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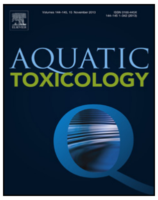

Separating the effects of water physicochemistry and sediment contamination on *Chironomus tepperi* (Skuse) survival, growth and development: A boosted regression tree approach

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ABSTRACT

More comprehensive ecological risk assessment procedures are needed as the unprecedented rate of anthropogenic disturbances to aquatic ecosystems continues. Identifying the effects of pollutants on aquatic ecosystems is difficult, requiring the individual and joint effects of a range of natural and anthropogenic factors to be isolated, often via the analysis of large, complicated datasets. Ecotoxicologists have traditionally used multiple regression to analyse such datasets, but there are inherent problems with this approach and a need to consider other potentially more suitable methods.

Sediment pollution can cause a range of negative effects on aquatic animals, and these are used as the basis for toxicity bioassays to measure the biological impact of pollution and the success of remediation efforts. However, experimental artefacts can also lead to sediments being incorrectly classed as toxic in such studies. Understanding the influence of potentially confounding factors will help more accurate assessments of sediment pollution.

In this study, we analysed standardised sediment bioassays conducted using the chironomid *Chironomus tepperi*, with the aim of modelling the impact of sediment toxicants and water physico-chemistry on four endpoints (survival, growth, median emergence day, and number of emerging adults). We used boosted regression trees (BRT), a method that has a number of advantages over multiple regression, to model bioassay endpoints as a function of water chemistry, sediment quality and underlying geology. Endpoints were generally influenced most strongly by water quality parameters and nutrients, although some metals negatively influenced emergence endpoints. Sub-lethal endpoints were generally better predicted than lethal endpoints; median emergence day was the most sensitive endpoint examined in this study, while the number of emerging adults was the least sensitive. We tested our modelling results by experimentally manipulating sediment and observing the impact on *C. tepperi* endpoints. For survival, experimental observations were accurately predicted by models, which highlighted the importance of conductivity and dissolved oxygen for this endpoint. In comparison, experimental median emergence day was poorly modelled, most likely due to the influence of a wider range of predictors identified as being important influences on this endpoint in models. To demonstrate how BRT model results compare to more traditional techniques, we analysed survival data using multiple regression. Both models yielded similar results, but boosted regression trees offer important advantages over multiple regression. Our results illustrate how boosted regression trees can be used to analyse complex ecotoxicological datasets, and reinforces the importance of water chemistry in sediment toxicology.

1. Introduction

Sediments provide important habitat for many aquatic organisms but they can also act as sinks for pollutants (Ingersoll et al., 1995; Townsend et al., 2009). Sediment pollution remains a serious concern in many areas of the world, and may affect aquatic organisms through decreased growth, survival and fecundity, delayed development and morphological deformities (de Bisthoven et al.,

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1998; Ford et al., 2003; Marshall et al., 2010; Pascoe et al., 1989; Townsend et al., 2009). Given that sediments can be sources for environmental toxins from which they dissolve after contamination in a given area has ended, sediment pollution can be a serious, and on-going environmental problem. Toxicity bioassays with benthic invertebrates exploit the negative effects on aquatic organisms to evaluate the biological significance of sediment-associated contamination (Burton, 1992), and such tests have become an essential tool in establishing acceptable concentrations of pollutants in both marine and freshwater sediments (ANZECC/ARMCANZ, 2000; MacDonald et al., 2000).

Assessing the effects of sediment pollution on aquatic ecosystems is a difficult task and requires the individual and combined effects of a range of natural and anthropogenic variables to be disentangled, often from within complex datasets. Traditionally, the effects of predictor variables have been separated using multiple regression followed by stepwise selection of important variables (Preston and Shackelford, 2002; Vanhattum et al., 1991), but these methods can produce spurious results due to collinearity between predictors (Mac Nally, 2000, 2002). There are also other potential drawbacks with using stepwise multiple regression, for example: biases in parameter estimation, inconsistencies in algorithms for model selection (e.g. influence of order and number of parameters entered), and an inappropriate reliance on finding the single 'best' model (Whittingham et al., 2006). Although the limitations of multiple regression for isolating the effects of multiple predictor variables are well-documented and accepted within the statistical literature (see Whittingham et al., 2006 and references within), the use of outdated analytical methods persists in ecotoxicology (Fox, 2010; Newman, 2008). As ecotoxicology moves towards more comprehensive ecological risk assessment procedures, better analytical tools are needed, particularly for analyses of large, complex datasets with many predictor variables (Delignette-Muller et al., 2011; Forfait-Dubuc et al., 2012; Fox, 2010; Newman, 2008).

In Australia, the endemic *Chironomus tepperi* (Diptera, Chironomidae) is commonly used for standardised sediment toxicity bioassays (following OECD and ASTM guidelines), and is sensitive to a wide range of toxicants commonly found in sediments (e.g. Choung et al., 2013; Phyu et al., 2005; Stevens, 1992; Stevens et al., 2004). Dipterans from the Family Chironomidae are particularly popular test organisms for freshwater sediment toxicity bioassays, due to the fact that they are diverse and abundant, can vary in terms of their sensitivity to environmental stressors, spend a considerable period of their life cycle in direct contact with sediment and also are relatively easy to culture and handle (Carew et al., 2007). Both acute (e.g.: survival to 4th instar, successful metamorphosis to adult) and chronic (e.g.: mean length of 4th instar larvae, development speed) endpoints are commonly used and have been illustrated to respond to contaminated sediments.

In this study, we compare the sensitivity of four commonly used endpoints (growth, survival, median emergence day and the proportion of larvae to develop into adults) incorporating potentially acute and chronic responses of *C. tepperi* to water quality parameters, water nutrients, geology and metals. Our aim was to examine the response of *C. tepperi* to metal pollution, and also to identify factors that could hinder our ability to detect these effects. Adverse effects on test organisms due to factors other than anthropogenic contaminants may result in sediments incorrectly being classed as toxic. These "false positive" results may be caused by a wide range of experimental artefacts such as the physicochemical characteristics of the test sediment (Ankley et al., 1994), sediment grain size (DeWitt, 1998), and the physicochemistry of the overlying water (ASTM, 1997; OECD, 2010).

We used boosted regression tree (BRT) models to examine potential relationships between each endpoint and predictor variables from a large dataset (>200 observations for each endpoint). Unlike traditional statistical approaches where a single parsimonious model is fitted, BRTs are an ensemble method whereby many simple models are combined to improve model performance (i.e. Boosting), using recursive binary splits to relate responses to predictor variables (i.e. Regression trees) (Elith et al., 2008). BRTs are robust to variable collinearity, variable outliers, and missing data, and can include both categorical and continuous variables. While their use has become increasingly popular in other fields, especially ecology (e.g. Elith et al., 2008; Leathwick et al., 2008; Perry et al., 2012), to our knowledge the only application of BRTs in the context of ecotoxicology was a recent assessment of the responses of bacteria to pollutants in estuaries (Sun et al., 2012). While BRTs have a number of obvious advantages, utilising these requires a shift in thinking from traditional statistical techniques (Elith et al., 2008). For example, combining many simple models to improve model performance differs from identifying one 'best' model containing few parameters. In comparison to conventional regression models, BRTs focus on predictive accuracy rather than *p*-values to indicate the significance of model coefficients. However, BRT models can be easily run within the free software package R, and there is a number of recent papers that discuss in detail the rationale behind and advantages of this approach, and provide excellent guides on how to undertake analyses (Elith et al., 2008; Leathwick et al., 2006, 2008). For one endpoint (survival), we also analysed data using multiple regression to act as a point of comparison between the two methods.

While BRT models offer promise as a flexible analytical tool, their performance in ecotoxicology has not been experimentally validated. To assess their potential utility and application, we validated our models by exposing *C. tepperi* to a range of manipulated conductivities in reference sediment and observed its effect on two endpoints. We hypothesised that endpoints strongly affected by conductivity in field sediments (i.e.: survival) would show similar responses in artificially manipulated sediments, while for endpoints not strongly affected by water conductivity (i.e.: median emergence day), no such response should be observed. The second main aim of this study was to highlight some of the potential advantages of boosted regression trees as a highly flexible analytical tool, and to encourage the use of more powerful analytical approaches in the ecotoxicological research community.

2. Materials and methods

2.1. Field methods

Sediment was collected from 203 sites located throughout much of the state of Victoria, Australia, sampled as part of on-going work (between 2009 and 2012) undertaken by researchers at the Centre for Aquatic Pollution and Identification (CAPIM) at the University of Melbourne (see Supplementary Table 1 for details of the sampling regime). Site geology was classified into three broad groups (sedimentary, igneous and metamorphic) based on the Geology of Australia GIS layer (www.ga.gov.au). Sediments were collected using a dip net or shovel from the surface (approximately top 2 cm) and filtered (<64 μm nylon sieve) to remove coarser particles (ANZECC/ARMCANZ, 2000). Filtered sediment was returned to the laboratory in rinsed polypropylene buckets, with supernatant water decanted the following day, and settled sediment stored in nitric acid and acetone treated jars at 4 °C until analyses and bioassays. All sediments were analysed for metals, total organic carbon, total Kjeldahl nitrogen and total phosphorus by a commercial laboratory (ALS Laboratories, Melbourne: www.alsglobal.com) using standard methods.