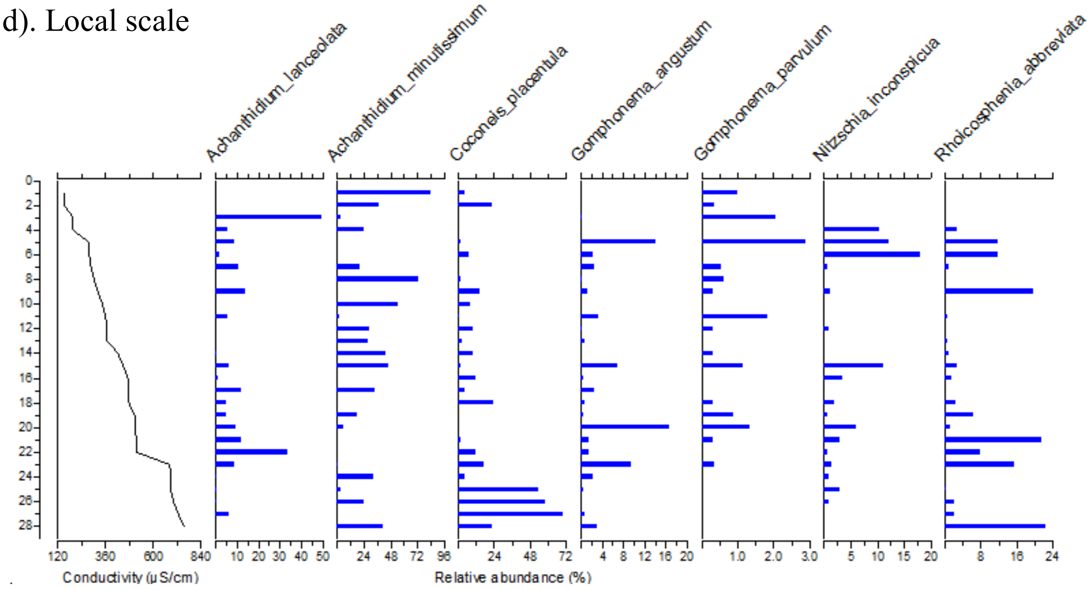
b). Regional scale:
(AMLR)



c). Subregional scale (WMLR)



d). Local scale



Appendix 4: Abundance trends of most abundant threshold indicator species along electrical conductivity (EC) gradient among spatial scales and between regions of South Australian streams; a) regional scale of South East region; b) regional scale of Adelaide & Mt. Lofty Region (AMLR); c) subregional scale within AMLR region i.e Western Mt. Lofty Region (WMLR) and; d) local scale, i.e. four streams within the WMLR.

Chapter 5:

Species-specific macroinvertebrate responses to climate and land use scenarios in a Mediterranean catchment revealed by an integrated modelling approach

Statement of Authorship

Title of Paper	Species-specific macroinvertebrate responses to climate and land use scenarios in a Mediterranean catchment revealed by an integrated modelling approach	
Publication Status	☑ Published ☐ Submitted for Publication	☐ Accepted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Sultana, J., Recknagel, F. and Nguyen, H. (2020). Species-specific macroinvertebrate responses to climate and land use scenarios in a Mediterranean catchment revealed by an integrated modelling approach.	

Principal Author

Name of Principal Author (Candidate)	Jawairia Sultana		
Contribution to the Paper	Formal analysis, SWAT scenario simulations, results preparation, writing, original draft preparation. Acted as the first and corresponding author. I hereby certify that statement of the contribution is accurate.		
Overall percentage (%)	80%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	31-07-2020

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Friedrich Recknagel		
Contribution to the Paper	Conceptualisation, resources, supervision, critical reviewing I hereby certify that statement of the contribution is accurate.		
Signature		Date	31/7/20

Name of Co-Author	Hong Hanh Nguyen		
Contribution to the Paper	Model calibration and reviewing I hereby certify that statement of the contribution is accurate.		
Signature		Date	17/08/2020

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Species-specific macroinvertebrate responses to climate and land use scenarios in a Mediterranean catchment revealed by an integrated modelling approach

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Climate change
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Gradient forest
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ABSTRACT

Climate and land use changes are altering flow and nutrient regimes in catchments that affect stream habitats and aquatic biodiversity. This study applied an integrated modelling approach to quantify the impacts of climate and land use scenarios on the macroinvertebrate community in Sixth Creek catchment, South Australia. Gradient forest (GF) determined flow as most important driver affecting macroinvertebrate assemblages, and identified most flow-sensitive species based on highest cumulative importance along flow gradients. A catchment model developed using the Soil and Water Assessment Tool (SWAT) simulated flow under the following scenarios: (1) deforestation, (2) reforestation, (3) climate change, and (4) a 10% increased urbanisation as projected by local authorities over the next 30 years. The urbanisation scenario predicted a 2% increase of average flow compared to 17% flow increase predicted by the deforestation scenario. In contrast, diminished monthly flow was suggested by both the climate change scenarios due to declining precipitation and higher temperatures, and the reforestation scenario most likely due to declining surface run-off. In reforestation and climate change scenarios, SWAT results predicted the highest decline in flow during late autumn and late spring, which may shift this unusual permanent flowing stream towards intermittency. The Hybrid Evolutionary Algorithm (HEA) was used to develop flow-driven models for population dynamics of taxa identified by GF as flow sensitive i.e. *Hydrobiidae* spp., *Austrocerca tasmanica, Offadens* spp., *Cloeon* spp., *Micronecta* spp. and *Tasmanocoenis tillyardi* based on 14 years of data monitored at a representative stream site of Sixth Creek. The HEA models achieved coefficients of determination r^2 between 0.85 and 0.97 for predicted vs. observed abundances. While the abundance of *Cloeon* spp. diminished in all scenarios, it was most affected by low flow predictions of the climate change scenario. *Hydrobiidae* spp. appeared most tolerant to altered flow conditions by reaching highest abundances at high flow conditions caused by deforestation and urbanisation. Overall, this study highlighted the non-linear response of species within the commonly used *Ephemeroptera, Plecoptera* and *Trichoptera* (EPT) grouping and a need to redirect focus of such studies from community to species level. The integrated modelling approach based on GF, SWAT and HEA proved to be suitable for simulating complex species-specific macroinvertebrate responses to relevant climate and land use scenarios. Learning more about these interrelationships is a prerequisite for sustainable catchment management.

1. Introduction

Worldwide, freshwater ecosystems are exposed to multiple stressors that adversely affect stream biota (Ormerod et al., 2010). Species diversity is declining due to climate change, urbanisation, agricultural activities and other stressors affecting the water resources. These changes alter the physical and chemical characteristics of streams resulting in loss of biodiversity (Dudgeon et al., 2006; Vörösmarty et al., 2010). Altered flow regimes have been widely recognised as major impact on river ecosystems (Dewson et al., 2007; Domisch et al., 2017). There is a large number of catchment models that allow prediction of altered stream flow and nutrient loads resulting from land use and climate scenarios (e.g. (Nguyen et al., 2018; Shrestha et al., 2017; Westra et al., 2014). Moreover, a number of studies quantified the response of stream biota to modelled flow (Hough et al., 2019; Kakouei et al., 2018; Kakouei et al., 2017; Pyne and Poff, 2017). While these

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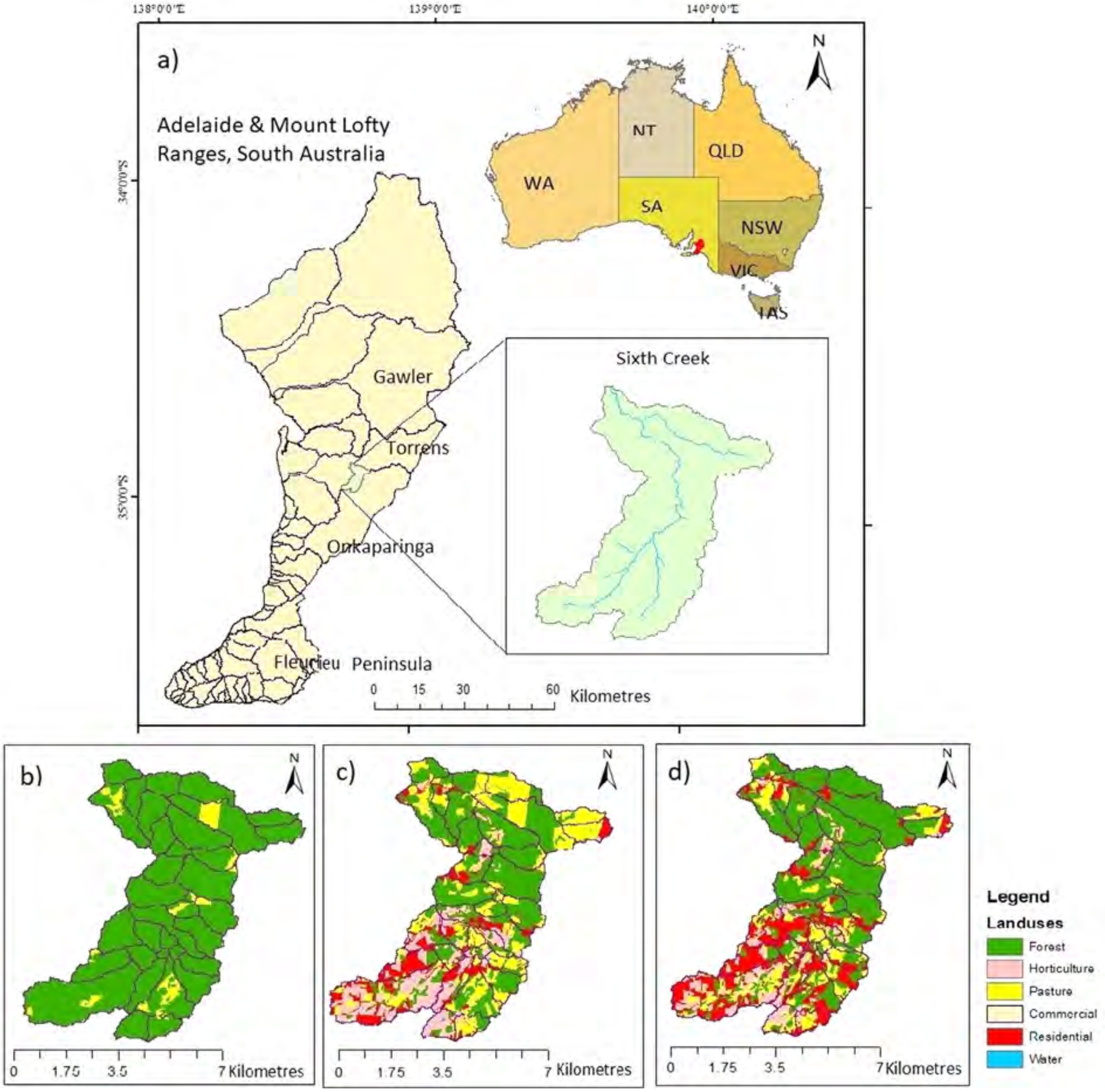

Fig. 1. a) Location of Sixth Creek catchment in South Australia. Land use maps of: b) pre-European; c) 2007 and; d) 2016.

studies focused on benthic macroinvertebrates at community or assemblage level, there is an obvious gap of knowledge regarding species-specific responses of macroinvertebrates to changing stream flow.

There is evidence that climate and land use change in combination will synergistically contribute to biodiversity loss at species, genetic or habitat level (Mantyka-Pringle et al., 2014; Nelson et al., 2009), but little is known whether biodiversity is shaped stronger by combined or indidual effects.

Benthic macroinvertebrates are successfully used as bioindicators of river health due to their sensitivity to a range of environmental stressors, ubiquitous distribution and relatively long life cycle (Cairns and Pratt, 1993; Chessman, 1995). Thus, short- and long-term monitoring programs for river health management regularly utilise macroinvertebrate analysis (Cortes et al., 2013). However, making complex catchment management decisions based on data generated by such monitoring efforts is still challenging. River health monitoring programs may find it difficult to obtain consistent trends from the data due to necessary changes of monitoring objectives from time to time (Goonan et al., 2012). Furthermore, to comprehend the complexity of ecosystems affected by multiple stressors may not be possible using a single model. To overcome such limitations, the analysis and synthesis of bioindication data using a combination of models appears to be a promising approach for forecasting the status of future river health.

Combination of models are increasingly applied to evaluate impacts of climate change induced flow alterations on macroinvertebrate communities (Kakouei et al., 2018). A Bayesian Belief Network has been used to identify a potential decline in macroinvertebrates and fish richness in catchments of south-east Queensland, Australia, as a result of changing land use and climate conditions (Mantyka-Pringle et al., 2014). In northern Germany, Guse et al. (2015) revealed impacts of simulated climate and land use changes on fish and macroinvertebrate communities by cascading eco-hydrological models where the output from one model was used as an input to another. Woznicki et al. (2016) combined the Soil and Water Assessment Tool (SWAT) with a Bayesian variable selection method to assess impacts of climate change on bioindicators of stream health at community level, in watershed of Michigan, USA. Segurado et al. (2018) applied the model SWAT together with an empirical model to address effects of land use, nutrient enrichment and water scarcity on fish, macroinvertebrates, macrophytes and phytobenthos in the Mediterranean basin of Central

Table 1

Summary statistics of the data from Sixth Creek monitored from1994 to2007.

Variables	Unit/description	Min	Max	Mean	Median
Water quality variables					
Flow	Average seasonal discharge (m^3/s)	0.033	0.742	0.144	0.075
Turbidity	NTU	0.3	11.2	2.1	1.5
pH	pH units	8.2	9.3	8.5	8.4
WT	Water temperature (°C)	9.2	22.4	14.9	15.1
EC	Electrical conductivity (μS/cm)	442	743	636	670
TotalP	Total phosphorus (mg/L)	0.005	0.040	0.016	0.016
TotalN	Total nitrogen (mg/L)	0.160	1.516	0.386	0.237
Macroinvertebrate community					
abundance		4993	47,216	17,406	14,775
diversity		1.027	3.210	2.493	2.763
richness		37	81	66	68

Portugal. Kakouei et al. (2018) projected potential impacts of changing flow patterns on macroinvertebrate assemblages of two catchments in Germany, by quantifying their preferences to a range of flow conditions based on hierarchical logistic regression and the IHA (indicators of hydrological alterations) metrics (Olden and Poff, 2003). One shortcoming of this approach is that it predicted increasing abundances of macroinvertebrate assemblages mainly consisting of 'generalists' that benefit from flow alterations (Kakouei et al., 2018) while stream conservation is primarily concernd about flow-sensitive 'specialists'. In addition, this integrated modelling approach is still at an early stage of development. Some studies considered biological responses at broader community level and used univariate community metrics like EPT taxa (Li et al., 2018; Nukazawa et al., 2018; Stefanidis et al., 2018), others have explored impacts of single drivers either land use change or climate change affecting the stream biota (e.g (Krynak and Yates, 2018; Mustonen et al., 2018; Woznicki et al., 2016). Overall, the above mentioned studies have in common that they do not allow to assess species-specific responses of site-specific macroinvertebrate species to altered flow projections affected by multiple drivers.

This study exemplifies an integrated modelling approach utilising stream-specific hydrological and biological data for predicting abundances of macroinvertebrate species for relevant future scenarios for Sixth Creek catchment in South Australia. Sixth Creek has been chosen for this case study because of its unusual permanent flows in a Mediterranean setting of the driest state in Australia. Other reasons include the availability of both long-term hydrological and biological data, and being a habitat of variety of sensitive, rare and flow-dependent macroinvertebrate species (www.epa.sa.gov.au/reports_water). This study aimed to test the feasibility of the proposed integrated modelling approach for quantitative assessment of the response behaviour of macroinvertebrate species to altered flow conditions. We addressed following research questions: 1. Is flow the key driver of macroinvertebrate assemblages in the Sixth Creek catchment? 2. What are the most flow-sensitive macroinvertebrate species in the Sixth Creek? 3. How is flow affected by possible future climate and land use? 4. How might flow-sensitive macroinvertebrate species respond to future climate and land use? Answering these questions may inform sustainable river management by providing insights to possible future impacts on macroinvertebrate species.

2. Material and methods

2.1. Study area

The study area comprises of Sixth Creek catchment (Fig. 1) that covers an area of 4300 ha east of Adelaide the capital of South Australia. The climate of the region is Mediterranean with extreme hot and dry summers and cool winters (Peel et al., 2007). Sixth Creek was selected as a case study because of its importance for water supply, long-term records of daily flows, inhabited by variety of sensitive, rare and flow-dependent macroinvertebrate species, and rated as good in terms of ecological status by South Australian Environmental Protection Authority (www.epa.sa.gov.au/reports_water/). Sixth Creek is characterised by high flows in spring and low flows in autumn. Three land use maps of Sixth Creek from pre-European settlement (Source: Dynamic Land Cover Database DLDC), 2007 and 2016 (Source: South Australian Water Corporation SA Water and Department of Environment and Water DEW) were used to extract land use data shown in Fig. 1. Catchment land use is dominated by stock grazing (52%) and native vegetation (16%). The Sixth Creek catchment is exposed to emerging pressures e.g. by invasive weeds in the riparian zone, non-point nutrient sources and declining flow due to water extraction and climate changes (Nguyen et al., 2017; Shrestha et al., 2016; Tibby et al., 2010).

2.2. Data sources

Benthic macroinvertebrate species and water quality data were provided by South Australian monitoring, evaluation and reporting program that was established in 1994 to assess the river health of South Australian waterways. Macroinvertebrates and water samples were collected from pool and riffle mesohabitats at Sixth Creek from same location every year in autumn and spring from 1994 to 2007 using a variant of the AusRivAS method (Simpson and Norris, 2000) and are summarised in Table 1. Macroinvertebrates were collected and preserved in the field using 10 m sweep nets. A 10% subsample was sorted in laboratory and identified to the species level resulting in 330 identified taxa. Flow data was obtained from the gauging station operated at the stream site where macroinvertebrate samples were collected, and has been downloaded from www.waterconnect.sa.gov.au.

2.3. Modelling approach

The integrated modelling approach used is outlined in Fig. 2. Firstly, we applied gradient forest (GF) (Ellis et al., 2012) to determine important drivers and flow-sensitive macroinvertebrate species. Secondly, we used a catchment model developed by Soil and Water Assessment Tool (SWAT) (Neitsch et al., 2011) to simulate flow alterations for future climate and land use scenarios. Thirdly, we developed flow-dependent models for the flow-sensitive macroinvertebrate species by means of the hybrid evolutionary algorithm (HEA) (Cao et al., 2013) to simulate the macroinvertebrate species responses to future climate and land use scenarios.

Gradient forest (GF) is a novel machine learning technique to identify change in species responses across multiple environmental stressors (Ellis et al., 2012; Roland Pitcher et al., 2012). It is based on regression trees and incorporates a bootstrap procedure to calculate drivers' importance and species responses along gradients. The species response curves allow to identify most responsive species based on R^2-weighted importance. GF finds advantage over other methods in determining the relative importance of environmental gradients affecting macroinvertebrate community, besides identifying change in species responses (Sultana et al., 2020). The R package 'gradientForest' (Ellis et al., 2012; R Development Core Team, 2018) was used to run GF.

SWAT is a modelling tool for river basins that includes mathematical descriptions of physical, hydrochemical and biogeochemical processes, and simulates catchment processes such as surface runoff and infiltration, percolation, evapotranspiration, nutrient and sediment transport (Neitsch et al., 2011). It has been calibrated and validated for Sixth Creek by dividing the catchment of Sixth Creek into 35 sub-basins and 173 Hydrological Response Units (HRUs), which represent its land use, slope and soil characteristics (Nguyen et al., 2019).

HEA allows development of predictive IF-THEN-ELSE-models from time-series data. It incorporates genetic programming (Koza, 1992) to optimise the model structure and differential evolution (Storn and

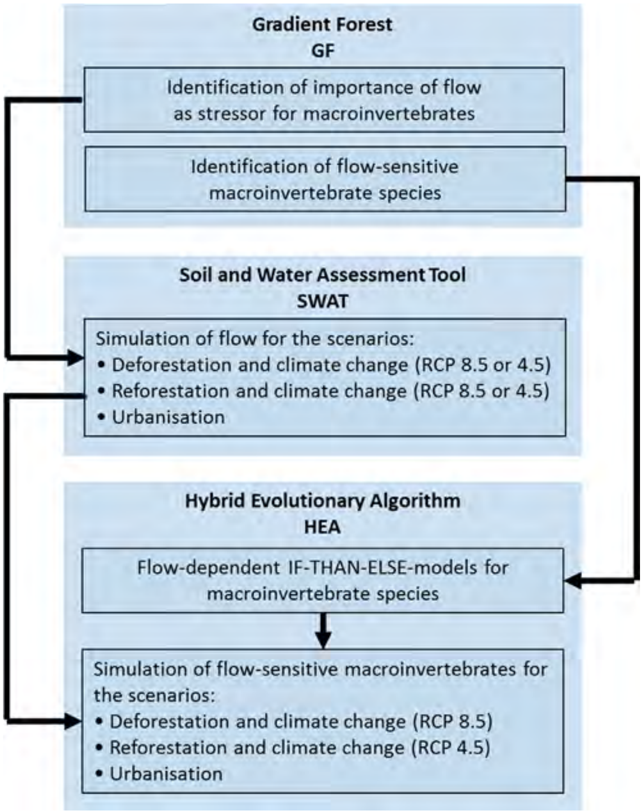

Fig. 2. Integrated modelling approach based on GF, SWAT and HEA for the prediction of macroinvertebrate responses to climate and land use scenarios.

Price, 1997) to optimise model parameters. The C++ version of HEA was applied on a Corvus supercomputer with a cyclic boot-strap scheme of 100 runs to develop flow-dependent models for simulating population dynamics of flow-sensitive macroinvertebrate species identified by GF. The models with $r^2 > 0.7$ were selected to forecast species-specific responses of macroinvertebrates to altered flow resulting from climate and land use scenarios by SWAT.

2.4. Design of the scenario analysis

The calibrated SWAT model was used to simulate scenarios on climate change, land use change and a combination of climate and land use change for 30 years in the future.

Data for future climate scenario were extracted from the Goyder Institute Water Research (GIWR) project developed for SA climate (GIWR, 2015). According to this project, statistical downscaling methods called Nonhomogeneous Hidden Markov Model (NHMM) were used to simulate and calibrate daily rainfall at multiple stations based on global climate models (GCMs). Fifteen Coupled Model Intercomparison Project phase 5 (CMIP5) were selected for downscaling project of South Australia, which were further shortlisted to the six best GCMs. This study used the ensemble of following six GCMs: CanESM2, CNRM-CM5, GFDL-ESM2M, IPSL-CM5B-LR, MIROC5 and MRI-CGCM3. These GCMs were used due to their ability to accurately model the important climate drivers for this region (GIWR, 2015). SWAT climate scenarios require data of five climate variables, namely precipitation, maximum and minimum temperature, solar radiation, and relative humidity. For precipitation, the study used data of the median realisation of 100 realisations from the six GCMs suggested by GIWR (GIWR, 2015). For other variables, data from the same selected realisation of precipitation variable was selected. The study used the Hargreaves method; thus wind speed input was estimated using a weather generator tool embedded in SWAT. The median realisation for climate data inputs was selected due to low uncertainty observed for each GCM projection as compared to a combination of 10th, 50th and 90th realisations (Nguyen et al., 2018). The time period of 30 years i.e. 1976–2005 was used to represent a historic time, while a time scale from 2016 to 2045 was used for scenarios to simulate future climate changes based on Representative Concentration Pathways (RCPs) 4.5 and 8.5, respectively (IPCC, 2013). The climate change scenarios were designed to use climate data under RCPs 4.5 and 8.5 for flow simulations by SWAT, whilst historic climate data from six global climate models was used as a reference.

Table 2
Definitions of scenarios simulated by SWAT and HEA.

Scenarios Description	SWAT	HEA
Climate: RCP 8.5	X	x
Climate: RCP 4.5	x	
[*1]Deforestation: 100% Forest to pasture	x	x
Deforestation: 50% Forest to pasture	x	
Deforestation and Climate: 100% Forest to pasture & RCP 8.5	x	
Deforestation and Climate: 50% Forest to pasture & RCP 8.5	x	
[*2]Reforestation: 100% Pasture to forest	x	x
Reforestation: 50% Pasture to forest	x	
Reforestation and Climate: 100% Forest to pasture & RCP 4.5	x	
Deforestation and Climate: 50% Forest to pasture & RCP 4.5	x	
Urbanisation: 10% Pasture to residential areas	x	x

x represent the scenarios used by each model.

[*1] Deforestation, in context of this study, it refers to vegetation clearance in the Sixth Creek catchment.

[*2] Reforestation, in context of this study, it refers to revegetation in the Sixth Creek catchment.

For land use change scenarios, strong and medium deforestation and reforestation and scenarios were used to evaluate the corresponding changes in flow (Table 2). Data for land use scenarios was guided by trends between: a historic 250 m land cover map of Australia from the Dynamic Land Cover Database (DLDC) of Geoscience Australia (Lymburner et al., 2010) with 95% native forest and 5% grassland before European settlement, a 10 m land use map of 2006 from the South Australian Water Corporation with 46.9% native forest, 23.8% pasture, 16.6% horticulture and 12.5% residential area, and a 10 m land use map of 2016 from the Department of Environment and Water (DEW) with 53.6% native forest, 13.8% pasture land, 10.8% horticulture and 21.5% residential area (Fig. 1). Table 2 summarises scenarios that were simulated by SWAT and HEA according to Fig. 2. Whilst the scenario analysis by HEA used only the extreme climate change scenario (RCP8.5), de- and reforestation by 100% of pasture to analyse the maximum differences on models (Kakouei et al., 2018). It also considered urbanisation, as the most likely case of expanding by 10% over the next 30 years due to inevitable increase in population as reviewed in '30 year Plan for Greater Adelaide report' (DPLG, 2010).

3. Results

3.1. Gradient forest modelling

Gradient forest (GF) identified flow (average seasonal discharge) and electrical conductivity (EC) as most important drivers for the macroinvertebrate community of Sixth Creek (Fig. 3). Species response curves identified 15 macroinvertebrate species that responded strongest to flow gradients (given as supporting information, Appendix 1). Out of these 15 most responsive species, six species with highest abundance in all samples and observed in more than 5 samples were selected (Fig. 4) i.e. the mollusc *Hydrobiidae* spp., the stonefly *Austrocerca tasmanica,* and the mayfly *Offadens* spp. were selected as positively responding, and the mayflies *Tasmanocoenis tillyardi* and *Cloeon* spp. as well as the backswimmer *Micronecta* spp. as negatively responding species. These six macroinvertebrate species were considered for predictive modelling by

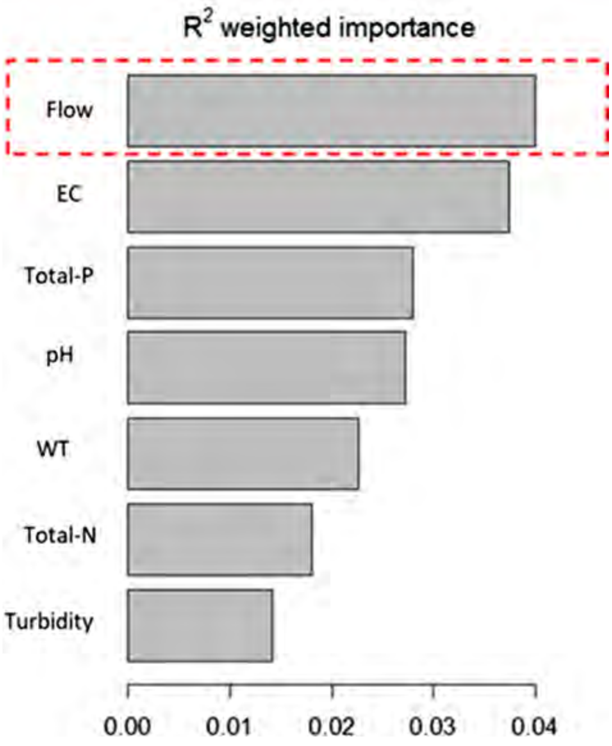

Fig. 3. Ranking of water attributes as stressors for the macroinvertebrate community of Sixth Creek.

HEA.

3.2. SWAT modelling

3.2.1. Validation

The study applied the calibrated parameters of the SWAT by (Nguyen et al. 2019) which were satisfactorily calibrated and validated

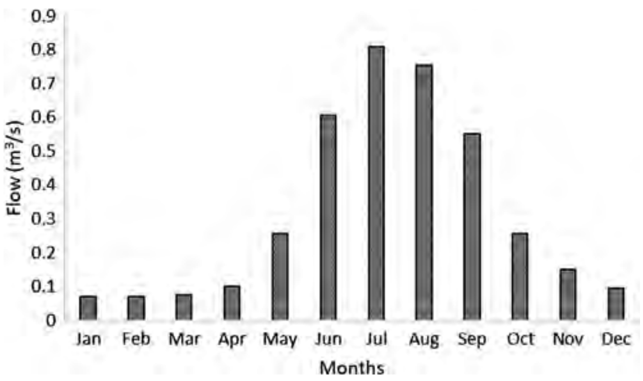

Fig. 5. Average monthly flow, 1981 to 2005 simulated by SWAT.

for the time period from 1997 to 2015 according to criteria defined by Moriasi et al. (2007). In order to extend the scenario analysis by SWAT to the macroinvertebrate community of the Sixth Creek, the daily flow data was further validated by SWAT. The simulation results for 25 years from 1981 to 2005 (Fig. 5) served as baseline for the subsequent scenarios, while the autumn and spring data of the years from 1994 to 2007 (Fig. 5) were important since macroinvertebrates have been sampled during this period. SWAT validation results achieved a very good Nash–Sutcliff Error (NSE), root mean square error (NSE = 0.81, RMSE = 0.07) and a satisfactory percent bias (PBIAS = 24.4) (Fig. 6).

3.2.2. Scenario analysis

The Fig. 7 summarises outcomes of the scenarios defined in Table 2. The simulation of a hypothetical climate change using the RCP 8.5 trajectory (Fig. 7 a, b) resulted in annual average flow decline by 7.2% and at RCP 4.5 in a flow decline by 4.3% in next 30 years time (Fig. 7c, d). When RCP 8.5 was combined with deforestation by 100% the flow increased by 10% (Fig. 7a), and combined with deforestation by 50% the flow increased by 3% (Fig. 7b). Contrarily, a hypothetical reforestation by 100% combined with RCP 4.5 showed a declined flow by 14.5% (Fig. 7c) whilst a reforestation by 50% combined with RCP 4.5

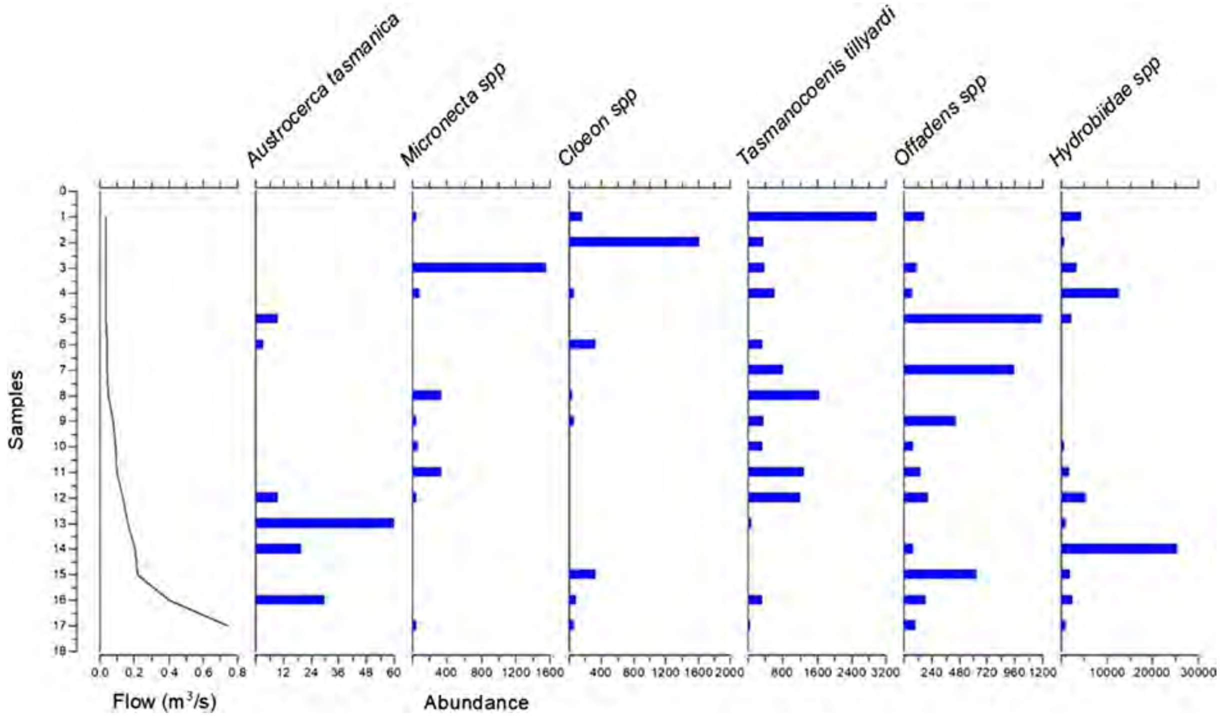

Fig. 4. Abundances of flow-sensitive macroinvertebrate species across the observed flow range of Sixth Creek.

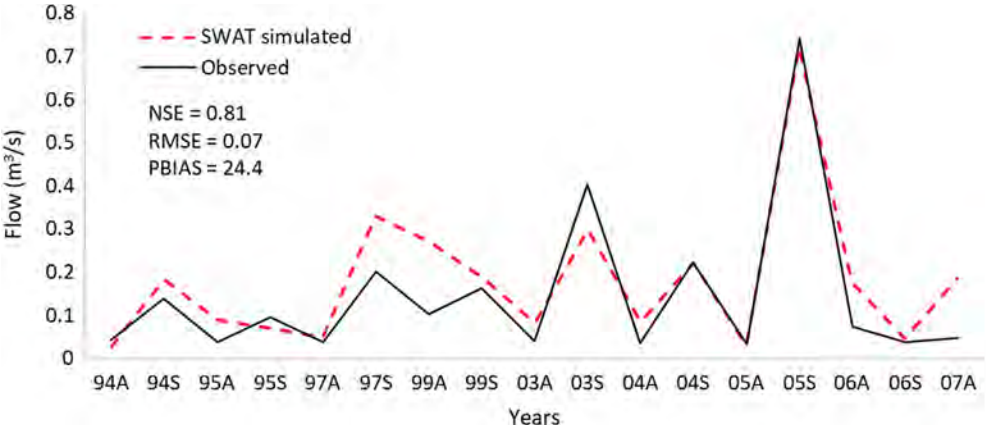

Fig. 6. Average seasonal flow (A = autumn, S = spring), 1994 to 2007 observed vs. simulated by SWAT.

resulted in a decline in flow of 9.6% (Fig. 7d). Fig. 5e indicates that a 10% expansion of residential areas may result in a 2% increase of flow.

As indicated in Table 2, the scenarios 'deforestation by 100% and climate RCP8.5′ (Fig. 7a), 'reforestation 100% and climate RCP4.5′ (Fig. 7c) and 'urbanisation' (Fig. 7e) were used in HEA models for macroinvertebrate. Fig. 8 illustrates average monthly flow resulting from these three scenarios. The scenario 'deforestation by 100% and climate RCP8.5′ predicted highest increases in flow in the autumn month April clearly dominated by hydrological effects of land clearing, while lowest flows were predicted for the winter months August and September (Fig. 8a). The scenario 'reforestation 100% and climate RCP4.5′ suggested strongly declining flow during late autumn in May and during late spring in November as cumulative effect of both processes (Fig. 8b). The scenario 'urbanisation' predicted highest increases in flow during autumn in April and during summer in December (Fig. 8c).

3.3. HEA modelling

The flow-driven IF-THEN-ELSE-models developed by HEA for the six flow-sensitive macroinvertebrate species (Fig. 4) achieved satisfying validation results with coefficients of determination $r^2 > 0.85$. The IF-THEN-ELSE-model for *Tasmanocoenis tillyardi* has been documented in Fig. 9a, and results of its validation and scenario analysis for the years 1994 to 2007 are shown in Fig. 9b. The documentation of the remaining five macroinvertebrate models is provided as Figures A2 to A6 of the appendix. *Tasmanocoenis tillyardi,* known for its preference of calm to slow-flowing waters, declined significantly in abundance during the deforestation scenario associated with swelling flow, but showed stimulation effects by the climate change scenario during the dry autumns in 1995, 1997 and 2004.

Results of the scenario analysis for all six macroinvertebrate species are summarised in Fig. 10. Abundances of *Cloeon* spp., *Tasmanocoenis tillyardi* and *Micronecta* spp. showed overall decreasing trends in relation to urbanisation, deforestation and reforestation. In contrast, *Hydrobiidae* spp. demonstrated increasing abundances to the four scenarios shown in Fig. 10 with the highest increase by 92% during the deforestation scenario. *Hydrobiidae* spp., *Austrocerca tasmanica* and *Offadens* spp. responded to the urbanisation scenario with increased abundances. *Offadens* spp. displayed declining abundances during the climate change and reforestation scenarios which are associated with low flow conditions.

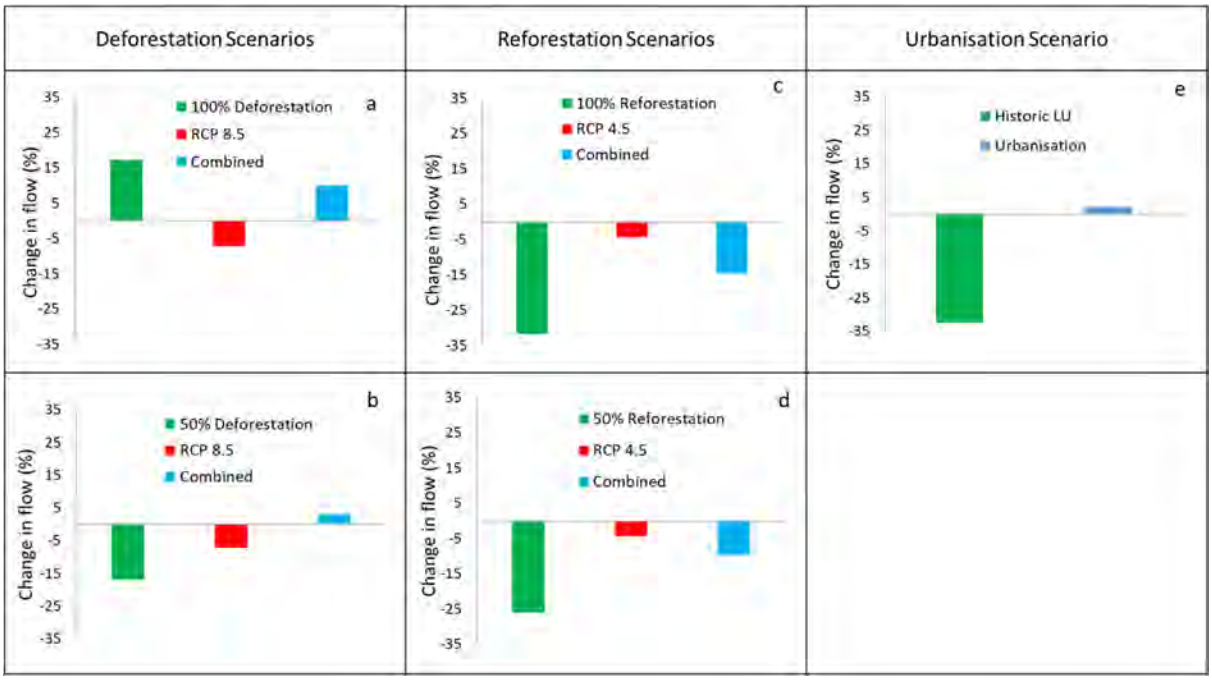

Fig. 7. Change in average annual flow resulting from scenarios of deforestation (a, b), reforestation (c, d) and urbanisation (e).

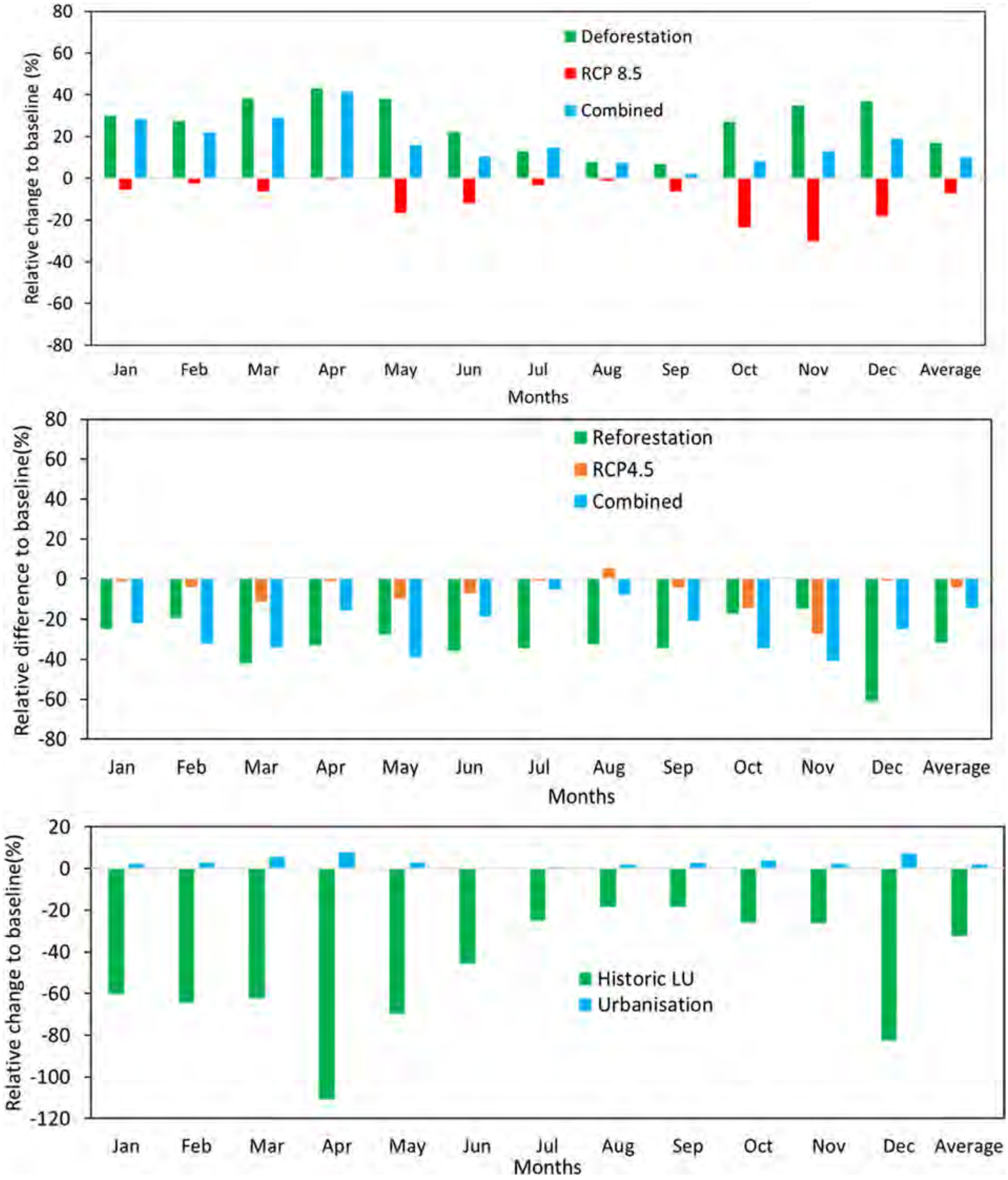

Fig. 8. Change in average monthly flow resulting from scenarios on deforestation (a), reforestation (b) and urbanisation (c).

4. Discussion

An integrated modelling approach has been applied to quantify impacts of hypothetical climate and land use changes on stream macroinvertebrates by scenario analysis.

GF modelling identified flow as most important driver as compared to other water quality variables in this subcatchment. This finding corresponded with other studies such as Lancaster and Hildrew (1993) and (Wills et al., 2006) that suggested fluctuating flows as key driver of the site-specific distribution of macroinvertebrates in streams. Another study on effect of altered flow regimes in northern Australia, suggested that flow permanence, regularity and wet-dry seasonality are among the key hydrological drivers affecting the habitat and species composition in rivers (Leigh and Sheldon, 2008). It has been found that flow and conductivity are most important in driving macroinvertebrate and diatom species responses for perennial flowing streams like Sixth Creek that are less impacted, as compared to the sites with high nutrient enrichment [(Sultana et al., 2020); Sultana et al., unpublished]. Among the flow-sensitive species identified by GF was *Hydrobiidae* spp. that Chessman (2009) characterised as rheo- and thermophilic, as well as *Micronecta* spp. (Gibbins et al., 2000; Smythe-McGuiness et al., 2012) described as being sensitive to minor changes in flow. May- and stoneflies such as *Offadens* spp., *Cloeon* spp., *Tasmanocoeni tillyardi* and *Austrocerca tasmanica* are considered as good indicators of stream

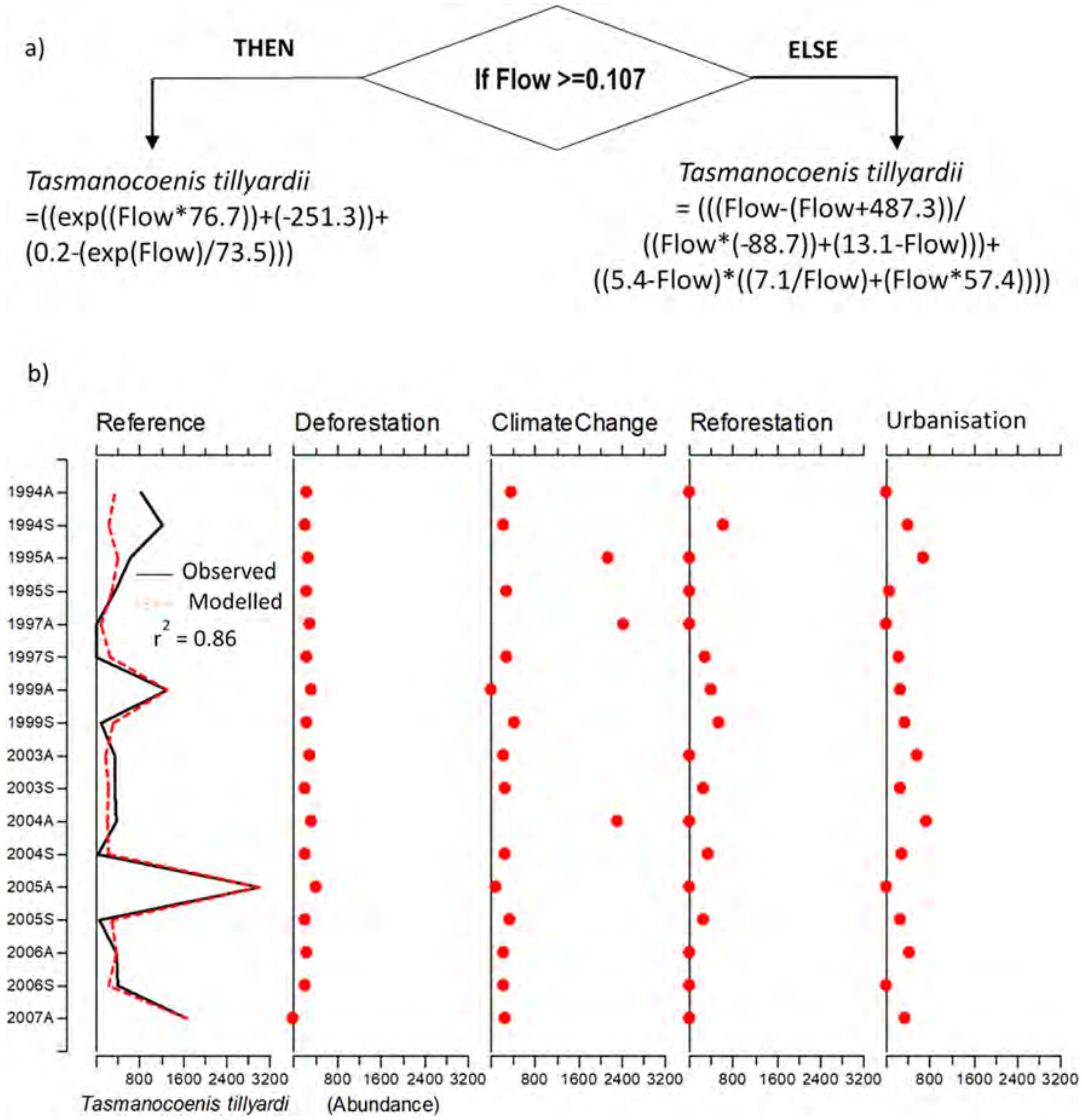

Fig. 9. Flow-dependent model for *Tasmanocoenis tillyardi.* a) IF–THEN–ELSE–model. b) model validation and scenario analysis. Scenarios are listed in Table 2.

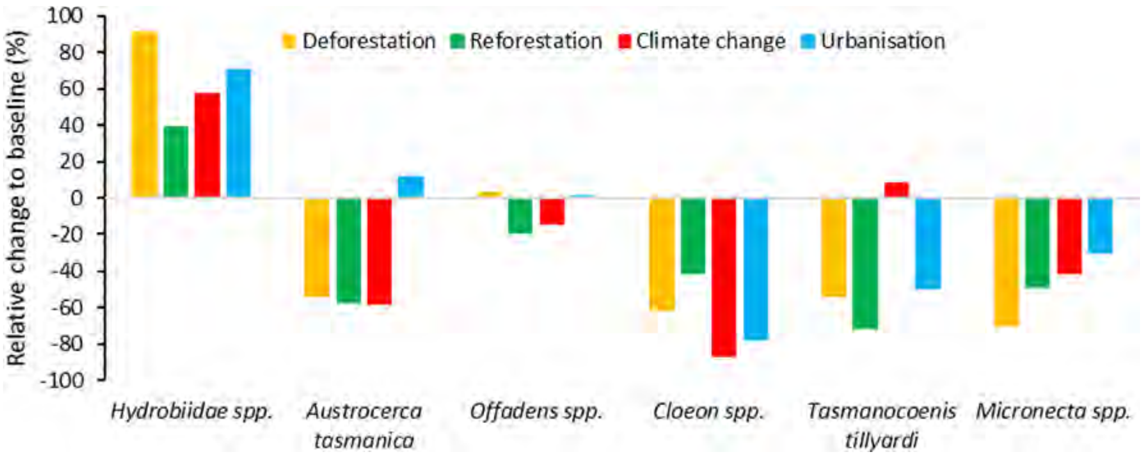

Fig. 10. Relative change in abundances of six macroinvertebrate species predicted by the scenarios deforestation, reforestation, climate change and urbanisation.

health even though their presence in South Australian ephemeral streams is limited (Suter and Bishop, 1990). This study focused on six key indicator species identified by GF to verify the combined modelling approach. Although the limited number of species constrains the possibility to extrapolate to other areas, however, this approach can facilitate the process of key indicator species selection for future predictive modelling. Future work will allow to apply the approach to larger sets of macroinvertebrate species with a broader range of variations in other geographical areas.

The eleven scenarios simulated by SWAT resulted in plausible flow patterns (Fig. 4). The global warming scenarios being associated with increased evapotranspiration by higher water and air temperatures (IPCC, 2013) suggested declining flow and more frequent droughts affecting stream biota in multiple ways. Climate change is likely to affect the frequency and intensity of extreme events like droughts (Trenberth et al., 2015) which threatens the survival of freshwater macroinvertebrates with vulnerable traits including rheophilic species requiring flowing, cool and well aerated water (Chessman, 2015). Opposite flow trends were predicted in response to alternative land use scenarios. Both conversion of forested areas either to pasture or to residential areas resulted in increased flow, whilst reforestation of pasture areas caused declining flow. By simulating gradually extending land use changes in percent terms, we were able to identify thresholds at which flow changed significantly. These thresholds provide crucial information for sustainable land use management. In case of urbanisation, results have shown that a predicted 10% extension of residential areas over the next 30 years may lead to a 7% increase in flow (Fig. 7). Sixth Creek, has unusual permanent flows, with high and low flows during spring and autumn respectively. In reforestation scenario with medium climate change (RCP 4.5), a decline in flow during late autumn and late spring can result in prolonged autumn flow decline in future. This future scenario pose a high risk to trigger this permanent flowing stream to shift towards intermittency. Other studies also highlighted that climate change can impact perennial nature of streams (Chiu et al., 2017) and similar findings were observed for small streams of North America, where it is likely that some perennial streams will shift to intermittent due to warming climate (Reynolds et al., 2015). Moreover, another study on rivers of northern Australia has shown a distinct variability in environmental and macroinvertebrate assemblage attributes of intermittent and regular flowing streams (Leigh et al., 2012).

The species-specific models for macroinvertebrate abundance developed by HEA achieved high coefficients of determination with flow. The mud snail *Hydrobiidae* spp. was predicted to increase in abundance under all changing conditions with highest abundance predicted in deforestation scenario, suggesting that it is relatively insensitive to flow. These findings were similar to another study, whereby *Hydrobiidae* spp. has been identified as tolerant to harsh habitat and climatic conditions in South Australian streams (Corbin and Goonan, 2010). Our results suggested that stonefly *Austrocerca tasmanica* exhibited highest decline in abundance due to low flow scenarios of reforestation and climate change, however, increased flows of urbanisation scenario may lead to slightly increased abundance, showing its preference to medium high flows. Other studies found that the ovoviviparous stonefly *A. tasmanica* occurs in South Australian streams at low to high flow typically during spring, showing its affinity towards wet habitat conditions (Corbin and Goonan, 2010; Towns, 1985). Furthermore, taxa of mayfly *Offadens* spp. showed highest decline in response to decreasing flow predicted by reforestation, however, slightly increased predicted abundance in response to deforestation and urbanisation scenarios show its affinity to high flow conditions. Sixth Creek is characterised by permanent flows and according to SA-EPA, *Offadens* is among the suite of taxa that are pollution sensitive and dependent on the near permanent flows observed at this creek (www.epa.sa.gov.au/reports). Another mayfly, *Cloeon* spp. and water boatman *Micronecta* spp. predicted a decline in all scenarios. Whilst *Cloeon* spp. is known to prefer slow flow conditions (Gibbins et al., 2000; Smythe-McGuiness et al., 2012), our results suggest an apparent insensitivity to flow reflecting that other factors like increased predation pressure due to high abundance of other flow tolerant species may affect their abundance. In contrast, another mayfly *Tasmanocoenis tillyardi* showed a decreasing trend in all scenarios with highest decline in reforestation scenario but a slightly increased abundance in response to climate change, suggest a preference for no to medium flow conditions, in concordance with another study from South Australian streams (Corbin and Goonan, 2010). However, less affinity of *T. tillyardi* to low flows of reforestation as compared to climate change scenario, can be due to more seasonal fluctuations whereby highest decrease in flow observed in spring season. Irrespective of individual species trends, overall responses of these six species to future climate and land use change appeared to be complex and nonlinear. Another study from New South Wales, Australia, at a broad spatial scale highlighted intrafamilial differences based on individual species responses to climate change, in addition to the main finding that suggested a more likely decline of macroinvertebrate families that favour faster-flowing habitats (Chessman, 2009). Similar findings were observed in our study showing that the EPT taxa *Austrocerca tasmanica* and *Offadens* spp. appeared tolerant to altered flow conditions in the urbanisation scenarios, whilst the EPT taxa *Tasmanaocoenis tillyardi* responded sensitive to flow alterations. Thus, a species-specific approach provides better understanding of nonlinear changes in the community structures that are typical of freshwater ecosystems.

The effects of flow alterations on riverine biota are expected to be complex and may not be explained using a single model (Leigh et al., 2012). The integrated modelling approach presented in this study allowed us to identify species-specific relationships of macroinvertebrates with flow in response to changing climate and land use, which cannot be revealed by either relying on one model, or models focusing on traits or assemblages as a univariate variable, such as commonly used EPT taxa.

5. Conclusions

This case study focused on the quantitative assessment of the response behaviour of flow-sensitive macroinvertebrate species to changed flow conditions forecasted for prospective climate and land use changes of the Mediterranean Sixth Creek catchment in South Australia. Based on stream- and climate-specific data, following conclusions can be drawn:

- The integrated application of the modelling techniques GF, SWAT and HEA allowed to determine consecutively flow-sensitive macroinvertebrate species, scenario-specific flow patterns and scenario-specific macroinvertebrate abundances.
- Climate change and reforestation scenarios suggested a prolonged decline in flow, which may shift the unusual permanent flowing stream of Sixth Creek towards intermittency in future.
- Macroinvertebrate species responded in a species-specific way to climate and land use change scenarios. This study has shown that species belonging to EPT responded distinctly different to altered flow conditions. Projections for the next 30 years showed that a possible 10% increase of urbanisation would affect negatively some mayflies and the back-swimmer *Micronecta* spp. However, mayflies with affinity to wet habitats e.g. *Offadens* spp. may survive high flows of urbanisation scenario. Overall, mayflies also exhibited variations in sensitivity to low flow scenarios of climate change. Furthermore, the mud-snail *Hydrobiidae* spp. may adapt successfully to possible climate and land use changes.
- The modelling approach indicated consequences of future developments in catchments taking into account trends of global warming and urbanisation. It can be applied to any river catchment provided that historical and current land use data, time-series of climate, hydrological and macroinvertebrate data are available.

Future research will examine trends of species responses to future scenarios by extending the set of indicator species for the same stream site, and testing the applicability of the proposed modelling approach to another river catchments. It will also focus on forecasting impacts of salinity dynamics on macroinvertebrate communities affected by climate and land use change.

CRediT authorship contribution statement

Jawairia Sultana: Conceptualization, Formal analysis. **Friedrich Recknagel:** Conceptualization, Resources, Supervision. **Hong Hanh Nguyen:** .

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2020.106766.

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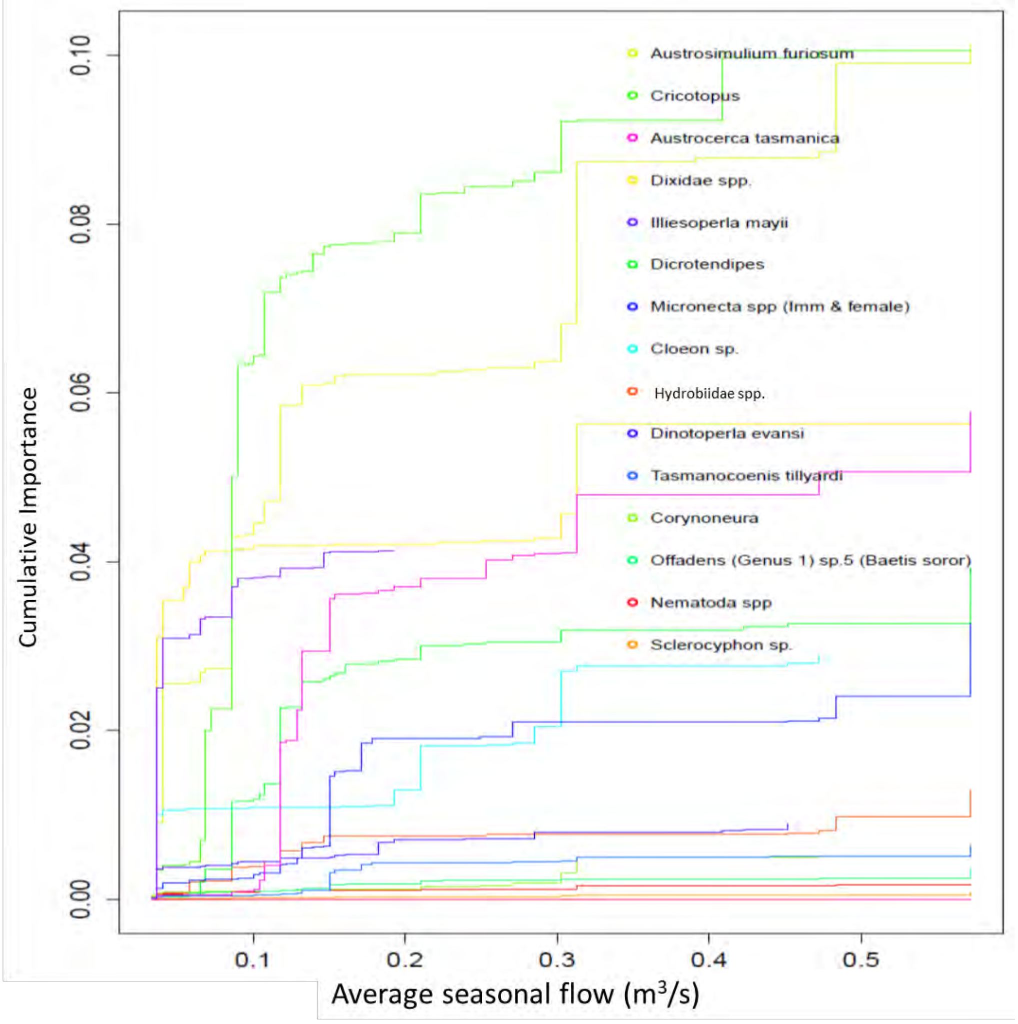
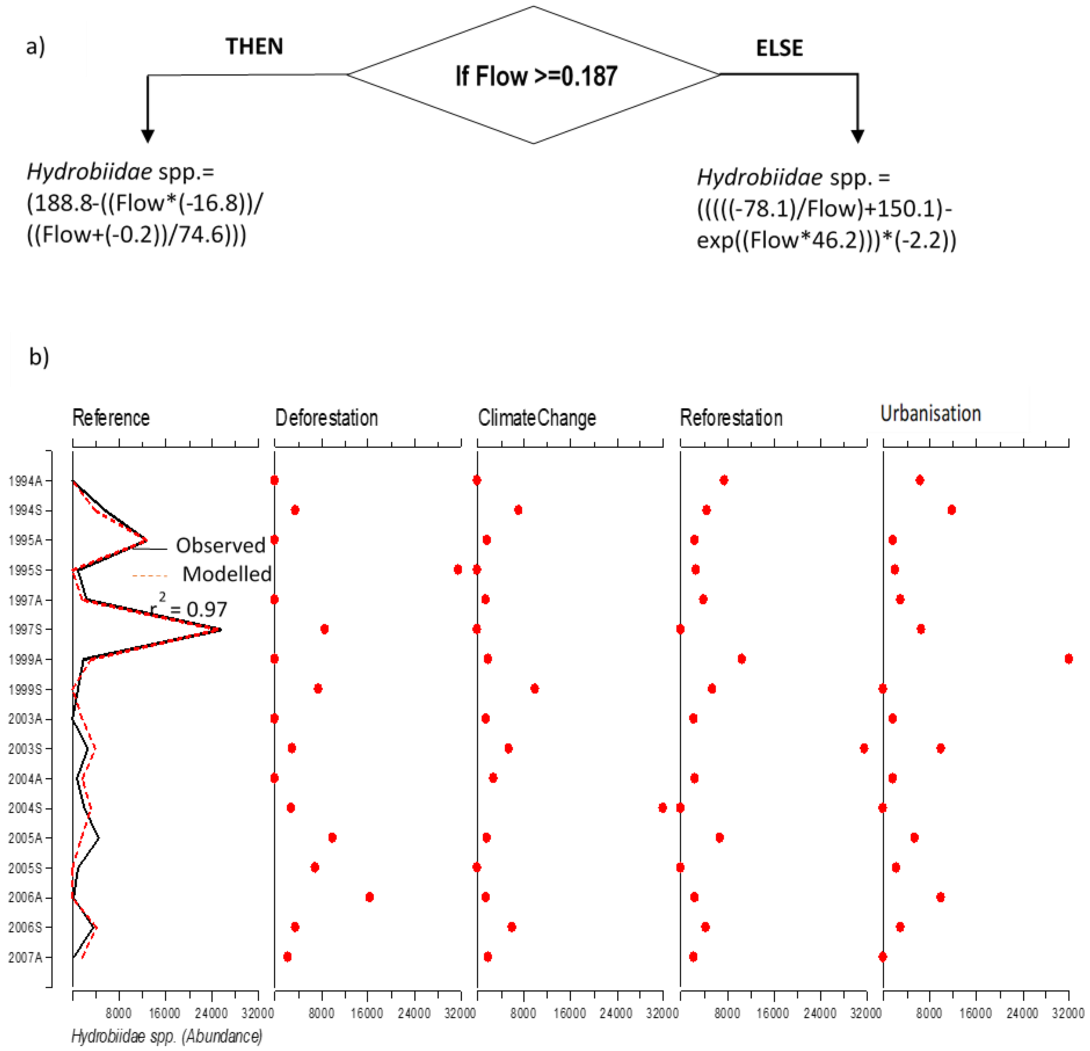
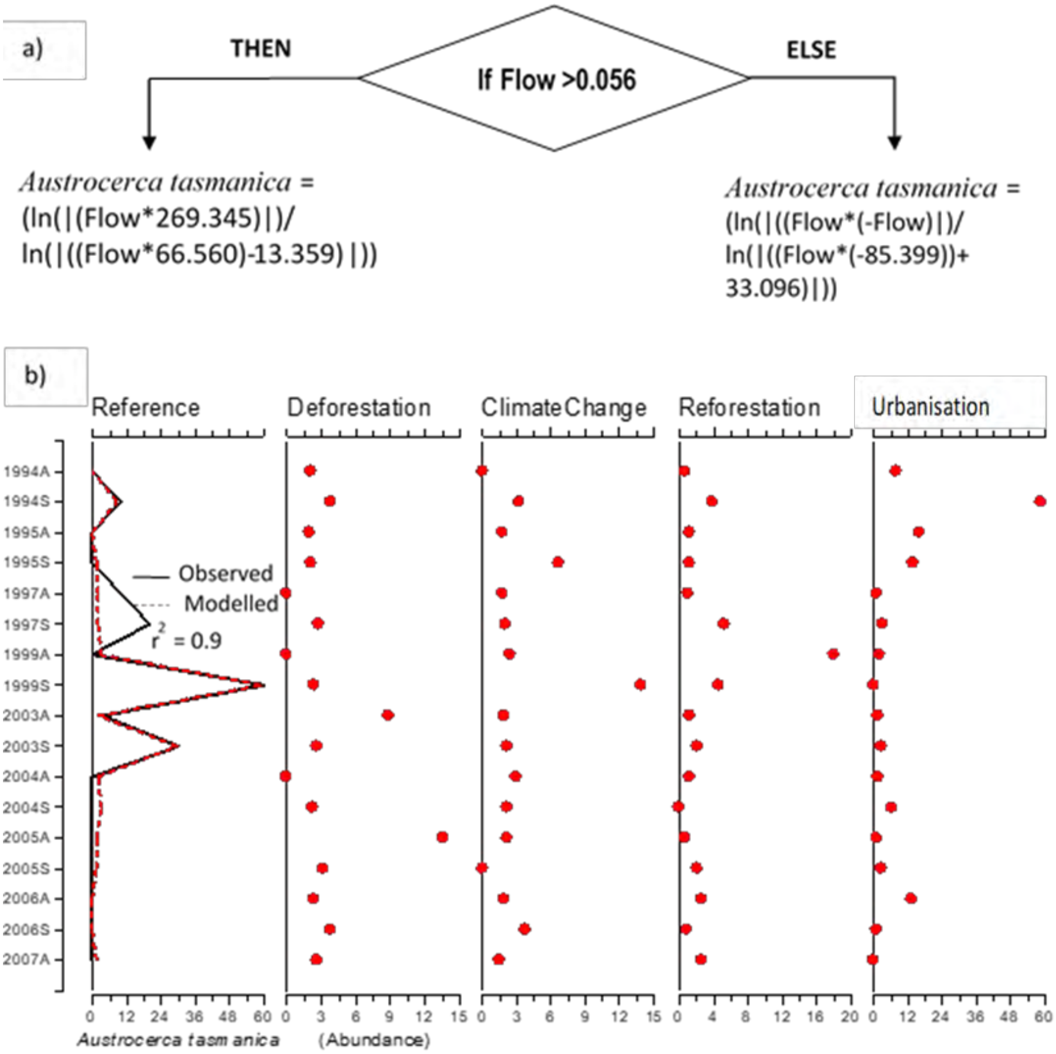
Supporting Information (Chapter 5)



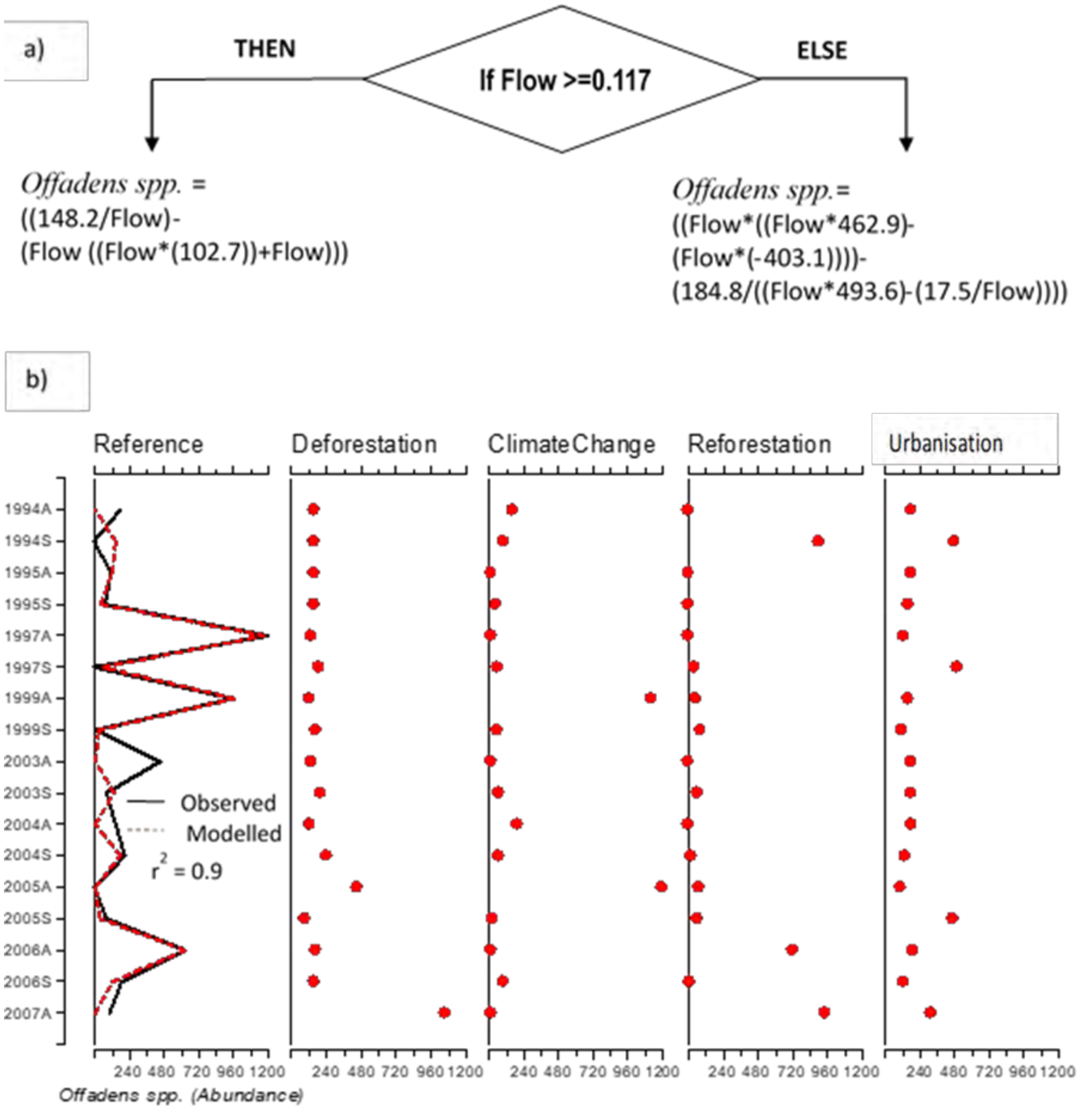
Appendix 1: Gradient Forest output with fifteen most responsive species across flow gradient.



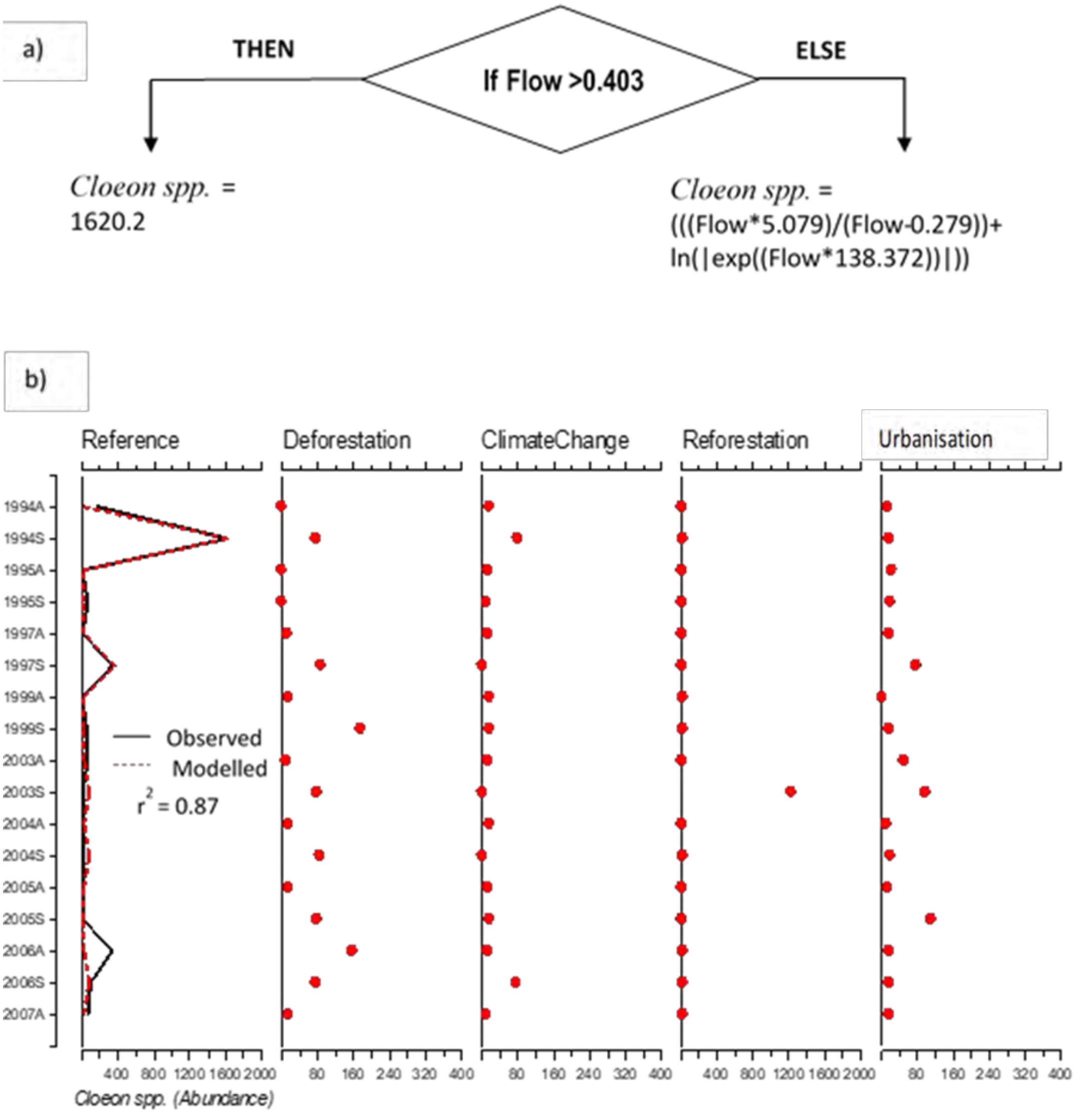
Appendix 2: HEA based predictive modelling for *Hydrobiidae spp.* when average seasonal flow (m^3 /s) was used as an input. a) HEA based IF-THEN-ELSE rule; b) model validation based on observed data (1994-2007) and future predictions in respective scenarios.



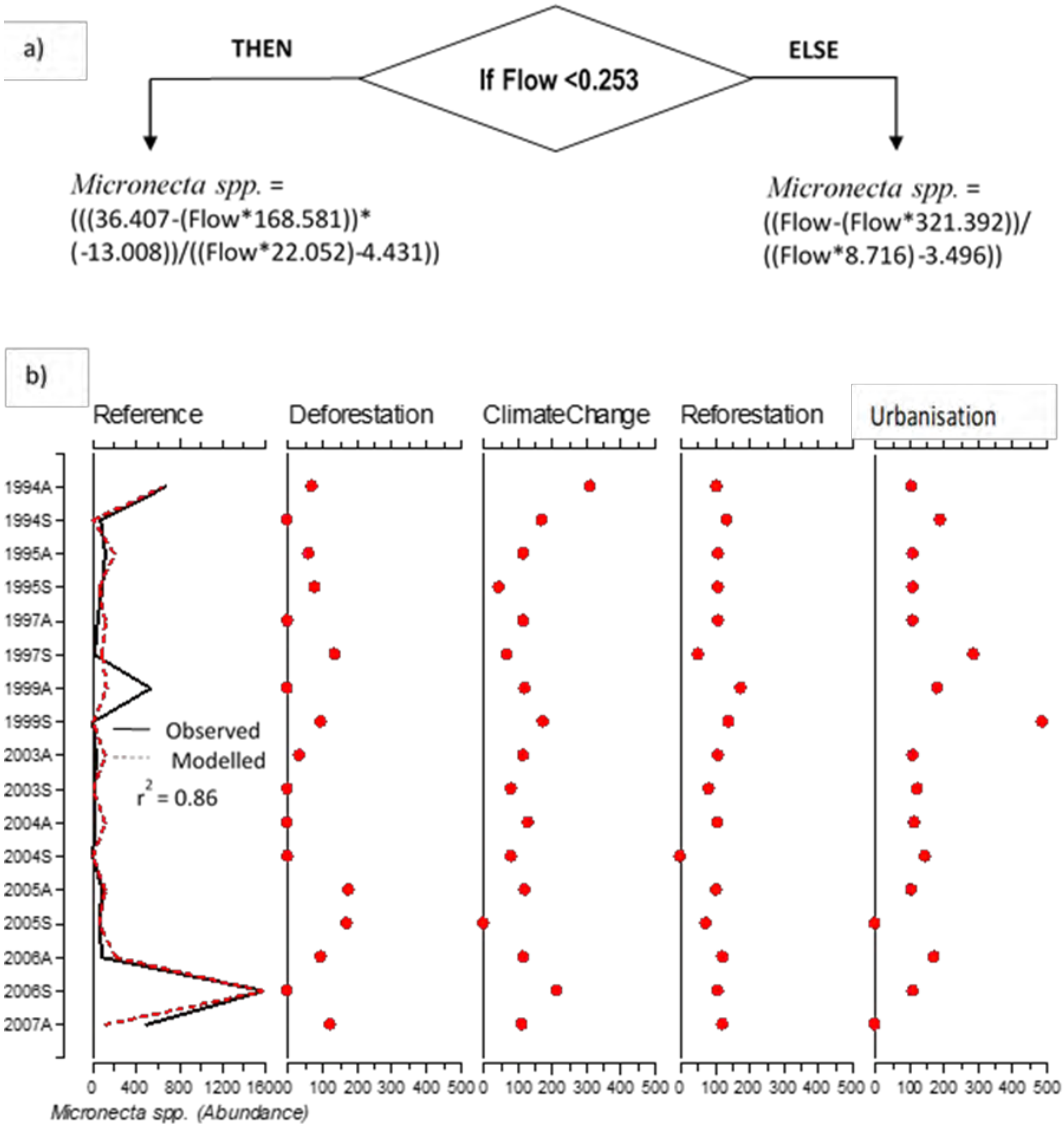
Appendix 3: HEA based predictive modelling for *Austrocerca tasmanica*.when average seasonal flow (m^3 /s) was used as an input. a) HEA based IF-THEN-ELSE rule; b) model validation based on observed data (1994-2007) and future predictions in respective scenarios.



Appendix 4: HEA based predictive modelling for *Offadens spp.* when average seasonal flow (m^3 /s) was used as an input. a) HEA based IF-THEN-ELSE rule; b) model validation based on observed data (1994-2007) and future predictions in respective scenarios.



Appendix 5: HEA based predictive modelling for *Cloeon spp.* when average seasonal flow (m^3 /s) was used as an input. a) HEA based IF-THEN-ELSE rule; b) model validation based on observed data (1994-2007) and future predictions in respective scenarios.



Appendix 6: HEA based predictive modelling for *Micronecta spp.* when average seasonal flow (m^3 /s) was used as an input. a) HEA based IF-THEN-ELSE rule; b) model validation based on observed data (1994-2007) and future predictions in respective scenarios.

Chapter 6: Conclusions and future research

The purpose of monitoring is “to gain information needed for management actions” (Downes et al., 2002; Gerber et al., 2005). However, it has been argued that long-term river health monitoring programs often find it challenging to define clear objectives which translate to management outcomes (Bunn et al., 2010; Downes et al., 2002; Goonan et al., 2012). This work integrated bioindicator monitoring and recent modelling approaches while addressing the associated challenges to inform river health management.

The potential contributions of modelling approaches to bioindicator monitoring are diverse, and so are the challenges. This research focussed on identifying appropriate modelling techniques and testing the application of ecological threshold models as tools for stream management. The variety of alternative approaches to identify ecological thresholds and the role of spatial dependence in identifying and applying these thresholds for conservation, present significant challenges to management. In the course of my PhD research I: (1) applied both Hybrid Evolutionary Algorithm and Threshold Indicator Taxa Analysis (TITAN) to determine water quality thresholds for the macroinvertebrate communities of the River Torrens and Onkaparinga River catchments, (2) compared the performance of the alternative ecological threshold methods GF and TITAN based on synthetic data as well as field data of macroinvertebrates and benthic diatoms, and evaluated the spatial dependence of these thresholds from local to regional to state-wide scale, and (3) developed an integrated modelling approach to predict species-specific responses of macroinvertebrates to projected future climate and land use. This final chapter draws the findings of my research in context with my key research questions, and discusses current limitations and future research directions.

6.1 Key outcomes and conclusions

(i) Catchment characteristics and the choice of model influence the detection of thresholds

Chapter 2 addressed the research question (1) (mentioned in Section 1.4) by comparing water quality thresholds for macroinvertebrates from the River Torrens and Onkaparinga River catchments identified by HEA and TITAN. While the change points suggested by both methods for electrical conductivity (EC) and total nitrogen (TN) were similar for the two catchments. Change points for total phosphorus (TP) in the Onkaparinga River catchment exceeded those of the River Torrens catchment by factor three, reflecting the intensive agricultural land use in this catchment.

Species-specific thresholds for TN and TP and EC identified by HEA were higher than community thresholds identified by TITAN in both catchments. This was likely because TITAN keeps the explicit individual values of drivers for data analysis whereby HEA looks at statistical means. Generally, despite the differences observed in thresholds based either on a catchment or the modelling technique, overall this study showed that South Australian water quality guideline values for the protection of freshwater systems appear to be too high.

(ii) Testing the performance of the two commonly used methods GF and TITAN for threshold analysis by means of synthetic and field data warns more caution

Ecological thresholds have become increasingly relevant for river health management. In the last two decades, there has been a growing number of applications of these techniques with much success in freshwater management (King et al., 2011; Wagenhoff et al., 2017a). A review of literature in Section 1.2.2 has shown that justification for the choice of selected methods and the interpretation of identified thresholds by considering the rationale of the method, is often ignored.

Chapter 3 compared the two frequently used ecological threshold methods Gradient Forest (GF) and TITAN using field and synthetic data. Both approaches have similar data requirements, are user-friendly and have similar aims in terms of supporting threshold identification. This study showed that both models identified the major thresholds in macroinvertebrate data at almost identical low concentrations of the most significant stressors EC, TP and TN. However, when applied to a data set of benthic diatoms, GF sometimes overestimated thresholds as it aggregated the response of positive and negative responding taxa. In experiments with synthetic data, both models accurately identified similar thresholds in uniformly distributed data, whereby GF outperformed TITAN in cases with multiple change points. Overall, it was found that while both models have weaknesses, they are robust in identifying change points in species responses. I recommended both GF and TITAN as appropriate tools for river health management, provided their limitations are taken into account.

(iii) Stream-specific diatom assemblages show distinct variability in relation to water quality variables

Diatoms collected at four different stream sites given as Appendix B, identified that diatom species from the two nutrient-rich stream sites responded primarily to total phosphorus (TP). Whilst diatom assemblages from the stream site, i.e. Sixth Creek with lower nutrient concentrations, electrical conductivity (EC) and flow appeared to be the most important driver. Self-organising maps (SOMs) revealed a distinct variability of diatom indicator species among observed sites. High abundances of tolerant indicator taxa identified by TITAN were observed at sites with highest TP and TN concentrations, e.g. Cox Creek in Onkaparinga River catchment. However, high abundance of sensitive species identified by TITAN was found at a pristine site with minimal human disturbance. Results from this study highlighted the significance of stream-specific findings where management actions based on broad spatial scale may not take into account specific characteristics of individual streams. This study was further extended to compare diatom threshold responses at multiple spatial scales in Chapter 4.

(iv) Water quality thresholds for diatoms differ between regions but exhibit a consistent trend within regions

My review of the literature identified that the scale dependence of thresholds is an important and often neglected aspect of their application to freshwater management. The study comparing threshold responses for diatoms among different hierarchical spatial scales and between regions, is presented in Chapter 4. This study indicated that when spatial resolution was increased from state to local scale within the same region, TITAN identified similar thresholds at subregional or local scale. Overall, local scale thresholds, in the particular streams studied, were lower than state scale. However, there were variations in diatom thresholds between regions, with higher thresholds observed in the South East region. These thresholds corresponded to higher TP and EC concentrations, than the Adelaide and Mount Lofty Ranges region. Therefore, it is recommended that thresholds derived at broad spatial scales alone are unlikely to be appropriate for finer-scale assessment. Thus, deriving thresholds at the finest scale for which there is an appropriate amount of data, or adjustment to the scale at which data is collected can inform appropriate management guidelines.

This study also suggested that *Nitzschia inconspicua* consistently responded positively, while *Achanthidium minutissimum* negatively in relation to TP and EC increases at multiple spatial scales. Reliable indicators of water quality may provide practical river health assessment, and this research showed that *N. inconspicua* and *A. minutissimum* might be used by managers to assess salinity and nutrient enrichment of South Australian streams, across multiple spatial scales.

(v) Combining GF, SWAT and HEA provides greater utility in modelling species-specific macroinvertebrate responses to changing land use and climate than any alone

To my knowledge, the here proposed integrated modelling approach is unique in quantifying species-specific responses of macroinvertebrate species under changing climate and land use conditions. It is consecutively (cascade-like) taking advantage of the capacity of (1) GF to identify macroinvertebrate species that respond strongest to the most important stressor streamflow in the data sets; (2) the process-driven model SWAT that allows to predict the streamflow under changing climate and land use conditions; (3) HEA that allows to develop predictive models for macroinvertebrate species driven by streamflow under different climate and land use conditions.

Findings from this integrated modelling approach presented in Chapter 5, suggested that flow i.e. average seasonal discharge was the most important driving factor affecting macroinvertebrate assemblages of the Sixth Creek in the River Torrens catchment. The scenario analysis with SWAT revealed the highest decline in river flow during late autumn and spring by reforestation and climate change, which may cause a prolonged flow decline in autumn and may shift Sixth Creek from a permanent to an intermittent flowing stream. The HEA model quantified population dynamics of flow-sensitive macroinvertebrate species identified by GF in response to the altered flow predicted by SWAT for the scenarios: (a) deforestation, (b) reforestation, (c) climate change, and (d) 10% increased urbanisation. Results revealed distinct nonlinear responses of species belonging to Ephemeroptera, Plecoptera and Trichoptera that are commonly considered as the group EPT with similar properties, suggesting potential benefits from redirecting the focus of such studies from the community- to species-level. The understanding of species-specific responses to changing stream habitat conditions will be further improved by studying alternative stream sites with similar conditions using the integrated modelling approach.

6.2 Research assumptions and limitations

Despite considerable increases in our understanding of the requirements for identifying and modelling thresholds, there are a number of limitations that warrant further research. First, current results related to spatial scale influences on thresholds analysis were limited to local scale evaluation of one region. In future, extending it to another region following a hierarchical monitoring design by replicating each spatial scale may enable to propose generalised conclusions on optimal spatial scales needed for management. Second, scenarios for simulating future land uses were developed using the land use update tool available in SWAT. This ignored the nonlinear criteria of land use changes. Therefore, simulation results related to changes in percentages of revegetation and deforestation may not happen in the same way in future. However, this limitation was masked by analysing the effect of extreme deforestation and reforestation changes. Third, future research is needed to evaluate possible sources of uncertainty in scenario outputs of the SWAT model. However, findings of the SWAT-based integrated modelling approach demonstrated its applicability to study complex ecosystem dynamics in combination with other models. Fourth, individual species responses simulated to future scenarios were based on limited long term data available from the Sixth Creek catchment, and future research will focus on extending the modelling approach to other similar catchments for generalising conclusions on individual species' trends. Thus this study emphasised the feasibility of the proposed integrated modelling approach by using Sixth Creek as a case study, rather than focusing only on individual species response to future scenarios. However, it became evident that species belonging to EPT taxa responded in a specific nonlinear ways rather than as a group.

6.3 Future research

Future research can focus on: 1) considering additional stressors, e.g. native vs. non-native vegetation cover in riparian zones and different flow matrices which are likely to influence the response of macroinvertebrate and diatom assemblages. It may improve the lines of evidence required to derive site-specific thresholds following the suggestions from revised water quality guidelines in Australia (ANZG, 2018). 2) Testing the performance of the advanced version of SWAT+ (www.swat.tamu.edu), a completely revised version of the model recently released, by extending its applications to other similar catchments and; 3) forecasting macroinvertebrate species responses to climate and land use changes by means of salinity predicted by HEA and SWAT corresponding to Bailey et al. (2019).

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Appendices

Appendix A

International conference paper and abstracts

Oral Presentation Australian Freshwater Sciences Society Conference 2018

Title: A rationale for threshold model selection and identification of ecological thresholds for macroinvertebrate assemblages: Comparison of two commonly used methods (#85)

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Abstract

Worldwide, freshwater ecosystems are exposed to multiple stressors that adversely affect the stream biota. In recent years, there has been a rapid expansion in methods to evaluate biotic threshold response to stressors. Despite the emergence of newly developed techniques for threshold analysis, optimal model selection has received little attention. This study has a twofold purpose: i). to identify macroinvertebrate stressor thresholds for river health based on monitoring data from South Australian catchments and ii). to compare the performance of two threshold models; i.e. Threshold Indicator Taxa Analysis (TITAN) and gradient forest (GF) using both real and synthetic data. We simulated different sample distributions (i.e. sample to environment distributions SEDs), and different patterns of species optima across a hypothetical environmental gradient to evaluate the bias in threshold location in the two methods. Results of 14 years of real data indicate that EC is the most prominent water quality variable affecting macroinvertebrate assemblages. There is a reasonable agreement between thresholds from the two models at low EC concentrations. However, GF thresholds at higher concentrations need both consideration and caution, i.e. thresholds are considered at this point when there is significant change in species response and caution is needed to avoid the overestimation of data irregularities. Based on synthetic data, we demonstrated that TITAN thresholds are more likely to be influenced by different SEDs than GF. In addition to SEDs, pattern of species optima is another important factor to decide the location of thresholds identified by the two models. Overall results indicate that TITAN provides realistic detection of thresholds on noisy data sets, however, GF performed better in the presence of outliers and unevenly spaced environmental data. Another advantage of GF is ranking of relative importance of each stressor in relation to macroinvertebrate species composition, therefore, can serve as a good starting point of threshold analysis. In conclusion, the nature of an ecological data set affects the performance of threshold models and hence threshold identification.

Keywords: Threshold indicator taxa analysis (TITAN), gradient forest (GF), sample to environment distributions (SEDs), species response, simulated data

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Integrated approach for predicting impacts of future climate and land use changes on macroinvertebrates in a Mediterranean catchment using GF, SWAT and HEA models

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Abstract: Climate and land use changes are expected to alter flow and nutrient regimes in catchments affecting stream habitats and aquatic biodiversity. Scenario analysis by an integrated modelling approach may assist to better understand after-effects of such projected changes on biodiversity. This study aimed to quantify the impacts of climate and land use changes on the macroinvertebrate community of the Torrens river catchments, South Australia. Gradient forest (GF) determined average seasonal flow as main factor affecting macroinvertebrate assemblages in this catchment. A catchment model developed by the Soil and Water Assessment Tool (SWAT) was used to simulate flow under following scenarios: 1) future climate change scenario (RCP 8.5) based on six global circulation models, 2) hypothetical land use change scenario of deforestation over the next 30 years, and 3) scenario combining land use and climate change. Results of the future climate change scenario suggested decreased monthly flow due to declining precipitation and increasing air temperature, in contrast to the future land use change scenario of deforestation that predicted increased monthly flows. The combined future scenario to some extent suggested a trade-off between projected climate and land use changes but indicated dominating land use impacts due to deforestation resulting in increased runoff and higher flows. The Hybrid Evolutionary Algorithm (HEA) was used to model flow-driven abundances of GF-identified key species *Hydrobiidae* spp. and *Tasmanocoenis tillyardi* over 14 years at a representative stream site. The coefficients of determination r^2 of the HEA models ranged between 0.88 and 0.96. Results indicated *Hydrobiidae* spp. to be tolerant and adaptive to altered high flows under the future combined scenario by showing higher abundance as compared to individual climate change and land use change scenarios. In contrast, *T. tillyardi* has shown affinity to low flow conditions and decreased abundance in future combined scenario. The integrated modelling approach based on SWAT and HEA proved to be suitable for studying stream health under the impact of projected future climate and land uses.

Keywords: *Climate change, land use change, gradient forest, SWAT, HEA, macroinvertebrates*

Jawairia Sultana, Integrated approach for predicting impacts of future climate and land use change on macroinvertebrates in a Mediterranean catchment using GF, SWAT and HEA models

1. INTRODUCTION

Worldwide, freshwater ecosystems are exposed to multiple stressors that adversely affect stream biota. Species diversity is declining due to climate change, urbanisation, intensive agriculture and other stressors affecting the water resources. These changes alter the physical and chemical characteristics of streams that result in loss of biodiversity and ecosystem services. Land use and climate change has shown an increased nutrient enrichment and varying flow trends affecting the overall river health (Nguyen et al. 2018, Shrestha et al. 2017, Walsh et al. 2005). Even though altered flow regimes have been widely discussed as an important variable affecting river ecosystems (Dewson et al. 2007, Domisch et al. 2017), only a few studies explored the impact on stream biota (Kakouei et al. 2018, Kakouei et al. 2017, Pyne and Poff 2017).

Among a variety of stream biota, macroinvertebrates are widely used as bioindicators by different river health monitoring programs due to their sensitivity to environmental conditions and ubiquitous distribution. A number of modelling approaches have been developed and applied to examine ecological impacts on stream biota (Baker and King 2010, Sonderegger et al. 2009). Despite these efforts, catchment managers face difficulty in communicating information from the database generated by river health monitoring programs. Most of the applied approaches are either limited to threshold analysis for updating water quality guidelines or biophysical models that evaluate changes in the environment (Peter Goonan 2012). To overcome these limitations an integration between bioindicator monitoring and different modelling approaches to predict river health is required.

Thus we proposed an integrated approach to develop and test a modelling framework which can be used to predict and monitor the status of river ecosystems under projected future conditions, saving time and cost. Our approach combines the inferential ecological models Gradient Forest GF and Hybrid Evolutionary Algorithm HEA with the process-driven hydrological model Soil and Water Analysis Tool SWAT to improve the understanding of complex eco-hydrological processes. This study was based on the following research questions: 1.What are the most important environmental stressors affecting the studied macroinvertebrate assemblages? 2. What are the indicator species affected by the most important environmental stressor? 3. How may projected variations in abiotic drivers (climate change and land use change) affect assemblages of selected indicator species?

2. MATERIAL AND METHODS

2.1 Study Site and Data

The study area comprises of Sixth creek, a sub-catchment of the Torrens River that covers an area of 4300 ha in the Mount Lofty region of South Australia. This sub-catchment is characterized by Mediterranean climate of dry summers and mild wet winters (Peel et al. 2007). It is associated with perennial stream flows that inhabits diverse macroinvertebrates with many rare, pollution sensitive and flow-dependent species (www.epa.sa.gov.au). This sub-catchment was selected as a case study because of its importance for water supply, long records of historical daily flows and near pristine conditions.

Macroinvertebrates and water quality data was provided by South Australian monitoring, evaluation and reporting Program (SA-MERP), that was established since 1994 to assess the river health of South Australian waterways. Flow data was obtained from existing gauging station at the same site macroinvertebrates data was collected. Future climate data was extracted from six Global Climate Models (GCMs) as mentioned in the Goyder Institute Water Research (GIWR) project developed for SA climate and run for high emission (RCP 8.5) and medium emission (RCP 4.5) scenario.

Associated considered related

2.2 Modelling design

Our integrated modelling approach for this study (Figure 1) started with the use of gradient forest (GF) for identifying the most important environmental stressor affecting the macroinvertebrate assemblages. Then two representative species among the most responsive species discovered by GF were selected as indicator taxa related to the identified stressor. GF is a regression tree based machine learning method. It uses bootstrap procedure to calculate predictor importance and identifies most responsive species to any gradient based on highest R^2 weighted importance. GF was used using R package “gradientForest”.

At the second step, the process-based hydrological model Soil and Water Assessment Tool (SWAT) was used to simulate future flow for the following 3 scenarios: 1) future climate change scenario based on six global circulation models, 2) hypothetical land use change scenario i.e forest to pasture over the next 30 years, and 3) scenario combining land use and climate change. SWAT is a river basin model that simulates catchment processes such as surface runoff/infiltration, percolation, evapotranspiration, nutrient and sediment transport (Neitsch et al. 2011). This study used a well-developed SWAT model for the Sixth creek and further details on model calibration and validation can be found in Nguyen et al. (2019).

Finally, the simulated time series output from SWAT and indicator species from gradient forest were used for developing predictive models by the Hybrid Evolutionary Algorithms (HEA). HEA has been developed to evolve predictive models in form of simple IF-THEN-ELSE rules. It incorporates genetic programming (Koza 1992) to find the optimum structure in combination with differential evolution (Storn and Price 1997)to parameterize the fittest models. The C++ version of HEA (Cao et al. 2013) was run on a Corvus supercomputer with a cyclic boot-strap scheme of 100 runs. The models with $r^2 > 0.7$ were selected for further evaluation.

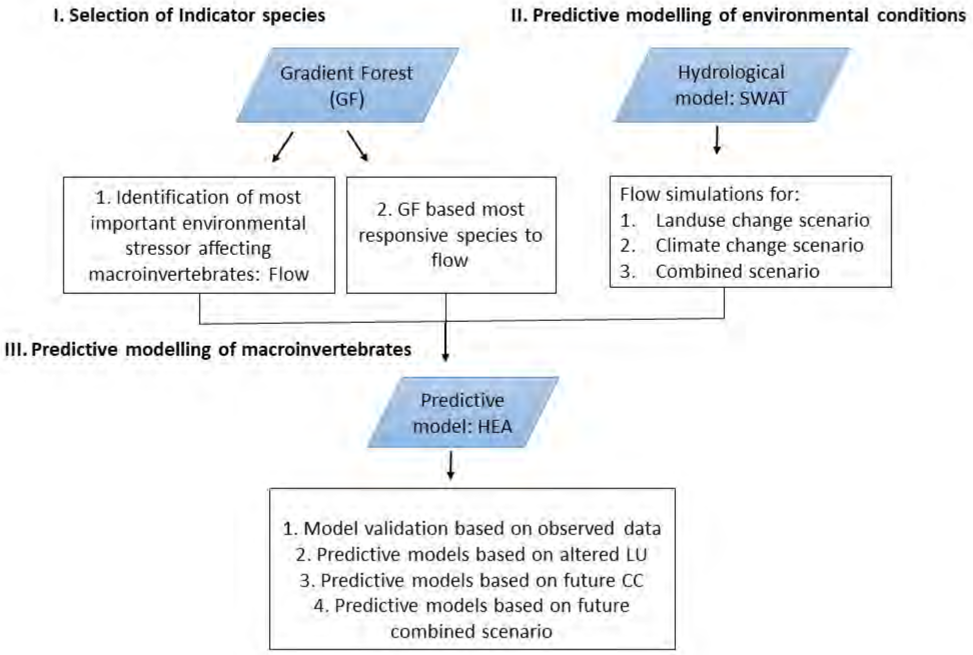

Figure 1. The overall modelling design

3. RESULTS AND DISCUSSION

3.1 Identification of important stressor and most responsive species

Among different environmental stressors, gradient forest (GF) determined average seasonal flows (ASF) as the most important factor affecting macroinvertebrates in the study area (Figure 2a). GF also identified the most responsive species to ASF (Figure 2b). Out of the most responsive species, two species were selected for predictive modelling i.e. *Hydrobiidae* spp. assuming its ubiquitous distribution irrespective of changing environmental or flow conditions (Davies et al. 2010) and *Tasmanocoenis tillyardi* due to its preference to still or slow flowing habitats (Gibbins et al. 2000).

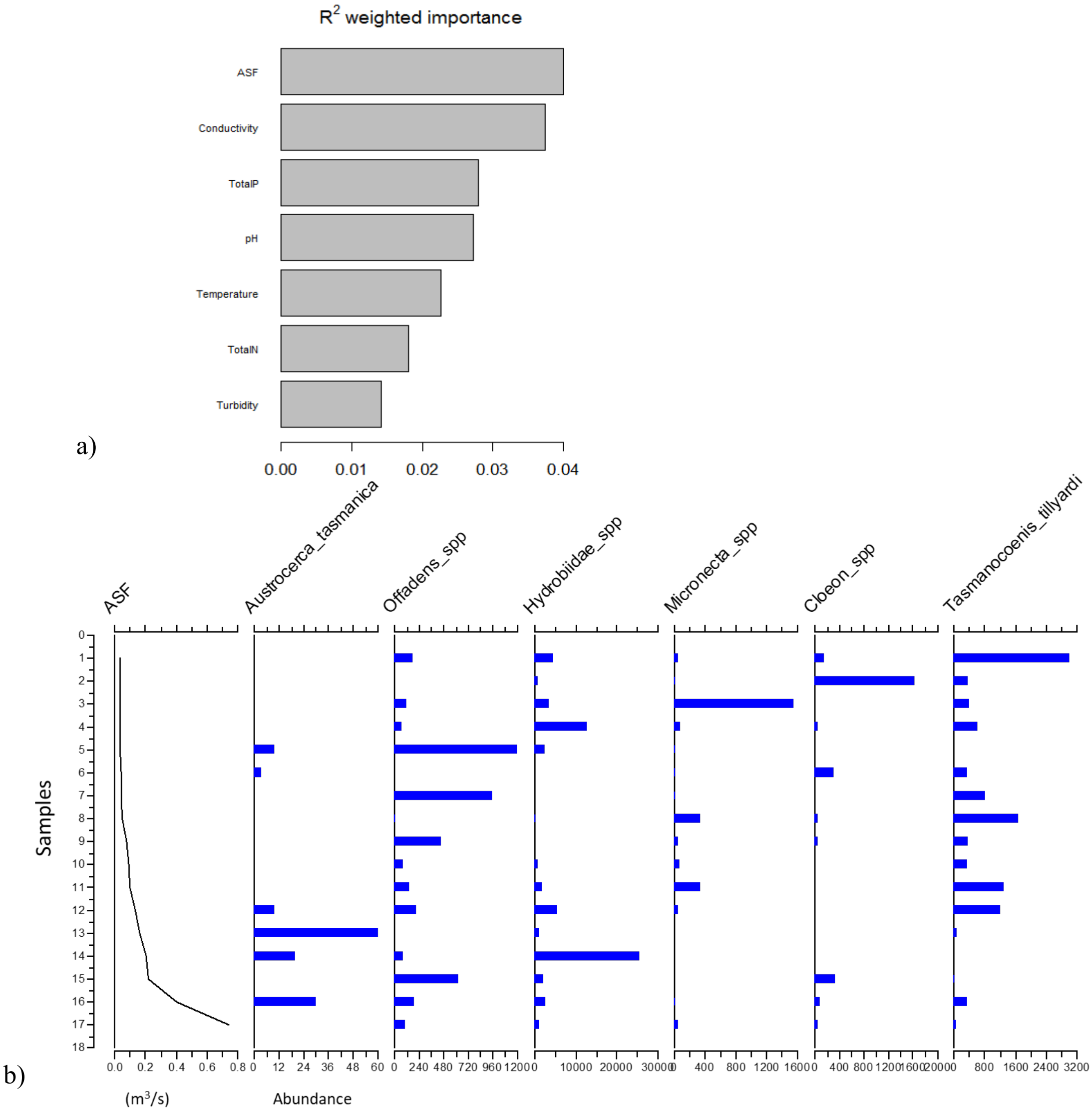

Figure 2. Gradient forest (GF) results: a) identifying Average Seasonal Flow (ASF) as most important variable affecting macroinvertebrates; b) abundance trends of six most responsive species in relation to ASF

3.2 Hydrological model simulations for flow under future scenarios

SWAT results are summarized as relative change to the baseline simulation using historic climate data (1981-2005) of six global climate models and constant land use (Figure 3). For scenario simulations, the highest relative increase in flow (14%) was observed in land use change scenario which corresponds to highest decrease (-12%) in climate change-induced flows. Sixth creek belongs to a forested catchment and future extensions of these land uses by pastures seemed to increase runoff that lead to ultimate high flows in receiving water bodies. The combined future scenario showed potential increasing trends in flow with largest increases (13%) observed during late winters (Figure 3). Similar trend of higher flows were predicted in these catchments based on hypothetical deforestation scenario over the next 30 years (Nguyen et al. 2018, Shrestha et al. 2017) with land use change of greater concern as compared to future climate change.

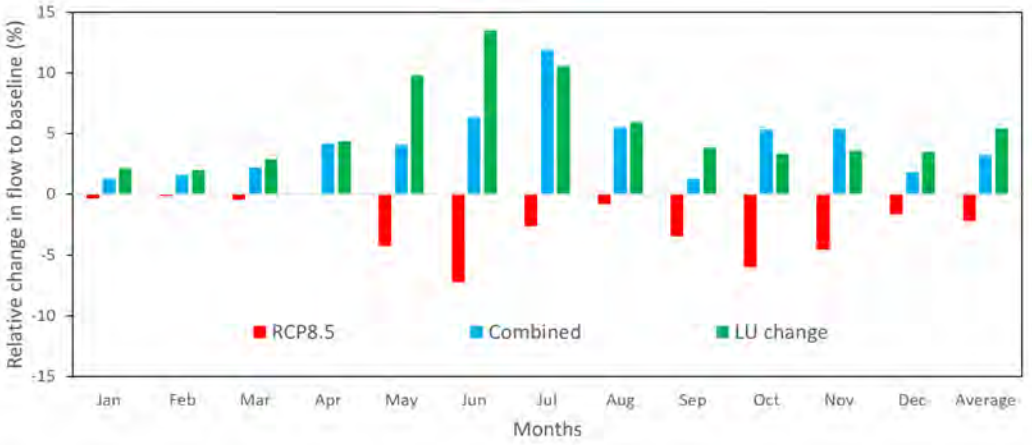

Figure 3. Soil and Water Assessment Tool (SWAT) results for relative change in mean monthly flow for climate change, land use change and combined future scenario compared to the baseline simulation for the time period 2016-2045.

3.3 Impacts of climate and land use change on selected species

The predicted changes in species abundance in relation to the observed baseline are shown in Figure 4. All these predictions were based on IF-THEN-ELSE-rules discovered by HEA, where validation was performed using observed data provided by South Australian Environmental Protection Authority (SA-EPA). The species abundance in relation to future flow conditions were different during individual scenarios and was species dependent. *Hydrobiidae* spp. appeared as tolerant or adaptive to future flow conditions with an increasing trend in all the simulated scenarios (Figure 4). In contrast *Tasmanocoenis tillyardi* has shown a decreasing pattern to the higher land use change-induced flows. A slight increase (7%) in abundance of *T. tillyardi* in climate change scenario indicated some affinity to low flow conditions (Figure 4). Overall, *Hydrobiidae* spp. abundance displayed ubiquitous distribution in all the future altered flow conditions.

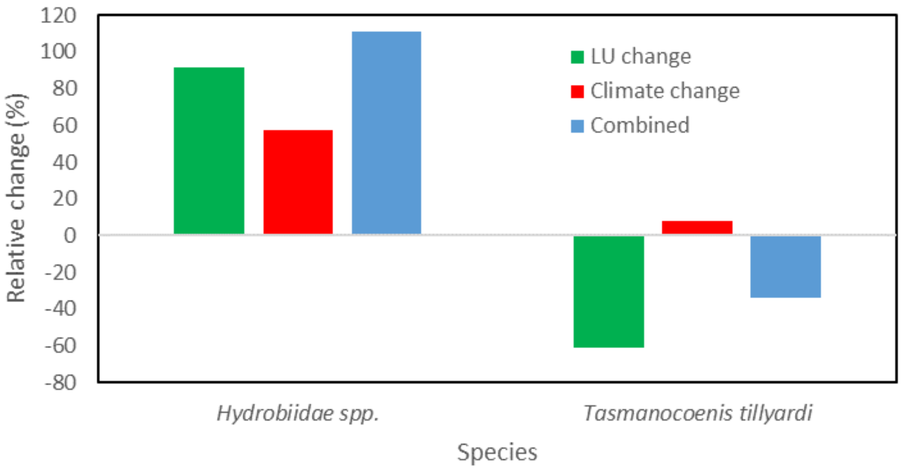

Figure 4. Hybrid Evolutionary Algorithms (HEA) results for relative change of selected macroinvertebrate species abundances of future scenarios compared to the baseline simulation for the period 2016-2045.

4. CONCLUSIONS

In this study, an integrated modelling approach was demonstrated to reveal the impacts of projected climate change and land use change scenarios on river biodiversity. It is applicable to any catchment system with community to species level consideration, enabling managers of informed decision and management. This approach can be useful to provide an insight in future habitat preferences of the targeted species. Following conclusion can be drawn from this specific study:

- Average seasonal flow is the most important factor affecting macroinvertebrate assemblages in these river catchments.
- Altered flow conditions for the individual and combined scenario simulations revealed highly species-specific responses.
- Flow tolerant species like *Hydrobiidae* spp. has shown an increased abundance at expense of other sensitive species of mayflies.

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Proceedings of the 10th International Conference on Ecological Informatics

Overall and site-specific response of the macroinvertebrate community of Swan Coastal Plain Wetlands (West Australia) to water quality gradients revealed by GF and HEA

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ABSTRACT:

The Swan Coastal Plain is situated along the Western Australian seaboard and accommodates a large number of permanently, seasonally and episodically flooded wetlands. Many of these wetlands are affected by eutrophication and hydrological changes. A systematic monitoring program has been conducted between 1989 and 1990 to assess the environmental status of 41 selected wetlands based on measurements of 19 physical-chemical attributes and the collection of 253 macroinvertebrate taxa samples (Davis et al. 1993). This study analysed 35 wetlands with consistent data collected in Nov 1989 and Nov 1990 by means of Gradient Forest (GF) and the Hybrid Evolutionary Algorithm (HEA). Whilst GF allows identifying macroinvertebrate taxa with the "strongest overall response" to gradients in "important" physical-chemical attributes, HEA allows to model population dynamics of the taxa depending on "important" attributes identified by GF along all 35 wetlands. HEA models are represented by IF-THEN-ELSE rules whereby IF-conditions disclose attribute thresholds that indicate changes in the species abundance across the wetlands. GF suggested different ranking of the attributes EC, TN and DIP for both years as well as different taxa assemblages for same attributes in 1989 and 1990. Since results for merged data were also different, only the year-by-year specific results have been taken into account. When inferential models have been built for the 4 species that responded "strongest" to EC, DIP and TN in 1989 and 1990 by HEA, the threshold conditions fall in the range of overall gradients of these attributes discovered by GF. GF and HEA proved to be complementary tools for identifying overall attribute gradients and species- and site-specific thresholds in complex ecological data sets.

KEYWORDS: Bio-indication, Wetlands, Macroinvertebrates, Gradients, GF, HEA

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Davis, J.A., Rosich, R.S., Bradley, J.S., Growns, J.E., Schmidt, L.G. and F. Cheal, 1993. Wetlands of the Swan Coastal Plain. Vol. 6: Wetland classification on the basis of water quality and invertebrate community data. Water Authority of Western Australia.

Appendix B

Data analysis using field monitored data

Status: Written in publication style but not submitted to any journal

Title: Spatial variability of stream-specific diatom assemblages in relation to water quality variables from River Torrens and Onkaparinga River catchments

Abstract: River ecosystems are affected by many factors ranging from stream microhabitat scale to catchment and global scale. This study aimed to highlight stream-specific spatial variations of diatom indicator species affected by important water quality variables. Gradient forest (GF) analysis of four years of data from four sites have shown that diatom species responses were mainly affected by total phosphorus (TP) and electrical conductivity (EC). However, a distinct dissimilarity using analysis of similarity (ANOSIM) (ANOSIM R-statistic = 0.77, p <5%) was observed among sites. Results indicated that diatom species responses in a stream with highest nutrient enrichment were primarily driven by TP. However, for the stream site with lower nutrient concentrations (Sixth Creek: mean TP= 21 µg/L, maximum= 36 µg/L), electrical conductivity (EC) and flow appeared to be the most important variables affecting diatom assemblages. Thresholds indicator taxa analysis (TITAN) assisted in identifying important indicator species, and self-organising maps (SOMs) revealed a distinct variability of diatom indicator species among observed sites. High abundances of tolerant indicator taxa (z+) identified by TITAN such as *Nitzschia inconspicua* were observed at sites with highest TP and TN concentrations (e.g. Cox Creek: mean TP= 213 µg/L and mean TN= 2.8 mg/L). In contrast, high abundance of sensitive indicator taxa (z-) identified by TITAN such as *Fragilaria capucina* were observed at a pristine site with minimal human disturbance (First Creek: mean TP= 11 µg/L and mean TN= 0.36 mg/L). Overall, results suggest significant variations among the four stream sites with similar geographical locations. These results highlight the significance of stream-specific findings, where management actions based on broad scale studies may not be taking into account specific limnological features of individual streams. Methods applied in this study may be useful for identifying important stressors, indicator species and their spatial variations among sites.

Keywords: spatial variations, indicator species, self-organising maps (SOMs), gradient forest (GF), threshold indicator taxa analysis (TITAN)

1. Introduction

Understanding the spatial distribution of organisms at different spatial scales has become an important paradigm in river ecology (Kuemmerlen et al., 2019; Schmidt et al., 2020; Schweiger et al., 2005). River ecosystems are controlled by many factors, where small scale systems are determined by the larger-scale systems of which they are part (Feld and Hering, 2007). To date, numerous studies have highlighted the importance of different variables affecting stream biota at different spatial scales varying from catchment to reach or microhabitats in river ecosystems (Brosse et al., 2003; Townsend et al., 2003). Some studies also highlighted that indicators of biodiversity can vary between ecosystems ranging from site to catchment scale (Feld and Hering, 2007). Thus understanding the variations of indicator species and factors affecting the response of these species at stream-specific scale cannot be ignored, where localized actions can lead to larger-scale benefits.

Among variety of bioindicators used for monitoring freshwater ecosystems, diatoms find its unique position because of their quick response to any physical, chemical or biological change, rapid growth owing to short life cycle, ubiquitous nature, less habitat dependent than macroinvertebrates and well addressed taxonomy (Reid et al., 1995; Stevenson et al., 2010). Since change in focus of European Water Directive Framework (European Union, 2000) from use of single bioindicator to several bioindicators for assessing river health, a number of studies directed their focus from widely assessed macroinvertebrates to incorporate other bioindicators including diatoms (Grenier et al., 2010; Hering et al., 2006). In Australia, diatom thresholds have been assessed to complement previously addressed macroinvertebrate thresholds to improve the water quality guidelines in South Australia (Tibby et al., 2019).

Studies on diatom community responses have shown a wide variety of factors responsible for shaping diatoms community structure that varies between different regions. Depending on the study objectives and spatial resolution, different studies focussed on various factors affecting diatom assemblages including local habitat heterogeneity (Chen et al., 2019), environmental and spatial factors (Falasco et al., 2019), riparian vegetation (Hlúbiková et al., 2014), trophic levels (Marra et al., 2018), water quality (nutrients and pH) (Mirzahasanlou et al., 2020), latitudinal and altitudinal variation of temperature (Potapova and Charles, 2002). There is a strong evidence that diatoms distribution is affected by hydrological variables (Sun et al., 2018), water quality and nutrient load of river ecosystems (Dalu et al., 2017). A study in south-east Australia suggested that geographic position plays an important role in diatoms composition at subregional level (Philibert et al., 2006). Contrary to this, another broad scale study from south-east Australian rivers discussed insignificant relationship of geographical positions on diatoms species index (Chessman et al., 2007), although diatoms species index at finer scale was strongly correlated with catchment urbanization in suburbs of Melbourne (Chessman et al., 2007; Sonneman et al., 2001). Other studies highlighted significant differences in diatom assemblages due to variations in water quality factors particularly pH and salinity (Negus et al., 2019; Tsoi et al., 2017). While multiple ecological stressors affect diatoms community structure in different regions, focus of stressors affecting diatoms community is dependent on spatial scales and objectives of study.

According to revised Australian water quality guidelines (revised in 2018 and available as online platform: www.waterquality.gov.au/anz-guidelines), deriving a site-specific criteria is preferred to the default guidelines based on regional criteria. Thus, this study aimed to evaluate site-specific variations of bioindicator species in relation to water quality variables. Following research questions were addressed: 1. Which water quality variables are most important in driving stream-specific diatoms response? 2. Is there any distinct variability of water quality variables among streams? 3. What are the key indicator diatom species affected by important water quality variables? 4. Is there any distinct variability of indicator species among streams? Answer to these questions can be useful to understand stream-specific dynamics of hydrological and biological variables highlighting trends of indicator species.

2. Materials and methods

2.1. Study Area

Study area comprises of the four tributaries First Creek, Sixth Creek, Cox Creek and Aldgate Creek of the Torrens and Onkaparinga river catchments. Diatoms and water quality samples were collected from these perennial flowing streams having similar geographical positions but considerable different land use trends. First Creek and Sixth Creek were nearly pristine in the forested Torrens river catchment, however, Aldgate and Cox Creek belong to agricultural land cover sites with possible nutrients enrichment in Onkaparinga river catchment. These four streams have been monitored by South Australian Environmental Protection Authority for many years, where macroinvertebrates as bioindicators were of main concern. However, there is no record of any long term monitoring focussing on diatoms as bioindicators.

For diatoms sampling, an artificial substrate was used to collect samples in both autumn and spring of 2017–2019. About 100–200 cm lengths of identical polypropylene ropes with 5cm frayed ends were deployed at each site for a period of over 6 weeks in both seasons. Ropes were collected after 6 weeks and the frayed ends were cut and preserved to process in laboratory. Rope samples were treated with 25% hydrogen peroxide to remove organic material and then washed with distilled water in the laboratory. The diatom slurry was mounted on slides using Naphrax mounting medium. A total of 300 diatom valves were counted on each slide using microscope (Carl Zeiss Axio Scope.A1) at a magnification of 1000X. Diatoms were identified to lowest taxonomic level using reference keys (Krammer, 1986, 1988, 1991; Sonneman et al., 2000). Two years collected data were merged with another two years from previous sampling study from same sites by recounting the slides. Sampling protocol and water quality analysis were repeated in a similar way to maintain consistency. During recounting, *Achanthidium lanceolatum* was reported instead of *Planothidium lanceolatum* in the previous study from South Australia (Tibby et al., 2019), both are synonyms used for same species.

Physicochemical parameters i.e. pH, electrical conductivity, dissolved oxygen and water temperature were measured at each site where ropes were deployed using a multi-sensor data logger YSI 6820. For nutrients i.e. total phosphorus (TP), total nitrogen (TN) and nitrates, water samples were collected from same sites and further analysed in laboratory using standard American Public and Health Association methods (APHA, 2005b).

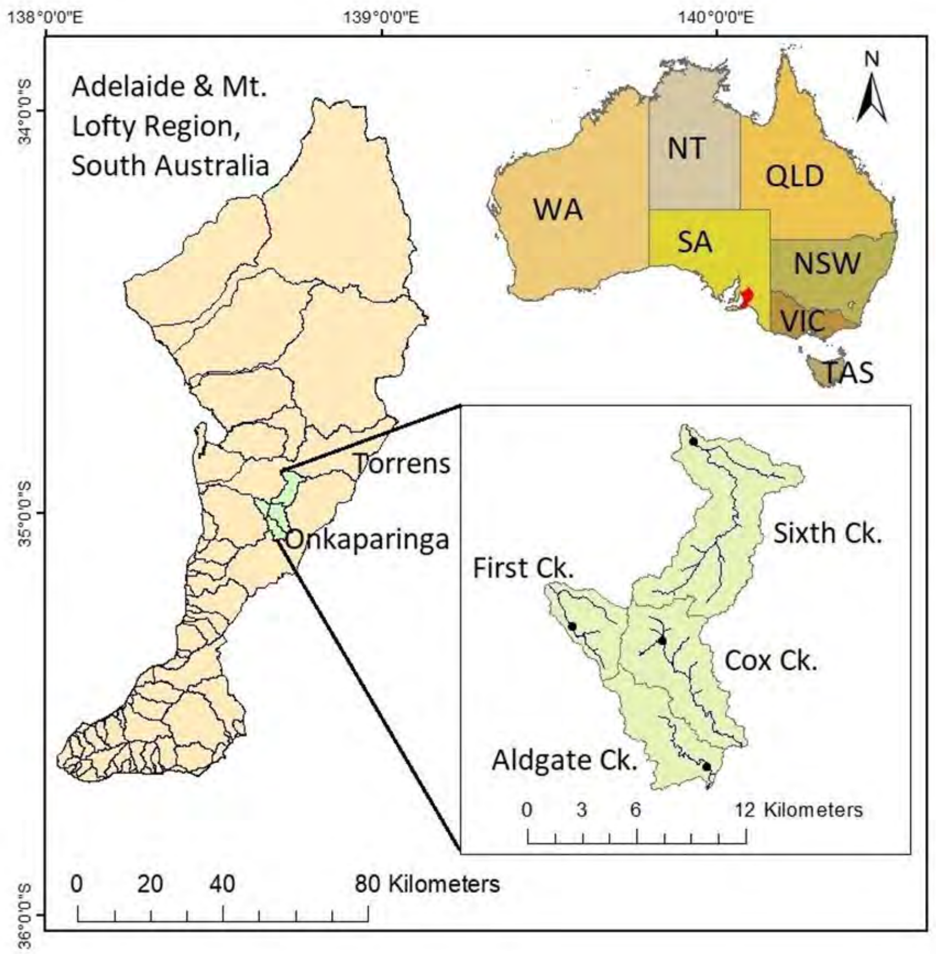

Figure 1: Location of sampling sites.

2.2.Data Analysis

Gradient Forest (GF) (Ellis et al., 2012) was used to identify most important stressors affecting diatoms community in these streams. GF is a novel, machine learning approach used to detect change in species responses along different environmental gradients (Ellis et al., 2012). GF is based on extended random forest (Breiman, 2001) approach using split function and bootstrap procedure to calculate predictors' importance and species response curves along gradients. The importance of each variable, expressed by the coefficient of variation is calculated for each predictor that is derived by maximizing the variance homogeneity for each subsequent split. As a result the following three outputs are provided: 1) R^2 weighted importance for each predictor; 2) Species turn over functions based on predictor splits and data density; 3) Overall compositional turnover based on individual species and the change in cumulative response. Gradient forest analysis was carried out using the R package “gradientForest” (Ellis et al., 2012). To meet the statistical requirements, percentage relative abundances were arcsine square root transformed.

Further change in species responses and identification of important indicator species was confirmed using thresholds indicator taxa analysis (TITAN). TITAN (Baker and King, 2010) distinguishes taxa responding positively or negatively to the specific predictor variable in terms of z scores. Change points or thresholds are detected when there is a synchronous change in the abundance of a number of taxa lying within a narrow range of the predictor variable. This method is based on IndVal (indicator values from species indicator analysis) (Dufrêne and Legendre, 1997) and incorporates a bootstrap procedure to find taxon responses that are pure and reliable. TITAN analysis was performed in the R package “TITAN 2” (Baker and King, 2010). Taxa with occurrences <3 were not considered following Baker and King’s (2010) recommended minimum criterion for TITAN.

For scope of this study, stream-specific thresholds were not considered and both methods were used only to identify important stressors and most responsive indicator species.

For spatial variations among sites, non-metric multidimensional scaling (NMDS) and self-organizing maps (SOMS) were applied. NMDS was used to assess the community composition based on environmental variables with the Bray-Curtis distance measure. Analysis of similarity (ANOSIM) was also used to assess significant difference among sites via the R-statistic to compare relative magnitudes of dissimilarity within grouped samples based on the rank dissimilarity. ANOSIM R-statistic values close to 1 indicate high dissimilarity among groups, while close to 0 indicate less dissimilarity. NMDS and ANOSIM was performed using PRIMER (v6.01). SOMs were used to assess spatial variation of important stressors and indicator species among streams. SOM approximates the probability of input variables through a nonsupervised learning algorithm and presents a non-linear projection of multivariate data in two-dimensional space (Kohonen, 2001). The clusters were obtained using the k-means method (Jain and Dubes, 1988). SOM analysis was performed in MATLAB using SOM toolbox (Vesanto et al., 1999).

3. Results

Summary statistics of water quality variables are given as Table 1 and 2. Overall, these four streams show TP enrichment (mean= 69.5 µg/L), whereby highest TP was contributed by Cox Creek (maximum= 326 µg/L and mean= 212.7 µg/L), highest TN levels were also observed at Cox creek (maximum= 5.46 mg/L and mean= 2.78 mg/L), followed by Aldgate Creek (Mean TP= 61.9 µg/L and TN= 0.84 mg/L). Sixth creek has shown only slight TP enrichment (mean= 36 µg/L), while first creek has shown no nutrient enrichment indicating minimal human disturbance.

For diatoms, thirteen most abundant species in decreasing order were: *Achanthidium minutissimum, Achanthidium oblongella, Cocconeis placentula, Achanthidium lanceolata, Rhoicosphenia abbreviata, Melosira varians, Nitzschia dissipata, Nitzschia inconspicua, Gomphonema angustum, Amphora pediculus, Navicula gregaria, Fragilaria capucina, Navicula veneta, Nitzschia palea.* The abundance trend of these species along TP gradient is shown in Figure 2. No significant trend of total abundance, richness and diversity indices (Shannon diversity index) was observed in relation to TP (Figure 2).

Table 1: Summary statistics of water quality variables collected from four streams (n=28), flow data was obtained from four respective gauging stations

Water quality variables	Description/Units	Min	Max	Mean	Median	ANZECC 2000
TotalN	Total nitrogen (mg/L)	0.08	5.46	1.02	0.6	1
TotalP	Total phosphorus (µg/L)	0.005	326	69.5	32	100
Temperature	(°C)	9.5	21.7	14.2	12.8	
EC	Electrical conductivity(µS/cm)	150	753	434.8	435.0	100-5000
DO	Dissolved oxygen (mg/L)	2.04	10.95	7.43	8.2	
pH	pH units	6.65	8.35	7.81	7.86	6.5-9.0
ASF	Average seasonal flow (m^3/s)	0.91	62.44	10.57	3.35	
ZFDs	No. of zero flow days	0	176	37	2	

ANZECC 2000 refers to Australia and New Zealand Environment and Conservation Council water quality guidelines.

Table 2: Summary statistics of water quality variables from each stream.

	Sixth Creek				First Creek				Cox Creek				Aldgate Creek			
Water quality variables	**Min**	**Max**	**Mean**	**Median**	**Min**	**Max**	**Mean**	**Median**	**Min**	**Max**	**Mean**	**Median**	**Min**	**Max**	**Mean**	**Median**
TotalN (mg/L)	0.20	0.99	0.45	0.35	0.08	0.64	0.36	0.33	0.94	5.46	2.78	2.78	0.51	1.29	0.84	2.78
TotalP (µg/L)	7.0	36.0	21.0	19.5	5.0	15.0	10.6	11.0	66.0	326.0	212.7	220.5	30.0	92.0	61.9	220.5
Temperature (°C)	9.52	20.77	14.71	14.85	10.20	21.70	14.87	12.70	11.60	18.06	13.84	13.51	10.30	20.40	13.32	13.51
Conductivity (µS/cm)	472.0	753.0	623.0	689.0	150.0	420.0	300.4	345.0	276.0	685.0	431.3	417.5	196.0	506.0	357.0	417.5
Dissolved Oxygen (mg/L)	2.04	10.95	7.34	8.00	4.78	10.17	8.27	8.30	3.44	8.59	7.08	8.05	2.60	10.33	6.98	8.05
pH	7.56	8.35	7.99	7.99	7.66	8.09	7.88	7.90	6.65	7.85	7.46	7.51	7.20	8.18	7.83	7.51
ASF	2.17	62.44	23.29	21.45	2.51	8.36	4.46	3.04	0.91	17.02	4.35	1.36	1.22	31.69	7.46	1.36
ZFDs	0	176	50	12	0	35	6	0	0	7	1	0	20	117	84	0

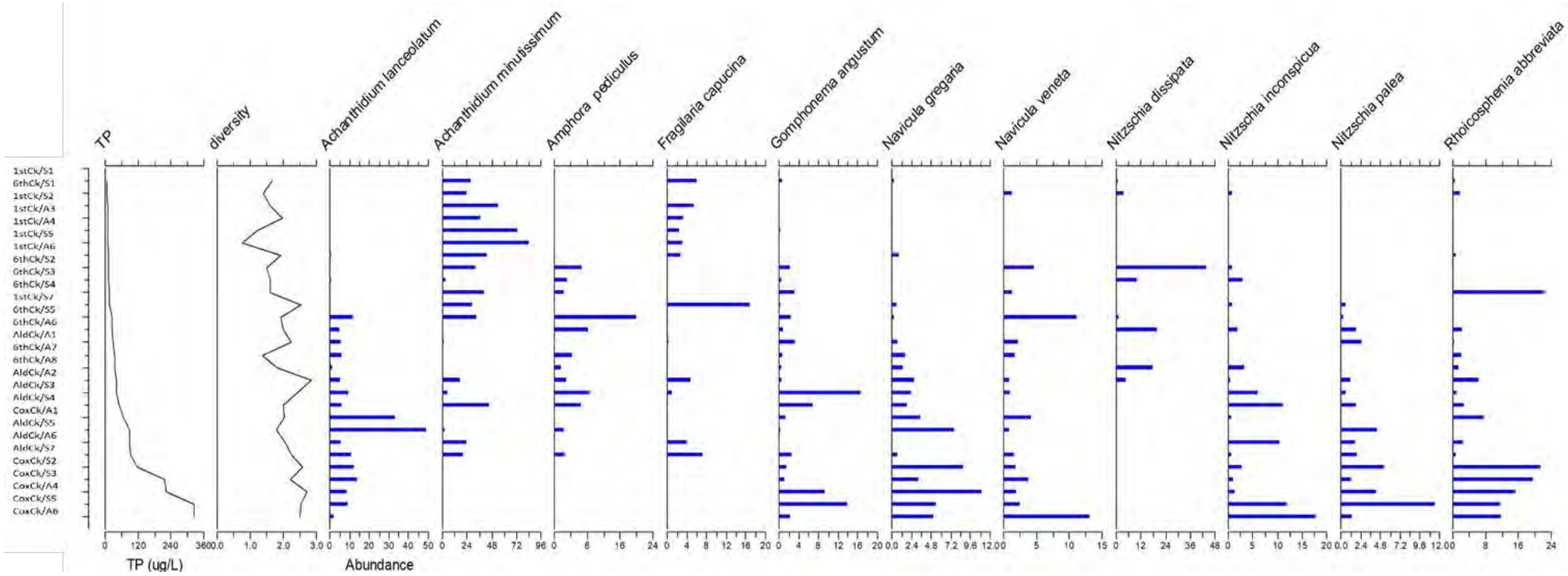

Figure 2: Abundance trend of 11 most abundant species (total abundance > 50%) along total phosphorus gradient.

3.1.Important variables affecting diatom assemblages and spatial variations

GF identified water quality variables i.e. TP and EC as the most important stressors affecting diatom assemblages (Figure 3). However other hydrological variables i.e. average seasonal flows (ASF) or number of zero flow days (ZFDs) were less important in driving diatoms responses of these perennial flowing streams (Figure 3). Further characterization of these streams has shown that diatom species responses at Sixth Creek are driven by conductivity and ASF, while species response at Cox Creek is affected the most by nutrients enrichment (Figure 4).

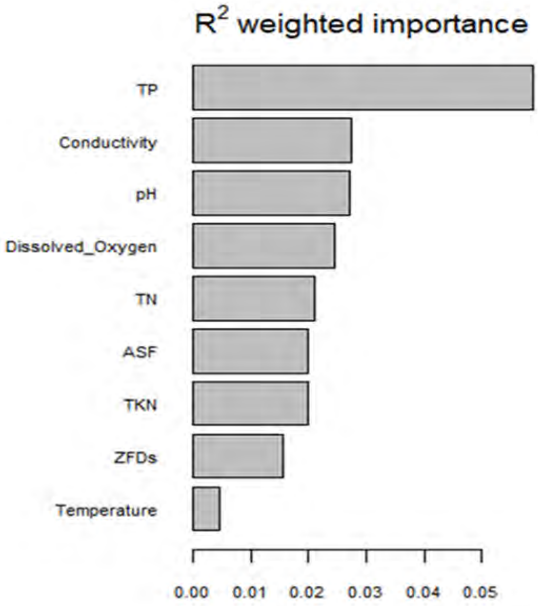

Figure 3: Relative importance of water quality and hydrological variables affecting diatom assemblages. Explanation of variables is given in Table 1.

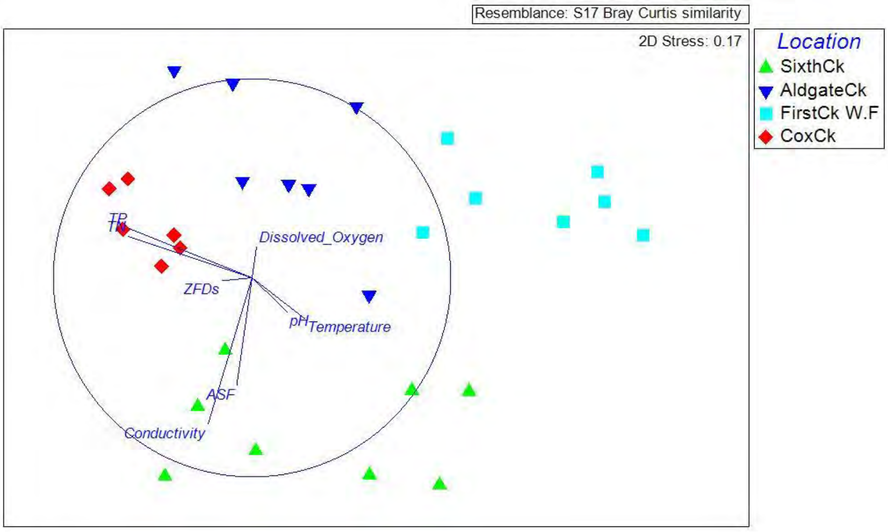

Figure 4: Nonmetric multidimensional scaling plots showing grouping among sites.

All the four sites have shown significant dissimilarity based on pairwise ANOSIM test with R = 0.77 and level of significance p= 0.1%. Highest dissimilarity was observed between Cox Creek and First Creek (R= 0.99 and p=0.2%). All the remaining site pairs have shown distinct dissimilarity with high level of significance <=0.2% (Table 3).

Table 3: ANOSIM results for dissimilarity among four streams. p values showing level of significance in percentage

Groups	R	p (%)	Possible permutations	Actual permutations
Sixth Ck, Aldgate Ck	0.663	0.1	6435	999
Sixth Ck, First Ck	0.787	0.1	6435	999
Sixth Ck, Cox Ck	0.689	0.1	3003	999
Aldgate Ck, First Ck	0.841	0.1	1716	999
Aldgate Ck, Cox Ck	0.716	0.1	1716	999
First Ck, CoxCk	0.991	0.2	1716	999

3.2.Spatial variations of indicator species in relation to important variables

TITAN identified indicator species with magnitude and direction of change in responses to TP and EC gradients (Figure 5). TTIAN identified some tolerant species as z (+) and sensitive as (z-) based on z scores.

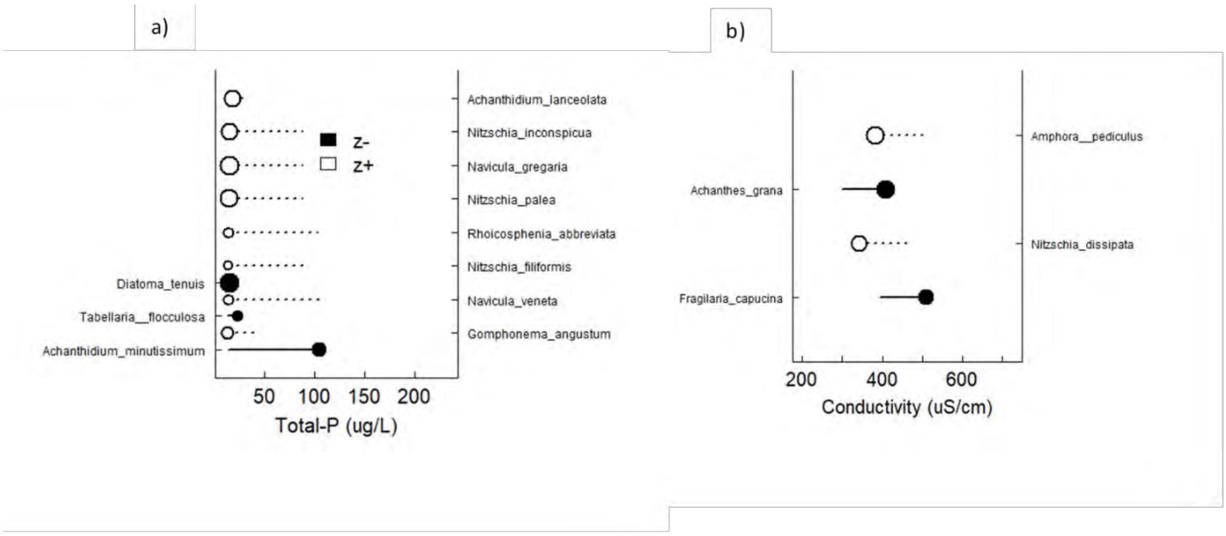

Figure 5: TITAN output with (z-) and (z+) taxa along: a) Total-P gradient and b) electrical conductivity gradient

Figure 6 shows different distributions on the SOMs according to the environmental preferences of indicator species identified by TITAN. Indicator species with highest abundance were considered for spatial variation trends. SOMs identified high to medium abundance of *Achanthidium lanceolatum, Gomphonema angustum, Navicula* *gregaria, Navicula veneta, Nitzschia inconspicua, Nitzschia palea, Rhoicosphenia abbreviata* found at sites of nutrient enrichment i.e. Cox Creek and Aldgate Creek. All these species correspond with the positive responding taxa (z+ species) identified by TITAN. Among *Nitzschia, Nitzchia inconspicua, Nitzschia veneta* and *Nitzschia palea* has shown medium to high abundance at this site. In contrast, lowest abundance of *Nitzschia dissipata* was observed at Cox Creek characterised by highest nutrient enrichment, but found in highest abundance at Sixth Creek (Figure 6).

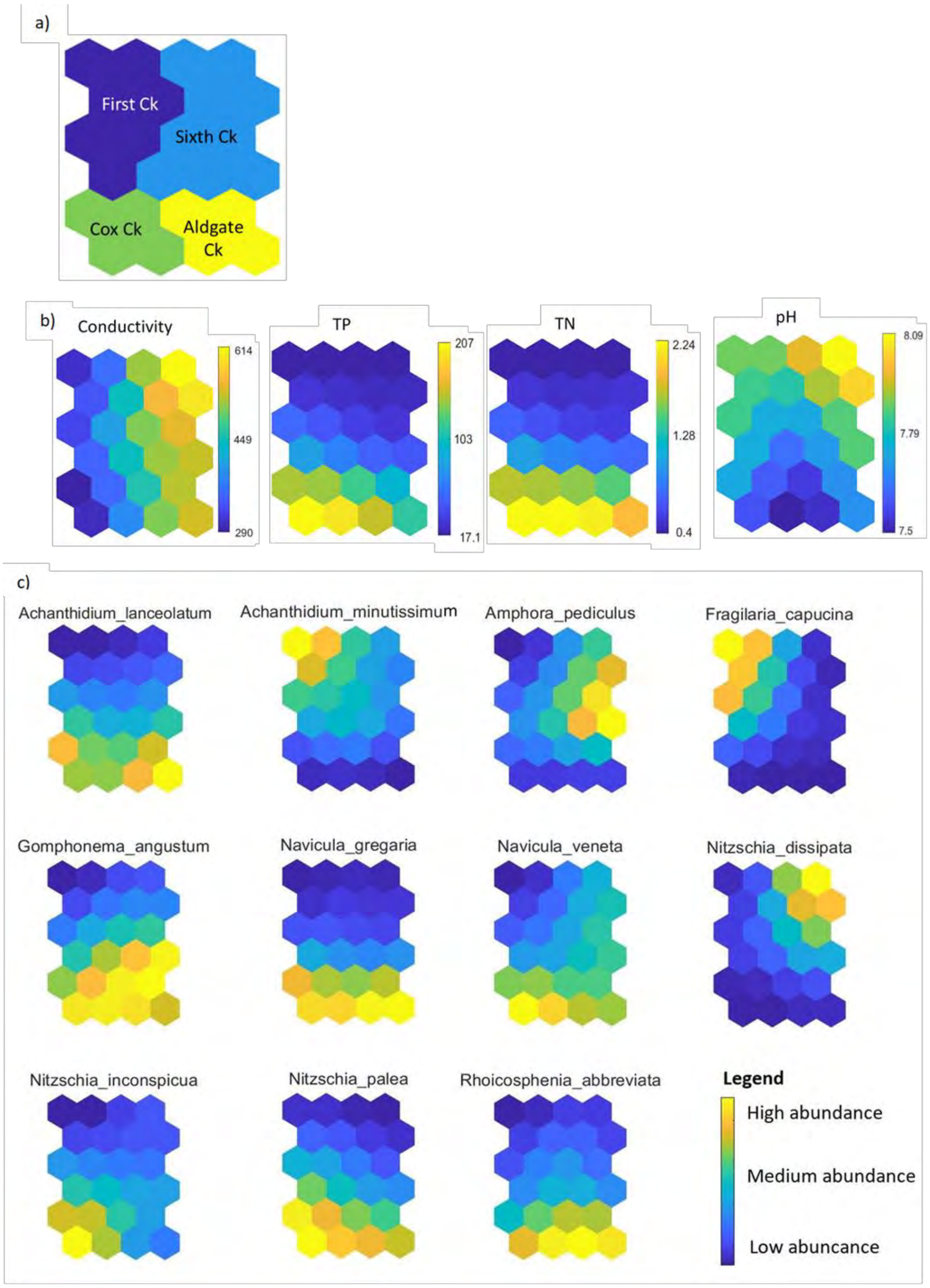

Figure 6: Self-organising maps showing clustering of sites (a) and spatial variability of b) water quality variables and c) indicator species among 4 streams.

4. Discussion

A distinct variability in water quality variables was observed among the studied perennial flowing streams, indicating impacts from different land uses. In impacted sites, TP enrichment is most important factor driving diatom species responses. In contrast, another site with only slight TP enrichment, was driven by conductivity and average seasonal flows. The fact that Cox Creek exhibited highest nutrient concentrations as compared to other sites from the same geographical area is due to differential land use changes in the subcatchment, pertaining to agricultural activities (Shrestha et al., 2017). However, Sixth Creek and First creek are forested subcatchments categorised as near pristine. This study has shown slight TP enrichment at Sixth Creek, however water quality at First Creek exhibited minimal signs of human disturbance. Importance of total phosphorus in explaining diatoms assemblage composition has been universally found in number of studies from different regions of the world ranging from big data sets across United States (Becker et al., 2018; Lee et al., 2019), eutrophic boreal lakes (Kauppila et al., 2002) and lakes of China (Wang et al., 2019; Yang et al., 2008) leading to widely studied diatom-inferred TP models (Juggins et al., 2013; Ponader et al., 2007; Tibby, 2004). Other studies from South Australia using diatoms as bioindicators also focussed on TP and conductivity for deriving ecological thresholds (Tibby, 2004; Tibby et al., 2019).

Distinct spatial variations of diatom indicator species was observed in concordance with water quality variables. Our results indicate that although diatom species responses were coherent to water quality variables (mainly TP and conductivity), but distribution pattern varied considerably among sites from same geographical area. Diversity indices (Shannon-diversity index), total abundance and richness varied considerably along TP gradient but we were unable to draw any ongoing trend, which is usual for such studies due to nonlinear behaviour of multiple species that contribute towards these univariate indices. Among indicator species identified by TITAN, most of the positive responding (z+) taxa in relation to TP were found at Cox Creek and Aldgate Creek e.g. *Achanthidium lanceolatum, Gomphonema angustum, Navicula gregaria, Navicula veneta, Nitzschia inconspicua, Nitzschia palea* and *Rhoicosphenia abbreviata*. Among these tolerant species, *Nitzschia inconspicua* has shown affinity only to Cox Creek by showing highest abundance whereas *Achanthidium lanceolatum, Gomphonema angustum, Navicula veneta* and *Nitzschia palea* has also shown medium to low abundance at other sites, showing their ubiquitous nature. In another study from South Australia at broad spatial scale, *Nitzschia inconspicua* has also exhibited positive response to increase TP and EC levels (Tibby et al., 2019). *Nitzschia inconspicua* has been found tolerant to range of salinity levels and organic or nutrient pollution and suggested as potential bioindicator of human disturbance in streams (Rovira et al., 2015; Sonneman et al., 2000). *Nitzschia dissipata* has shown high abundance to the site with only slight TP enrichment. However, high abundance of *Fragilaria capucina* found only in pristine water conditions show its sensitivity to pollution loads. Different varieties of *Fragilaria* has shown a negative response to TP and EC in South Australian streams (Tibby et al., 2019). Thus, a suite of positive and negative responding species and their abundance trends vary among different sites.

Findings from this study reflect unique dynamics of every site. For example, among forested subcatchments with similar land uses, Sixth Creek has shown slight TP enrichment and high abundance of specific indicator species, which is different to another site with similar land use. However, another site with highest nutrient enrichment was reflective of agricultural land use trends in the area. We recommend adaptive management strategies for individual sites from same geographical area but different ecological status. Understanding the unique dynamics of each site with differential land use trends can be useful to predict important indicator species. Depending on unique characteristics of site, not only land use but also other factors like riparian zone vegetation and habitat heterogeneity should be considered. We recommend to extend such monitoring efforts to generate baseline data for deriving site-specific thresholds, which may vary from one stream to another stream. Future work will compare and evaluate site-specific thresholds with broad scale sampling.

5. Conclusions

By evaluating response of stream-specific diatom assemblages and their spatial distribution in relation to water quality variables, following conclusions can be made:

- TP enrichment was the most important stressor driving diatoms responses in nutrient enriched perennial flowing streams, however in less impacted streams diatom species responses were affected by conductivity and flow.
- Highest TP and TN enrichment was observed in a stream with intense agricultural activities.

- Abundance trend of indicator species vary among streams with similar land use trends and geographical positions.

Results from this study highlighted the importance of stream-specific response of diatom species. Future work will focus on that how site-specific findings can be extrapolated in a broader regional scale context.

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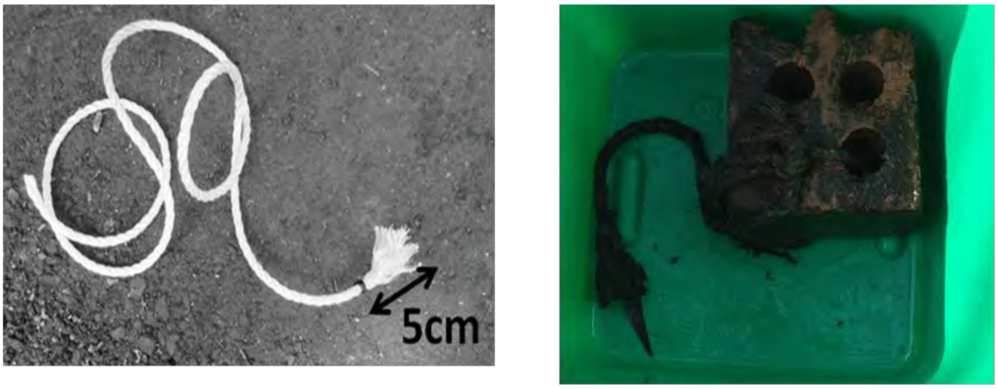


a) b)

Rope substrate used for diatoms collection; a) before deploying; b) six weeks after deploying

Diatoms substrate collection from First Creek, River Torrens catchment

Sampling location at Aldgate Creek, Onkaparinga River catchment

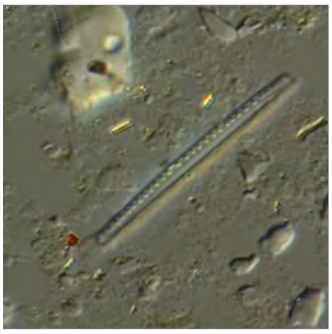
Surface water run-off near sampling location at Cox Creek, Onkaparinga River catchment

Achanthidium lanceolatum

Achanthidium minutissimum

Amphora pediculus

Gomphonema angustum



Nitzschia filiformis

Navicula gregaria

Navicula veneta

Nitzschia inconspicua

Rhoicosphenia abbreviata

Tabellaria flocculosa (Girdle view)

Some of the threshold indicator diatom species identified (at 1000X magnification) from the study sites.