CRANFIELD UNIVERSITY

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Towards a conceptual model of the impact zone ecology in rivers

School of Environment, Energy and AgriFood

MSc by Research
Academic Year: 2014

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ABSTRACT

In many regions of the world, untreated wastewater is discharged directly into rivers containing sanitary determinands including ammonia, nitrite and organic matter which places a demand on dissolved oxygen in the water. The wastewater may also contain chemical ingredients of home and personal care products. When sewage treatment is lacking, often in developing regions, these sanitary determinands and down-the-drain chemicals may be present at high concentrations in surface waters which may adversely impact the ecological communities present downstream of the effluent outfall. Some studies have studied these ecological effects by sampling the taxa present at regular intervals downstream of an wastewater outfall, from which a common pattern in terms of macroinvertebrate species richness, dominance and diversity throughout the impact zone is evident. The aim of the project was to develop a conceptual model in order to predict the ecological composition downstream of an effluent outfall, as a result of multiple stressors' concentration gradients. The model combines water quality data and toxicity data of the stressors on aquatic organisms, in the form of species sensitivity distributions (SSDs) to predict this impact. The model was based on selected stressors: ammonia, nitrite and dissolved oxygen which are present, in particular, in untreated wastewater; and two chemical ingredients used in home and personal care products which are washed down-the-drain. The model was applied to data from a field study on the South Elkhorn Creek in Kentucky, USA. Predicted effects on taxonomic composition were in line with field observations, although further enhancements to the model could incorporate more environmental realism. This was a useful step in the direction to creating a conceptual model of the impact zone ecology in rivers.

Keywords:

Species sensitivity distributions (SSDs), predictive modelling, untreated wastewater, species richness, traits.

ACKNOWLEDGEMENTS

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STP	Sewage Treatment Plant
PEC	Predicted Environmental Concentration
PNEC	Predicted No-Effect Concentration
NH_3	Unionised ammonia
NO_2^-	Nitrite
NO_3^-	Nitrate
HPC	Home and Personal Care
BOD	Biochemical Oxygen Demand
DO	Dissolved Oxygen
SSD	Species Sensitivity Distribution
PAF	Potentially Affected Fraction
CDF	Cumulative Frequency Distribution
EQC	Environmental Quality Criteria
HC_5	Hazard Concentration at which 5% of the population are adversely affected
msPAF	Multi-Substance Potentially Affected Fraction
SPEAR	SPEcies At Risk
CA	Concentration Addition
RA	Response Addition
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
TU	Toxic Units
NOEC	No Observed Effect Concentration
DF	Dilution Factor
TCS	Triclosan
LAS	Linear Alkyl Benzene Sulphonate
OD	Oxygen Deficit
TOC	Total Organic Carbon
USGS	United States Geological Survey

1 INTRODUCTION

1.1 Context

The chemical ingredients used in many domestic cleaning and personal care products (e.g. laundry detergents, surface cleaners, soaps, shampoos, etc.) and in pharmaceuticals often end up in wastewater. If the wastewater is treated in a sewage treatment plant (STP), the concentrations of these chemicals can be significantly reduced. However, the emission of untreated wastewater still occurs. The risks posed by these "down-the-drain" chemicals are assessed by conducting an environmental risk assessment in which the predicted environmental concentration (PEC) is compared to the predicted no-effect concentration (PNEC), which is derived from laboratory ecotoxicity studies. If the PEC is greater than the PNEC, the chemical is considered to pose a risk to the ecosystems of receiving water bodies. This "conventional" risk assessment method works well where there is sewage treatment. However, where wastewater is emitted into surface waters without treatment which is common practice in many regions of the world, the concentrations of other substances may be high enough to cause severe ecological effects. These substances may include "sanitary determinands" (e.g. ammonia (NH_3), nitrite (NO_2^-), organic carbon). The presence of these sanitary determinands poses a challenge for conventional risk assessment of the chemicals, as they may be at concentrations high enough to cause toxicity to aquatic organisms with the impact of the chemicals unknown (Finnegan *et al.*, 2009). However, it is not clear which components of this mixture of stressors cause toxicity to particular organisms.

The "impact zone" is a term used to describe the length of a river downstream of an effluent outfall in which concentrations of sanitary determinands are high enough to cause toxicity to organisms present. There are potential impacts on individuals, species, diversity, community structure and ecological function. A resulting main hypothesis is that after the end of the impact zone the biological community will recover to its original composition and structure (Finnegan *et al.*, 2009). The chemicals

in the discharge may have varying effects throughout the impact zone depending on their degradation rates and toxicity to aquatic organisms.

The chemicals considered in this research project are present in home and personal care (HPC) products which are washed down the drain after use and disposal by consumers. Chemicals in HPC products should not inhibit the natural recovery (sometimes called self-purification) of the river (McAvoy *et al.*, 2003) and should not cause toxic effects downstream after the end of the impact zone. The emissions of sanitary determinands and HPC chemicals occur continuously over time. Hence any adverse impact is likely to be chronic (Mason, 2002). It is, however, not practical to generate field data for every chemical across multiple river systems. Therefore a modelling approach to predict the ecological structural changes in the impact zone would be beneficial.

1.2 Literature review

There are just a few sources in the literature which report changes in ecological composition, immediately downstream of wastewater effluents on a scale relating to effects on the biological community (Birge *et al.*, 1989; Avery, 1970; Ortiz *et al.*, 2005).

1.2.1 The Impact Zone

The impact zone is the length of river between an initial site of impact (e.g. untreated wastewater discharge) and the point where the biological community recovers to an expected composition had the sewage not been discharged (McAvoy *et al.*, 2003; Limlette III Workshop, 1995). In the absence of sewage treatment, the impact zone is typically exposed to high levels of stressors which include suspended solids, biochemical oxygen demand (BOD) which depletes dissolved oxygen (DO) levels, NO_2^-, NH_3 and synthetic organic chemicals present in the effluent (Finnegan *et al.*, 2009). Self-purification occurs in the impact zone via a number of processes, including biodegradation, nitrification, volatilization, sorption and settling. It can even be thought of as a sort of natural sewage treatment facility where a sufficient oxygen concentration is required for biodegradation to occur (McAvoy *et al.*, 2003).

Anthropogenic chemicals should not inhibit this recovery process (Finnegan *et al.*, 2009).

1.2.1.1 Nitrogenous compounds in the impact zone

In the impact zone, the concentration of ammoniacal nitrogen (NH_4^+ + NH_3) peaks shortly after discharge while the NO_2^- concentration is low. NO_2^- concentration then increases downstream as ammonium is nitrified by bacteria such as *Nitrosomonas* species (Figure 1-1) (McAvoy *et al.*, 2003; Jensen, 2003). Further nitrification of NO_2^- is mediated by other bacteria such as *Nitrobacter* species to nitrate (NO_3^-) (Jensen, 2003). This causes the concentration of NO_2^- downstream to decrease (Finnegan *et al.*, 2009).

$$\mathrm{NH_3} \leftrightarrows NH_4^+ \xrightarrow{Nitrosomonas\ spp.} NO_2^- \xrightarrow{Nitrobacter\ spp.} NO_3^-$$

Figure 1-1 Nitrification of ammonia.

Within the impact zone a number of changes occur as a result of changing concentrations of components present in the effluent (Figure 1-2). With the introduction of degradable organic effluent, the demand on oxygen (BOD) due to biodegradation increases, thereby reducing the concentration of dissolved oxygen. Concentrations of NH_3 and NO_2^- are linked: as NH_3 is oxidised, NO_2^- forms, thereby causing them to peak at different distances downstream. Concentrations of organic chemicals (discounting metabolites) peak at the beginning of the impact zone and steadily decrease downstream as they are degraded. Stressor degradation rates may be influenced by the concentration of oxygen, temperature, the presence of competent micro-organisms and chemical-specific properties (e.g. susceptibility of bonds to cleavage by enzymes).

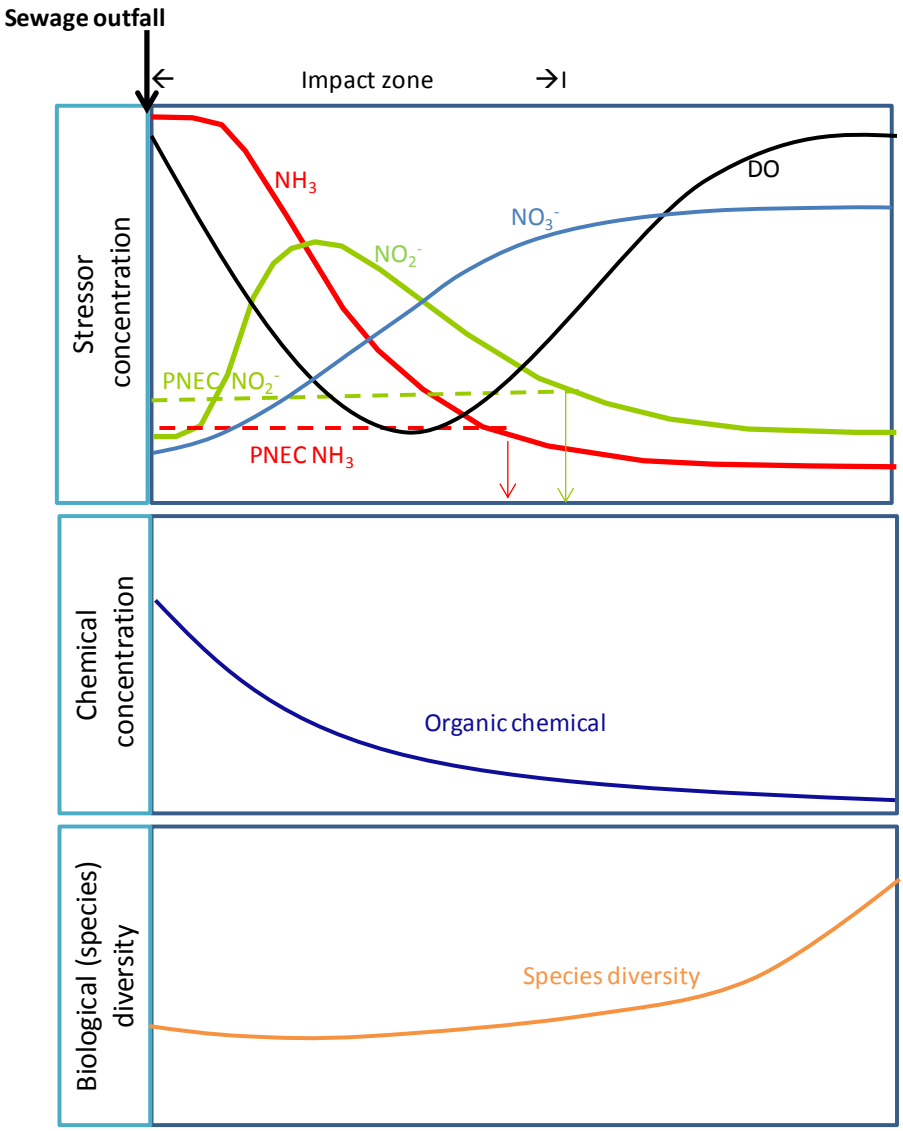


Figure 1-2 Stressor concentration, chemical concentration and species diversity changes in the impact zone, adapted from Finnegan *et al.*, 2009; Whelan *et al.*, 2007;(2007) Dyer *et al.*, 2003.

Although called the impact zone in more recent literature, after the term was introduced at the Limlette III Workshop (Whelan *et al.*, 2007; Finnegan *et al.*, 2009; McAvoy *et al.*, 2003; Limlette III Workshop, 1995), this concept has existed for quite some time. Gaufin and Tarzwell (1956), for example, classified three zones in Lytle Creek in Ohio, USA, following a sewage outfall: clean water zone, septic zone and the zone of recovery. This was based primarily on the concentration of DO along the system (Gaufin and Tarzwell, 1956). Even earlier work by Richardson in 1928 on the

Illinois River, in Illinois, USA, produced ideas on zones of degradation and recovery in terms of the impacts of organic pollution on the macroinvertebrate community (Hynes, 1994).

1.2.1.2 Oxygen in the impact zone

Oxygen is essential for the survival of many aquatic species. It enters the water course mainly by diffusion from the air. The DO concentration is influenced by temperature, current speed, turbulence, turbidity (suspended solids), groundwater, in-stream vegetation and altitude (Giller and Malmqvist, 1998). At higher temperatures, oxygen molecules have more energy and tend to exist preferentially in the gas state so the saturation concentration of oxygen is lower. Figure 1-3 shows the relationship between the saturation concentration (equilibrium) of DO and temperature.

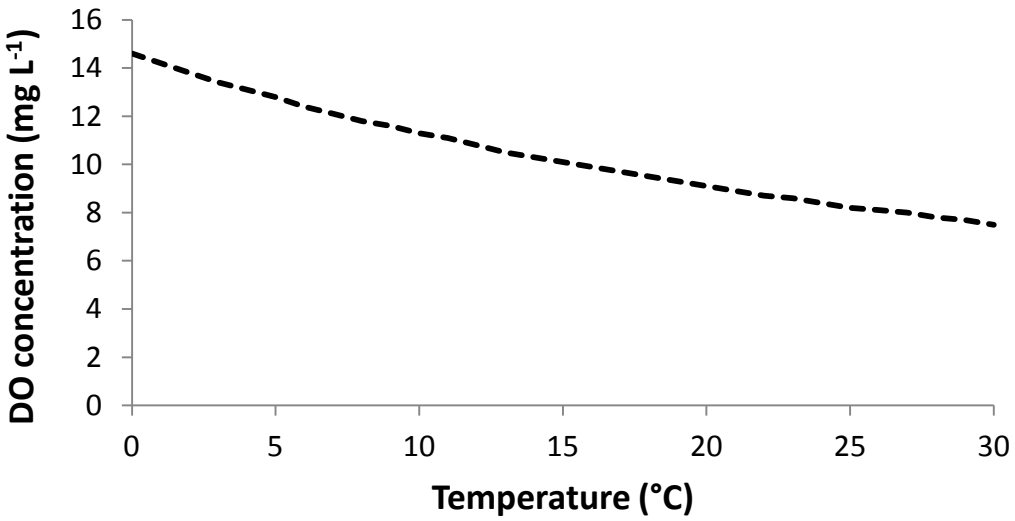


Figure 1-3 Relationship between DO saturation concentration and temperature. Values are based on air pressure of 1 atmosphere and are taken from Gray (1999).

In the impact zone, DO concentration can fall or "sag", as the BOD is high with aerobic microbes breaking down organic matter (Figure 1-2). The extent of the oxygen sag, is dependent on a balance between oxygen consumption (i.e. the rate at which BOD is reduced) and reaeration (i.e. the rate at which DO can be replaced in the water). Both sets of processes are affected by temperature, dilution and the number and types of micro-organisms (Giller and Malmqvist, 1998). Low DO concentration acts as a stressor

to organisms, with different species having varying requirements and tolerances for DO concentration.

1.2.1.3 Ecology in the impact zone

Following the discharge of wastewater, sensitive species will disappear as they are no longer able to survive (Keup, 1966). This is the result of direct toxicity from wastewater constituents and/or indirect effects such as loss of their food source or competition for resources. Since the concentrations of the stressors follow a pattern of effect and recovery in the impact zone, there is also a change in ecological structure and function. As self-purification proceeds, species diversity also recovers.

The length of the impact zone along any individual river will vary, with river discharge which velocity is associated to. The end of the impact zone is the point downstream where the concentrations of all major sewage related stressors fall below their toxic thresholds (Finnegan *et al.*, 2009). At high flow, when velocities are higher, this point will be further downstream than at low flow because the processes of degradation and reaeration are related to flow time rather than distance. Similarly, the impact zone may expand at low temperatures because degradation rates are limited and stressor concentrations remain high for a greater distance downstream (Welch, 1992). In the example in Figure 1-2, nitrite concentration determines the end of the impact zone, where its concentration is has fallen to its PNEC. After this point nitrite no longer has a significant effect on aquatic organisms. The stressor which determines the end of the impact zone will depend on a number of factors relating to the stressors themselves including degradation rate and initial concentration, and to the system including dilution (discharge), velocity and temperature. The prevailing pattern of ecological effects is as follows: tolerant organisms are initially dominant, then decline in number as more sensitive species start to reappear downstream as the system recovers. This is presented in Figure 1-4.

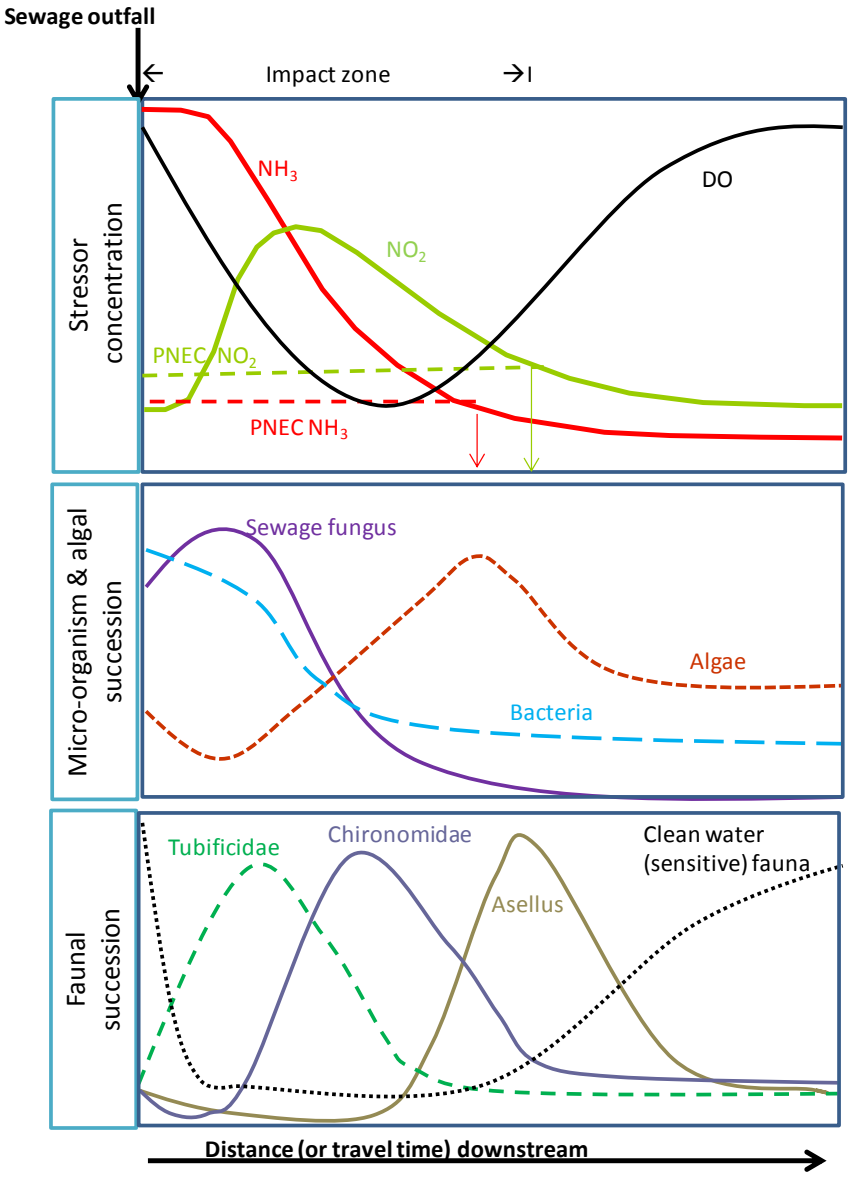


Figure 1-4 Ecological changes in the impact zone. Adapted from Hynes (1960).

Secondary ecological effects can also occur because sensitive species, which could be predators or prey to other organisms, are no longer present. Tolerant prey species can become more abundant as a result of a reduction in the populations of their predators (Welch, 1992). Conversely, tolerant predators may disappear if their prey succumb to toxic stress.

The extent of the impact on communities is dependent on the waste type and load, dilution and turbulence causing reaeration. Gaufin and Tarzwell (1956) for example, demonstrate the variation in seasonal abundances of species upstream and

downstream of a sewage outfall (Figure 1-5) (Welch, 1992). Flow and dilution were low in late summer and early winter when observed effects were greater. The zone with the minimum number of taxa was stretched in winter when temperatures were lower, reducing the rate of degradation.

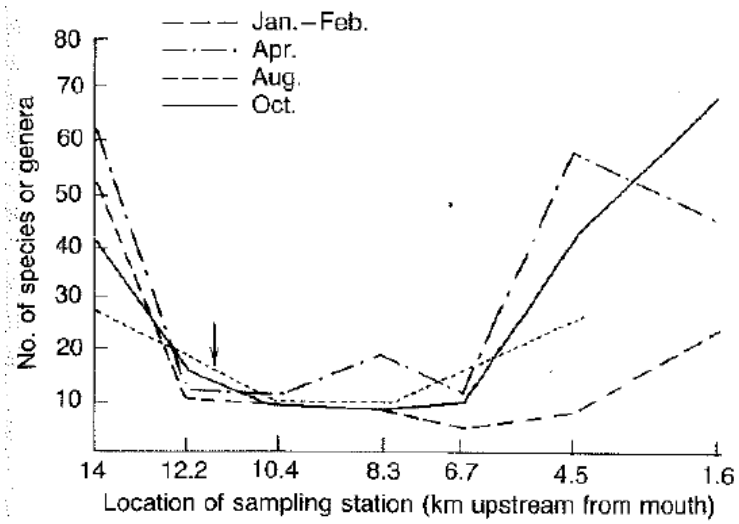


Figure 1-5 Distribution of species abundance at Lytle Creek, Ohio (Welch 1992). Original data from Gaufin and Tarzwell (1956).

1.2.2 Use of macroinvertebrates to assess water quality

The presence of some macroinvertebrates is considered to be a good ecological indicator of water quality because their sensitivity to organic pollution varies, they are relatively easy to sample, they are mainly sedentary, their fluctuations in biomass and species composition are lower than in plankton and their longevity is greater than fish (Resh, 1995; Welch, 1992). Common patterns are often seen in macroinvertebrate data in the impact zone, where diversity and species richness decrease as sensitive species cannot survive, while fewer more tolerant species survive in abundance (Birge *et al.*, 1989; Ortiz *et al.*, 2005).

Biological indices are widely used and appear to be a valuable tool in monitoring macroinvertebrate response to both unimpacted (reference conditions) and anthropogenic disturbances in rivers (Lewin *et al.*, 2013). The indices combine the relative tolerance of macroinvertebrates present or absent with a numerical

expression of the community structure or indicator populations (Welch, 1992). The indices are broadly one of three types: biotic (pollution), diversity or similarity. The biotic indices require a judgement on the relative tolerance of the taxa identified, and the distribution is then weighted. Diversity and similarity indices relate more to community structure rather than relative tolerance; they consider the number of species and individuals (Welch, 1992).

1.2.3 Fundamentals of Ecotoxicology

The toxicological effects of chemical stressors on species are studied in the field of Ecotoxicology. The ultimate aim of ecotoxicology is to determine effects of stressors in the environment at large spatial scales (Beketov and Liess, 2012).There are standard laboratory assays which provide information on the direct toxic effects of a substance on standard species (European Commission, 2003). They usually span trophic levels so that effects at different levels of biological organisation are known and therefore some relevance to the environment is derived (SCENIHR *et al.*, 2012). The aquatic organisms which are usually tested are algae (primary producers), *Daphnia magna* (primary consumers) and fish (predators). Studies are conducted either on a short term (acute) or long term (chronic) basis, the latter covering multiple generations and identifying effects other than lethality such as changes to reproduction, feeding habits and mobility. From these studies, the concentration of a stressor which causes an adverse effect on the replicates of a species is determined. The endpoint for acute studies is usually LC50 or EC50, which is the concentration at which the chemical causes lethality (LC) or an effect (EC) to 50% of the population. The endpoint for chronic studies is normally a NOEC (No Observed Effect Concentration). There is some debate about how accurate and ecologically relevant extrapolating toxicity data from a few species in the lab under standard conditions to multiple species in the field under environmental conditions is (SCENIHR *et al.*, 2012; Calow, 2009; Seitz and Ratte, 1991). This is discussed in more detail in Chapter 5.

1.2.4 Species Sensitivity Distributions

A Species Sensitivity Distribution (SSD) is a statistical distribution describing the variation in toxicity among a set of different species by a given compound (Posthuma *et al.,* 2002) (see Figure 1-6). It is based on the recognition that not all species are equally susceptible to the same toxicants. The sensitivity values represented in an SSD are LC50/EC50s or NOECs from Ecotoxicology studies. Each dot on the curve in Figure 1-6 represents a species. Moving up the *y* axis, the potentially affected fraction of species (PAF) increases. A PAF value of 1 means that all species are affected. Along the *x* axis, the (log) EC50/NOEC concentration increases. At lower concentrations, only the most sensitive species are affected while at higher concentrations of the stressor, a greater fraction of species are affected. The SSD can usually be described by a mathematical function (a cumulative distribution function - CDF) which can take one of various forms (e.g. Vose (2000)). It is common practice to fit a CDF to the data in an SSD.

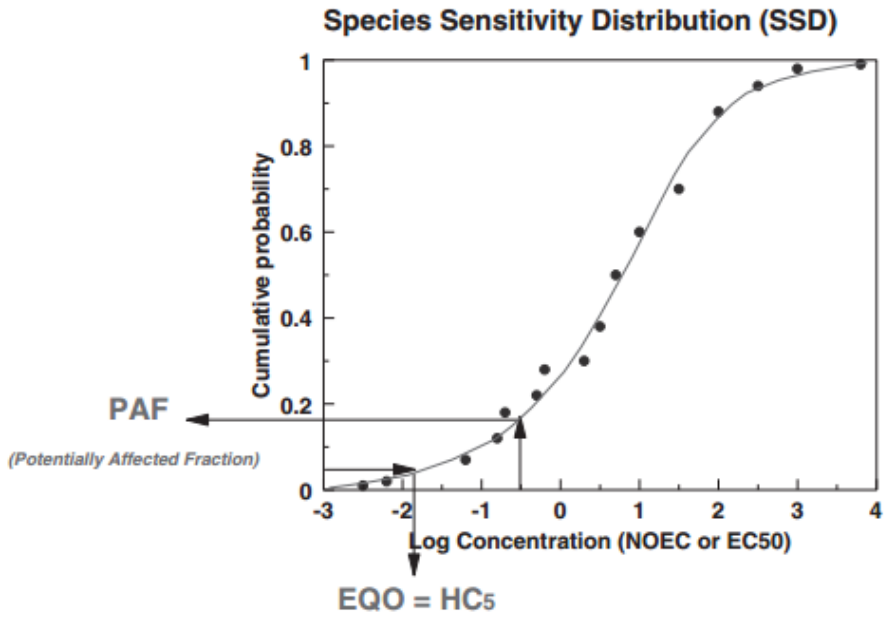


Figure 1-6 An example of a species sensitivity distribution (SSD) (Vighi *et al.*, 2006).

There are a number of assumptions made when compiling an SSD:

1. The SSD is modelled well by the selected distribution (CDF);

2. The sensitivity of species tested in the laboratory approximates the sensitivity of species in the field;
3. The species in the SSD are weighted equally i.e. their importance to the ecosystem is similar (Forbes and Calow, 2002);
4. The number of species in the SSD is adequate (Forbes and Calow, 2002). For use in European risk assessment, the guideline is to use at least ten long-term toxicity values covering a range of trophic levels (European Commission, 2003);
5. The sample of species is a random (or at least a representative) sample (Versteeg *et al.*, 1999; Pinto *et al.*, 2010).

Interactions between stressors or effects at the community-population level are not taken into account (Ippolito *et al.*, 2010; van den Brink *et al.*, 2006). However, SSDs are a useful tool in representing the toxicity of a substance to many species in probabilistic terms. Versteeg *et al.* (1999) determined that SSDs are good predictors of effects at community and ecosystem levels of organisation.

SSDs were originally used in the USA in the 1970's to propose environmental quality criteria (EQC) which sets a "safe" concentration for a given stressor, above which a risk to the environment (e.g. in rivers, soil, sediment) occurs. Their use extended into ecological risk assessment in the 1980's and is still used in both of these applications (Posthuma *et al.,* 2002). For both of these purposes, SSDs are used to derive a threshold concentration at which a given percentage of species (*p*%) will be affected. This is usually referred to as the hazardous concentration (HC_p), the most commonly used is the HC_5, that is when 5% of species will be affected. Despite being constructed using results from single species toxicity studies, the HC_p is applied to natural ecosystems with multiple species (Maltby *et al.*, 2005). This is set when identifying protection goals for the ecosystem being considered (Maltby *et al.*, 2005), for example when deriving a predicted no effect concentration (PNEC). The protection goal of a PNEC derived from an SSD is community structure (Forbes and Calow, 2002) rather than function. However it is assumed implicitly that functional protection will be afforded by structural integrity. In this project, SSDs are used in an alternative

approach as the basis for a conceptual ecological model which also incorporates water quality parameters.

1.3 Aim and objectives

The aim of the project was to develop a conceptual model to describe the changes in the ecology of rivers (community composition) along multiple stressor gradients downstream of a wastewater discharge.

The specific objectives were:

- To review ecological data in the literature describing field biomonitoring studies in the impact zone in order to inform the conceptual basis for the model;
- To devise a conceptual modelling approach to determine changes in community composition throughout the impact zone due to the effects of varying stressor concentrations, taking into consideration properties of the river system being assessed;
- To model in parallel concentration changes and associated ecological effects of sanitary determinands and HPC product ingredients;
- To validate the model using field-based ecological data from the literature.

2 ANALYSING AVAILABLE ECOLOGICAL DATA

2.1 Sources of ecological data

There are few published studies reporting data on changes in the ecological composition in the impact zone downstream of either treated or untreated effluent discharges to rivers. Some papers describe the ecological changes as a result of treated effluent emission in which a zone of impact and recovery is evident, e.g. Ortiz *et al*. (2005), Birge *et al*. (1989) and Avery (1970). The majority of studies in the literature describe the ecology in terms of abundance of particular species at intervals downstream of an effluent. Control (or reference) sites which can be upstream or in a similar type of stream or river are useful in biomonitoring for detecting effects as they experience similar factors caused by changes in current, elevation, temperature and substrata (Welch 1992). A number of studies were reviewed and described in the following sections.

2.2 Biological community change in UK streams – Hynes (1960)

The pattern of effects of organic effluents on the ecology of rivers has been known for some time. It was described in one of the earliest publications of impact zone ecology by Hynes (1960). An idealised plot of changes in water quality and invertebrate ecology downstream of a sewage effluent discharge point, derived from studies of Hynes (1960) in the impact zone, is shown in Figure 2-1.

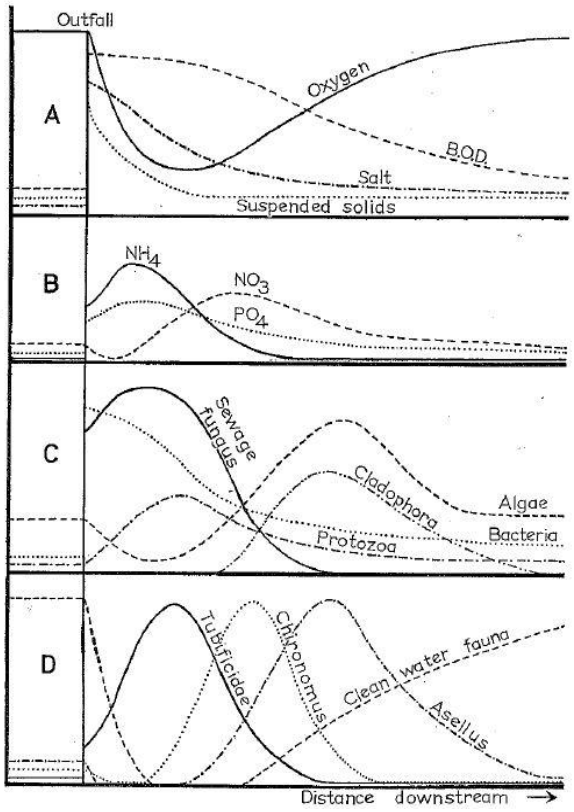


Figure 2-1 Idealised representation of effects of organic effluent from the study of Hynes (1960). Types of changes are A & B: Physical and Chemical, C: Micro-organisms, D: Larger animals.

The physical and chemical changes shown in parts A and B of Figure 2-1 describe changes in the impact zone which includes the oxygen sag curve, the initial increase in ammonia which then decreases, followed by an increase in nitrate. Parts C and D illustrate the general changes in ecology.

Hynes (1960) presented ecological data from two biomonitoring studies, the first was on an unnamed river and the second was conducted on the Welsh River Dee.

2.2.1 Biomonitoring study on unnamed river

The first study reported by Hynes (1960) was conducted on a river in the UK receiving "mild" pollution. When data from Hynes' field study were examined in detail, there were no biomonitoring data for *Asellus* or *Tubificidae* so a direct comparison with Figure 2-1 is not presented. Tabular data were used to construct graphs of ecological changes

with distance (Figure 2-2). The biomonitoring station 91 metres (0.09km) downstream of the effluent discharge on the same bank as the outfall exhibited the greatest difference from reference sites upstream of the outfall, in terms of the types and abundance of macroinvertebrates present. Mayflies and stoneflies were reduced in numbers following the effluent outfall while more tolerant organisms, such as non-biting midges, were much less affected. Caddisflies and biting midges (Ceratopogonidae) did not appear to be affected by the presence of the effluent.

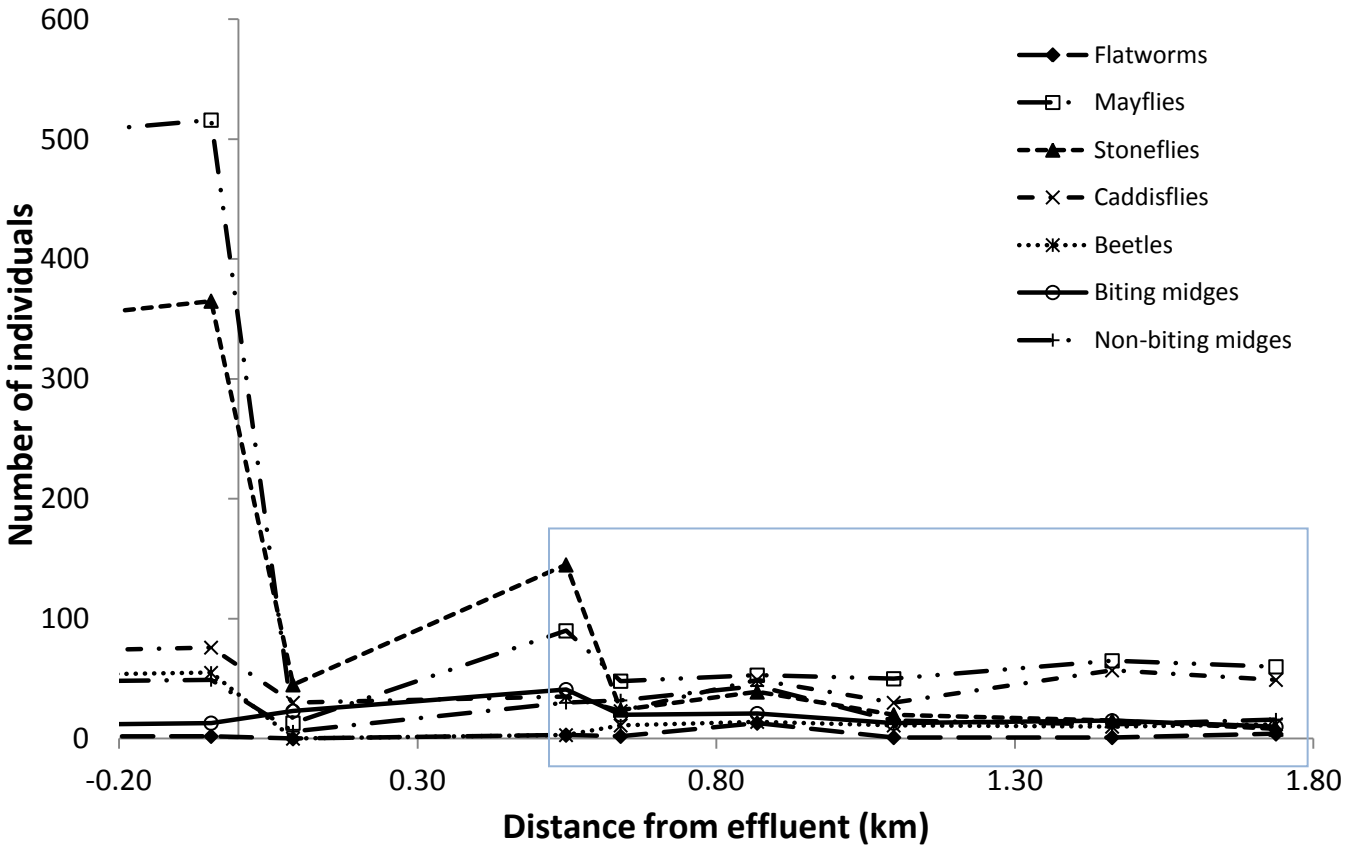


Figure 2-2 Changes in the observed abundance of different macroinvertebrate taxa with distance downstream of a sewage effluent outfall on a UK stream (Hynes, 1960). Data shown for stations upstream of 0km are considered to be reference sites.

As the data points in Figure 2-2 from 0.5km downstream (where mixing was complete) are closely grouped, this area of the graph is shown in more detail in Figure 2-3.

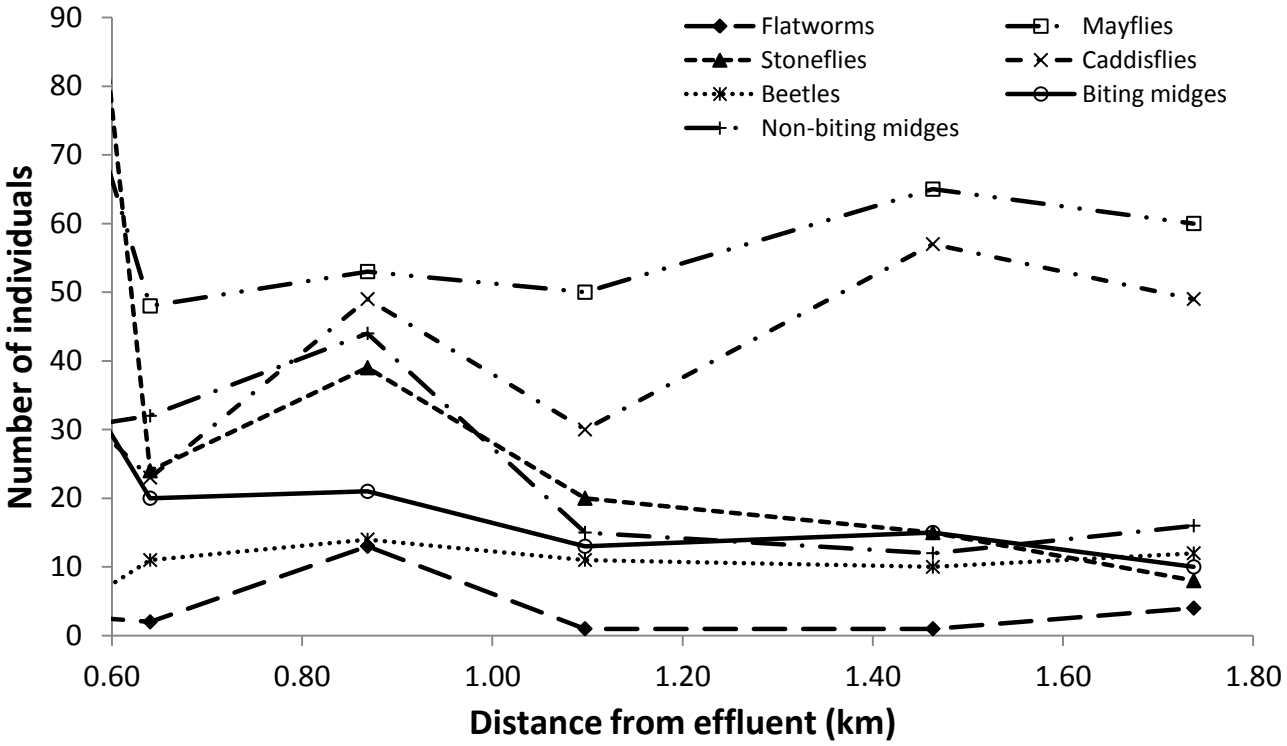


Figure 2-3 Changes in the observed abundance of different macroinvertebrate taxa with distance downstream, from 0.6km to 1.8km downstream of a sewage effluent outfall in a UK stream (Hynes, 1960).

2.2.2 Biomonitoring study on Welsh River Dee

The second study reported by Hynes (1960) was conducted on the Welsh River Dee in January 1956 and April 1957 receiving mild organic pollution. This study was conducted over a longer distance, up to 26km downstream of the effluent outfall. Between 0.3 and 0.6km upstream of the effluent, there was also a smaller effluent from a dirty brook and a rural sewage works. This outfall did not impair the taxa present in terms of composition, but some taxa decreased in numbers e.g. stoneflies and caddis worms. The abundance of macroinvertebrates from the 1957 study is shown in Figure 2-4. In the summer of 1956, a STP was installed near the effluent outfall, so these data are a result of ecological effects from treated effluent. This suggests that the treatment efficiency was limited.

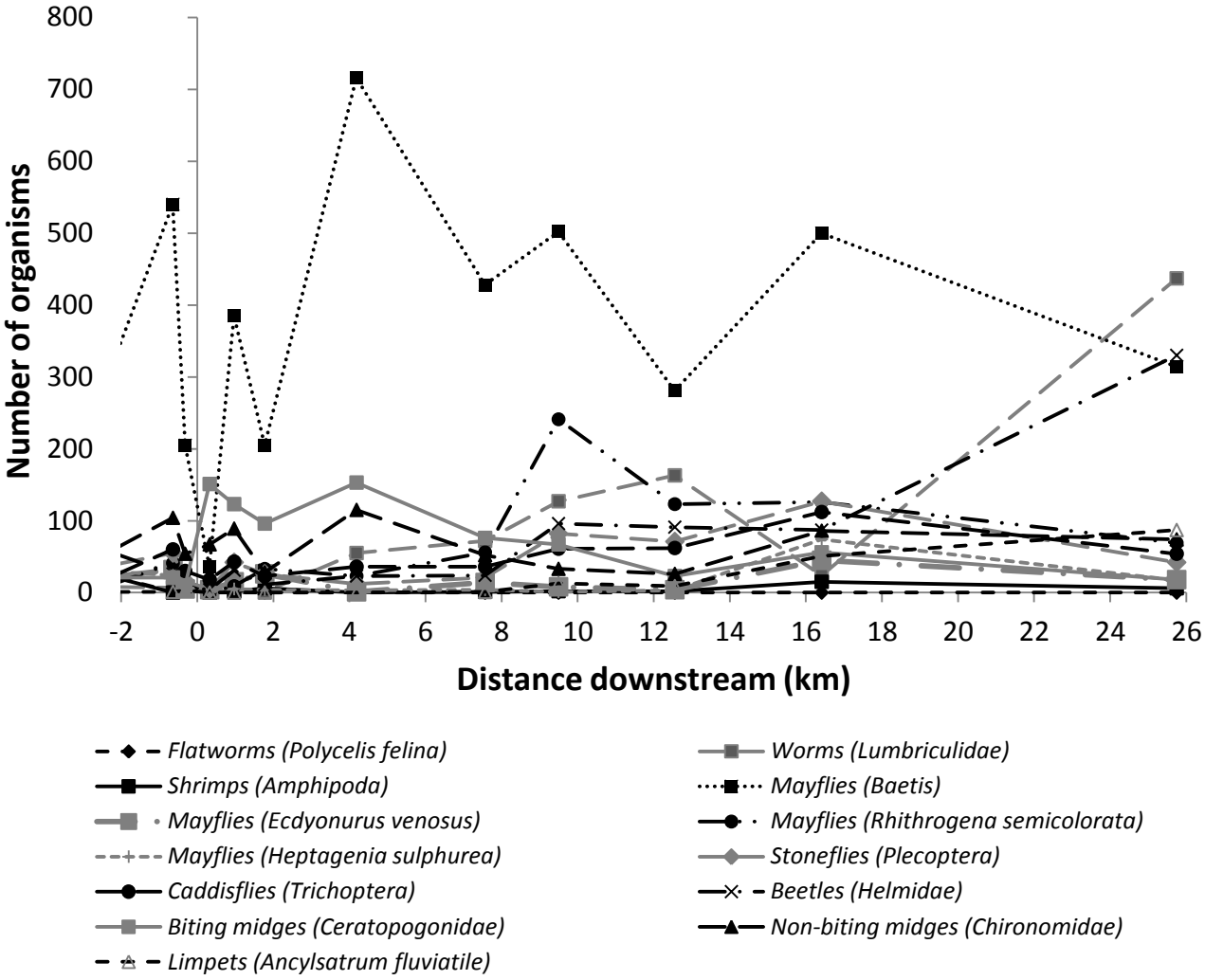


Figure 2-4 Changes in the observed abundance of macroinvertebrate taxa with distance following an effluent outfall on the Welsh River Dee in Hynes' study in 1957 when flow was low (Hynes 1960). Negative distances indicate they are upstream of the effluent outfall. The effluent was treated by an STP which was installed in the summer of 1956.

Figure 2-4 does not reflect Hynes' idealised curves of Figure 2-1. To ascertain if this is reflected in specific taxa, the only taxa labelled in both graphs, chironomids, were examined in more detail in Figure 2-5. In the idealised curve, the family Chironomidae displays a normal distribution downstream, starting from a distance below the effluent outfall. In Figure 2-5, the measured numbers of chironomids (*Chironomus* genus) reflect a peak in number a short distance downstream, but there are other peaks and declines also.

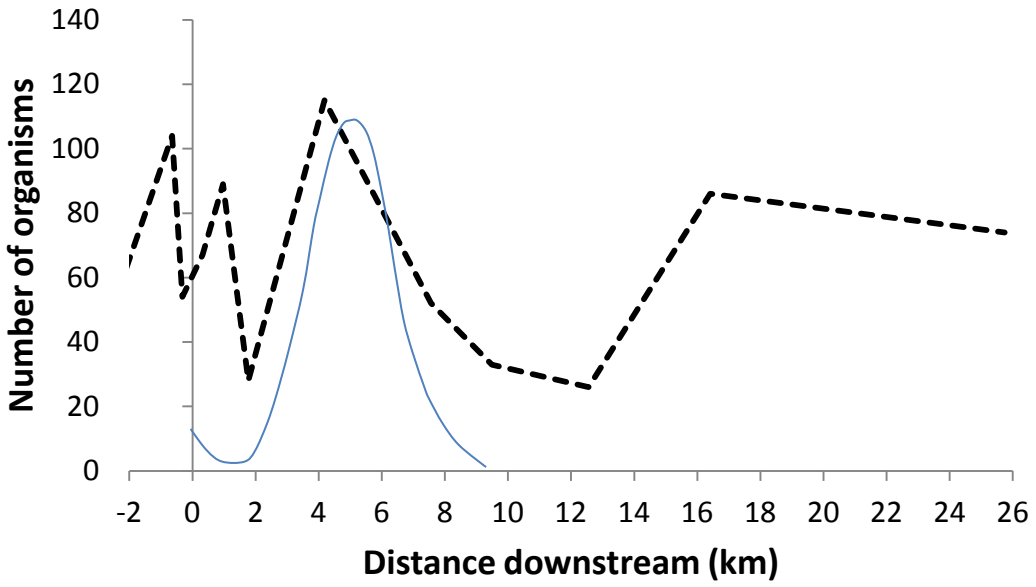


Figure 2-5 Changes in abundance of non-biting midges (Chironomidae) along the Welsh River Dee in Hynes' study in 1957 (Hynes, 1960). The idealised curve for *Chironomus* is superimposed (in blue), although distance and number of organisms are unknown.

Despite the lack of analysis on the effluent, these studies of Hynes (1960) described ecological changes in the impact zone at a time when little was known of it.

2.3 South Elkhorn Creek – Birge *et al*. (1989)

A study by Birge *et al.* (1989) was conducted on the South Elkhorn Creek which is part of the Elkhorn basin in north-central Kentucky, USA in 1983 (Figure 2-6). The only major outfall in the system in this study was the Town Branch STP in Lexington, between biomonitoring stations TB1 and TB2, on the tributary Town Branch Creek (Birge *et al.*, 1989; Laflin, 1970). Birge *et al.* (1989) observed that contributions from Town Branch Creek adversely impacted the ecology of the middle and lower reaches of the South Elkhorn Creek. The ecology of the upper reaches of the South Elkhorn Creek, which were unaffected by the Town Branch tributary, had higher species richness and diversity. This suggested that Town Branch Creek was the main source of the impaired quality and associated poor ecological status in downstream reaches, although there were some lower impact emissions from other sources such as agriculture.

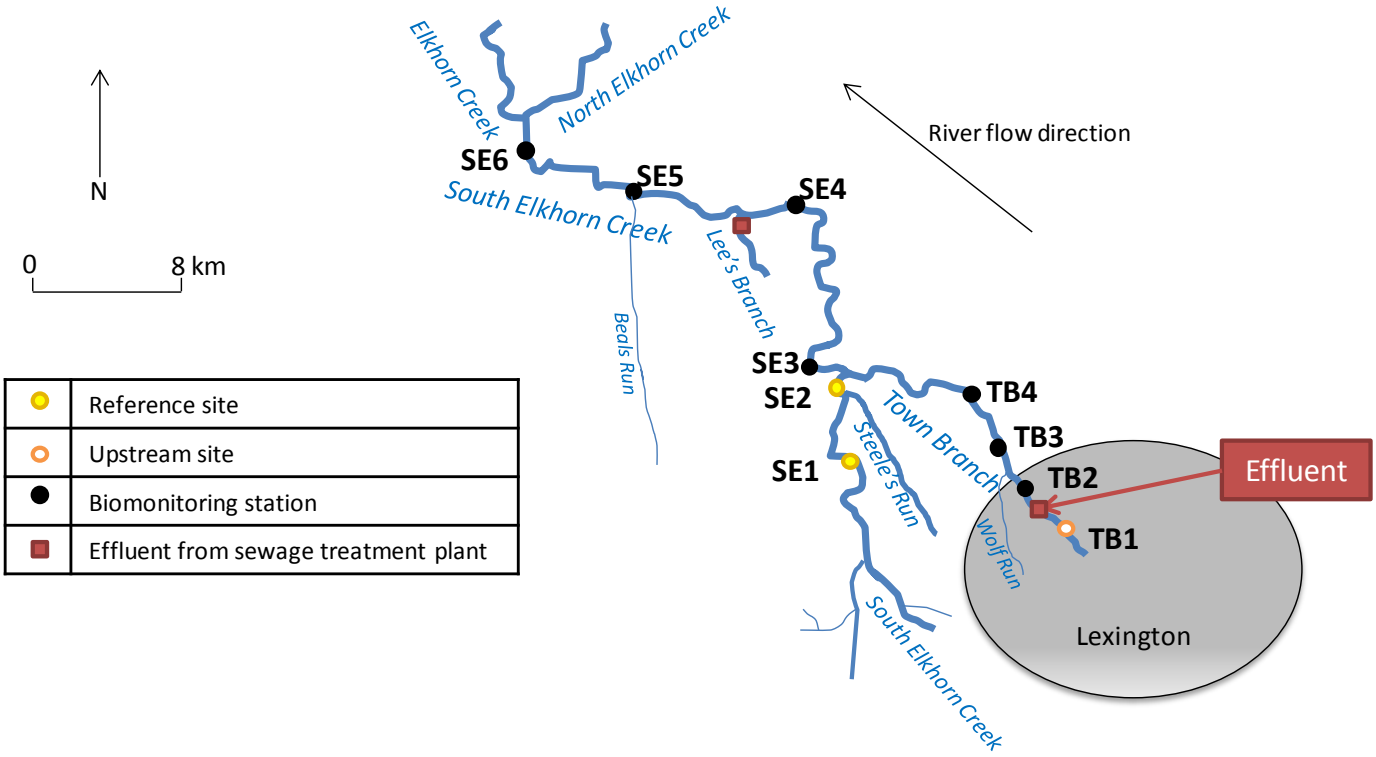


Figure 2-6 The South Elkhorn Creek showing the location of sampling stations, the effluent outfall in Lexington and the location of reference sites from Birge *et al.* (1989).

Upstream of biomonitoring site TB1 storm water run-off entered Town Branch Creek, so the system was already ecologically impaired before the STP effluent outfall between TB1 and TB2 (Environmental Quality Committee, Town Branch Trail Inc., 2001; Birge *et al.*, 1989). Therefore TB1 was not suitable as a reference site. SE1 and SE2 on the South Elkhorn Creek which were not impacted, and therefore represented reference conditions better than TB1.

Effects of the effluent discharged from the Town Branch STP are reflected in observed macroinvertebrate data (Figure 2-7). These data suggest that the ecosystem was severely impaired after the STP but that the ecology recovered with distance downstream, in line with expectations from the impact zone concept. Macroinvertebrate species richness appeared to be the most sensitive ecological endpoint (Birge *et al.*, 1989) since macroinvertebrates were more adversely affected than fish, taking longer to recover downstream.

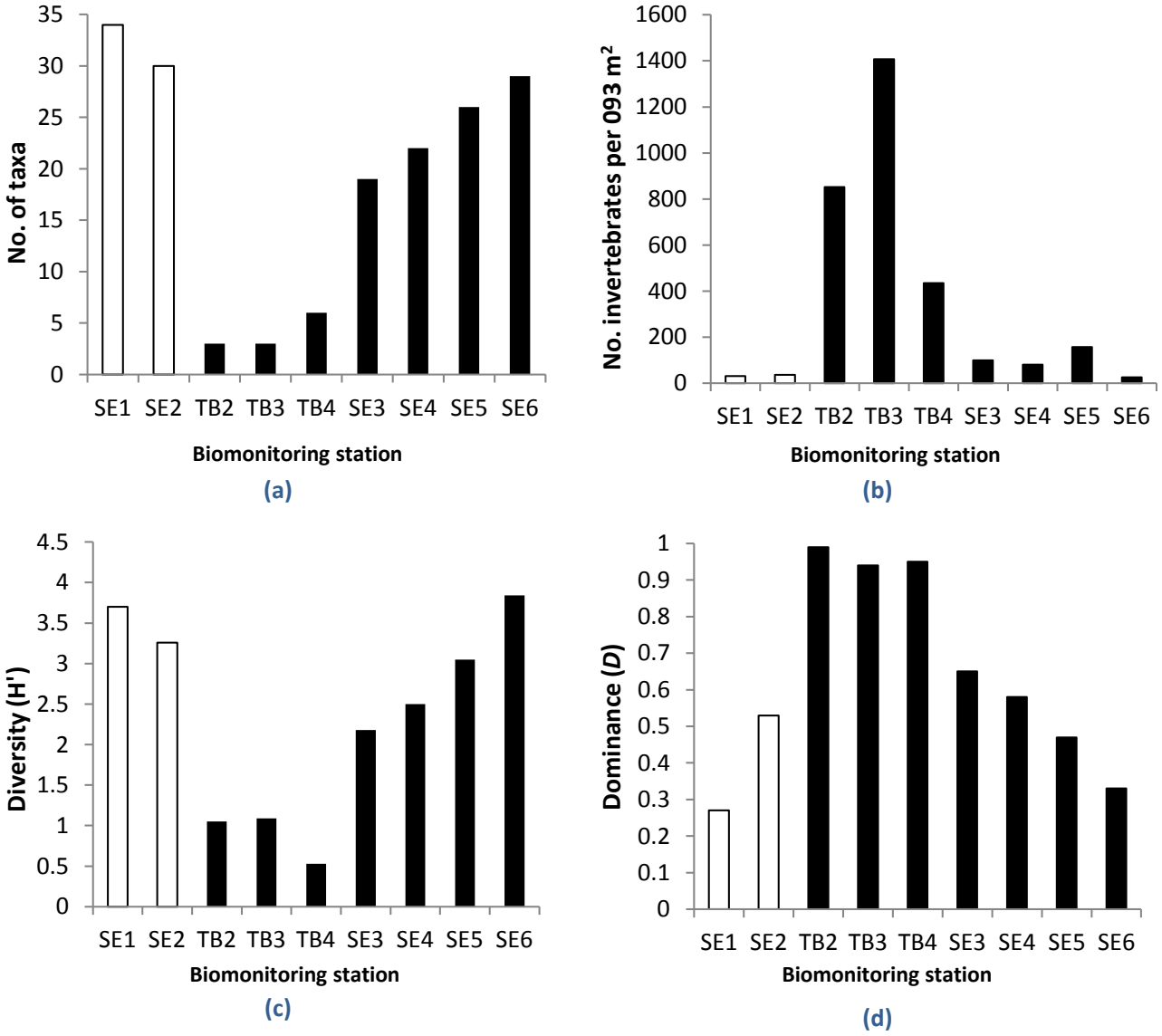


Figure 2-7 Observed changes in macroinvertebrate community structure in the South Elkhorn Creek expressed in terms of (a) species richness, (b) density, (c) diversity and (d) dominance in Birge *et al*. (1989). Biomonitoring stations are presented in order of their position downstream. Reference sites in are white; downstream sites are in black.

Macroinvertebrate species richness (Figure 2-7(a)) showed a marked decrease following effluent discharge, but steadily increased throughout the impact zone. Likewise species diversity (Figure 2-7(c)) was adversely affected but by the end of the impact zone recovered to a similar extent as seen in reference sites (SE1 and SE2). Simultaneously, macroinvertebrate density (Figure 2-7(b)) and dominance (Figure 2-7(d)) increased as a

small number of species, best adapted to these conditions, increased in number. This was, in part, a result of the decline of sensitive species, which may have reduced competition and predation. It may have been assisted by nutrient inputs from the wastewater and the tolerance of these taxa to the conditions.

Birge *et al*. (1989) reported macroinvertebrates in terms of functional feeding groups which give more insight into the changes taking place to ecosystem function and the types of invertebrates which appear to show sensitivity or tolerance to the effluent (Figure 2-8). This classification is based on the organisms' food source and how they obtain it. Shredders are detritivores which feed on coarse particulate organic matter; scrapers (or grazers) consume biofilm or algae by scraping them from rocks or other surfaces; predators engulf, pierce or suck their prey; collector-filterers feed on fine particulate organic matter using a filter e.g. net of silk or mucous and collector-gatherers gather fine particulate organic matter using mouth brushes or other modifications (Richardson and Moore, 2010).

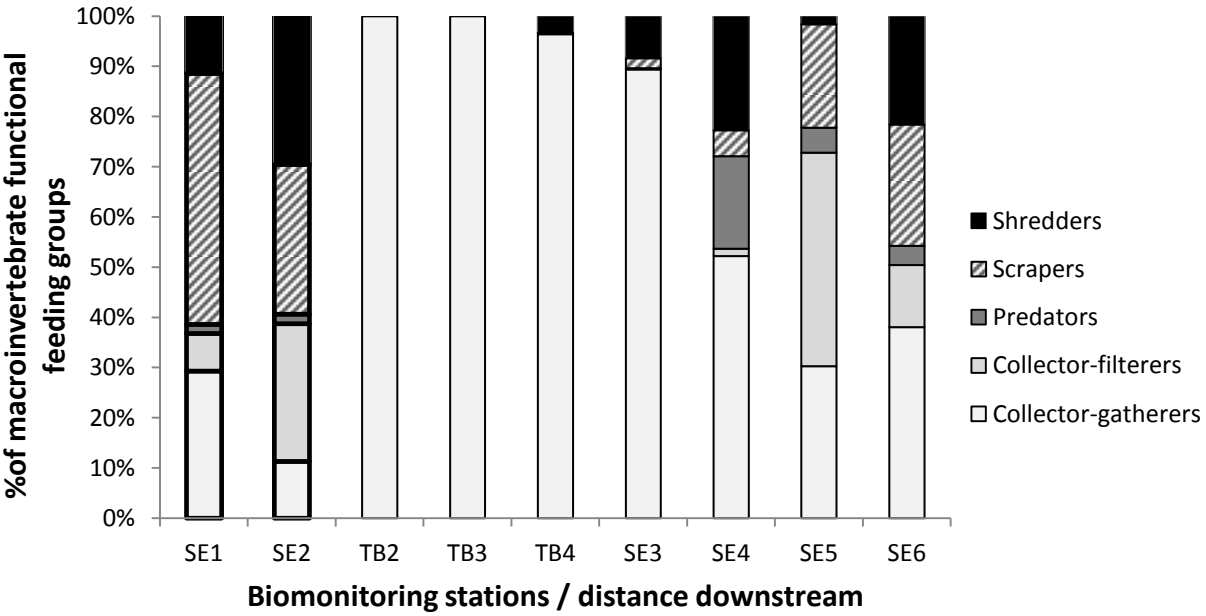


Figure 2-8 Distribution of observed macroinvertebrates into feeding groups at different stations along the South Elkhorn Creek (Birge *et al*., 1989). Biomonitoring stations SE1 and SE2 are reference sites.

Immediately downstream of the effluent outfall, only collector-gatherers survived which were predominantly Oligochaete worms and Chironomids. Chironomids can tolerate sudden changes in habitat conditions and can build populations up rapidly (Solimini *et al.*, 2003). They are tolerant of low DO conditions as they contain a substance similar to haemoglobin which has a high affinity for oxygen so they can temporarily store it, and they can also become dormant during low DO conditions (Rasmussen, 1996; U.S.EPA, 2009). Collector-gatherers are generalists who have a broader selection of food sources they are often referred to as being more tolerant to pollution than specialists whose food source may have disappeared (AQEM consortium, 2002).

At TB4, shredders and scrapers returned in very low numbers, but the ecological composition only returned to its original composition approximately 50-60km downstream (around SE5 to SE6) of the discharge.

Classification by functional feeding group is useful as it can overcome the patchy distribution of individual species within and between different habitats (Baird and Burton, 2001). However, when classifying field data into functional feeding groups there is a danger of incorrect classification if conditions are not ideal or food availability varies. Some taxa can be opportunistic feeders or can have generalised feeding habits (Baird and Burton, 2001).

An overall finding by Birge *et al.* (1989) was that species richness was the most sensitive indicator of perturbation demonstrated by macroinvertebrates in the South Elkhorn Creek system.

2.4 East Gallatin River – Avery (1970)

Avery (1970) presented data on aquatic insects in the East Gallatin River in Montana, USA at one site upstream of a STP, and four sites downstream as far as 20km of the STP. The data were presented in terms of numbers (per $0.9m^2$) and volumes (as cm^3 per m^2) of the Orders Tricoptera, Ephemeroptera, Diptera, Plecoptera and Coleoptera (Avery, 1970). Sampling was conducted twelve times in total, from September 1967 until November 1968 to determine if there were seasonal differences.

Although the effluent was treated it is likely that treatment efficiencies were relatively low as a zone of impact was seen in the data. At the second station, 0.72km downstream of the sewage outfall, numbers and volumes of Trichoptera, Ephemeroptera, Plecoptera and Coleoptera larvae were significantly lower than at the first station (0.56km upstream of the outfall). The number of Diptera organisms more than doubled at the second station, but the volume decreased to less than half that at the first station. This was because larger Diptera organisms were not so prevalent downstream whereas a larger number of larvae were present following the effluent outfall where conditions for them were favourable. Overall, total numbers of organisms increased downstream with some fluctuations at the two sites immediately downstream of the STP. Total volumes decreased between the first two sites and then increased at the remainder of sites downstream. Average numbers and volumes are shown in Figure 2-9 and Figure 2-10 to illustrate the overall trend downstream.

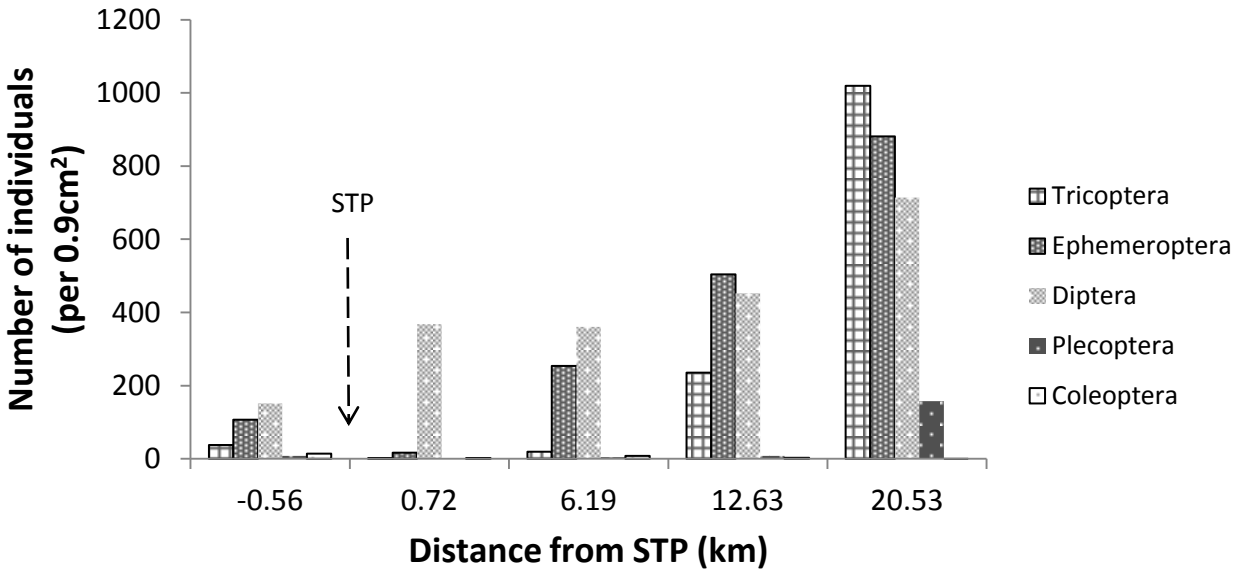


Figure 2-9 Insect Orders (as average number of organisms from ten sampling times) present upstream and downstream of the Bozeman STP in the East Gallatin River (Avery, 1970). Negative distance indicates station is upstream of outfall.

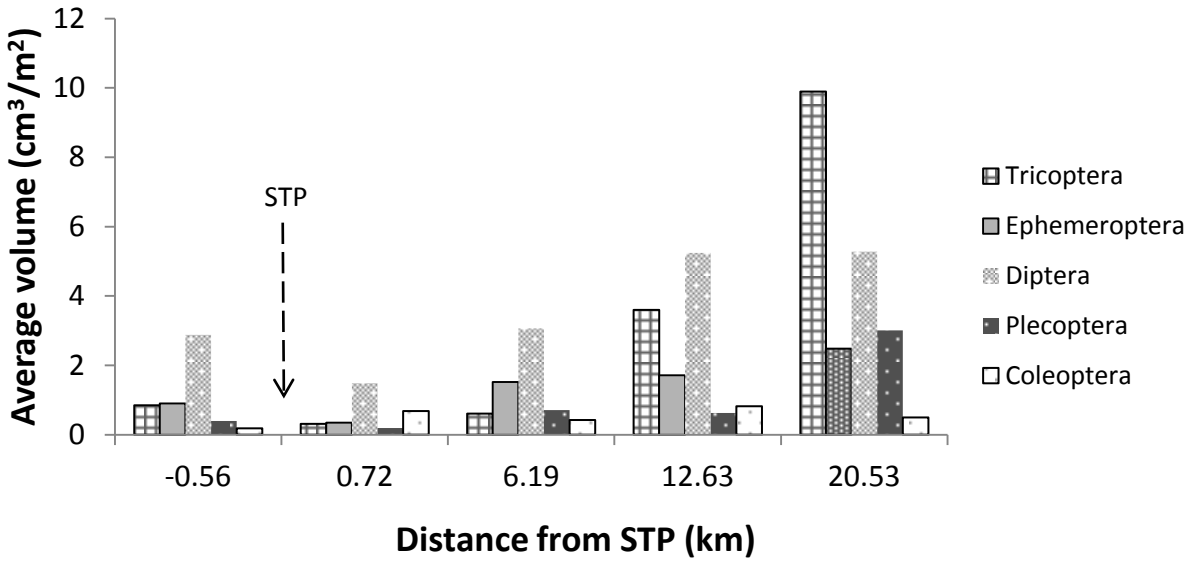


Figure 2-10 Average volumes of insect Orders of ten sampling events, present upstream and downstream of the Bozeman STP on the East Gallatin River (Avery, 1970). Negative distance indicates station is upstream of outfall.

Some seasonal variation was evident in the taxa present. Overall numbers of organisms were highest in September 1967 and August 1968. Overall volumes of organisms were highest in November of both sampled years when organisms had grown in size. These patterns varied for individual orders although seasonal variations were still evident. The numbers and volumes of Diptera over the course of the study are presented in Figure 2-11 and Figure 2-12. Peak numbers were observed in September 1967 and in August 1968. Peak volume was observed in November in both years.

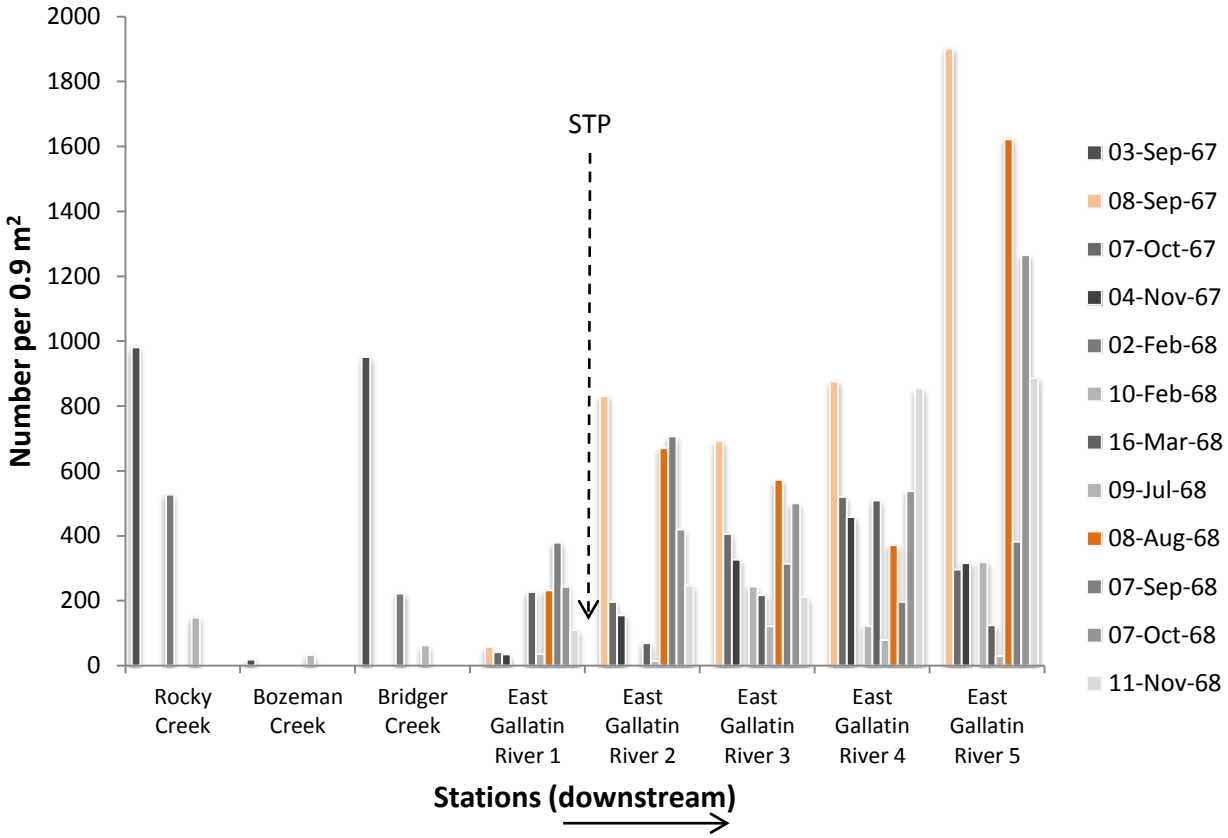


Figure 2-11 Seasonal variation in numbers of Diptera organisms. Rocky, Bozeman and Bridger Creeks are upstream tributaries of East Gallatin River. The STP is located between East Gallatin River 1 and East Gallatin River 2. Numbers were highest in September 1967 and August 1968 (highlighted in orange).

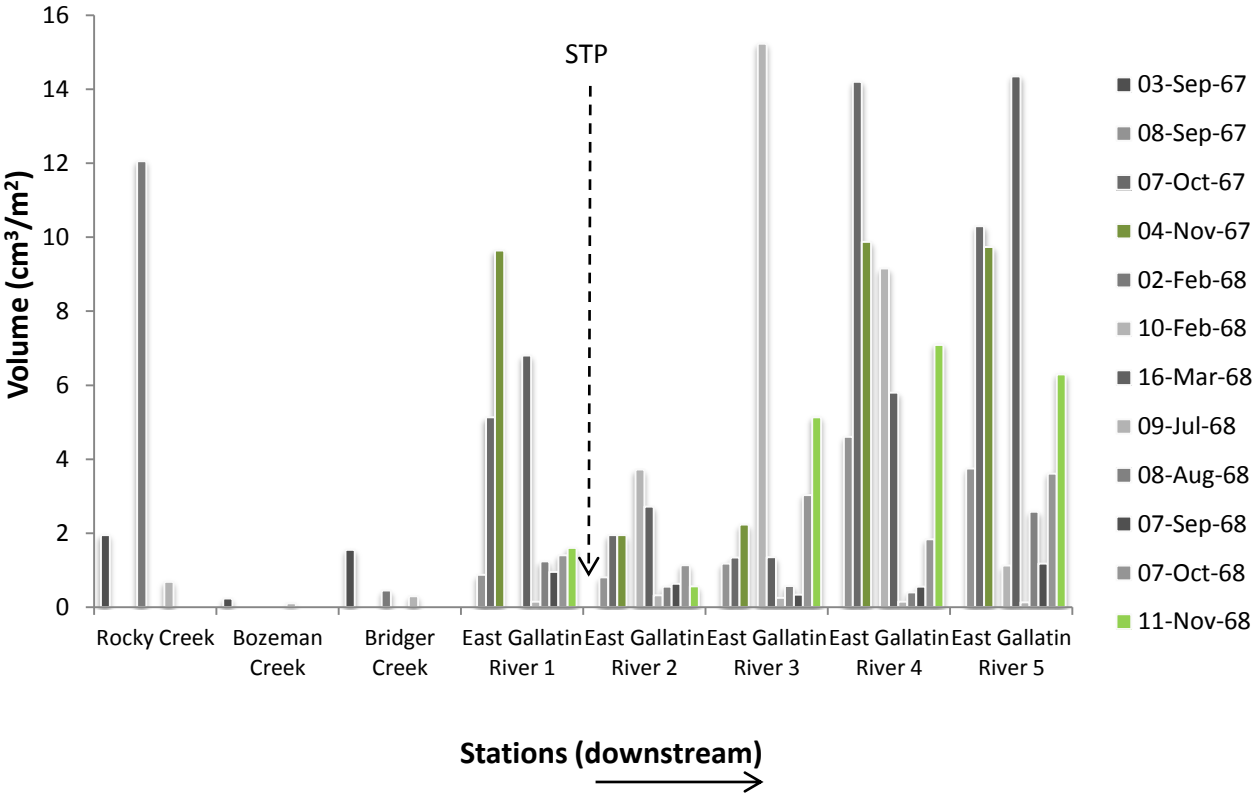


Figure 2-12 Seasonal variation in volumes of Diptera organisms. Rocky, Bozeman and Bridger Creeks are upstream tributaries of East Gallatin River. The STP is located between East Gallatin River 1 and East Gallatin River 2. Peak volume was observed in November 1967 and in November 1968 (highlighted in green).

2.5 Balatuin River – Dyer *et al*. (2003)

Most of the studies conducted which assess the effects of organic pollution on ecological composition in the impact zone are conducted in temperate regions. Dyer *et al*. (2003) conducted a study in tropical regions to study the effects of untreated wastewater on ecological communities in the Balatuin River, Philippines. There were a number of effluents being discharged into the river until about 6km downstream of the initial sampling site. From 6km downstream, there were no further inputs, velocity increased and the river became deeper and wider. Macroinvertebrate samples were collected from artificial substrates which were glass slides placed in the river. The results presented in Figure 2-13 represent the macroinvertebrate samples collected after 14 days exposure and illustrate the decline in water quality around 6km downstream,

where the community is dominated by oligochaetes and chironomids. Self-purification of the river occurred as taxa absent immediately downstream of the effluent outfalls, began to re-appear downstream e.g. decapoda. Known pollution-sensitive taxa e.g. mayflies and caddisflies, were only present at the first and penultimate sites. DO concentrations increased as self-purification occurred. From 13km downstream DO concentrations were higher than those at 0km.

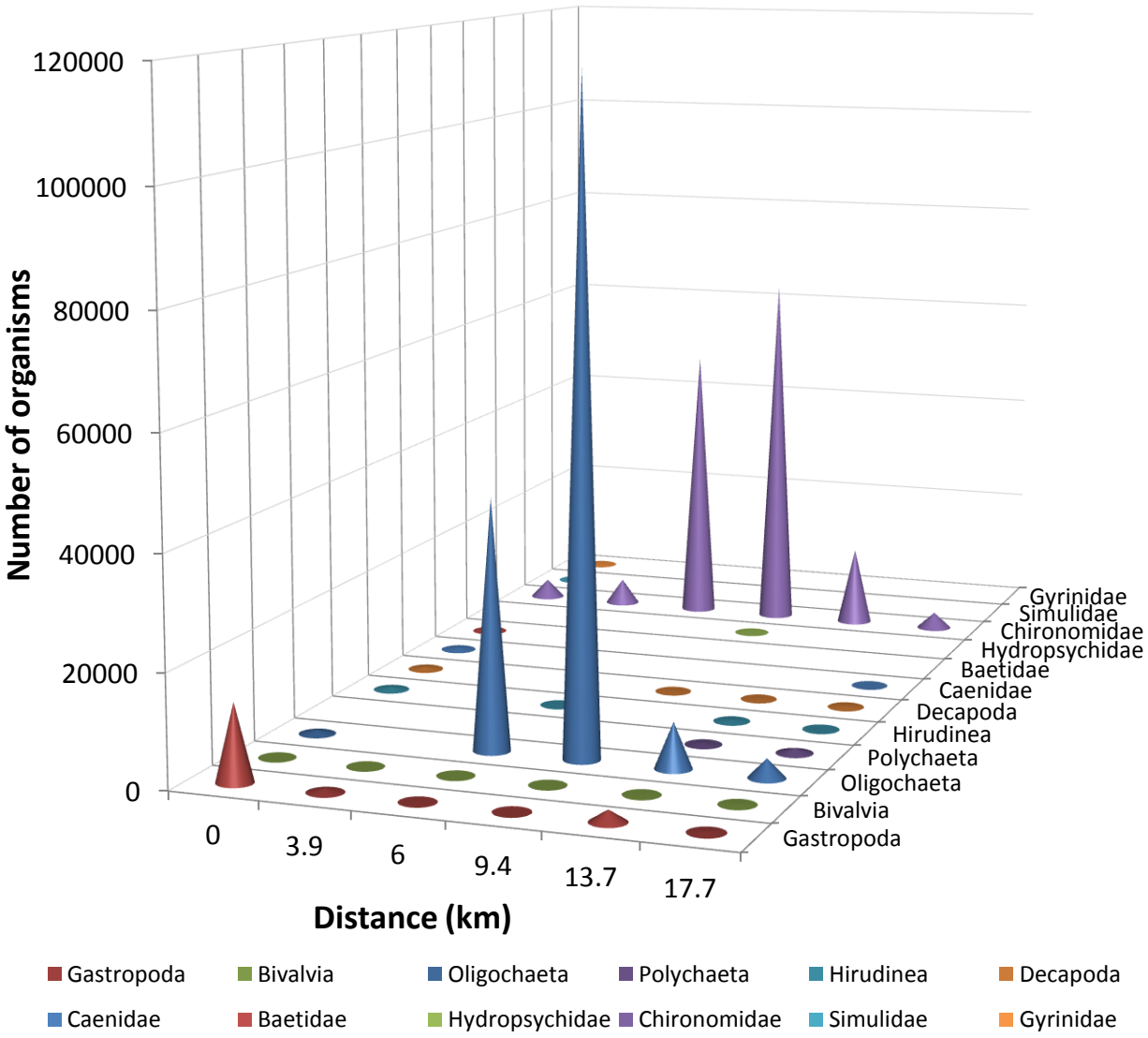


Figure 2-13 Macroinvertebrate taxa densities (organisms/m^2) in artificial substrates in the Balatuin River, Philippines, after exposure time of 14 days.

Concentrations of sanitary determinands and the HPC ingredient LAS were measured. The concentration of LAS was found to be lower than its PNEC, which was derived as a HC_5 from an SSD. This supports the idea that an alternative risk assessment method for ingredients in consumer products is required as the presence of sanitary determinands was of higher risk than LAS to the aquatic communities.

2.6 La Tordera stream – Ortiz *et al*. (2005)

Ortiz *et al*. (2005) studied the effects of a STP effluent on La Tordera stream in Catalonia, Spain. There were three biomonitoring sites, one 1km upstream and two, 60m and 500m, downstream from the STP. The STP effluent contributed to the river flow, as the discharge and velocity were much higher at the downstream sites. The downstream DO concentration (4.4 mg L^{-1}) was about half of the upstream concentration (8.7 mg L^{-1}). A diurnal pattern in DO concentration was seen with night-time concentrations around 3 mg L^{-1}. The water temperature of 20°C may have played a part in this. Macroinvertebrate abundance increased significantly downstream, as shown in Figure 2-14. The downstream sites were dominated by chironomids and oligochaetes. Both taxa richness and EPT (Ephemeroptera, Plecoptera, Tricoptera – known sensitive Orders) richness decreased after the effluent outfall (Figure 2-14). The increase in the relative percentage of collector-gatherers and concurrent decrease in the relative percentage of shredders and predators, reported in the study of Birge *et al*. (1989), was also seen by Ortiz *et al*. (2005).

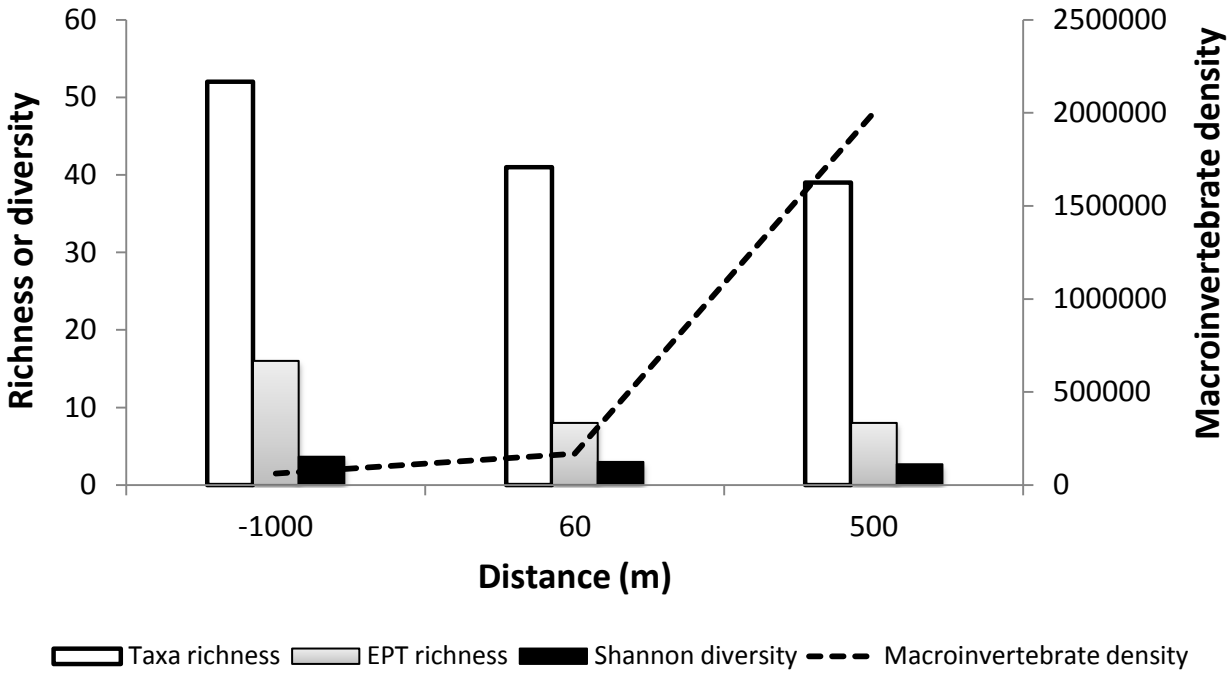


Figure 2-14 Changes in macroinvertebrate density, richness, diversity and EPT richness in La Tordera stream, Spain, based on data from Ortiz *et al*. (2005).

The STP effluent also added nutrients and organic matter which enhanced respiration, which in turn caused low DO concentrations in particular at night-time. An unexpected recovery in the macroinvertebrate community was seen at 80-90m downstream of the STP but this was shortlived as this was not evident at 500m. This may indicate that the self-purification capacity was overwhelmed (Ortiz *et al.*, 2005).

2.7 Cedar Run – Kondratieff *et al*. (1984)

Kondratieff *et al*. (1984) conducted a macroinvertebrate biomonitoring study on a second-order stream, Cedar Run, in Virginia, USA. A reference site (upstream of an STP) and two reference sites on a tributary, Wilson Creek, were included in the study, along with four sites downstream of the STP. There was a second effluent outfall from an electroplating plant between site numbers 2 and 3 (Figure 2-15).

Macroinvertebrates were classified by functional feeding group in Figure 2-15, with similar patterns evident as in previous studies (e.g. Birge *et al*., 1989, Ortiz *et al*., 2005).

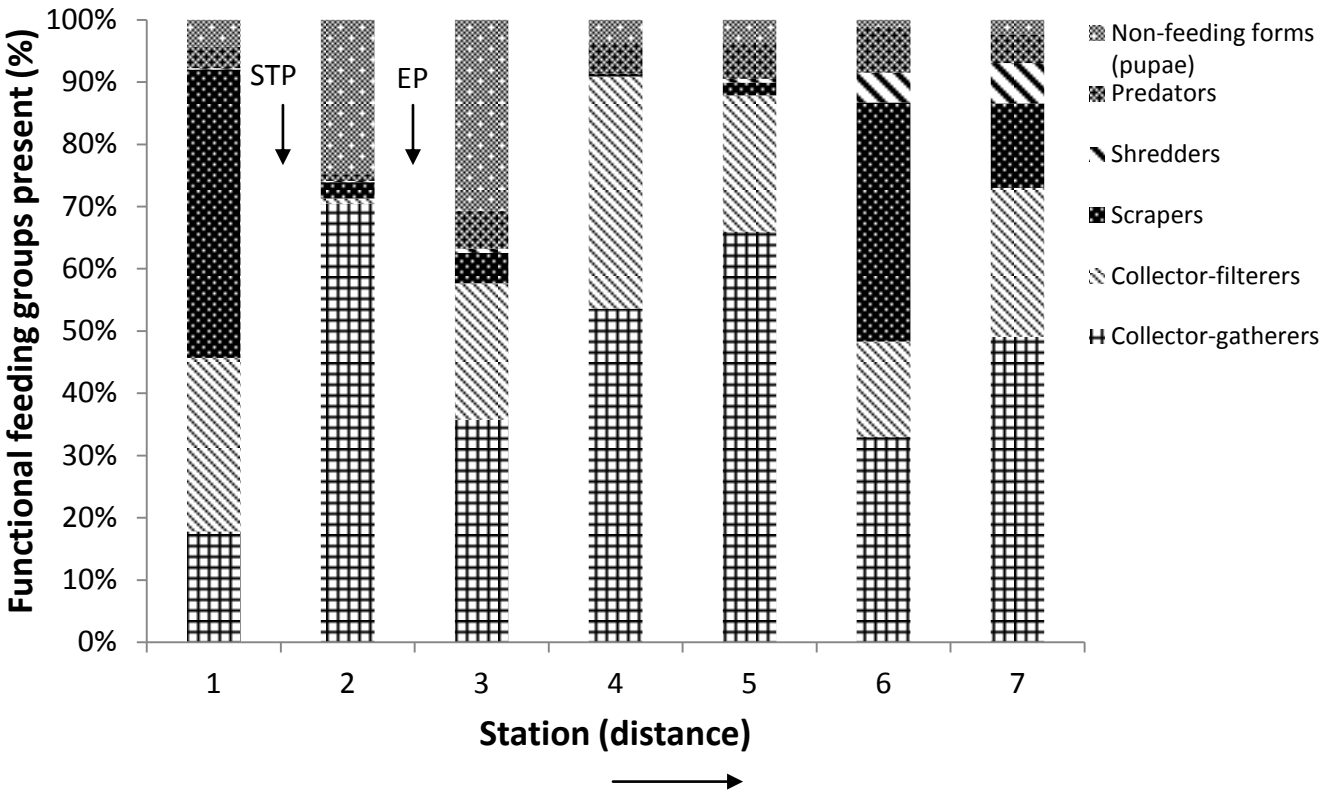


Figure 2-15 Macroinvertebrates present as a percentages functional feeding groups present at biomonitoring stations. Station 1 is an upstream reference site; stations 6 and 7 are reference sites on a tributary, Wilson Creek. Station 2 was downstream of a STP effluent outfall and station 3 was downstream of an electroplating plant (EP) effluent outfall (Kondratieff *et al.*, 1984).

Collector-gatherers almost completely dominated the community with primarily Chironomid and Psychodid flies and oligochaete worms. Overall taxa numbers decreased at stations 2 and 3, where the concentration of stressor peaked. Further downstream, the abundance of these taxa decreased while scrapers and shredders reappeared (Kondratieff *et al.*, 1984).

2.8 Methods of combining effects of stressors

Guidance on assessing the risks of multiple stressors has been developed by ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) (2011). There have been efforts to develop frameworks to assess the effects of multiple stressors on ecological communities, for example, msPAF and SPEAR.

2.8.1 msPAF

msPAF (multi-substance Potentially Affected Fraction of species) was proposed to predict the risk for direct effects of chemical stressors on the composition of species assemblages and biodiversity (De Zwart and Posthuma, 2005). SSDs are used to calculate the "toxic pressure" which is analogous to the toxic stress or ecological risk (Harbers *et al.*, 2006). The PAF values for individual chemicals are aggregated, based on the application of two toxicological models, which are concentration addition (CA) and response addition (RA) (van Zelm *et al.*, 2007; Harbers *et al.*, 2006). CA is applied to stressors with the same mode of action (e.g. ester narcosis), and RA is applied to those with different modes of action to predict their combined effect.

Harbers *et al*. (2006) concluded that msPAF is a useful tool to measure the relative likely impacts of stressors but does not yet predict absolutely the toxic effects on species assemblages. Most studies on mixture toxicity have been conducted on binary mixtures, so the effects of more complex mixtures are unknown (De Zwart and Posthuma, 2005). De Zwart and Posthuma (2005) concluded that there is still a large difference between a mechanistic and a probabilistic (e.g. SSDs are a probabilistic approach) of mixture toxicity and the risk posed by mixtures. The disadvantages associated with the use of msPAF are similar to those of SSDs, discussed in Chapter 5.

2.8.2 SPEAR

SPEAR (SPEcies At Risk) is a bioindicator index which is based on biological traits or characteristics of organisms and various contaminant types in freshwater. Three types of SPEAR indicators currently exist; $SPEAR_{pesticides}$, $SPEAR_{salinity}$ and $SPEAR_{organic,}$ the latter is most relevant to HPC chemicals (Liess and von der Ohe, 2005; Beketov and Liess, 2008; Schafer *et al.*, 2011). SPEAR is based on the ratio of physiologically sensitive species in the macroinvertebrate community (von der Ohe *et al.*, 2009), in other words, the fraction of abundance of sensitive individuals in a community for a specific stressor (Schafer *et al.*, 2011). It was originally developed to detect the adverse effects of pesticides on macroinvertebrates in agricultural streams (von der Ohe *et al.*, 2009).

The $SPEAR_{organic}$ index is calculated as the arithmetic mean of species' acute sensitivities to the crustacean *Daphnia magna*, $S_{organic,}$ weighted by the log-transformed abundance of the respective species. The sensitivity is that of a taxon to organic toxicants in general, rather than to specific organics (Beketov and Liess, 2008). A higher $SPEAR_{organic}$ value indicates a higher proportion of sensitive species to organic toxicants present (Bunzel *et al.*, 2013).

This index is dependent on the chemical toxicants mainly and largely independent of longitudinal factors, while other biological indices (e.g. Taxa richness, EPT taxa richness and Shannon's diversity index) are dependent on longitudinal environmental factors in a river system (Beketov and Liess, 2008). $SPEAR_{organic}$ aims to detect chronic exposure and combines the effects of various toxicant stressors, but lacks the possibility of discriminating the effects of individual toxicants (SCENIHR *et al.*, 2012). It is a measure of sensitivity against continuous organic pollution. The only trait which $SPEAR_{organic}$ is based on is that of taxon-sensitivity to organic toxicants.

Beketov and Liess (2008) in their study in a Siberian stream found it difficult to distinguish between the effects of organic toxicants and ammonium and nitrite, however the concentrations of the latter two contaminants were below their toxic threshold. So far, the only successful application of $SPEAR_{organic}$ was in the Siberian system (Bunzel *et al.*, 2013).

2.8.2.1 Advantages and disadvantages of using $SPEAR_{organic}$ for this project

The advantages of using $SPEAR_{organic}$ in this project were:

- It is based on biological traits rather than taxonomic composition or abundance (Beketov *et al.*, 2009);
- It is recommended for assessing the effects from chronic exposure to organic toxicants (von der Ohe *et al.*, 2009);
- This index is dependent on the chemical toxicants, rather than other stressors.
- It gives similar results across different ecoregions (von der Ohe *et al.*, 2009);

- It is specific for toxicants with a relatively constant exposure regime e.g. surfactants and other chemicals which we wish to risk assess (Beketov *et al.*, 2009)
- It takes recovery into account (Beketov *et al.*, 2009), however this is dependent on the user having sampling data further downstream demonstrating so

However, there were disadvantages associated with the use of $SPEAR_{organic}$ in this case:

- The abundance of species at various sampling points is required but is not informed by the conceptual model (further detail in Chapter 5), only presence or absence of species is known;
- It is relevant for macroinvertebrate organisms only;
- Toxic stress (sensitivity) is considered and not other stressors (von der Ohe *et al.*, 2009), so it is not fully a multi-stressor approach;
- The user needs to enter data on recovery in binary form, i.e. if recovery occurred or not.

2.9 Gap in the literature

There are only a few published sources of ecological data with distance along stressor concentration gradients in the impact zone, in terms of species diversity and abundance. Some more recent studies report only one or two biomonitoring sites in the impact zone e.g. Ortiz *et al*. (2005). The mechanisms behind the tolerance or sensitivity of species are not explained in any of the studies cited. A better understanding of the mechanisms of impairment would help to predict the impact of effluents in rivers. Integration of the fields of ecology and ecotoxicology, which have evolved separately (Relyea and Hoverman, 2006), would progress this understanding, and has been suggested by many authors in recent years (Calow, 1996; Relyea and Hoverman, 2006; Beketov and Liess, 2012). The bottom-up approach of ecotoxicology which predicts effects in the environment from small-scale experiments should be merged with a top-down macroecological approach, of ecological effects at large scales (Beketov and Liess, 2012).

The majority of studies in the literature reporting ecological communities downstream of an effluent outfall, report numbers (abundance) and types of macroinvertebrates present. However, they do not give details about the mechanisms of action of the stressors on the aquatic organisms or the mechanisms of recovery. There is a lack of ecologically based evidence in modelling to inform this. In the literature, studies on recovery appear to focus on a particular group of stressors which is plant protection products (e.g. herbicides, insecticides). The type of exposure of these stressors to communities differs to that of down-the-drain chemicals. They are often applied at particular times of the year (e.g. pre-emergent herbicides) or multiples times throughout a growing season (e.g. many insecticides) (Relyea and Hoverman, 2006).

Regular monitoring of the concentrations of down-the-drain chemical ingredients at several locations in a river system is time consuming and expensive. Predictive modelling can help to evaluate their impact on the receiving system if the models can be shown to provide a good description of the processes operating. McAvoy *et al.* (2003) predicted the concentration of LAS and the recovery of an impacted system in the Philippines using the QUAL2E water quality model. The impact on the ecology was analysed by the biomonitoring survey by Dyer *et al*. (2003) but a predictive method on the ecological side was not developed.

3 METHOD AND RESULTS FOR THE CONCEPTUAL MODEL

3.1 Method for a conceptual model of ecological change in the impact zone

The aim of this project was to develop a conceptual model to describe ecological changes in river impact zones. This model can also be used to assess the risks associated with particular chemicals used in HPC products and to determine the relative contribution of individual chemicals to any ecological impact.

3.1.1 Model overview

The model calculates the concentration of every defined stressor at each time and distance step downstream of a single effluent. The ecological composition is predicted in terms of the presence or absence of particular taxa along these concentration gradients. The model has been coded in Microsoft Visual Basic for Applications 6.5 using data in Microsoft Excel 2007. The code is in Appendix A.4.

3.1.2 Assumptions

A number of assumptions were made when developing the model:

- There is only one point source input of effluent in the system;
- The effluent mixes instantaneously i.e. there is no mixing zone;
- Stressor removal or reduction via processes other than dilution and degradation e.g. removal by sorption to sediment or volatilisation, are not included;
- Ecosystem composition can be represented by the taxa for which toxicity data are available;
- There are no indirect ecological effects i.e. interactions between trophic levels such that toxic effects in one level propagate through to changes in taxa in another level;
- Changes in stressor concentrations occur via first order kinetics, except for dissolved oxygen which is described using the Streeter-Phelps model (Streeter and Phelps, 1925).

3.1.3 Scenarios

The model can be applied to a fictitious generic scenario, or it can be parameterised to represent a real river system. A generic scenario was established, based on the European scenario set out in Part II of the Technical Guidance Document (TGD) for Environmental Risk Assessment in the EU (European Commission, 2003). This standardised risk assessment approach considers a hypothetical region of 200km by 200km with a population of 20 million. Within this region each STP serves a population of 10,000 people with each inhabitant using 200 L d^{-1} of water. The effluent is discharged from the STP into a river which is assumed in the TGD to have a 1 in 10 dilution (Figure 3-1).

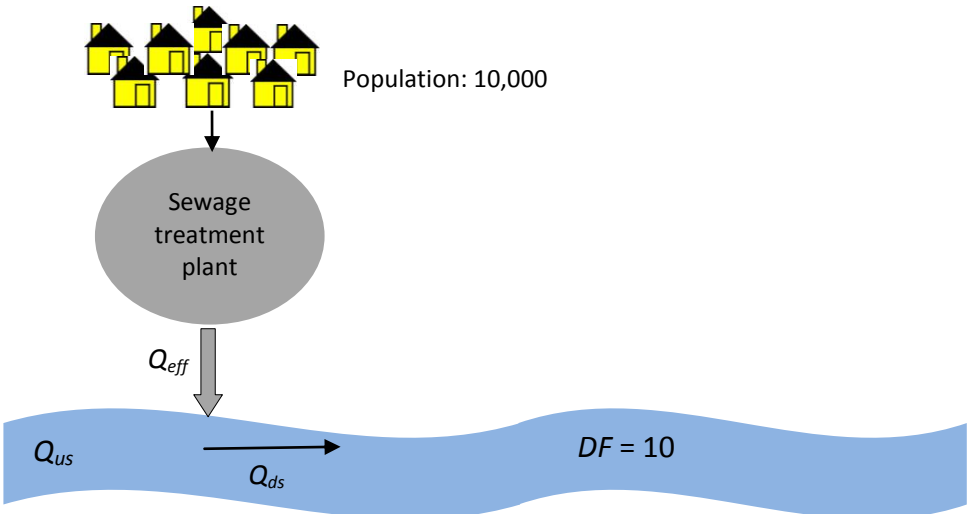


Figure 3-1 Illustration of the generic emission scenario based on the EU exposure assessment guidelines (European Commission, 2003).

The TGD scenario primarily considers a situation in which all wastewater passes through secondary sewage treatment. However, two scenarios are considered here, one assuming the discharge of treated wastewater and another assuming the discharge of untreated wastewater.

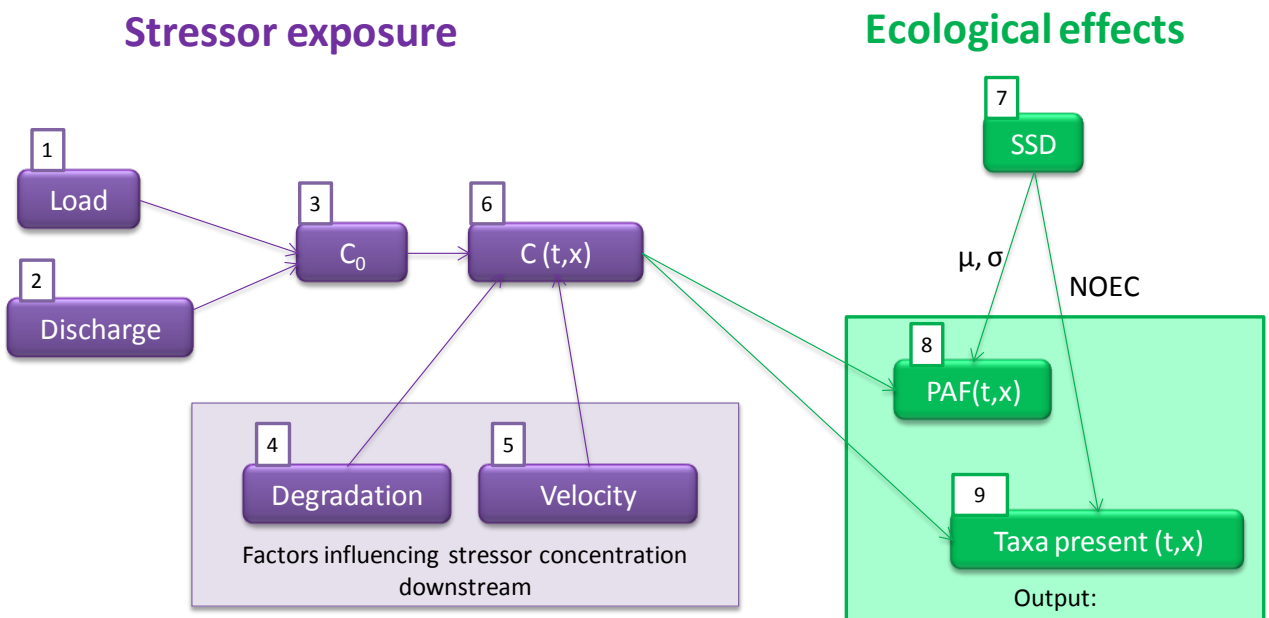
3.1.4 Model description

The model has two main components:

1. Stressor exposure assessment, and

2. Predictions of ecological effects resulting from this exposure.

This is illustrated in Figure 3-2 and explained in the subsequent sections.



Key :

1. Load (kg d^{-1})
2. Discharge (L s^{-1})
3. C_0: Concentration of stressor in receiving water at time 0 (µg/L)
4. Degradation (h^{-1})
5. Velocity (m s^{-1}) which is used to calculate travel time (h)
6. C(t,x): Concentration of stressor at time *t* and distance *x* (µg/L)
7. SSD: Species Sensitivity Distribution characterised using µ (mean of lognormal distribution) and σ (standard deviation of lognormal distribution) of NOECs (no observed effect concentration) (µg/L)
9. PAF: Potentially Affected Fraction of taxa in the system
10. Taxa present (t,x) at time *t* and distance *x*

Figure 3-2 Schematic illustration of the different components of the model showing the variables influencing exposure in purple (left hand side) and the variables describing the ecological effects in green (right hand side).

3.1.4.1 Load

The load refers to the mass of stressor per unit time discharged into the system via wastewater at the start of the impact zone. The concentration of each stressor in the receiving system will be the quotient of the load and the discharge of the water body downstream of the emission, assuming negligible upstream concentration of all stressors.

3.1.4.2 Discharge

Discharge (Q) is the volume of water moving down a channel past a given point per unit time. It varies with the size of the drainage basin and the rate of run-off. The discharge in the river after effluent is discharged (Q_{ds}) is the sum of the effluent (Q_{eff}) and upstream (Q_{us}) discharges:

$$Q_{ds} = Q_{us} + Q_{eff} \quad \textbf{(3-1)}$$

and the dilution factor (DF) is defined as:

$$DF = \frac{Q_{ds}}{Q_{eff}} \quad \textbf{(3-2)}$$

River width, depth and velocity will vary with discharge, with velocity being determined by a combination of the water surface slope and substrate roughness (Giller and Malmqvist, 1998).

Table 3-1 Parameters associated with discharge (European Commission, 2003).

Parameter (units)	Parameter description	Value
pop	Population connected to STP in the hypothetical "sewer shed"	10000
W_u (L cap^{-1} day^{-1})	Water use per capita per day	200
DF	Dilution factor	10
Q_{eff} (L s^{-1})	Discharge from sewage treatment plant (STP). To calculate multiply Wu by pop, then convert to seconds. Equation (3-3)	23.15
Q_{us} (L s^{-1})	Discharge upstream of effluent input Equation (3-4)	208.33
Q_{ds} (L s^{-1})	Discharge downstream of effluent input. Equation (3-1)	231.48
BOD_{us} (mg L^{-1})	BOD concentration upstream of effluent input	2
$Q_{(x)}$	Mean discharge at any point *x* in the system	Calculated by model

$$Q_{eff} = \frac{W_u * pop}{24 * 3600} \quad (3\text{-}3)$$

$$Q_{us} = Q_{eff}(DF - 1) \quad (3\text{-}4)$$

Discharge generally increases downstream (Gray, 1999) and this should be built into the model to increase its realism. However, the TGD makes no assumptions about changes in discharge downstream through a river system. Data on mean discharges for 1996-2000 were taken from the UK's National River Flow Archive for the river Great Ouse at Newport Pagnell (site A) in Buckinghamshire and downstream at Bedford (site B).

Table 3-2 Parameters used to calculate increasing discharge downstream.

Parameter (units)	Parameter description	Value	Reference
Q_A (L s^{-1})	Mean discharge at site A	4870	Centre for Ecology and Hydrology (2003)
Q_B (L s^{-1})	Mean discharge at site B	10200	Centre for Ecology and Hydrology (2003)
F	Fraction of discharge changed (total)	2.09	Equation (3-5)
$Dist_{tot}$ (m)	Total distance between sites A and B	60,000	Google Maps – Appendix A.1
f	Fraction of discharge changed per metre	0.000035	Equation (3-6)

$$F = 1 + \left(\frac{Q_B - Q_A}{Q_A}\right) \quad (3\text{-}5)$$

$$F = 1 + \left(\frac{10200 - 4870}{4870}\right) = 2.09$$

$$f = \frac{F}{dist_{tot}} \quad (3\text{-}6)$$

$$f = \frac{2.09}{60000} = 0.000035$$

Here it is assumed that the increase in discharge downstream is linear. Discharge ($Q_{(x)}$) at any location downstream is calculated by:

$$Q_{(x)} = Q_{ds} + (f * (x * dx)) \quad (3\text{-}7)$$

3.1.4.3 Initial concentration of stressors in the receiving water body (C_0)

Stressors include chemical toxicants and other factors which cause stress in an ecosystem. These include increased temperature, food limitation, increased salinity, low oxygen, UV radiation, predation, competition, lack of food, sanitary determinands and anthropogenic chemicals (SCENIHR *et al.*, 2012). As a case study, five stressors were considered in the model. Three are ubiquitous in all wastewater effluents; these are ammonia, nitrite and low DO. Two are ingredients used in HPC products, namely triclosan and LAS.

To calculate the starting concentration of a stressor (C_0) in the receiving water immediately after the effluent outfall, the concentration in the effluent (C_{eff}) was firstly calculated, using parameters in.

Table 3-3.

Table 3-3 Parameters used to calculate initial concentrations for different stressors in treated and untreated scenarios.

Parameter (units)	Parameter description	Stressor	Value		Reference
			Treated	Untreated	
C_{raw} (µg L^{-1})	Chemical concentration in raw sewage	TCS	13.70		Calculated by equation (3-8)
		LAS	12500		Calculated by equation (3-8)
$Chem_u$ (µg cap^{-1} d^{-1})	Chemical usage per capita per day	TCS	2739.73		Capdevielle *et al.* (2008)
		LAS	2,500,000		OECD SIDS (2005a)
r	Removal fraction in wastewater treatment	TCS	0.9	0	Capdevielle *et al.* (2008)
		LAS	0.95	0	HERA (2009)
C_{eff} (µg L^{-1})	Chemical concentration in sewage effluent. In an untreated scenario $C_{eff} = C_{raw}$	Ammonia (total)	4400	27000	Treated: Gray (2004) Untreated: Finnegan *et al.* (2009)
		Nitrite	800	6900	Treated: Gray (2004) Untreated: Finnegan *et al.* (2009)
		TCS	1.37	13.70	Calculated by equation (3-9)
		LAS	625	12500	Calculated by equation (3-9)

Ammonia and nitrite C_{eff} values for treated and untreated effluents were obtained from the literature.

Concentrations of triclosan and LAS in treated and untreated effluents were calculated from consumer usage figures, as these figures were readily available. To calculate the starting concentration of these stressors from usage data, the following equations were used:

$$C_{raw} = \frac{Chem_u}{W_u} \quad \textbf{(3-8)}$$

$$C_{eff} = (1 - r) * C_{raw} \quad \textbf{(3-9)}$$

The terms for these calculations are all defined previously or in.

Table 3-3.

Triclosan usage is currently estimated to be approximately 0.0028 g cap^{-1} d^{-1} in the UK (Capdevielle *et al.*, 2008) and 0.0062 g cap^{-1} d^{-1} in the USA (De Zwart *et al.*, 2006). In the generic scenario, the UK value is used as it fits best with the European situation. The concentrations in the treated situation are:

$$C_{raw} = \frac{2739.73}{200} = 13.70 \text{ µg L}^{-1}$$

$$C_{eff\ treated} = (1 - 0.9) * 13.70 = \ 0.1 * 13.70 = 1.37 \text{ µg L}^{-1}$$

The concentrations in the untreated scenario are:

$$C_{raw} = \frac{2739.73}{200} = 13.70 \text{ µg L}^{-1}$$

$$C_{eff\ untreated} = (1 - 0) * 13.70 = \ 1 * 13.70 = 13.70 \text{ µg L}^{-1}$$

LAS usage is currently estimated to be 2.5 g cap^{-1} d^{-1} in Western Europe (OECD SIDS, 2005a) and 3.14 – 3.56 g cap^{-1} d^{-1} in the USA (De Zwart *et al.*, 2006; OECD SIDS, 2005a). The value for Europe is used in the model for the generic scenario in line with TGD parameters. Concentrations in the treated scenario are:

$$C_{raw} = \frac{2500000}{200} = 12500 \text{ µg L}^{-1}$$

$$C_{eff\ treated} = (1 - 0.95) * 12500 = \ 0.05 * 12500 = 625 \text{ µg L}^{-1}$$

Concentrations in the untreated scenario are:

$$C_{raw} = \frac{2500000}{200} = 12500 \text{ µg L}^{-1}$$

$$C_{eff\ untreated} = (1 - 0) * 12500 = \ 1 * 12500 = 12500 \text{ µg L}^{-1}$$

Using C_{eff} and parameters in Table 3-1 and 3-3, C_0 is calculated in equation (3-10). It is assumed that some degradable organic matter is present upstream of the effluent, in the river.

$$C_0 = \frac{Q_{us} * BOD_{us} + Q_{eff} * C_{eff}}{Q_{us} + Q_{eff}} \quad \textbf{(3-10)}$$

Parameters relating to DO are unique to this stressor as are the relevant calculations. The initial DO concentration in the river immediately after full mixing of the effluent (DO_O) is:

$$DOo = \frac{DO_{us} * Q_{us} + DO_{eff} * Q_{eff}}{Q_{us} + Q_{eff}} \quad \textbf{(3-11)}$$

where DO_{us} and DO_{eff} are the concentrations of DO upstream and in the effluent respectively. From this the initial OD at the same point (ODo) is calculated as:

$$ODo = DO_{sat} - DO_o \quad \textbf{(3-12)}$$

where DO_{sat} is the DO equilibrium (saturation) concentration at the temperature of the mixed receiving water.

Table 3-4 Stressor initial concentrations calculated in the model.

Parameter (units)	Parameter description	Stressor	Value	
			Treated	Untreated
C_0 (µg L^{-1})	Starting concentration of stressor after mixing	Ammonia (total)	442	2702
		Nitrite	82	692
		Triclosan	2	3
		LAS	64	1252

3.1.4.4 Degradation

Degradation of a stressor within the system can be characterised in terms of its half life if we assume first order kinetics. Chemical half life ($t½$) is the time required for the concentration of a chemical to decrease to half its initial. The concentration of a stressor at flow time t was calculated (Morrall *et al.*, 2004) as follows:

$$C = C_0 * \exp(-kt) \quad \textbf{(3-13)}$$

where k is:

$$k = \frac{\ln(2)}{t_{\frac{1}{2}}} \quad \textbf{(3-14)}$$

In the case of ammonia, losses are not strictly via degradation. Rather they occur due to nitrification which is the process by which ammonium is oxidised to nitrite and, subsequently, to nitrate by chemoautotrophic bacteria (Finnegan *et al.*, 2009). Nitrite is also removed from the system via nitrification to nitrate.

Table 3-5 Degradation parameters.

Parameter (units)	Parameter description	Stressor	Value	Reference
DT_{50} (h)	Chemical half life (t ½)	Ammonia	29.7	NH_4 zero order half life. Whelan *et al.* (1999)
		Nitrite	9.0	Finnegan *et al.* (2009)
		Triclosan	11.3	Morrall *et al.* (2004)
		LAS	12.0	HERA (2009)
k (h^{-1})	Biokinetic rate constant of oxidation	Ammonia	0.02	Equation (3-14)
		Nitrite	0.08	
		LAS	0.06	
		Triclosan	0.06	

3.1.4.5 Velocity

The velocity of the river (m s^{-1}) is influenced by discharge. In general, river velocities increase with discharge because of the reduced effect of bed and bank resistance on the flow. The travel time (t) of a stressor downstream to a distance (d) is:

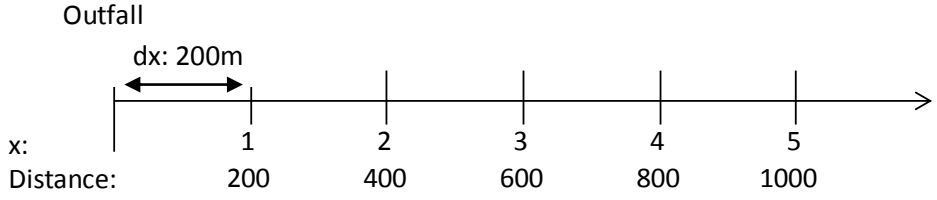
$$t = \frac{d}{v} \tag{3-15}$$

where velocity (v) is calculated using the empirical equation from Round *et al.* (1998):

$$v = 10^{-0.593} * {Q_{ds}}^{0.283} * \left(\frac{Q_{(x)}}{Q_{ds}}\right)^{0.495} \tag{3-16}$$

3.1.4.6 Concentration of stressor at time *t* and distance *x* steps

The distance interval (dx) is arbitrarily assigned as 200m meaning that each point along the river system (x) is equally spaced apart by 200m.



The distance (*d*) from the effluent outfall is measured at each location, *x*, in metres and can be calculated:

$$d = x * dx \quad \textbf{(3-17)}$$

The concentration of a stressor at each point is calculated in equation (3-13) from the initial stressor concentration, the rate of degradation and flow time (Morrall *et al.*, 2004), where *C* is the concentration at that point (µg L^{-1}), C_0 is the starting concentration (µg L^{-1}), *k* is the degradation rate constant (h^{-1}) and *t* is flow time (h).

Ammonia

In surface waters, an equilibrium is assumed to be reached between ionised ammonium (NH_4^+), unionised ammonia (NH_3) and hydroxide ions (OH^-):

$$NH_3 + H_2O \rightleftharpoons NH_4^+ + OH^- \quad \textbf{(3-18)}$$

The equilibrium, and therefore the proportion of NH_3 and NH_4^+ present are pH and temperature dependent (Hellawell, 1986). As pH and temperature increase, the equilibrium is shifted towards the NH_3 species (Broderius *et al.*, 1985; U.S.EPA, 1986). NH_3 is more toxic than NH_4^+, so the proportion of NH_3 present determines the toxicity of total ammonia (De Zwart *et al.*, 2006).

Therefore, the concentration of ammonia at *t* and *x* is converted from that of total ammonia (unionised NH_3 and ionised NH_4^+) to the concentration of NH_3 (Finnegan *et al.*, 2009). The temperature and *pKa* of the system are required to calculate this.

Table 3-6 Parameters in NH_3 calculation.

Parameter (units)	Parameter description	Value	Reference
α	Fraction of N in NH_4^+	0.78	Finnegan *et al*. (2009)
temp(°C)	Water temperature	12	Standard temperature – European Commission (2003)
pKa	Temperature dissociation constant. Equation (3-20)	9.66	US E.P.A. (2013)
pH	Measure of acidity or basicity	7	Standard pH – European Commission (2003)

$$C_{NH_3} = \frac{C_{NH_4}}{\alpha}\left(\frac{1}{1 + 10^{(pKa-pH)}}\right) \quad (3\text{-}19)$$

where C_{NH_4} is the concentration of total ammoniacal nitrogen (µg N L^{-1}).

$$pKa = 0.09018 + \left(\frac{2729.92}{273.2 + temp}\right) \quad (3\text{-}20)$$

where *temp* is temperature in °C.

3.1.5 SSD construction

SSDs were constructed for all stressors using data from toxicity studies on aquatic organisms from the literature. The methods for creating the SSDs themselves are described in section 3.1.6. The first step in creating the SSDs was to obtain toxicity data for the five stressors in the model.

3.1.5.1 Ammonia

Ammonia is an inorganic stressor. Unionised ammonia exerts toxicity to aquatic organisms, including the following effects in fish (McKenzie *et al.*, 2003; Camargo and Alonso, 2006):

- asphyxiation resulting from damage to the gill epithelium;
- reduction in blood oxygen-carrying capacity;

- disruption of blood vessels and osmoregulatory activity affecting the liver and kidneys;
- repression of the immune system increasing susceptibility to bacterial and parasitic diseases;
- lower nervous system function;
- hyperexcitability.

Chronic or long term, toxicity data are available in the EPA's report on ambient quality criteria of ammonia (U.S.EPA, 2013) and are reported as EC20 values which is the concentration at which 20% of the population are adversely affected (Table 3-7). Lower EC20 values suggest that the species is more sensitive to ammonia. These data demonstrate that ammonia is most toxic to bivalves and least toxic to other invertebrates.

Table 3-7 Ammonia toxicity data (U.S.EPA, 2013). The EC20s were normalised to pH7, and to 20°C for invertebrates.

Group	Common name	Species	EC20 (µg Total Ammoniacal Nitrogen L^{-1})
Invertebrate (Bivalve)	Wavy-rayed lampmussel	*Lampsilis fasciola*	1408
Invertebrate (Bivalve)	Fatmucket	*Lampsilis siliquoidea*	3211
Fish	Bluegill	*Lepomis macrochirus*	3273
Invertebrate (Bivalve)	Rainbow mussel	*Villosa iris*	3501
Fish	Rainbow trout	*Oncorhynchus mykiss*	6663
Invertebrate (Bivalve)	Long fingernail clam	*Musculium transversum*	7547
Invertebrate (Mollusc)	Pebblesnail	*Fluminicola sp.*	7828
Fish	Fathead minnow	*Pimephales promelas*	9187
Fish	Sockeye salmon	*Oncorhynchus nerka*	10090
Fish	Smallmouth bass	*Micropterus dolomieui*	11070
Fish	White sucker	*Catostomus commersonii*	11620
Fish	Green sunfish	*Lepomis cyanellus*	14630
Fish	Common carp	*Cyprinus carpio*	16530
Fish	Northern pike	*Esox lucius*	20380
Fish	Channel catfish	*Ictalurus punctatus*	21360
Fish	Lahontan cutthroat trout	*Oncorhynchus clarkii henshawi*	25830
Invertebrate (Amphipod)	Amphipod	*Hyalella azteca*	29170
Invertebrate (Water flea)	Water flea	*Daphnia magna*	41460
Invertebrate (Water flea)	Water flea	*Ceriodaphnia dubia*	45080
Invertebrate (Water flea)	Water flea	*Ceriodaphnia acanthina*	64100
Insect	Stonefly	*Pteronarcella badia*	73740

3.1.5.2 Nitrite

Nitrite is formed by the nitrification of ammonia by the bacteria *nitrosomonas* species (Figure 1-1) in aquatic systems. In freshwater aquatic organisms, particularly fish and crayfish, nitrite exerts toxicity by entering the organism via the gills and then converting oxygen-carrying pigments so that they are incapable of carrying oxygen

resulting in hypoxia and ultimately death. In fish, iron atoms are oxidised (Fe^{2+} is converted to Fe^{3+}) when nitrite enters the red blood cells and functional haemoglobin is converted to methaemoglobin which cannot release oxygen into tissues as the dissociation constant is high. In crayfish, copper atoms are oxidised (Cu^{+} is converted to Cu^{2+}) resulting in haemocyanin being converted into methemocyanin which is unable to bind reversibly to oxygen (Camargo and Alonso, 2006). Other effects of nitrite toxicity are evident in fish and crayfish (Camargo and Alonso, 2006; Jensen, 2003) and include:

- effects on neurotransmission, skeletal muscle contractions and heart function
- formation of mutagenic and carcinogenic N-nitroso compounds;
- damage to the mitochondria in liver cells causing tissue oxygen shortage;
- repression of the immune system;
- hyperventilation.

Fish and crayfish can recover from nitrite exposure by eliminating it via the gills and urine if the exposure is reduced significantly or eliminated (Jensen, 2003).

There are limited chronic toxicity data available for nitrite, in particular for organisms other than fish. Invertebrates are, however, sensitive to acute nitrite exposure suggesting there is another mechanism of action other than via gills (fish and crayfish) (Soucek and Dickinson, 2012). Insects were found to be more sensitive than crustaceans which in turn were more sensitive than molluscs (Soucek and Dickinson, 2012) suggesting that the chronic toxicity dataset (Table 3-8) is not complete. The data presented are in terms of NOECs. As with EC20 values, lower NOEC values represent higher toxicity. The toxicity values for nitrite were converted to equivalent units to ammoniacal nitrogen values i.e. in terms of nitrite-nitrogen (NO_2-N).

Table 3-8 Nitrite toxicity data.

Group	Common name	Species	NOEC (NO_2-N) (µg L^{-1})	Reference
Fish	Rainbow trout	*Oncorhynchus mykiss*	3	Kroupova *et al.* (2008)
Fish	Common carp	*Cyprinus carpio*	2130	Kroupova *et al.* (2010)
Fish	Fathead minnow	*Pimephales promelas*	2730	Adelman *et al.* (2009)
Fish	Silver perch	*Bidyanus bidyanus*	2780	Frances *et al.* (1998)
Fish	Topeka shiner	*Notropis topeka*	4450	Adelman *et al.* (2009)
Fish	Zebrafish	*Danio rerio*	12174	Voslářová *et al.* (2008)
Algae	Green algae	*Desmodesmus subspicatus*	20290	OECD SIDS (2005b)

3.1.5.3 Triclosan

Triclosan (TCS) is a broad spectrum anti-microbial agent present in consumer products (Capdevielle *et al.*, 2008; ECETOC, 2007). Triclosan exerts a toxic effect beyond a baseline narcotic mode of action. It has many intracellular and cytoplasmic target sites and may influence transcription of genes associated with amino acid, carbohydrate and lipid metabolism. In bacteria, it blocks lipid biosynthesis by specifically inhibiting the enzyme enoyl-acyl-carrier protein reductase, which is involved in the fatty acid synthesis, FASII, enzyme system. Fatty acid synthesis pathways are similar in plants (Health Canada and Environment Canada, 2012). The FAS II enzyme is not present in higher vertebrates and based on available data is also not thought to be present in aquatic invertebrates (ECETOC, 2007). This supports observations that TCS does not appear to be as toxic to these organisms as it is to bacteria and algae. TCS does, however, act on less specific targets such as the cell membrane (Nietch *et al.*, 2013). The differences in sensitivity of algae and bacteria to fish are evident from the toxicity data shown in Table 3-9.

Table 3-9 Triclosan toxicity data.

Group	Common name	Species	NOEC (µg L^{-1})	Reference	Primary Reference
Algae		*Pseudokirchneriella subcapitata*	0.2	Lyndall *et al.* (2010)	Yang *et al.* (2008)
Protozoa/ metazoa	Wheel animals	*Rotifer sp.*	0.5	Health Canada & Environment Canada (2012)	Lawrence *et al.* (2009)
Macrophytes	Devil's Beggartick	*Bidens frondosa*	0.6	Health Canada & Environment Canada (2012)	Stevens *et al.* (2009)
Macrophytes	Coffeeweed	*Sesbania herbacea*	0.6	Health Canada & Environment Canada (2012)	Stevens *et al.* (2009)
Cyanobacteria		*Anabaena flos-aquae*	0.67	Lyndall *et al.* (2010)	Orvos *et al*. (2002)
Algae		*Scenedesmus subspicatus*	0.69	Lyndall *et al.* (2010)	Orvos *et al*. (2002)
Cyanobacteria		*Lyngbya sp.*	1	Lyndall *et al.* (2010)	Lawrence *et al.* (2009)
Cyanobacteria		*Microcystis aeruginosa*	1	Lyndall *et al.* (2010)	Lawrence *et al.* (2009)
Cyanobacteria		*Oscillatoria tenius*	1	Lyndall *et al.* (2010)	Lawrence *et al.* (2009)
Algae		*Scenedesmus quadricauda*	1	Lyndall *et al.* (2010)	Lawrence *et al.* (2009)
Algae		*Dunaliella tertiolecta*	1.6	Lyndall *et al.* (2010)	DeLorenzo and Fleming (2008)
Macrophytes	False daisy	*Eclipta prostrata*	2.2	Health Canada & Environment Canada (2012)	Stevens *et al.* (2009)
Invertebrate	Amphipod / lawn shrimp	*Hyalella azteca*	5	Dussault *et al.* (2008)	—
Algal & bacterial community		Algal & bacterial community	10	Health Canada & Environment Canada (2012)	Lawrence *et al.* (2009)
Algae		*Ulothrix sp.*	10	Lyndall *et al.* (2010)	Lawrence *et al.* (2009)
Algae		*Navicula pelliculosa*	10.7	Lyndall *et al.* (2010)	Orvos *et al*. (2002)

Group	Common name	Species	NOEC (µg L^{-1})	Reference	Primary Reference
Fish	Rainbow trout	*Oncorhynchus mykiss*	34.1	Lyndall *et al.* (2010)	Orvos *et al*. (2002)
Invertebrate	Water flea	*Daphnia magna*	40	Lyndall *et al.* (2010)	Orvos *et al*. (2002)
Invertebrate		*Brachionus calyciflorus*	50	Lyndall *et al.* (2010)	Ferrari *et al.* (2002)
Fish	Mosquitofish	*Mosquitofish*	76.6	Health Canada & Environment Canada (2012)	Raut and Angus (2010)
Insect	Midge	*Chironomus tentans*	80	Health Canada & Environment Canada (2012)	Dussault *et al.* (2008)
Algae		*Ankistrodesmus falcatus*	100	Lyndall *et al.* (2010)	Lawrence *et al.* (2009)
Cyanobacteria		*Glaucocystis nostochinea*	100	Lyndall *et al.* (2010)	Lawrence *et al.* (2009)
Cyanobacteria		*Nostoc sp.*	100	Lyndall *et al.* (2010)	Lawrence *et al.* (2009)
Algae		*Synedra sp.*	100	Lyndall *et al.* (2010)	Lawrence *et al.* (2009)
Fish	Japanese medaka	*Oryzias latipes*	156	Lyndall *et al.* (2010)	Ishibashi *et al.* (2004)
Fish	Zebrafish	*Danio rerio*	160	Lyndall *et al.* (2010)	Tatarazako *et al.* (2004)
Algae		*Closterium ehrenbergii*	250	Lyndall *et al.* (2010)	Ciniglia *et al.* (2005)
Invertebrate	Midge	*Chironomus riparius*	440	Lyndall *et al.* (2010)	Memmert (2006)

3.1.5.4 LAS

Linear alkyl benzene sulphonate (LAS) is a surfactant used globally in household detergents (Boeije *et al.*, 2000). The mode of action of LAS is not specific, but via general narcosis, or baseline toxicity, causing a non-specific disturbance to the structure and function of cell membranes (Zhang *et al.*, 2010; Escher *et al.*, 2011). Recovery often occurs in organisms affected by baseline toxicity, although slow respiration can limit diffusion to excretory membranes and increase the time to

recovery (Escher *et al.*, 2011). A review of available chronic toxicity data are presented in terms of NOECs (HERA, 2009) in Table 3-10. The range of sensitivity of LAS is different to other stressors e.g. algae are sensitive to triclosan but less sensitive than other organisms to LAS.

Table 3-10 LAS toxicity data (HERA, 2009)

Group	Species	NOEC (µg L^{-1})
Fish	*Tilapia mossambica*	250
Fish	*Oncorhynchus mykiss*	340
Algae	*Microcystis spec*	800
Fish	*Pimephales promelas*	870
Crustacean	*Daphnia magna*	1400
Fish	*Brachydanio rerio*	2300
Insect	*Chironomus riparius*	2800
Crustacean	*Ceriodaphnia spec.*	3200
Fish	*Poecilia reticulata*	3200
Insect	*Paratanytarsus parthenogenica*	3400
Algae	*Chlorella kessleri*	3500
Algae	*Selenastrum spec.*	3800
Algae	*Scenedesmus subspicatus*	7700
Algae	*Chlamydomonas reinhardi*	12000
Algae	*Plectonema boryanum*	15000

3.1.5.5 Dissolved oxygen

Uptake of oxygen by aquatic invertebrates from the water generally occurs by passive diffusion, which is driven by the difference in oxygen partial pressure between the animal (internal) and the water (external) (Rostgaard and Jacobsen, 2005). The discharge of organic wastewater places a demand on DO in the system (Dunnivant, 2004; Welch, 1992) (Figure 3-3). A measure of this demand is the amount of DO utilised to degrade the organic matter present in a sample in a given period of time at a given temperature i.e. the BOD. In streams, reaeration is a function of current

velocity and water depth (U.S.EPA, 2012). The oxygen deficit (OD) is DO concentration in relation to the oxygen saturation at that temperature (Gray, 1999).

The Streeter-Phelps model describes the change in OD in a river via two primary mechanisms which influence the concentration of DO, deoxygenation and reoxygenation (Streeter and Phelps, 1925). Deoxygenation occurs as a result of biochemical oxygen demand resulting from the decomposition of organic matter by microbes in the system (Gotovtsev, 2010; Nas and Nas, 2009). Reaeration (reoxygenation) results from oxygen input from the atmosphere to the water which is enhanced by turbulence. Photosynthesis can also introduce oxygen into the water column during the day (Nas and Nas, 2009). Although this can be an important process, particularly in the tropics, it is not considered explicitly here. Initially following effluent discharge, the rate of deoxygenation exceeds the rate of reaeration. Further downstream deoxygenation equals reaeration and subsequently the rate of reaeration exceeds the deoxygenation rate resulting in recovery (Gray, 2004). The pattern is known as the DO sag curve and is illustrated schematically in Figure 3-3. The Streeter-Phelps model relates to a steady state scenario i.e. concentrations do not change with time, with one dimensional flow (Noutsopoulos and Kyprianou, 2014).

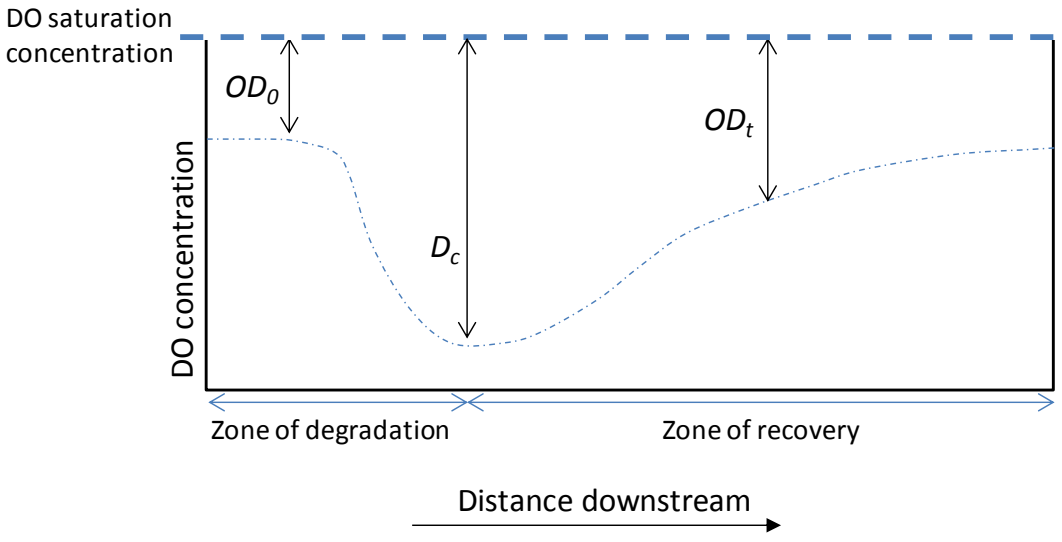


Figure 3-3 Streeter-Phelps DO sag curve. OD_0: oxygen deficit in river after mixing of effluent in river; D_c: critical/minimum DO concentration (i.e. maximum deficit); OD_t: oxygen deficit at any point at flow time *t* downstream. Adapted from Dunnivant (2004) and Gray (1999).

It is assumed that the reaeration rate is directly proportional to the size of the deficit. Deoxygenation is assumed to be proportional to BOD (Nas and Nas, 2009).

The oxygen deficit is described by Streeter-Phelps (1925) as

$$\frac{d(OD_t)}{dt} = k_{BOD} * BOD - k_{reaerate}.D_t \quad \textbf{(3-21)}$$

which has the solution:

$$D_t = \frac{k_{BOD}BOD_0}{k_{reaerate} - k_{BOD}} \left(exp(-k_{BOD}\tau) - \exp(-k_{reaerate}\tau)\right) + OD_0\exp(-k_{reaerate}\tau) \quad \textbf{(3-22)}$$

where

- OD_t is the oxygen deficit (mg L^{-1}) at any point in the flow at flow time *t* downstream of a point pollution source
- τ is the flow time (distance travelled / mean velocity) in days
- BOD_0 is the BOD (mg L^{-1}) immediately after full mixing of the effluent and the river (mixing is assumed to be instantaneous and complete)
- OD_0 is the oxygen deficit in the river (mg L^{-1}) immediately after full mixing of the effluent
- k_{BOD} is the degradation rate constant for BOD (day^{-1})
- $k_{reaerate}$ is the rate coefficient for re-oxygenation (day^{-1})

Both k_{BOD} and $k_{reaerate}$ are assumed in the Streeter-Phelps model to be constant (Gray, 2004).

Although other processes such as oxidation of sediment deposits and sedimentation can also impact the oxygen concentration, deoxygenation and reoxygenation are often the predominant processes (Gray, 2004). Lower DO concentrations are more toxic due to respiratory stress (U.S.EPA, 2012). In the absence of chronic toxicity data for DO in the literature, acute data in the form of LC50/EC50s were obtained (see Table_A 1 in Appendix A.2 for toxicity data, corresponding temperatures and references).

As the studies were conducted at different temperatures, which will have influenced the DO concentration, the LC50/EC50s were calculated in terms of OD from saturation at the reported temperature. The oxygen saturation concentration of DO at a given temperature, at 1 atmosphere in freshwater, was calculated from the equation used in the US EPA's river and stream water quality model, QUAL2K (Chapra *et al.*, 2008):

$$DO_{sat} = \exp\left(-139.3441 + \left(\frac{1.575701 \text{ x } 10^5}{temp_a}\right) - \left(\frac{6.642308 \text{ x } 10^7}{(temp_a)^2}\right) + \left(\frac{1.2438 \text{ x } 10^{10}}{(temp_a)^3}\right) - \left(\frac{8.621949 \text{ x } 10^{11}}{(temp_a)^4}\right)\right) \quad \textbf{(3-23)}$$

where $temp_a$ is the absolute temperature (in Kelvin (*K*)), which is the temperature in degrees Celsius plus 273.15.

The measured DO concentration was subtracted from the oxygen saturation concentration, giving the oxygen deficit (Gray, 1999).

$$[OD] = [DO_{sat}] - [DO_{toxicity}] \quad \textbf{(3-24)}$$

The OD toxicity values are in Table 3-11.

Table 3-11 Oxygen Deficit toxicity data.

Common name	Species	DO saturation (mg L^{-1})	OD (mg L^{-1})
Mayfly	*Ephemerella subvaria*	10.75	6.85
Stonefly	*Acroneuria lycorias*	10.75	7.15
Mayfly	*Baetisca laurentina*	10.75	7.25
Mayfly	*Ephemerella doddsi*	12.45	7.25
Caddisfly	*Hydropsyche betteri*	10.76	7.86
Mayfly	*Callibaetis montanus*	12.45	8.05
Stonefly	*Pteronarcys dorsata*	10.75	8.55
Mayfly	*Leptophlebia nebulosa*	10.75	8.55
Stonefly	*Pteronarcys californica*	12.45	8.55
Caddisfly	*Neophylax sp.*	12.45	8.65
Amphipod crustacean	*Gammarus pseudolimnaeus*	10.74	8.83
Stonefly	*Diura knowltoni*	12.45	8.85
Caddisfly	*Hydropsyche sp.*	12.45	8.85
Stonefly	*Arcynopteryx aurea*	12.45	9.15
Stonefly	*Nemoura cinctipes*	12.45	9.15
Diptera	*Simulium vittatum*	12.45	9.25
Midge	*Chironomus tentans fabricius*	10.94	9.34
Mayfly	*Hexagenia limbata*	10.75	9.35
Mayfly	*Ephemerella grandis*	12.45	9.45
Fish	*Deltistes luxatusi*	10.80	9.53
Midge	*Chironomus dilutus larvae*	10.85	9.85
Stonefly	*Pteronarcella badia*	12.45	10.05
Crustacean	*Daphnia pulex*	10.81	10.11
Amphipod crustacean	*Hyallela azteca*	10.81	10.51
Crustacean	*Daphnia magna*	11.24	10.59
Caddisfly	*Drusinus sp.*	12.45	10.65
Amphipod crustacean	*Gammarus lacustris*	11.17	10.67
Caddisfly	*Neothrema alicia*	12.45	10.75
Stonefly	*Acroneuria pacifica*	12.45	10.85

3.1.6 Creating the SSDs

SSDs represent the toxicity of one stressor to many different species. The SSDs were compiled in Microsoft Excel. The parameters in each SSD are stressor specific.

Table 3-12: Parameters in the SSDs.

Parameter	Parameter description
mu (μ)	Mean of log-transformed data in a lognormal distribution
sigma (σ)	Standard deviation of log-transformed data in a lognormal distribution
NOEC (μg L^{-1})	No observed effect concentration. Measure of chronic toxicity. Ammonia toxicity values are reported as EC20 and DO as LC50 or EC50. Generally in the code, these are all referred to as NOECs.
PAF	Potentially affected fraction of species
Observed PAF	PAF calculated from species data
Model PAF	PAF calculated using a lognormal distribution
RMSE	Root Mean Square Error – square root of the average of all error square values

Briefly, NOEC (or relevant toxicity) values were sorted in ascending order and a rank was assigned to each value from the rank. The observed PAF was calculated from the rank:

$$Observed\ PAF = \frac{rank}{number\ of\ species + 1} \quad (3\text{-}25)$$

The Model PAF was obtained by fitting a lognormal distribution to the observed data.

The error squared of the Model PAF and Observed PAF was then calculated:

$$Error\ squared = (Model\ PAF - Observed\ PAF)^2 \quad (3\text{-}26)$$

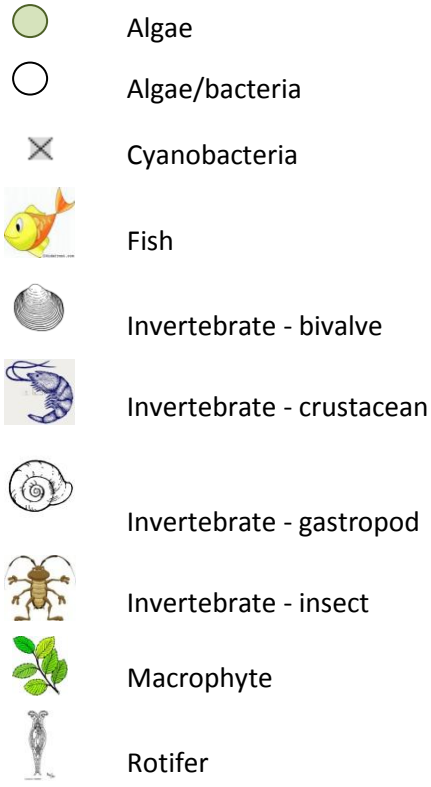
The RMSE was calculated by taking the square root of the average of all error square values. This is a measure of how similar the observed and model PAFs are.

The Solver tool in Microsoft Excel was used to minimise the RMSE (Root Mean Square Error) by altering mu (subject to being ≥0) and sigma (subject to being ≥0) in a trial and error optimisation.

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(M_i - O_i)^2} \quad \textbf{(3-27)}$$

where n is the number of species, i is the rank, M_i is the model PAF and O_i is the observed PAF.

Observed and model PAFs are shown in Figures 3-4, 3-5, 3-6, 3-7 and 3-8, for ammonia, nitrite, TCS, LAS (note log scales) and OD respectively. Data points were labelled by family name to illustrate that families can appear multiple times in a dataset or across datasets. The datapoints were classified by organism type to enable any patterns in sensitivity to be more easily identified.



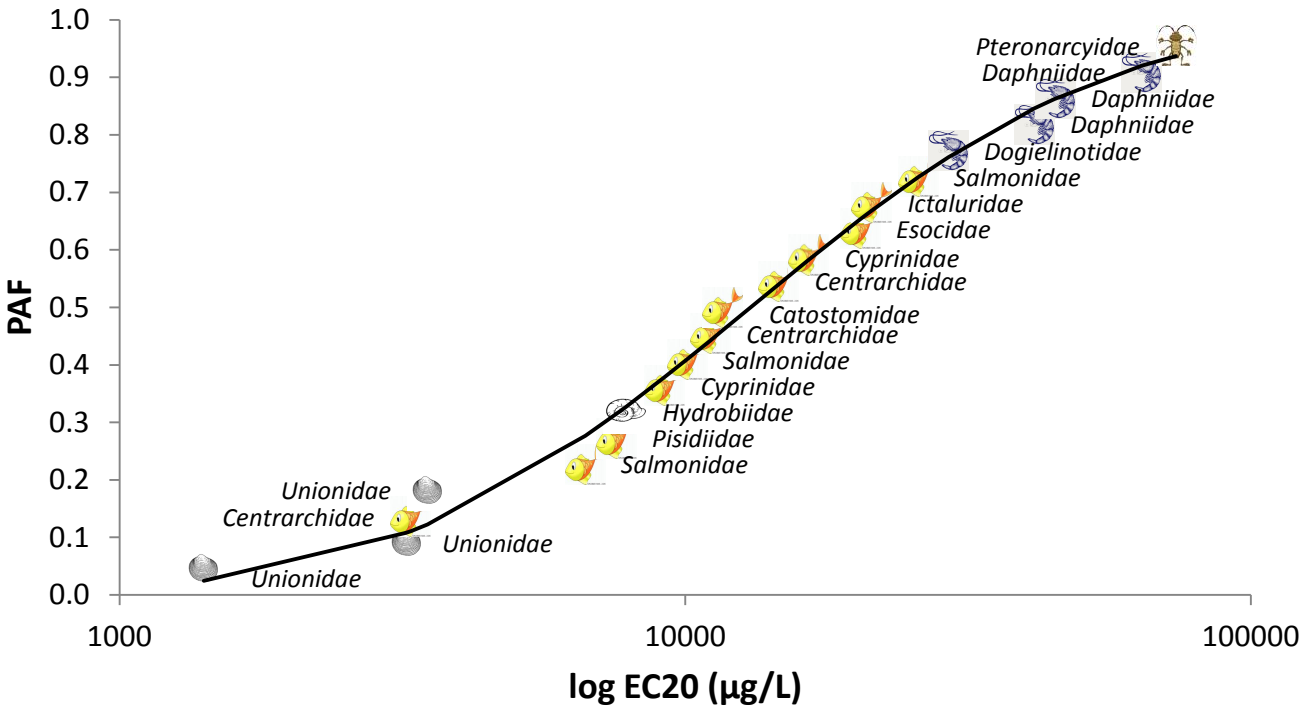


Figure 3-4 SSD for total ammoniacal nitrogen. The line shows the best fit cumulative log normal distribution (model PAFs). The data points (observed PAFs) are labelled by organism type and data labels by family.

It is clear from the ammonia SSD that a pattern of sensitivity exists with bivalves being more sensitive than fish, and fish in turn more sensitive than arthropods.

In the model, the EC20 values were converted into values for NH_3 to be comparable to the concentrations at each step downstream. The original EC20s were represented as µg total ammoniacal Nitrogen L^{-1}, therefore the contribution from the ammonium ion (NH_4^+) or unionised ammonia (NH_3) was not known. The EC20s were converted using equation (3-19). The results of the toxicity studies (Table 3-7) were normalised to pH7, and 20°C for invertebrates.

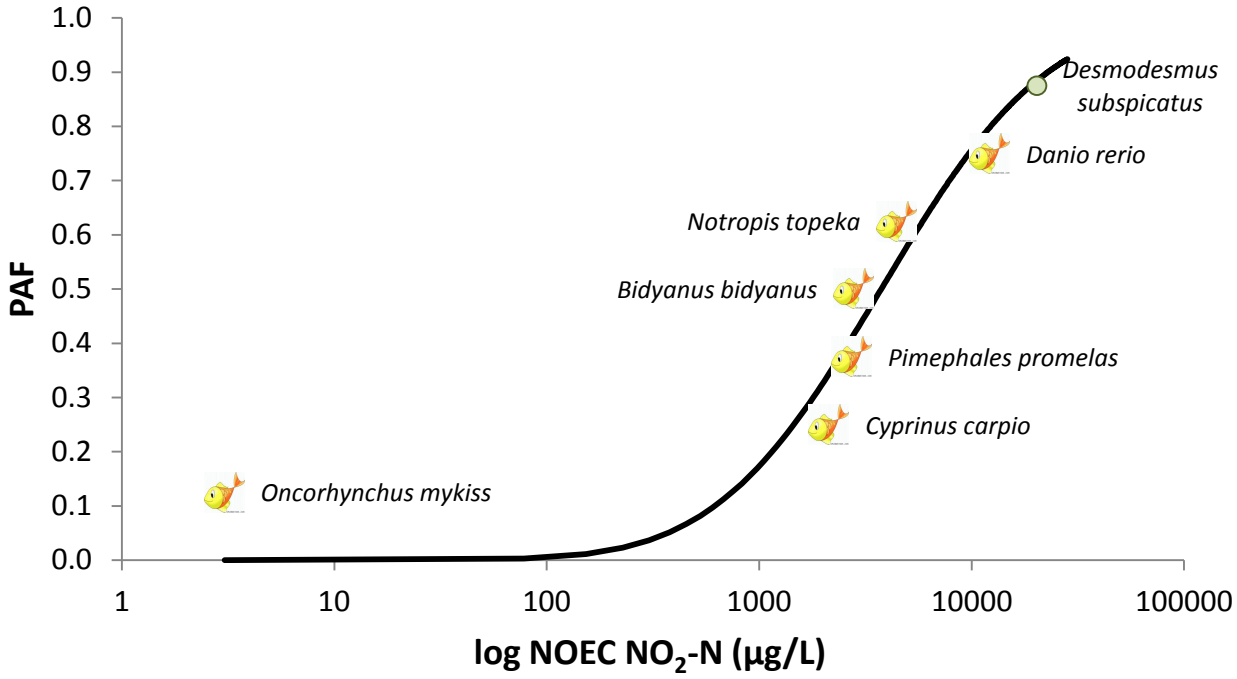


Figure 3-5 SSD for nitrite. The line shows the best fit cumulative log normal distribution (model PAFs). The data points (observed PAFs) are labelled by organism type and data labels by family.

The low availability of chronic toxicity data for nitrite creates a data sparse SSD dominated by fish.

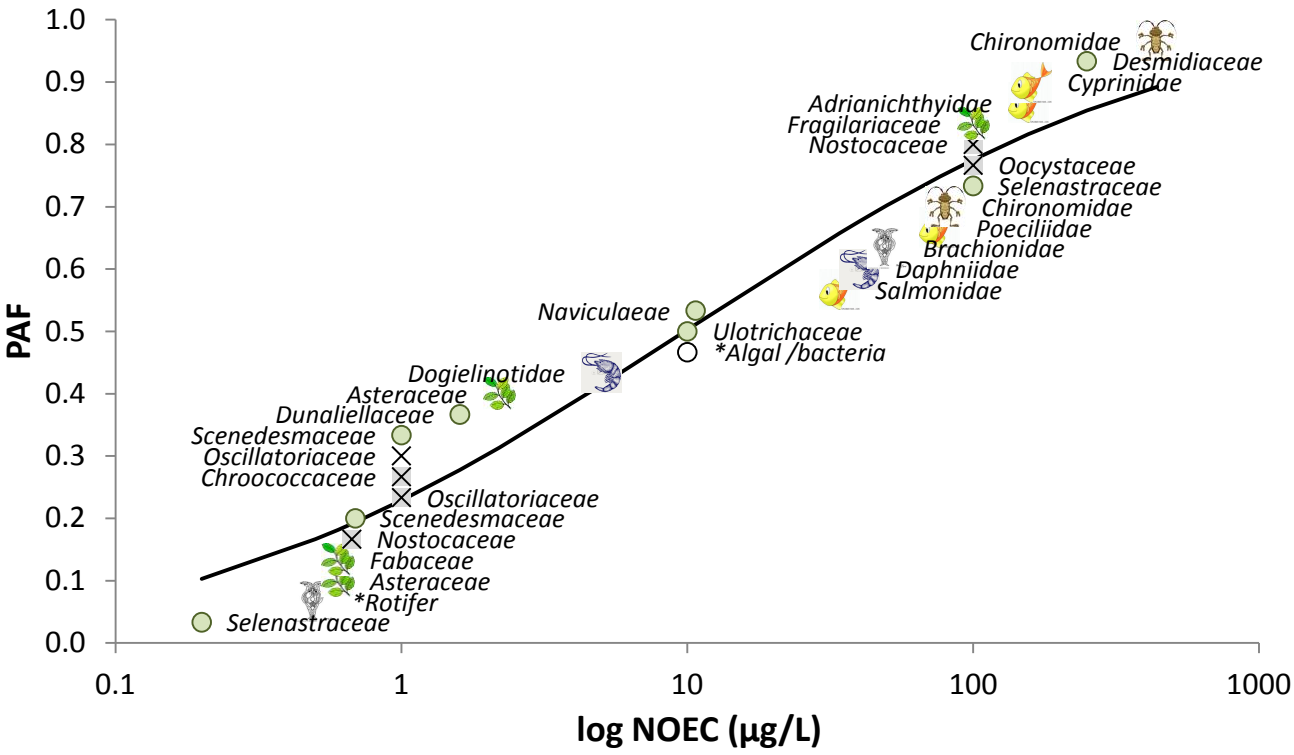


Figure 3-6 SSD for triclosan. The line shows the best fit cumulative log normal distribution (model PAFs). The data points (observed PAFs) are labelled by organism type and data labels by family.

The toxicity of triclosan to aquatic organisms varies by about four orders of magnitude. Macrophytes, cyanobacteria and algae are generally most sensitive to triclosan while fish and invertebrates demonstrate higher tolerance. However, there is some variation among the sensitive groups indicating there are more specific characteristics of the former groups which influence sensitivity.

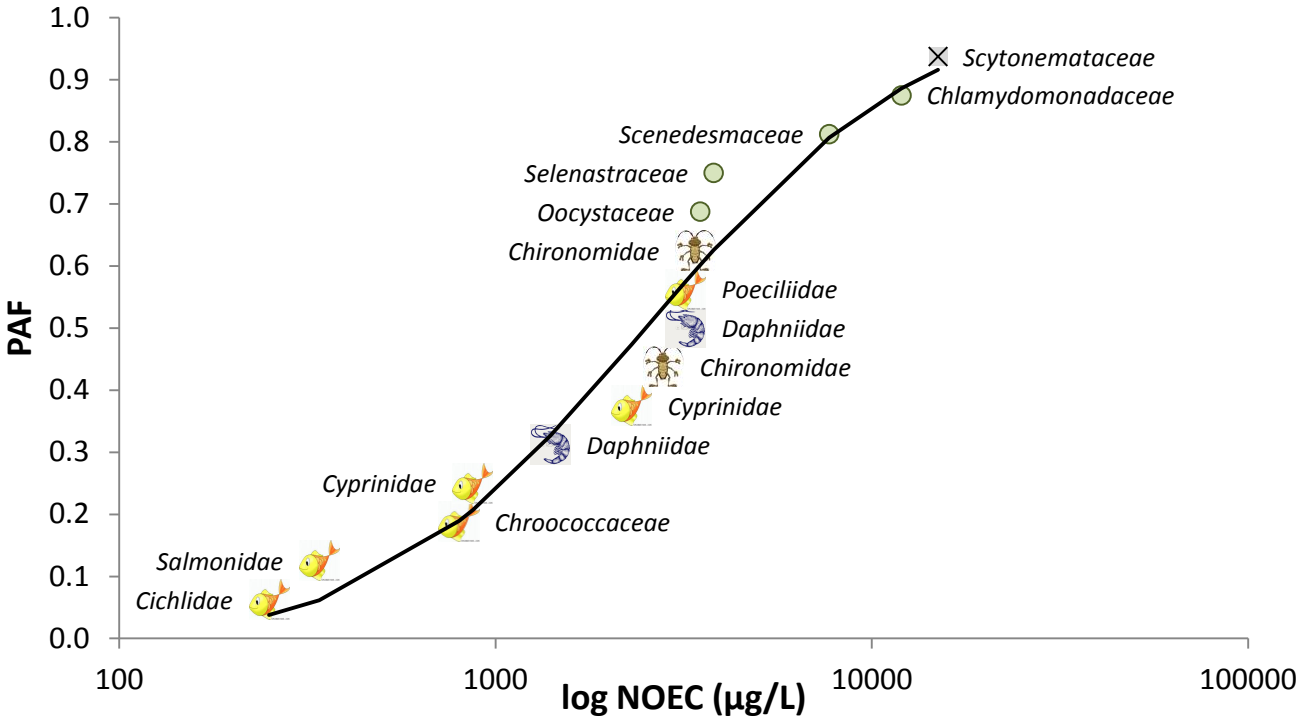


Figure 3-7 SSD for LAS. The line shows the best fit cumulative log normal distribution (model PAFs). The data points (observed PAFs) are labelled by organism type and data labels by family.

Despite the fact that LAS does not have a specific mode of action there is still a pattern in terms of its toxicity to different types of organisms. Fish are generally most sensitive and algae are generally more tolerant.

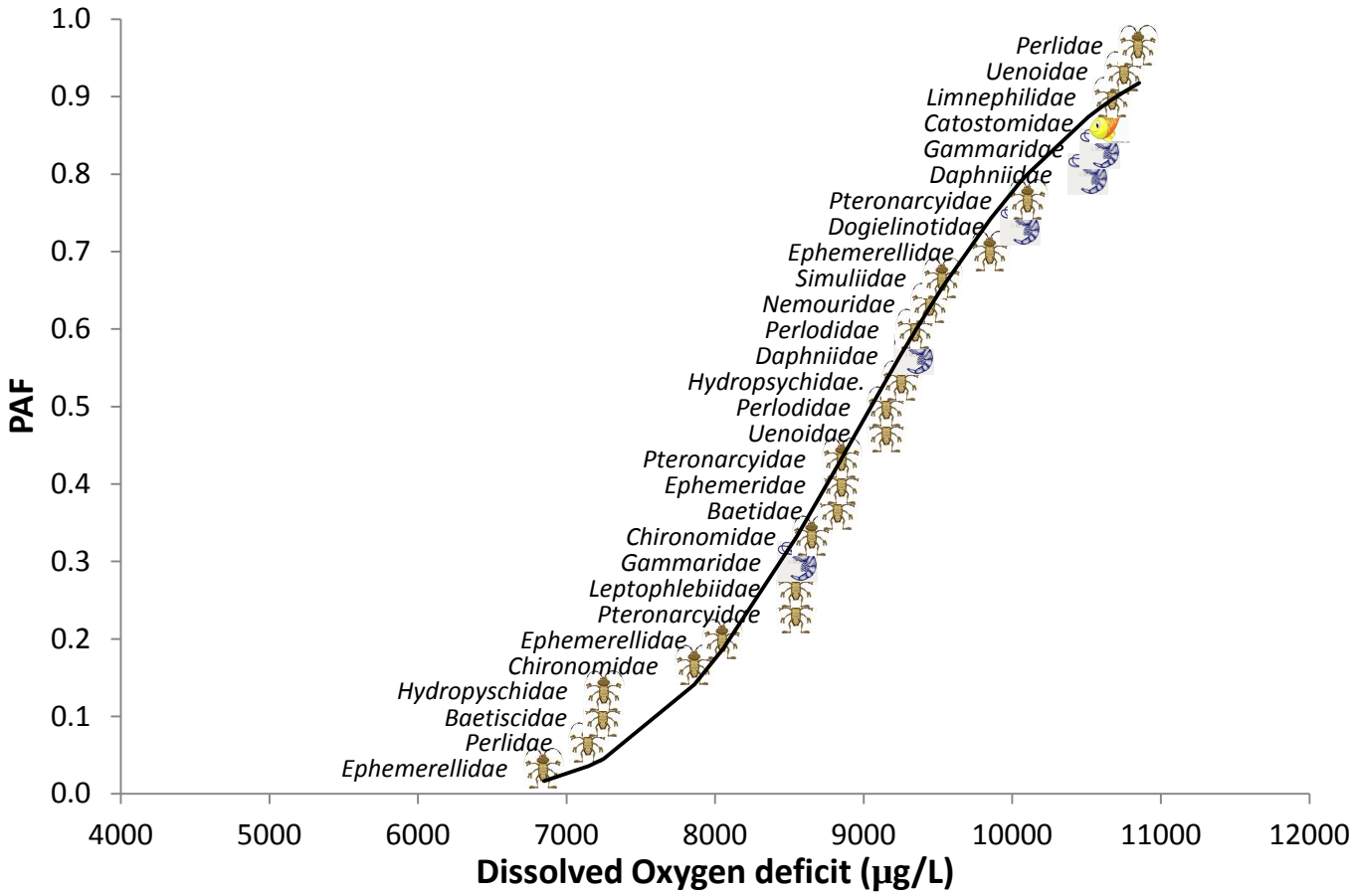


Figure 3-8 SSD for oxygen deficit (OD). The line shows the best fit cumulative log normal distribution (model PAFs). The data points (observed PAFs) are labelled by organism type and data labels by family. Note that the *x* axis values (OD) are not logtransformed.

The SSDs for all five stressors represented by species names, rather than family names, are in Appendix A.3.

3.1.6.1 Potentially Affected Fraction (PAF) of Species

In the model, predicted PAF for a given stressor concentration at location *x* is calculated from the following equation (Press, 2007):

$$PAF = \frac{1}{2} . erfc. (\frac{1}{\sqrt{2}} \left[\frac{\log(C_x) - \mu}{\sigma}\right]) \quad \textbf{(3-28)}$$

which can be simplified to:

$$PAF = \frac{1}{2} . erfc. (y) \quad \textbf{(3-29)}$$

where *erfc* (*y*) is complementary error function integral between y and infinity (∞).

In the model *y* is always set as negative and Microsoft Excel 2007 cannot calculate the *erfc* of a negative number. This has been corrected in subsequent versions of Microsoft Excel. However, *erfc*(–*y*) = –*erfc*(*y*) which can be used as a workaround. A sub routine to take the *erfc* of positive *y*, and then to make the result negative is written into the model to work around this error.

3.1.6.2 Taxa present at time *t* and location *x*

Model outputs at each step downstream include the distance (metres) downstream and corresponding flow time (hours), stressor concentration (µg L^{-1}) and predicted PAF. The species present at each step downstream are also predicted from the SSD. Moving downstream, more species are predicted to reappear as stressor concentrations decrease. This is illustrated schematically in Table 3-13.

Table 3-13 Schematic illustration of model outputs in terms of the taxa present at given stations in the impact zone, based on the toxicity of a single stressor. ✓ = species present.

Distance (m)	Time (h)	Stressor conc. (µg L^{-1})	PAF	Species 1	Species 2	Species 3	Species 4	Species 5	Number of species present
200	0.02	32	0.80					✓	**1**
400	0.04	29	0.60				✓	✓	**2**
600	0.06	25	0.40			✓	✓	✓	**3**
800	0.08	21	0.35			✓	✓	✓	**3**
1000	0.10	15	0.25		✓	✓	✓	✓	**4**
1200	0.12	9	0.20		✓	✓	✓	✓	**4**
1400	0.14	2	0.05	✓	✓	✓	✓	✓	**5**

3.1.7 Streeter-Phelps model

DO concentrations, expressed as OD, are predicted at points downstream using the Streeter-Phelps model. The parameters used in the Streeter-Phelps part of the model are presented in Table 3-14.

Table 3-14 Parameters used in Streeter-Phelps model.

Parameter (units)	Parameter description	Value	Details	Reference
BOD_{eff} (mg L^{-1})	BOD concentration in effluent	800	Untreated scenario. 190 (Metcalf and Eddy 2003); 200-800 (Hellawell 1986; Gray 1999). Lowest of range taken.	Hellawell (1986), Gray (1999) and Metcalf and Eddy (2003)
		20	Treated scenario. 3-50 (Hellawell 1986; Gray 1999). Design of STPs in Ireland and UK based on Royal Commission with standard effluent of 20 mg L^{-1} (Gray 1999).	Hellawell (1986) and Gray (1999)
BOD_{us} (mg L^{-1})	BOD concentration upstream	2	For relatively healthy streams.	University of Wisconsin (2005)
DO_{us} (mg L^{-1})	DO concentration upstream	8.1	Average of 2 cleanest sites in Belgian rivers. Close to 7 mg L^{-1} limit of EU Freshwater Fish Directive for salmonids (Gray 1999).	Comte *et al*. (2010)
DO_{eff} (mg L^{-1})	DO concentration in effluent	1	Untreated effluent. If effluent is kept moving, it should maintain minimum of 1-2 mg L^{-1} DO.	Gray (1999)
		4	Treated effluent	Birge *et al*. (1989)
k_{BOD} (h^{-1})	First order rate constant for BOD biodegradation	0.015	Range of values over varying discharge and temperature: 0.0008 - 0.015 h^{-1}. Mean of 0.015 h^{-1} in Sacramento River.	Bansal (1975) Padgett (1978)
$k_{reaerate}$ (h^{-1})	Rate constant for reaeration	0.03	0.03 h^{-1} in Sacramento River.	Padgett (1978)

BODo is the initial BOD concentration in the river after mixing. It is assumed that some degradable organic matter is assumed to be present in the river water upstream of the effluent. *BODo* is calculated in the model as follows:

$$BODo = \frac{Q_{us} * BOD_{us} + Q_{eff} * BOD_{eff}}{Q_{us} + Q_{eff}} \quad \textbf{(3-30)}$$

$k_{reaerate}$ is temperature dependent in reality and could be converted but this was not done in the model as the $k_{reaerate}$ value was not known with a high degree of uncertainty for the scenario being considered.

The travel time between distance steps ($\Delta\tau$; h) is calculated in the model as:

$$\Delta\tau = \frac{dx}{vel_{(x)} * 3600} \quad \textbf{(3-31)}$$

where $vel_{(x)}$ is the velocity at a distance step.

The BOD concentration at each location ($BOD_{(x)}$) is calculated in the model as:

$$BOD_{(x)} = BOD(x-1) * \exp(-k_{BOD} * \Delta\tau) \quad \textbf{(3-32)}$$

The OD concentration at each location ($OD_{(x)}$) is calculated from:

$$OD_{(x)} = \left(\frac{k_{BOD} * BOD(x-1)}{k_{reaerate} - k_{BOD}} * \exp(-k_{BOD} * \Delta\tau) - \exp(-k_{reaerate} * \Delta\tau)\right) + OD(x-1) * \exp(-k_{reaerate} * \Delta\tau) \quad \textbf{(3-33)}$$

The model output for OD is similar to that of the other stressors, here the stressor concentration given is in terms of OD (Table 3-15). It is expected that the minimum number of species will not be at the initial distance points as for the other stressors, but further downstream reflecting an oxygen sag curve in the system.

Table 3-15 Schematic illustration of model outputs in terms of species present at given stations in the impact zone based on exposure to OD.

Distance (m)	Time (h)	OD (µg L^{-1})	PAF	Species 1	Species 2	Species 3	Species 4	Number of species present	BOD (mg L^{-1})	DO (mg L^{-1})
200	0.02	2000	0.40		✓	✓	✓	3	23	8
400	0.04	2050	0.45			✓	✓	2	20	5
600	0.06	3200	0.55				✓	1	30	1
800	0.08	2000	0.40			✓	✓	2	20	5
1000	0.10	1400	0.20	✓	✓	✓	✓	5	12	10

The effect on the taxa was expressed in the output at each location downstream in terms of the fraction of species present (1-PAF) and species richness (number of species). To calculate species richness, the duplicate species were removed when they were present in more than one SSD and predicted to be present at that location as a result of the concentration of more than one stressor. In order the calculate the PAF at a particular location, as a result of all stressors in the model, the maximum PAF of the five PAFs (for each stressor) was assumed to be the overall effect on the taxa (PAF_{max}).

3.2 Conceptual model results

Along with the predicted taxa present with distance downstream, the proportional influence of each stressor on the species richness and the combined effect of all stressors were also predicted.

Figure 3-9 shows the predicted stressor concentrations with distance downstream for the untreated discharge scenario.

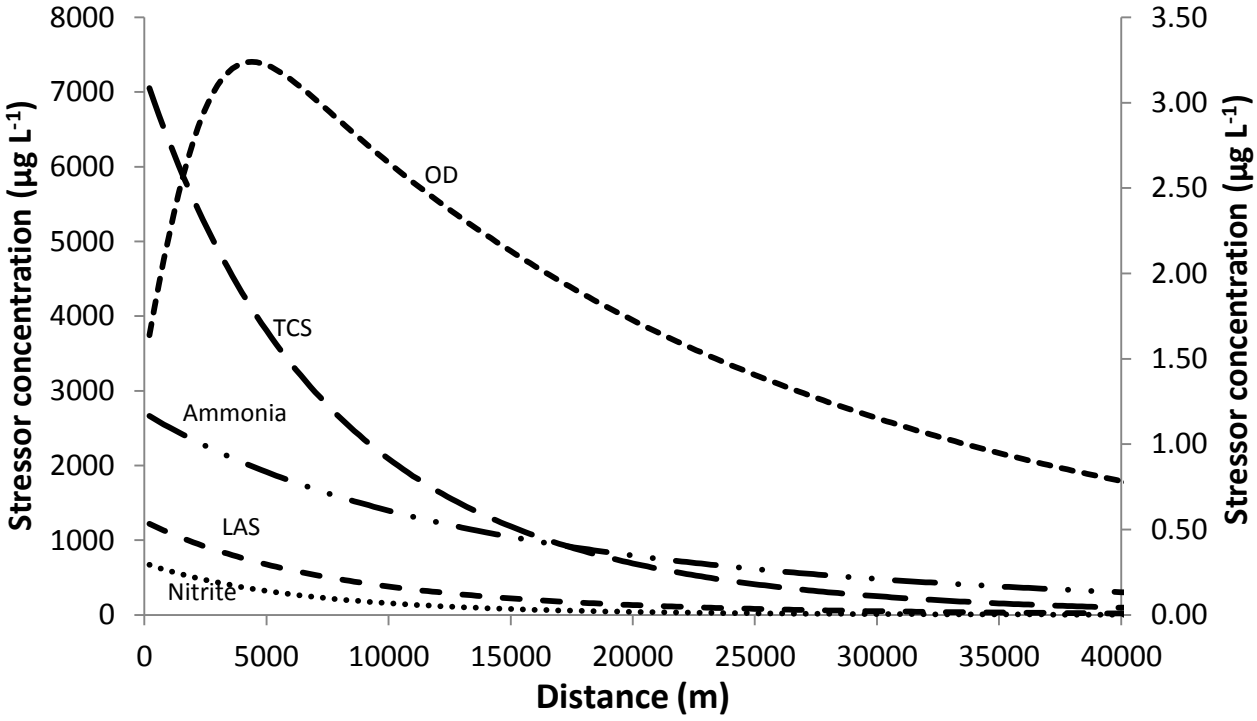


Figure 3-9 Stressor concentrations downstream of the effluent outfall in the untreated discharge scenario. Note secondary *y* axis for TCS.

The concentrations of stressors vary according to their initial concentrations and degradation rates. The impact of these stressor concentrations on taxa will depend on their NOECs. OD has the highest concentration, which increases further downstream due to the effect of the oxygen sag, before decreasing (see Figure 3-10 for more on oxygen concentrations). In order of concentration starting at the next highest are ammonia, LAS, nitrite and TCS. Of these last four stressors, the concentration of ammonia is higher than the others further along the impact zone also. The concentration of TCS falls to 0 mg L^{-1} at 65,000m downstream. The end of the impact zone is determined by the stressor concentration where no toxic effects are experienced by taxa.

In Figure 3-10 are the outputs in terms of oxygen: OD, DO concentration and BOD. BOD declines from the effluent outfall downstream. OD on the other hand increases in the first few kilometres (up to 4600m) before decreasing as the reaeration rate exceeds the deoxygenation rate.

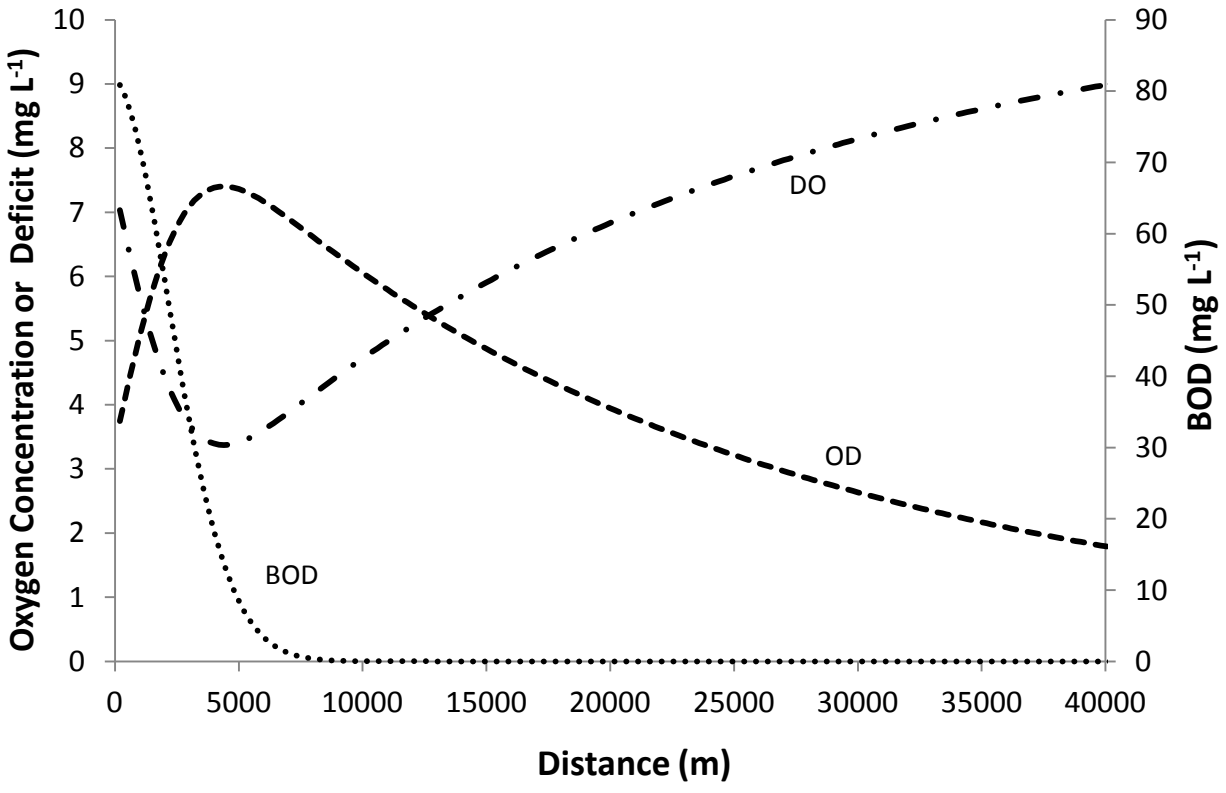


Figure 3-10 Predicted changes in the concentrations of deficit (OD), DO concentration and BOD downstream from the untreated discharge scenario.

The predicted PAFs as a result of each stressor with distance downstream are shown in Figure 3-11. In this case, triclosan causes a greater affect further distance downstream than the other stressors, despite its concentration being lower than the other stressor concentrations (Figure 3-9). This is a result of both its exposure, influenced by its initial concentration and degradation, and its toxicity to aquatic organisms. The exposure of each stressor downstream varies due to the relative rates of change as a result of their different half lives (degradation parameters in Table 3-5). The half-lives of NO_2^-, TCS and LAS are relatively similar at 9, 11.3 and 12 hours respectively. The half-life of ammonia at 29.7 hours, is much longer, therefore it is likely to be present either at higher concentrations and/or further downstream than the other stressors as it persists for longer.

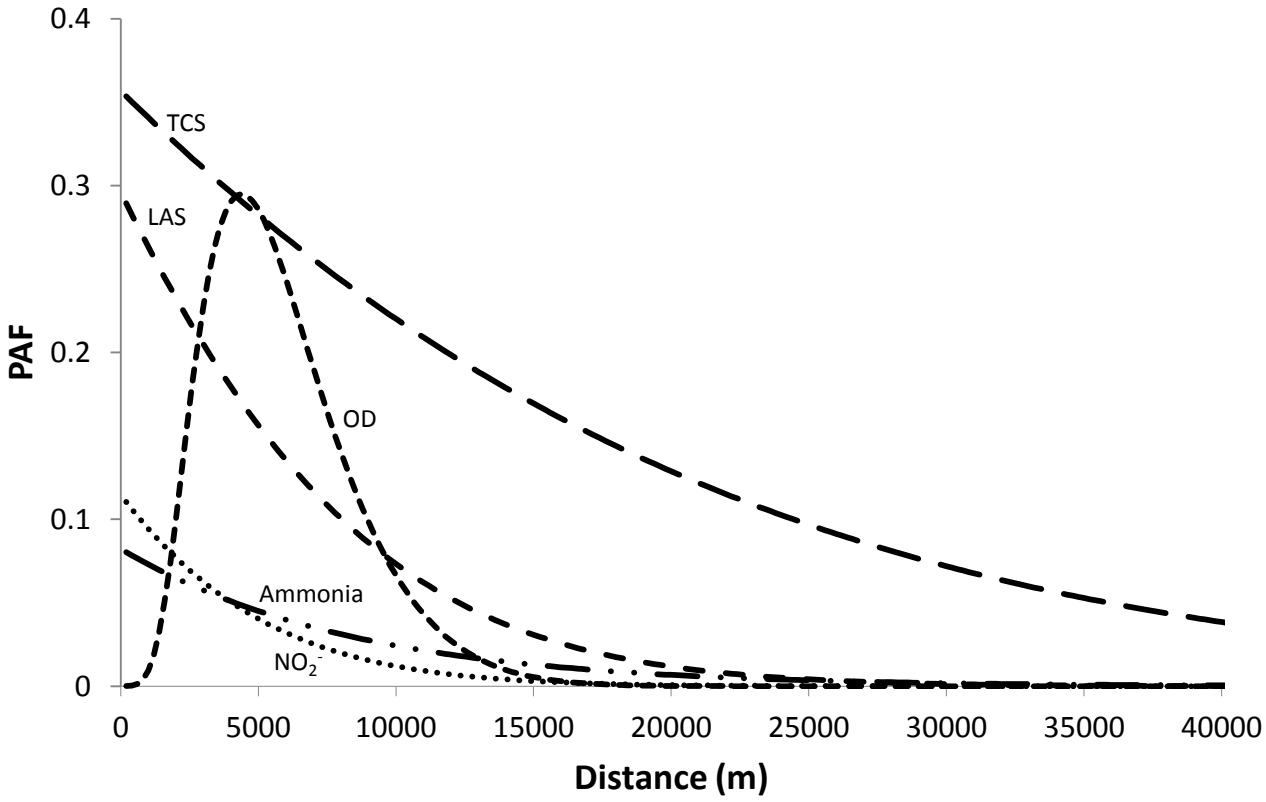


Figure 3-11 Predicted PAF from each individual stressor in the untreated discharge scenario.

Considering the overall impact on the ecology, the PAF_{max} is presented in Figure 3-12.

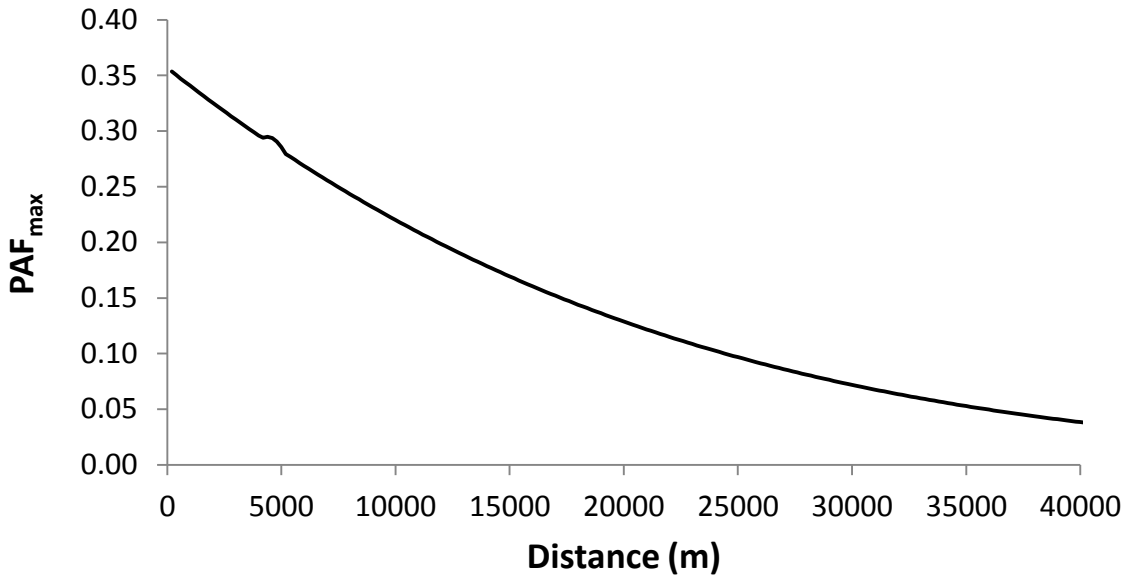


Figure 3-12 *PAF$_{max}$* (maximum PAF) as a result of all five stressors in the untreated discharge scenario.

The *PAF$_{max}$* decreases steadily along the impact zone as stressor concentrations decrease, therefore the adverse effects on taxa are also reduced. Between 4,000 and 5,000m downstream, *PAF$_{max}$* increases and falls again as a result of the increase in OD, due to the DO sag.

Expected changes in the ecology with distance downstream can also be expressed in terms of fraction of speices present, which is predicted to increase with distance downstream reflecting an expected increase in biodiversity associated with a decrease in stressor concentrations away from the effluent. The predicted fraction of species present as a result of each stressor is in Figure 3-13.

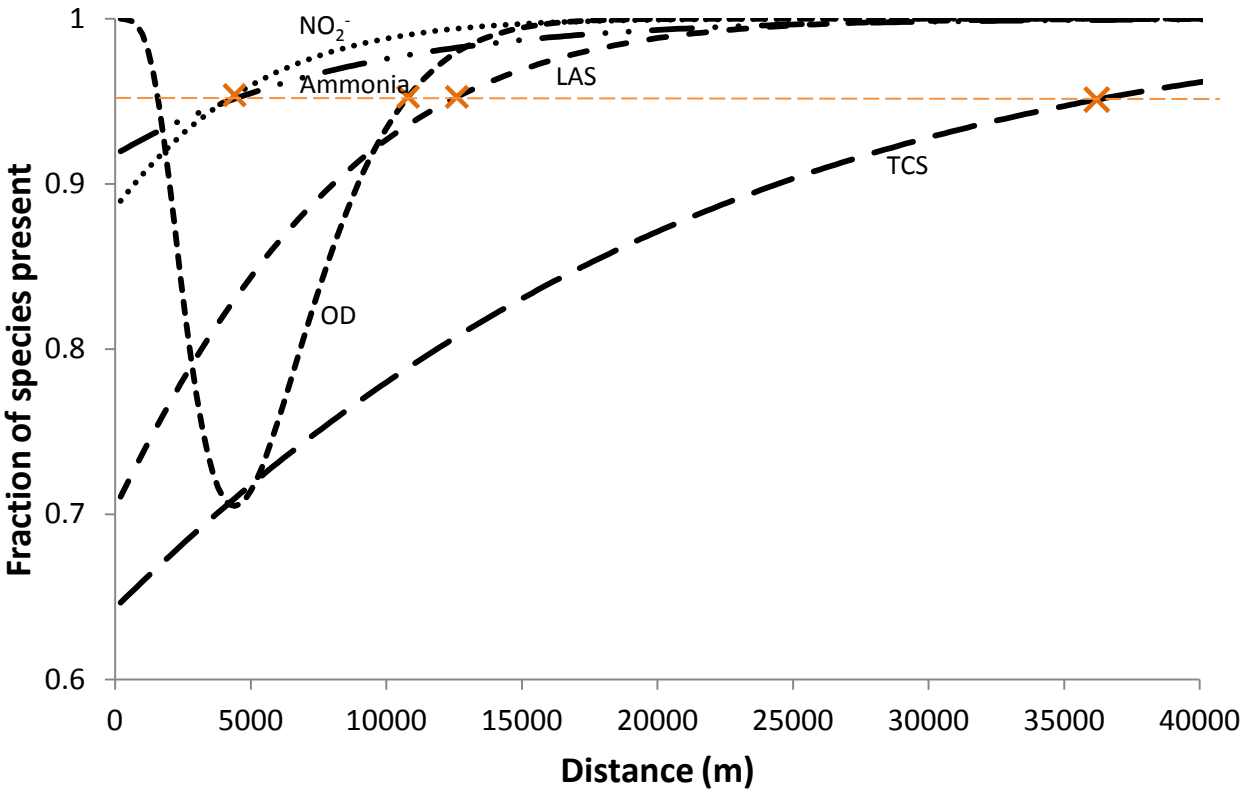


Figure 3-13 Predicted fraction of species present as a result of each stressor in the untreated discharge scenario. The point at which 95% of species in each dataset returns is highlighted (orange line and *x* points). The end of the impact zone is determined by TCS.

The effect of the oxygen sag on species present is evident. Despite the concentration of TCS being lower than the other stressors, its impact on species presence is greatest. At 36,000m downstream species richness returns to 95%.

Species present can also be expressed in terms of species richness which yields slightly different results (see Figure 3-14).

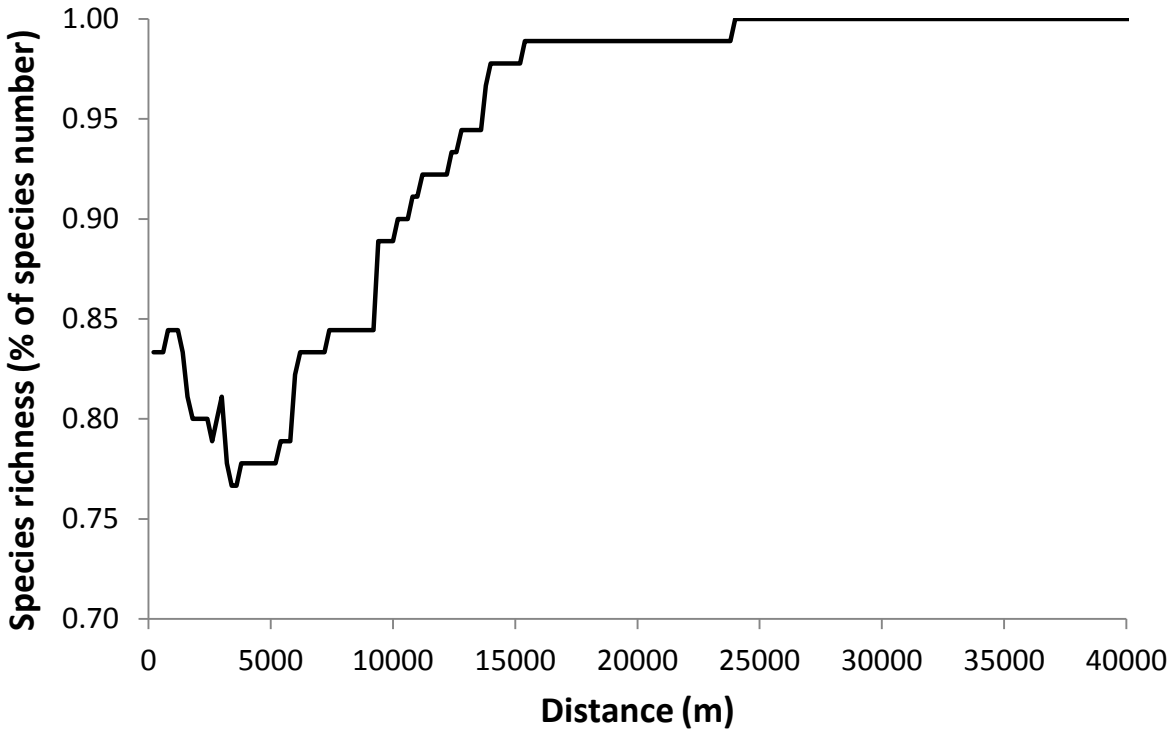


Figure 3-14 Species richness (as a percentage of the number of species) in the untreated discharge scenario.

This illustrates the combined effect of the stressors on the ecological community, where species diversity is generally reduced (with fluctuating numbers of species) until 3,600m downstream where the effects of the oxygen sag can be seen. After this point, species diversity and richness increases again. At 24,000m downstream of the effluent outfall, all species returned, therefore full recovery was evident here.

3.3 Results of treated vs untreated TGD scenarios

The impact of untreated effluent on riverine ecology in a generic TGD scenario is shown in section 3.2. These outputs can be compared with respective figures for treated effluent given in Appendix B.

In contrast to the untreated scenario, in the treated scenario DO starts at a higher concentration and quickly recovers as the demand placed on the system by the organic matter from the effluent is lower (Figure 3-15).

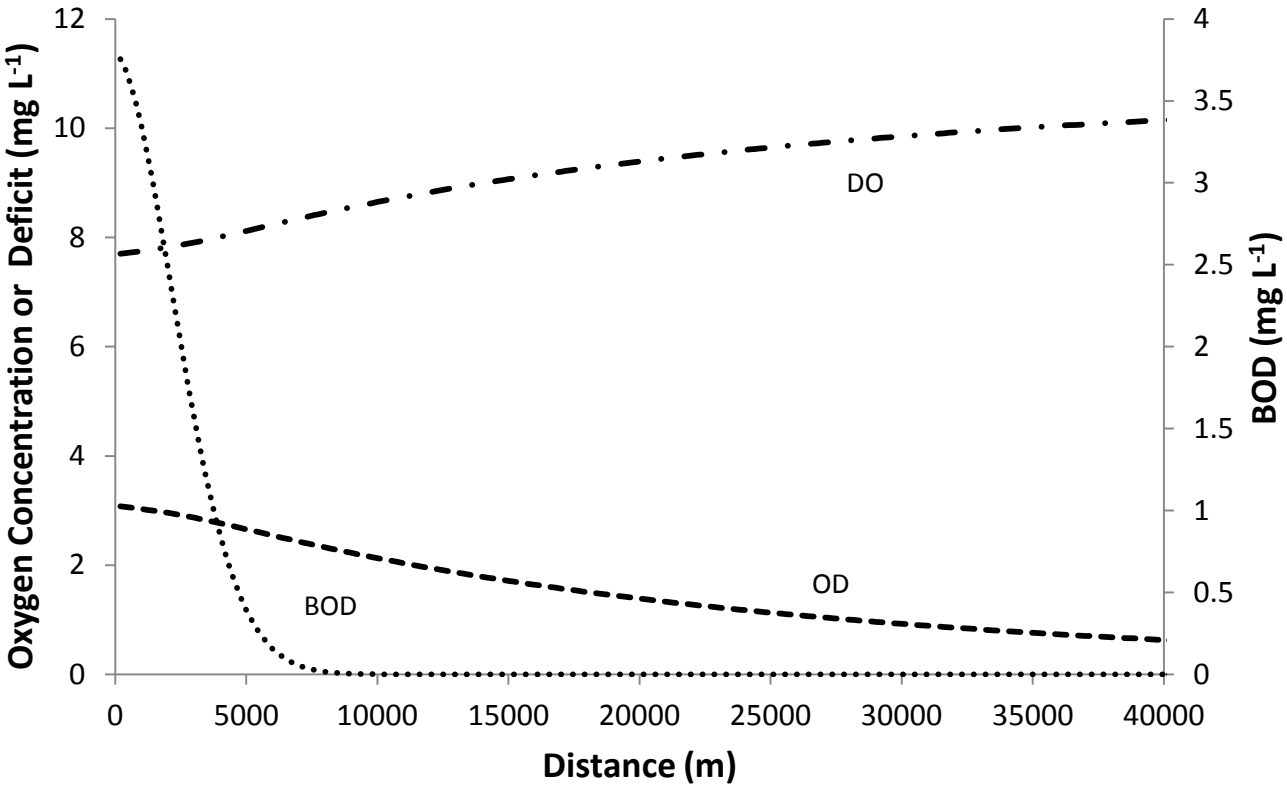
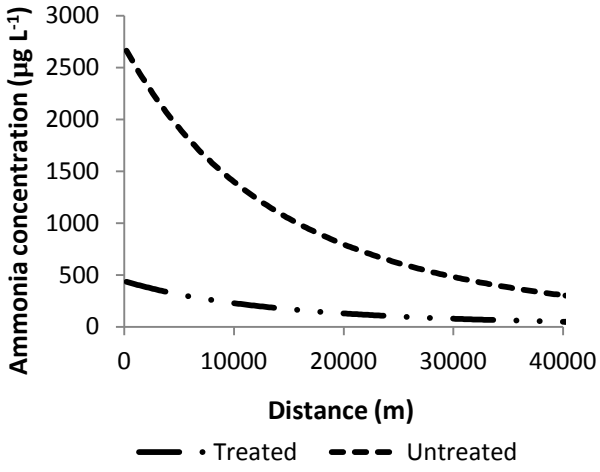


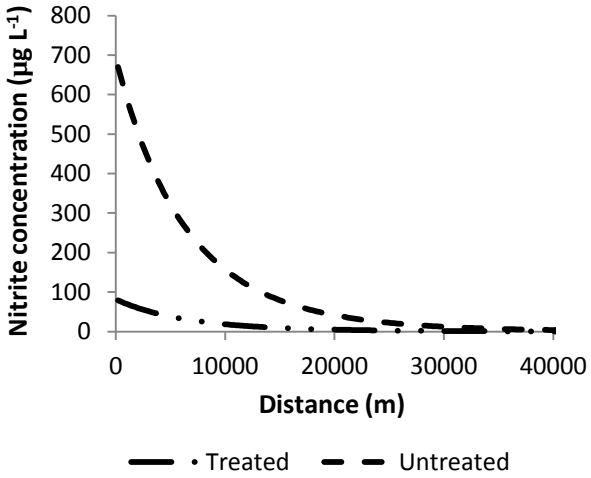
Figure 3-15 Oxygen output in terms of deficit, concentration and BOD in the treated discharge scenario.

The oxygen sag is much less pronounced, if apparent at all. The corresponding output for the untreated scenario was presented in Figure 3-10.

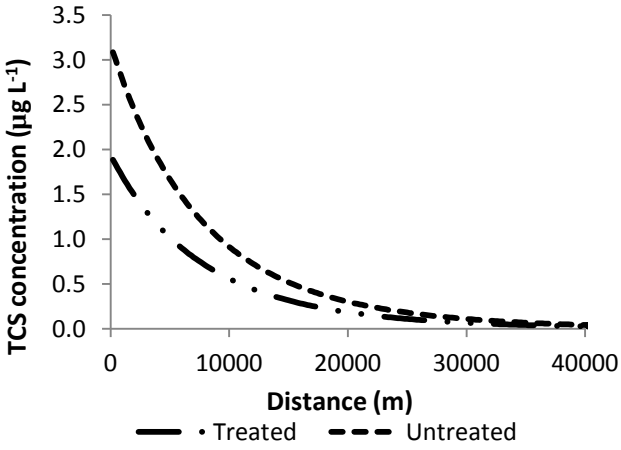
Sewage treatment removes a large fraction of these stressors. Their concentrations in river water after discharge of treated effluent are therefore much lower than in the respective untreated scenario.



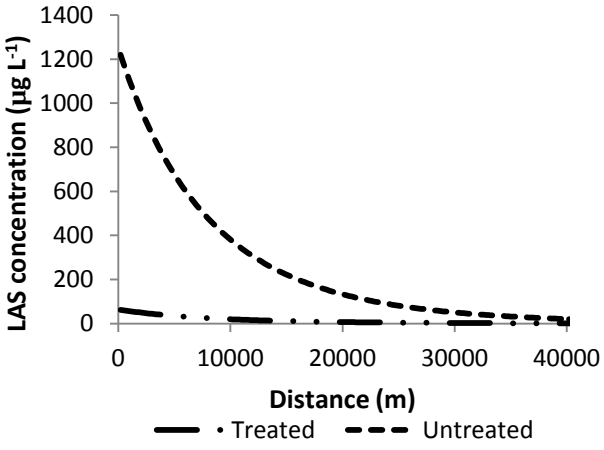
(a)



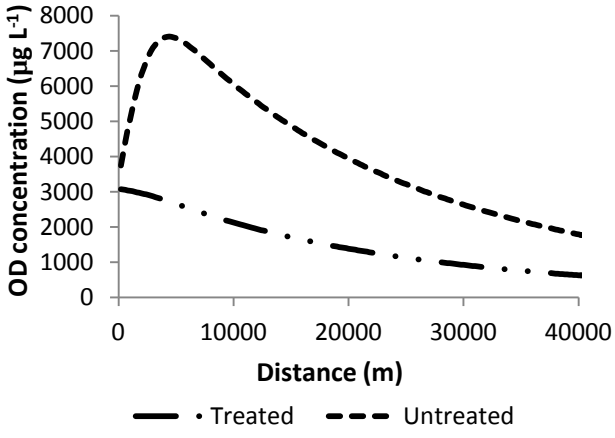
(b)



(c)



(d)



(e)

Figure 3-16 Stressor concentrations in treated vs untreated scenarios; (a) ammonia, (b) nitrite, (c) TCS, (d) LAS and (e) OD.

The difference in the overall impact resulting from these two scenarios is shown in Figure 3-17 in terms of species richness. The expected impact of the untreated effluent is significantly greater than for the treated scenario with recovery predicted to occur further downstream.

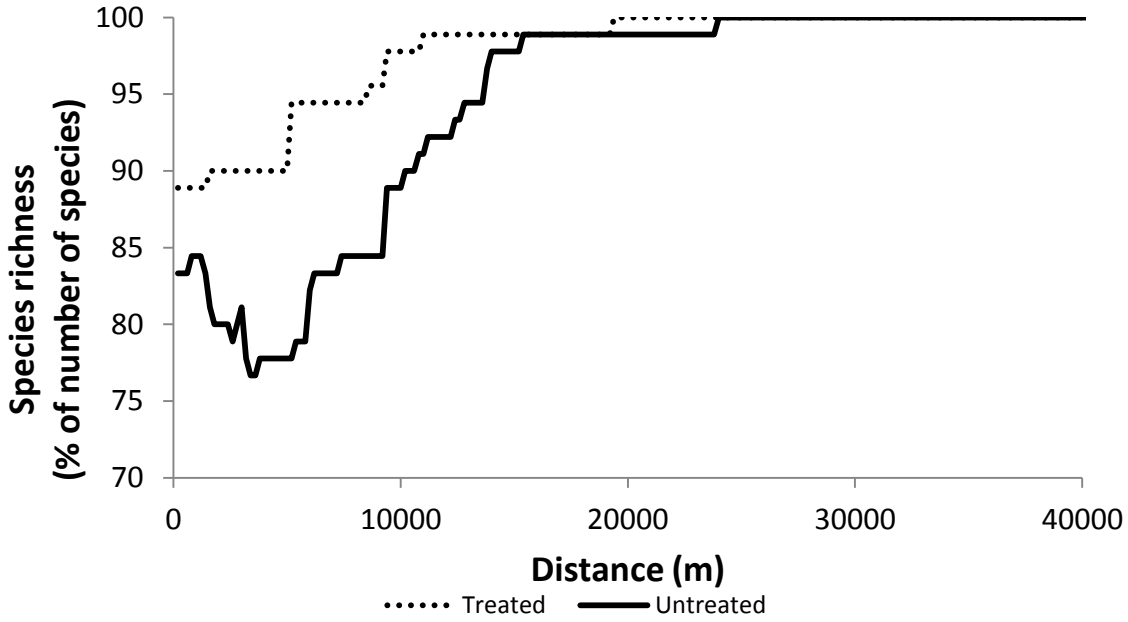


Figure 3-17 Predicted overall impact from all stressors in the treated and untreated scenarios, in terms of species richness, as a percentage of the number of species present.

In the scenario with untreated effluent, the effect on the taxa is more pronounced in terms of the magnitude of the impact and the duration (distance downstream). The impact of the oxygen sag is significant in the untreated scenario with the number of species decreasing from 1,200 downstream until 3,600m downstream of the effluent outfall. Conversely in the treated scenario, the number of species present steadily increases immediately downstream of the effluent throughout the impact zone. Where the species richness falls to 76% at 3,600m in the untreated scenario, at this same location in the treated scenario 90% of the species have returned. In the treated scenario, all species recover at 19,400m while in the untreated scenario complete recovery occurs at 24,000m downstream. This demonstrates that discharge of untreated wastewater is likely to have a significant impact on the ecological composition and function of the receiving water bodies.

4 APPLICATION OF THE MODEL TO A CASE STUDY

A rare example of reported data on the changes in community composition along stressor gradients downstream of wastewater emission points is Birge *et al*. (1989). The authors report a field biomonitoring study conducted in 1983 in the South Elkhorn Creek which rises near Kentucky, USA (map in Figure 4-1). The model was parameterised for the South Elkhorn Creek and the results were compared to benthic invertebrate assemblage data reported by Birge *et al*. (1989) in order to assess the validity of the predictions made.

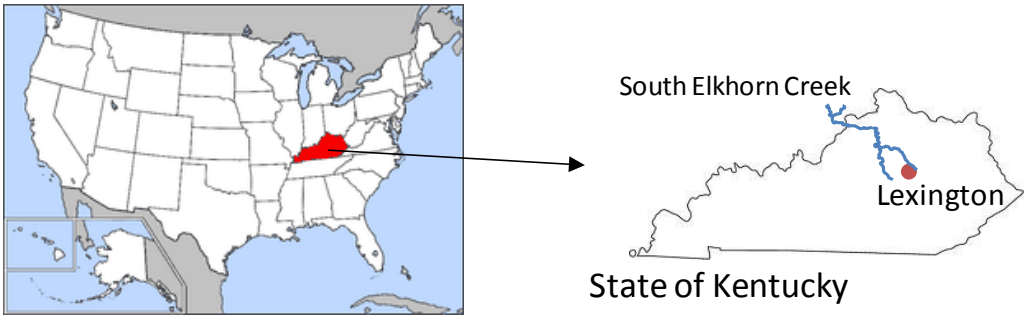


Figure 4-1 Location of Lexington in Kentucky, USA.

4.1 Methods of applying the model to South Elkhorn Creek

South Elkhorn Creek is one of the largest and most populated watersheds in the Kentucky River Basin. Land use in the catchment is 80% agricultural and 20% urban (Arthur, 2004). It is a warm water fourth order stream with an average gradient of 1.1m per km (Birge *et al.*, 1989). Town Branch Creek is a major tributary which flows into the South Elkhorn Creek approximately 14km downstream of the city of Lexington.

4.1.1 Study area

The study area on the South Elkhorn Creek is illustrated in Figure 4-2.

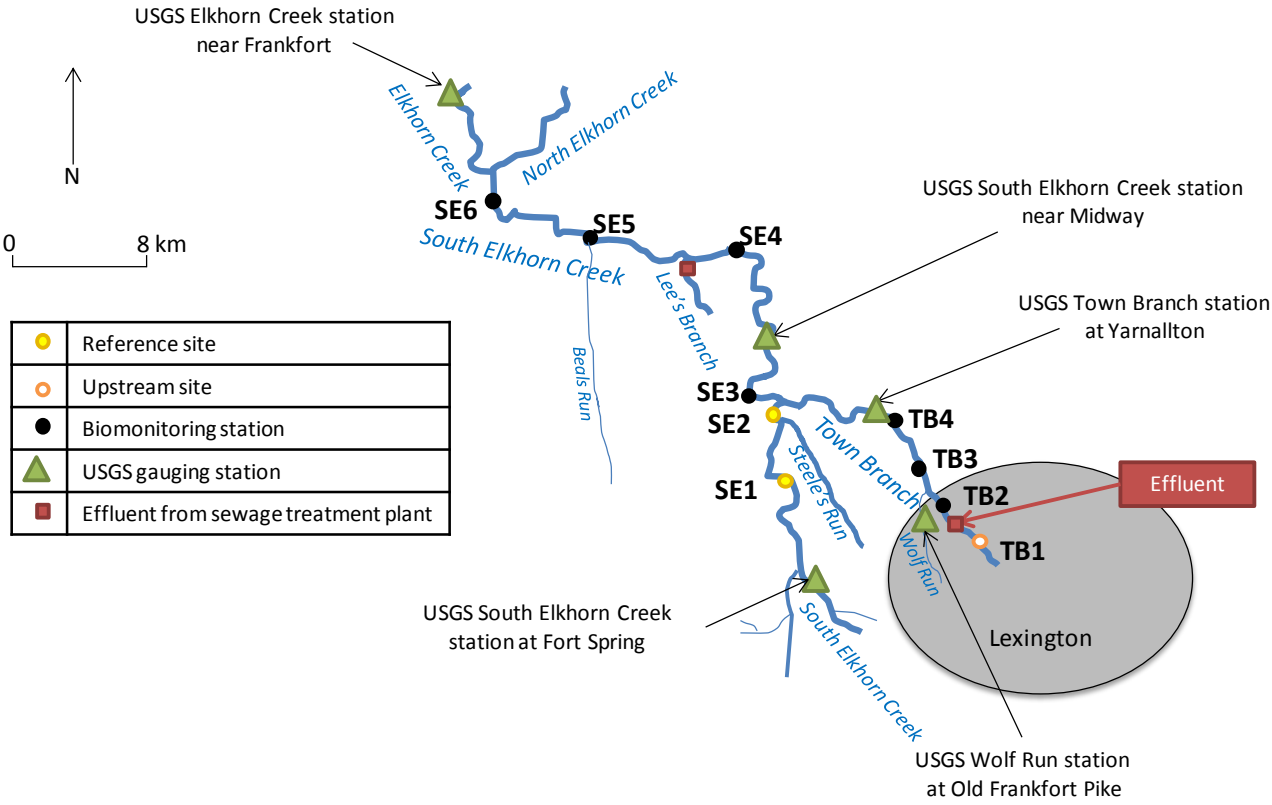


Figure 4-2 Schematic map of South Elkhorn basin showing the location of the biomonitoring stations, gauging stations and effluent.

The only major outfall in the study system was the effluent from the Town Branch STP in Lexington, between biomonitoring stations TB1 and TB2 and was the main source of the organic enrichment in the system according to Birge *et al*. (1989). Some minor physical and ecological effects were seen beyond 54km downstream of the treatment plant implying other minor sources of effluent may have been present (Birge *et al.*, 1989). Agriculture, urban runoff, storm sewers and alterations to stream flow are possible additional sources of impaired water quality in Town Branch Creek, and ultimately also in South Elkhorn Creek (Environmental Quality Committee, Town Branch Trail Inc., 2001).

The study area included biomonitoring and reference sites along with U.S. Geological Survey (USGS) biomonitoring stations. Upstream of biomonitoring site TB1, stormwater inputs to the stream were already likely to cause stress to the system before Town Branch STP discharged effluent. Therefore, TB1 was not a suitable

reference site, so SE1 and SE2 on South Elkhorn Creek were used to derive reference conditions better. These sites are all mapped in Figure 4-2.

Lee's Branch joins the South Elkhorn Creek 2.8km below SE4. On Lee's Branch 1.4km from the confluence with the South Elkhorn Creek was a small STP serving the town of Midway. Birge *et al.* (1989) refer to this as a "minor discharge" but there is no information from the time of the study in 1983 on this plant. Town Branch STP currently treats >100 times more wastewater than the Midway plant currently does (Midway Messenger, 2013; Woodford County KY, 2011; Lexington Fayette, Urban County Government, 2010), so it is assumed that in 1983 the effect from the Midway plant was two orders of magnitude lower than Town Branch.

Table 4-1 Biomonitoring sites on the South Elkhorn Creek (Birge *et al.*, 1989). Discharge is expressed as the mean of two mean measurements taken at the beginning and end of the 34 day study.

Station type	Biomonitoring station	Distance from effluent (km)	Flow time from effluent (h)	Velocity (m s^{-1})	Discharge (L s^{-1})	Percent effluent	Further information
Upstream	TB1	0.3	—	—	140	0	Unsuitable as ecological reference site due to urbanization and low flow
Effluent	Effluent	0	—	—	1020	100	
Downstream	TB2	0.2	0.1	0.56	1160	88	
	TB3	1.9	0.9	0.59	970	106	
	TB4	8.5	4.1	0.58	1390	73	Close to USGS station: Town Branch station at Yarnallton
	SE3	14.8	7.5	0.55	1700	60	~0.5km downstream of confluence of Town Branch and South Elkhorn
	SE4	37.5	16.9	0.62	1920	53	2.8km upstream of confluence of Lee's Branch and South Elkhorn
	SE5	54.1	24.6	0.61	—	—	Reproducible flow measurements could not be taken but Birge *et al*. (1989) state as consistent with USGS data. However, it is not close to USGS station
	SE6	67.6	30.6	0.61	3110	33	
Reference	SE1	—	—	—	—	—	Secondary reference site for ecology. Site used in study by Logan *et al.* (1983). 61.5km downstream of river source. 7.0km downstream of USGS gauging station at Fort Spring
	SE2	—	—	—	410	0	Primary reference site for ecology, hydrology and chemistry

4.1.2 Discharge data

Birge *et al.* (1989) measured discharge at each biomonitoring station at the beginning and end of the study at different locations. During each of these monitoring periods,

measurements were taken in triplicate from which mean values were calculated. One value was reported for each biomonitoring station, as the mean of these two measurements (Figure 4-3). No further details on exactly when the measurements were taken or the method used were reported by Birge *et al*. (1989). The raw data were not presented either. Discharge increased downstream after the effluent entered Town Branch Creek. However, it decreased at TB3 to 970 L s^{-1} (see Figure 4-2). This was not expected as this was downstream of the confluence with Wolf Run. The historical average discharge of Wolf Run is 509 L s^{-1} (U.S. Geological Survey, 2014). The discharge at TB3 may appear to decrease for a number of possible reasons, which are most likely to be:

i. Measurement errors: velocity and cross-sectional area measurements are subject to errors which combined can result in typical errors in discharge of approximately 20%, or more, depending on the irregularly of the cross section.
ii. Temporal differences in measurement at TB2, TB3 and in the effluent. Sewage discharge has a well known and regular temporal variability relating to the diurnal pattern of human activity in the sewershed. If discharge was measured at TB3 at a time corresponding to low discharge in the effluent, lagged by the travel time in the river, it could have resulted in an apparent decrease in flow. Similar phenomena were reported by Whelan *et al*. (1999) for the River Lambro.

Downstream of TB3, discharge progressively increases.

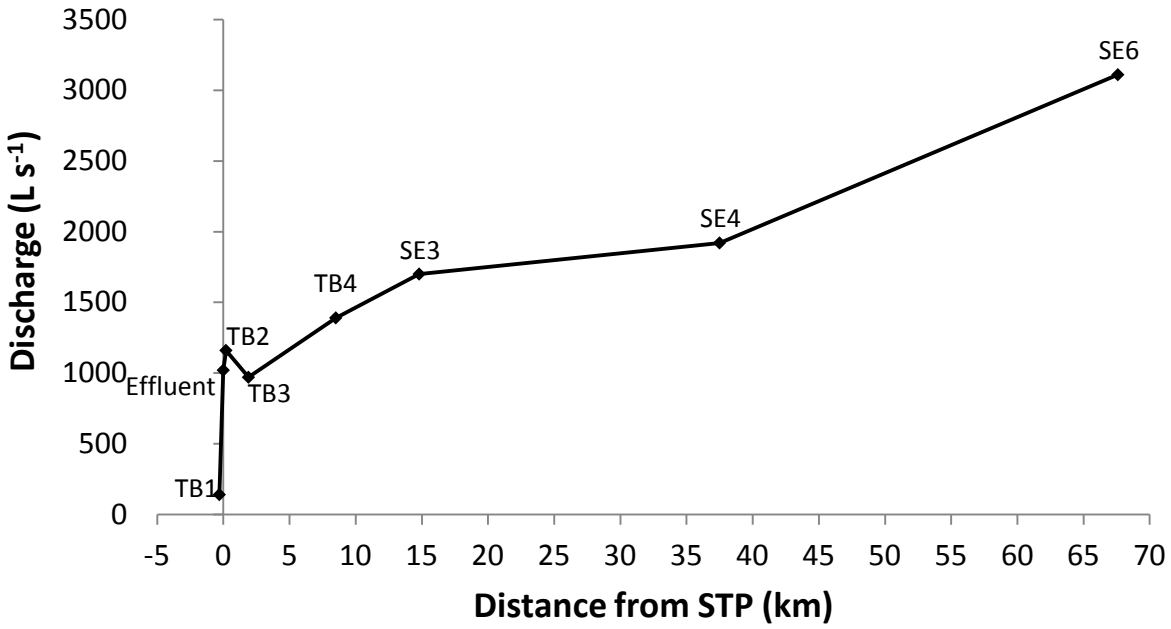


Figure 4-3 Discharge measured by Birge *et al*. (1989) from 0.3km upstream to 60km downstream of Town Branch STP.

The discharge data reported by Birge *et al.* (1989) were compared with USGS discharge data for the same system (Figure 4-3) to investigate the reliability of the data reported by Birge *et al*. (1989). In the study is there was also a reference to USGS data calculated by an alternative method, as the average daily flow for 31 days during the study period. The study period was 34 days. Calculating discharge using these two methods yielded different results however (Table 4-2). Two discharge datasets were incorporated into the model to determine the impact of organic effluent in the South Elkhorn Creek. They were:

i. historical long term averages obtained from the USGS were used to model long term effects of exposure to chemical stressors;
ii. measurements taken by Birge *et al*. (1989) during the study to validate the model's prediction of the impact on ecology compared to the measured impact in terms of species present. During the study, which took place in August and September, the flows were considered to be relatively low (U.S. Geological Survey, 2009), so the impact of these low flow conditions can be assessed.

Table 4-2 USGS monitoring data in South Elkhorn system.

Site	Stream	Location	Discharge ($L\ s^{-1}$)	Reference	Details
Fort Spring	South Elkhorn	7km upstream of SE1	290	Birge *et al.* (1989)	Average daily flow for 31 days during study period
			225	USGS Water Data (2014)	Average of two mean measurements taken at beginning and end of study period
			423		Average of average daily flows for the 34 day study period (14.93 $ft^3\ s^{-1}$)
			1002		Average for 1951-2013 (35.38 $ft^3\ s^{-1}$)
Near Midway	South Elkhorn	7.5km downstream of SE3; 15km upstream of SE4	1730	Birge *et al.* (1989)	Average daily flow for 31 days during study period (1.73 $m^3\ s^{-1}$)
			1741	USGS Water Data (2014)	Average of two mean measurements taken at beginning and end of study period
			2639		Average of daily flows for the 34 day study period (93.18 $ft^3\ s^{-1}$)
			5017		Average for 1983-2012 (177.19 $ft^3\ s^{-1}$)
Old Frankfort Pike[#]	Wolf Run	Approx. 1km upstream of confluence with South Elkhorn, between TB2 and TB3	509	USGS Water Data (2014)	Average for 1998-2013 (17.96 $ft^3\ s^{-1}$)
Yarnallton[#]	Town Branch	Close to TB4. Approx. 9.5km downstream of Town Branch STP	2579	USGS Water Data (2014)	Average for 1998-2013 (91.08 $ft^3\ s^{-1}$)

[#]USGS discharge data not available for the study period as the gauges did not exist then

Fort Spring

At Fort Spring, USGS discharge quoted by Birge *et al*. (1989) (290 L s^{-1}) was lower than the historical long term USGS average (1002 L s^{-1}). However, for the study period, there is a discrepancy in the USGS data. The calculated average daily discharge for 34 days is 423 L s^{-1}, however Birge *et al*. (1989) quote this value for 31 days as 290 L s^{-1}. Fort Spring is upstream of SE1, for which Birge *et al*. (1989) do not measure discharge data. The discharge reported at SE2 was 410 L s^{-1} which is expected to be higher than at Fort

Spring as there are a number of tributaries to the South Elkhorn downstream of Fort Spring.

Near Midway

Like Fort Spring, discharge was lower during the study than the historical long term average. Again, the USGS value quoted by Birge *et al*. (1989) (1730 L s^{-1}) was lower than that calculated from daily average flows for the 34 days (2639 L s^{-1}), from USGS Water Data (2014). However, the values in Birge *et al.* (1989) do correlate better with their measurements at the nearby sites SE3 (1700 L s^{-1}) and SE4 (1920 L s^{-1}). When calculating an average from the USGS data using discharge data from the first and last day (1741 L s^{-1}), agreement with Birge *et al*. (1989) is better.

In order to model the ecological effects of Town Branch STP, discharge data was normalised using long term historical data from the USGS biomonitoring site at Yarnallton, which is 1km from TB4 (National Renewable Energy Laboratory, 2014). A more detailed map is given in Appendix C.1 and details of the calculations are given in section 4.1.3.1.

Many chemical analyses were not reported by Birge *et al*. (1989), for example polar organics were not separated. Stressors such as ammonia and nitrite and other chemicals were not monitored. However, prior to the study, Logan *et al*. (1983) performed comprehensive analyses and an ecological survey on the same system in the same year. Unfortunately, this report is not available.

4.1.3 Model parameterization for the South Elkhorn Creek

The model described in Chapter 3 was altered to reflect the scenario in the South Elkhorn Creek and these aspects are presented. Parameters not described are unchanged from the original model.

4.1.3.1 Discharge

Firstly, the discharge data measured by Birge *et al*. (1989) was taken. Discharge at TB3 was lower than at TB2 which was unexpected, as discharge usually increases

downstream (Chapter 3) and it was not measured at SE5 in the study. To correct and fill these gaps, discharge values for these two sites were extrapolated from previous upstream sites i.e. for TB3 from TB2, and for SE5 from SE4.

Next, discharge at all stations was normalised to mean flow using long-term USGS data. USGS discharge data was not available for Yarnallton for the study period as the gauge was installed in 1998. The long term average of daily flows for 1998-2013 was used to normalise the data in Table 4-1. To calculate the normalised discharge at each site, the fraction of the discharge relative to the USGS discharge near TB4 was calculated, which was 1.86.

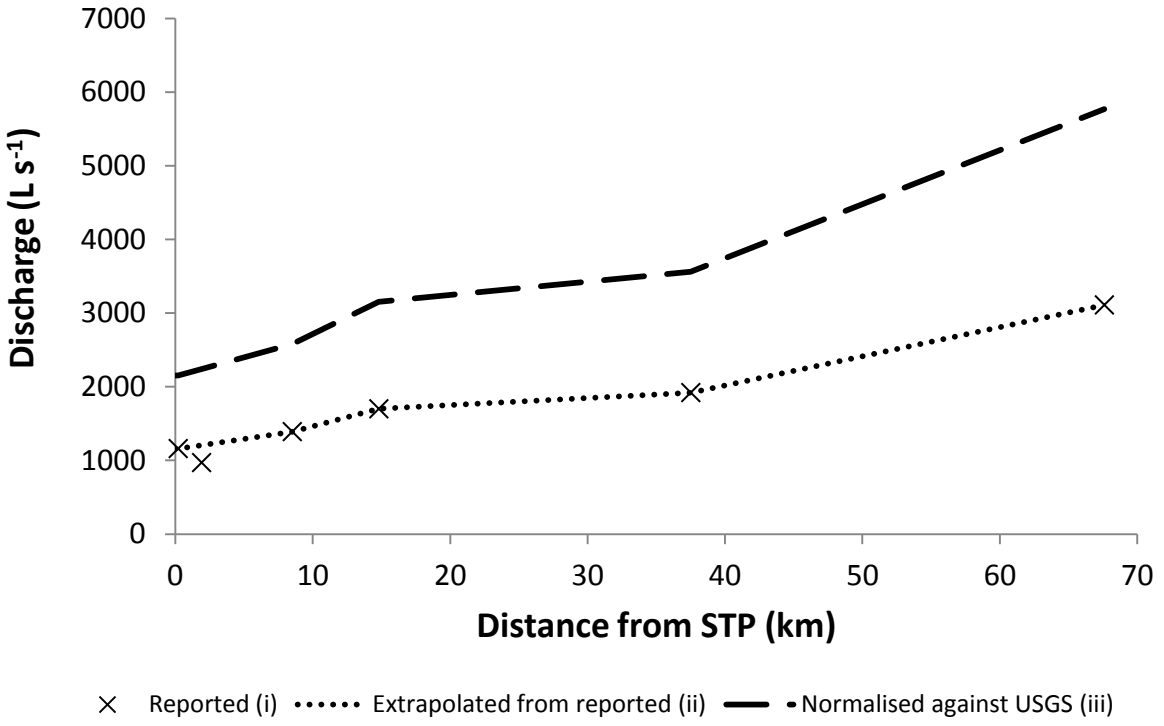


Figure 4-4 Discharge data: (i) reported by Birge *et al*. (1989), (ii) reported by Birge *et al*. (1989) with missing and unexpected values extrapolated and, (iii) calculated by normalising the data from Birge *et al*. (1989) using USGS data.

Table 4-3 Parameters associated with discharge used in the model from Birge *et al*. (1989). Downstream values ($Q_{(x)}$) from Figure 4-4 are also used in the model.

Parameter (units)	Parameter description	Value
Q_{eff} (L s^{-1})	Discharge from STP	1020
Q_{ds} (L s^{-1})	Discharge downstream of effluent input. Equation (3-1)	1160
Q_{us} (L s^{-1})	Discharge upstream of effluent input	140

4.1.3.2 Initial concentration of stressors in the receiving water body (C_0)

The starting concentrations were calculated by the same method as in the generic model for ammonia, nitrite, triclosan and LAS. The actual concentrations differ in the South Elkhorn Creek scenario as consumer usage figures for triclosan and LAS, and water usage per person, in the USA were used to represent the South Elkhorn Creek scenario. The water usage value for the USA was available from the USGS Water Census conducted in 2005 (American Water Works Association, 2014). DO concentrations at reference sites reported in Birge *et al.* (1989) were used to calculate the concentration after mixing. More details on DO in the model are in section 4.1.3.4.

Table 4-4 Parameters used to calculate initial stressor concentrations (C_0).

Parameter (units)	Parameter description	Stressor	Value	Reference
C_{raw} (µg L^{-1})	Chemical concentration in raw sewage	TCS	16.71	Calculated by equation (3-8)
		LAS	9596	Calculated by equation (3-8)
$Chem_u$ (µg cap^{-1} d^{-1})	Chemical usage per capita per day	TCS	6200	De Zwart (2006)
		LAS	3,560,000	OECD SIDS (2005a)
W_u (L cap^{-1} day^{-1})	Water use per capita per day in the USA	—	371	American Water Works Association (2014)
r	Removal fraction in wastewater treatment	TCS	0.9	Capdevielle *et al.* (2008)
		LAS	0.95	HERA (2009)
C_{eff} (µg L^{-1})	Chemical concentration in sewage effluent	Ammonia (total)	4400	Treated value (Gray, 2004)
		Nitrite	800	Treated value (Gray, 2004)
		TCS	1.67	Calculated by equation (3-9)
		LAS	480	Calculated by equation (3-9)

The higher and therefore more conservative of the available US figures for TCS usage was taken. C_{raw}, C_{eff} and C_0 were calculated using equations 3-8, 3-9 and 3-10.

$$C_{raw} = \frac{6200}{371} = 16.71 \text{ µg L}^{-1}$$

$$C_{eff} = (1 - 0.9) * 16.71 = 0.1 * 16.71 = 1.67 \text{ µg L}^{-1}$$

The highest of available usage values for LAS usage in the USA was used as a conservative estimate.

$$C_{raw} = \frac{3560000}{371} = 9596 \text{ µg L}^{-1}$$

$$C_{eff} = (1 - 0.95) * 9596 = 0.05 *= 480 \text{ µg L}^{-1}$$

Table 4-5 Assumed stressor starting concentrations downstream of the Town Branch STP.

Parameter (units)	Parameter description	Stressor	Value
C_0 (µg L^{-1})	Starting concentration of stressor after mixing	Ammonia (total)	3879
		Nitrite	714
		Triclosan	12
		LAS	432

4.1.3.3 Concentration of stressor at time *t* and location *x*

The only deviation from the original model in calculating downstream stressor concentrations, related to the concentration of NH_3 which was calculated by the same method, but the average temperature and pH (and therefore *pKa*) from the downstream biomonitoring sites in the study were used. When the proportion of NH_3 present was calculated, the temperature and pH of the system were 16.2°C and 7.3 respectively.

4.1.3.4 Dissolved oxygen model

BOD data are required by the Streeter-Phelps model but were not reported in Birge *et al.* (1989). Total organic carbon (TOC) values are presented however, and in the

literature their relationships with BOD have been presented (e.g. Dubber and Gray, 2010; Constable and McBean, 1979; Rene and Saidutta, 2008). BOD and TOC are both measures of the organic matter present in the system. TOC is a measure of the total organic matter while BOD is the portion of this which degrades and hence has a demand for oxygen.

In the South Elkhorn Creek, TOC concentrations decreased downstream as the organic matter was degraded (plotted in Figure 4-5). However, a slight increase was seen at SE5. This may be due to run-off from agricultural land and the input from Lee's Branch.

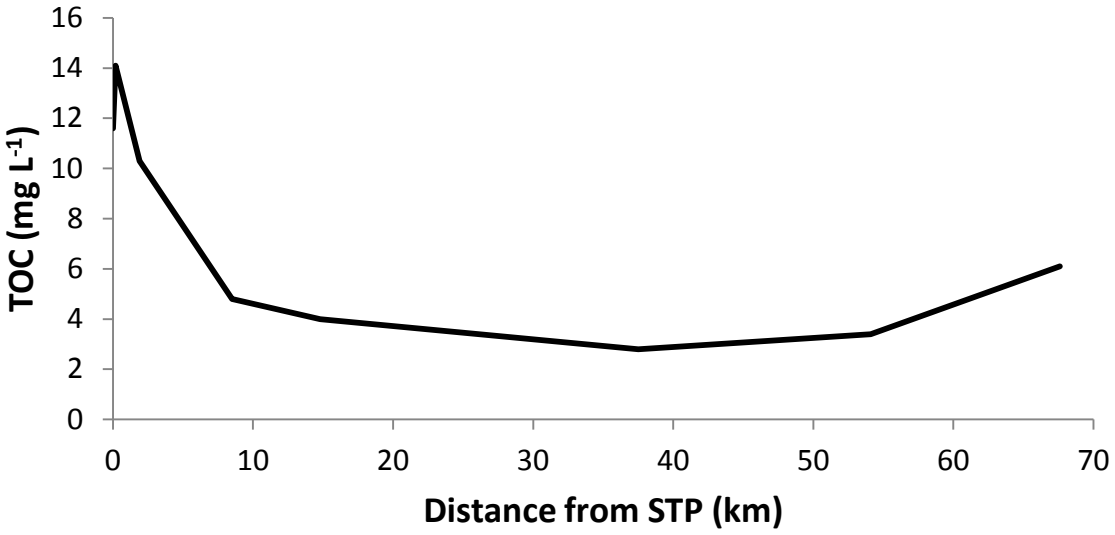


Figure 4-5 TOC (mg L^{-1}) measured by Birge *et al.* (1989).

There are few relationships between BOD and TOC published in the literature (Rene and Saidutta, 2008). BOD:TOC ratio varies depending on the quality of wastewater and the quality of treatment so it varies from system to system (Aziz and Tebbutt, 1980). Those available are given in Table 4-6.

Table 4-6 BOD-TOC relationships in wastewater available in the literature

BOD- TOC Relationship	Details	Reference
$BOD_5 = 23.7 + 1.68 * TOC$	Influent to STP, no correlation in treated effluent. P<0.001 highly significant linear relationship	Dubber and Gray (2010)
$BOD_5 = 11.6 + 1.875 * TOC$	Domestic wastewater	Schaffer *et al*. (1965) in Constable and McBean (1979)
$BOD_5 = 86.15 + 0.84 * TOC$	Domestic wastewater	Chandler *et al*. (1976) in Constable and McBean (1979)
$BOD_5 = 1.8954 + 1.4228 * TOC$	As a result of regression analysis	Rene and Saidutta (2008)

The BOD values required to input into the model are BOD in the effluent (BOD_{eff}) and BOD upstream (BOD_{us}). All of the estimated values based on TOC-BOD relationships were used in the model in turn, to optimise the pair of values which reflected the scenario best. These were the values from Chandler *et al*. (1976) (see Table 4-7).

Table 4-7 Predicted BOD_{eff} and BOD_{us} values in the South Elkhorn Creek based on relationships published in the literature.

	TOC (mg L^{-1})	BOD (mg L^{-1})			
	Observed values in Birge *et al*. (1989)	**Dubber and Gray (2010)**	**Schaffer *et al.* (1965)**	**Chandler *et al.* (1976)**	**Rene and Saidutta (2008)**
Effluent (TOC/BOD_{eff})	11.6	43.2	33.4	95.9	18.4
Reference sites (TOC/BOD_{us})	1.8	26.7	15.0	87.7	4.5

The remaining parameters used in the Streeter-Phelps model are given in Table 4-8.

Table 4-8 Parameters used in Streeter-Phelps model applied to the South Elkhorn Creek reported by Birge *et al*. (1989).

Parameter (units)	Parameter description	Value	Details
DO_{us} (mg L^{-1})	DO concentration upstream	9.4	Average of 2 reference sites. DO concentration of upstream site not presented.
DO_{eff} (mg L^{-1})	DO concentration in effluent	4	
DO_{sat} (mg L^{-1})	Saturation oxygen concentration at given temperature Equation (3-23)	Calculated in model	Calculated for average temperature of the downstream sites (16.2°C).

The rate constants for BOD biodegradation (k_{BOD}) and reaeration ($k_{reaerate}$) values were available in the literature. However, many factors such as the temperature, nature of the effluent and velocity of the river affect these rates (Negulescu, 1985; Gray, 2004), so they are just an estimate for the rates in the South Elkhorn Creek. Therefore the model was run with these values and was then optimised to reflect the situation in the South Elkhorn Creek more accurately. The reason for optimising these values was that the parameters should simulate the situation in Birge *et al*. (1989).

4.2 Results of applying the model to scenario in Birge *et al*. (1989)

4.2.1 Predicted results of the model

The model was applied to the South Elkhorn Creek scenario reported by Birge *et al*. (1989) with the scenario based on long term mean discharge rates, unless specified otherwise.

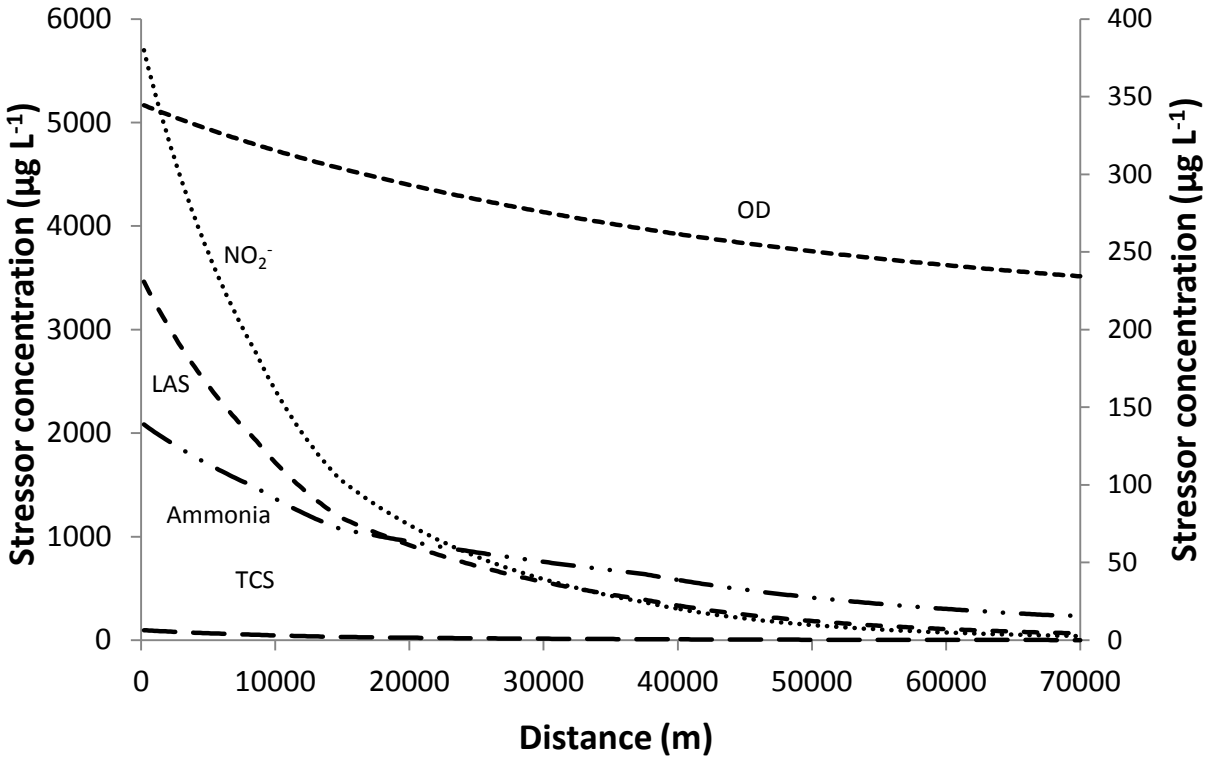


Figure 4-6 Stressor concentrations downstream using long-term mean discharge. Note second *y*-axis for LAS, TCS and nitrite.

The predicted concentrations of the different stressors in the South Elkhorn Creek vary both in terms of their initial concentrations and downstream concentrations as a result of different degradation rates. Their predicted effect on the species in the system is obviously linked to their toxicity thresholds (NOECs) for example triclosan has an impact despite its concentration being significantly lower than the other stressors (Figure 4-7).

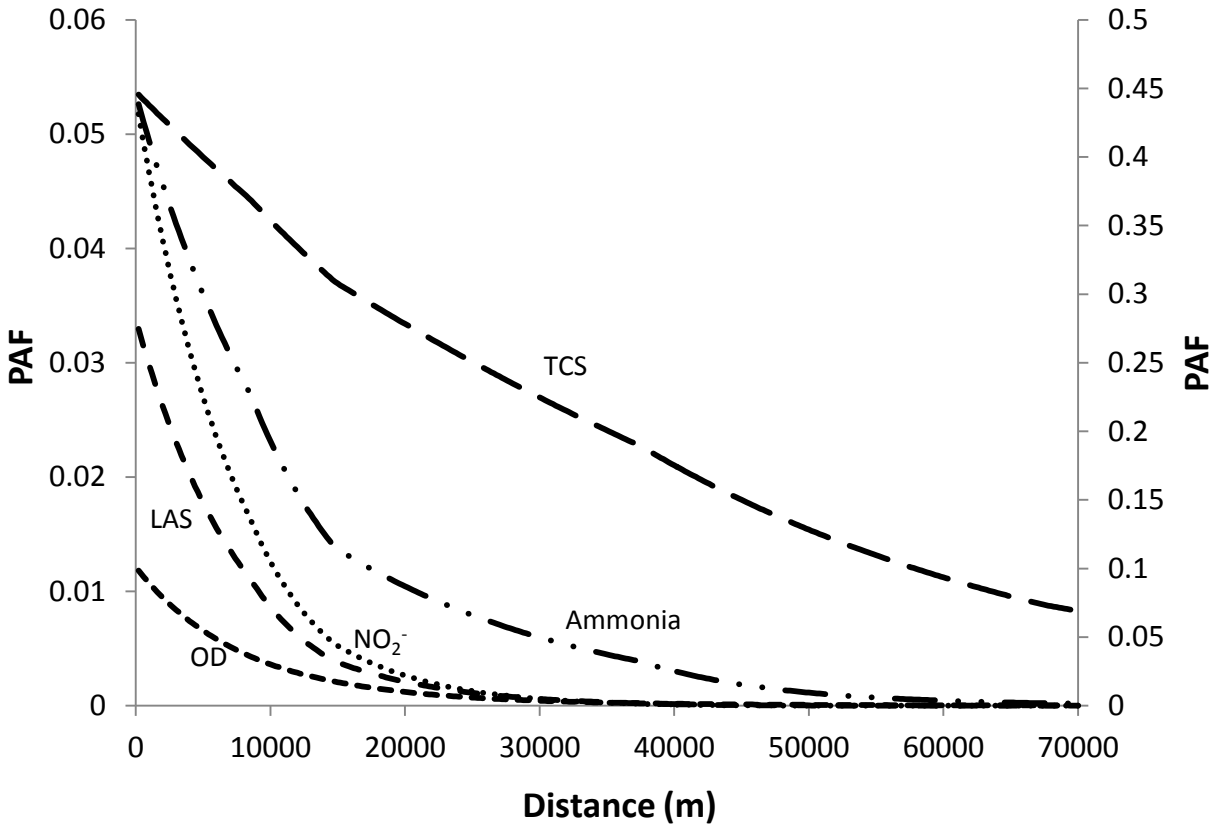


Figure 4-7 PAFs resulting from each stressor's concentrations in the system under long-term mean discharge conditions. Note TCS is on the secondary *y*-axis.

TCS was predicted to affect nearly half of the species in its SSD dataset (PAF of 0.44). The PAFs resulting from the remaining stressors were relatively low, around 0.05 and under. In this case, TCS determines the species richness downstream of the effluent outfall.

The predicted species richness as a result of all five stressors throughout the system is in Figure 4-8.

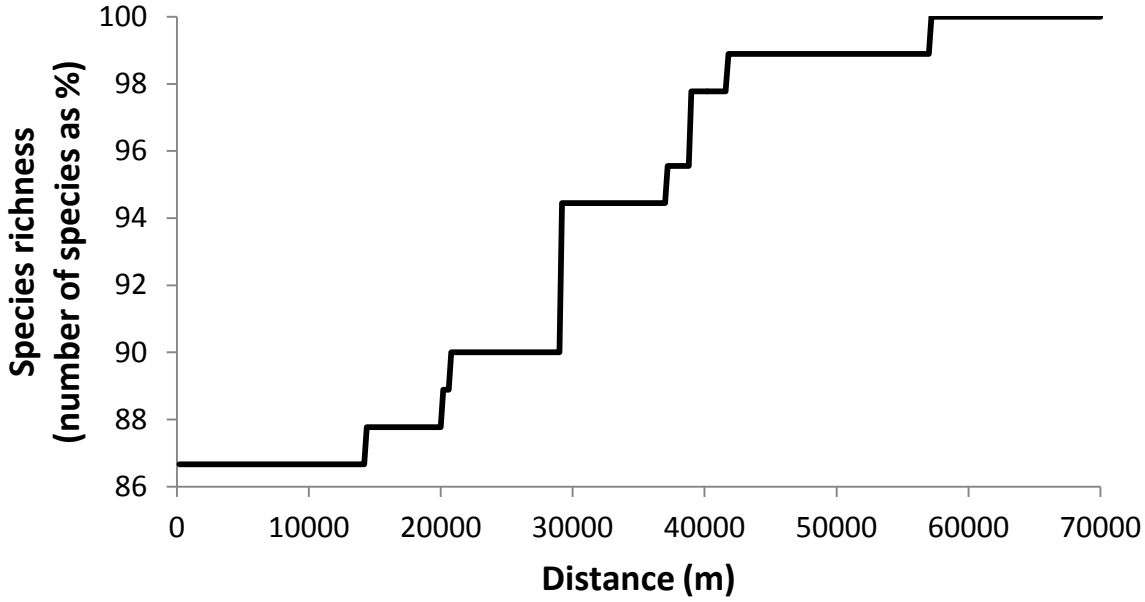


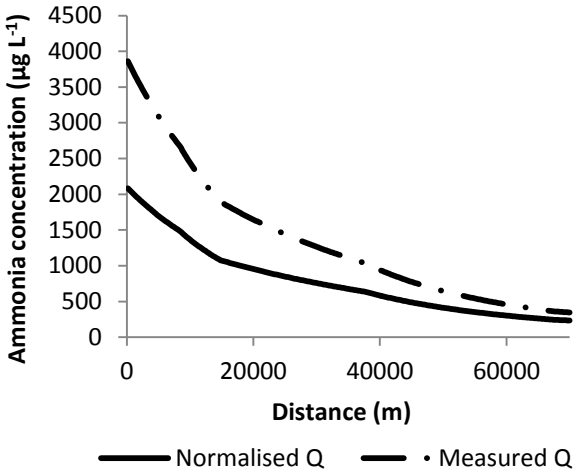
Figure 4-8 Predicted species richness (percentage of the number of species) present along the system using long-term mean discharge data.

As the stressor concentrations decrease downstream, the number of species present increases until all of the species in the SSD datasets are unaffected by the stressor concentrations. This occurred at 57.2 km downstream of the effluent discharge in the model.

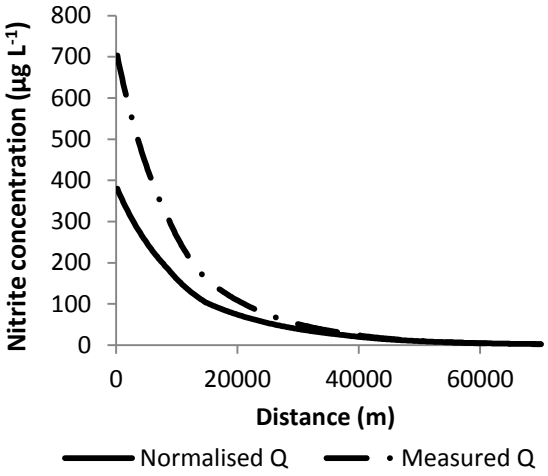
The results presented were based on the long term normalised discharge rates which affects the ecological composition over time rather than from short term exposure or low flow conditions.

4.2.2 Results of normalised vs measured discharge conditions

The long-term mean discharge data normalised by USGS data, were higher than discharge measured during the study which experienced low flows. The effect of the discharge can be seen in Figure 4-9.



(a)



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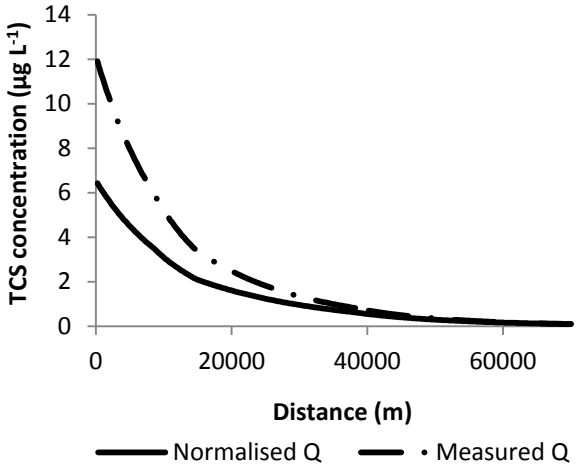
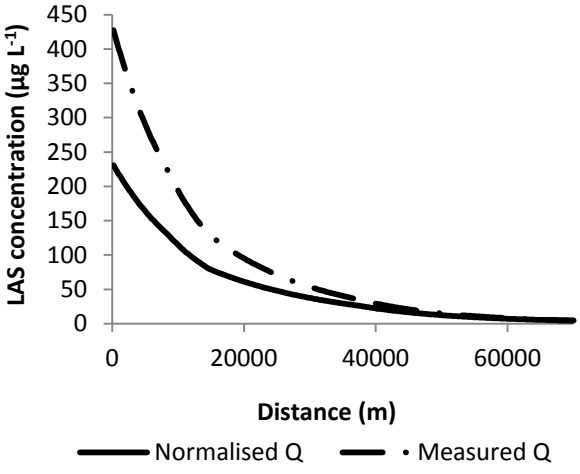
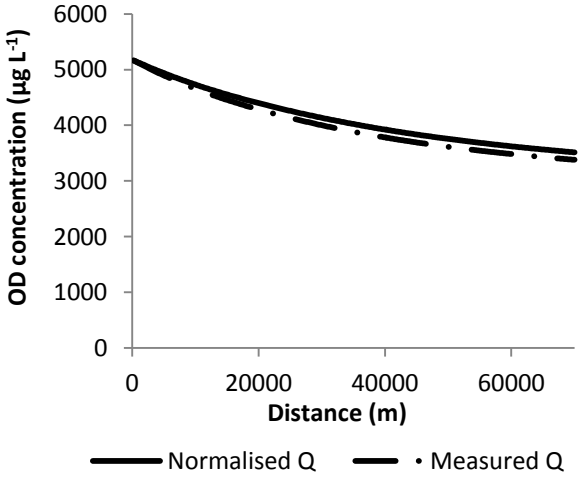


(c)



(d)



(e)

Figure 4-9 Stressor concentrations in long-term mean normalised discharge (Q) and measured discharge conditions; (a) ammonia, (b) nitrite, (c) TCS, (d) LAS and (e) OD.

As discharge was lower in the measured scenario, the stressor concentrations were higher as dilution was lower than in the normalised scenario. However, more species were present in the early part of the impact zone in the long-term mean discharge scenario than during the low flow conditions during the study itself. However, complete recovery i.e. the re-appearance of all species occurred in both scenarios at 12,800m downstream of the effluent outfall as seen in Figure 4-10.

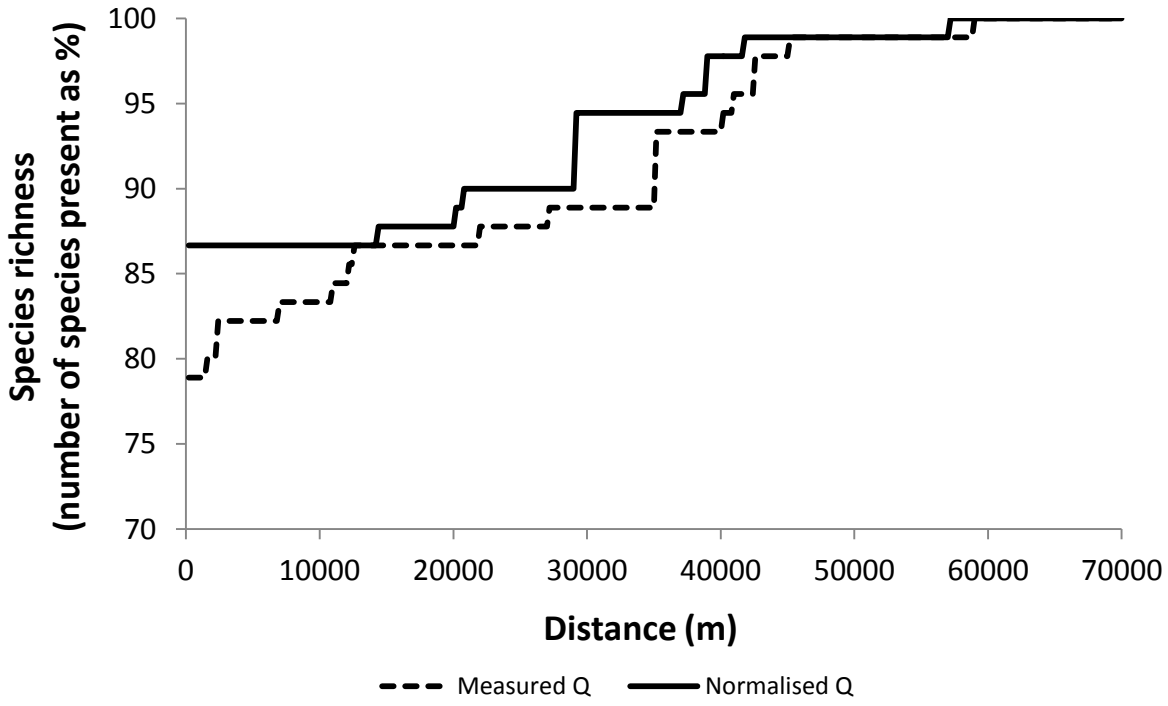


Figure 4-10 Species richness (percentage of the number of species present) in the measured discharge conditions of the study and in the long-term normalised mean discharge scenario. Duplicate species from SSD datasets were removed.

4.2.3 Modelled vs measured outputs

In order to determine the utility of the predicted results, those based on discharge measured during the study by Birge *et al*. (1989) were compared to the biomonitoring results in their study.

4.2.3.1 Dissolved oxygen

The stressor (OD), DO and BOD concentrations according to the Streeter-Phelps part of the model is presented in Figure 4-11, with the measured DO concentrations from Birge *et al*. (1989) also plotted.

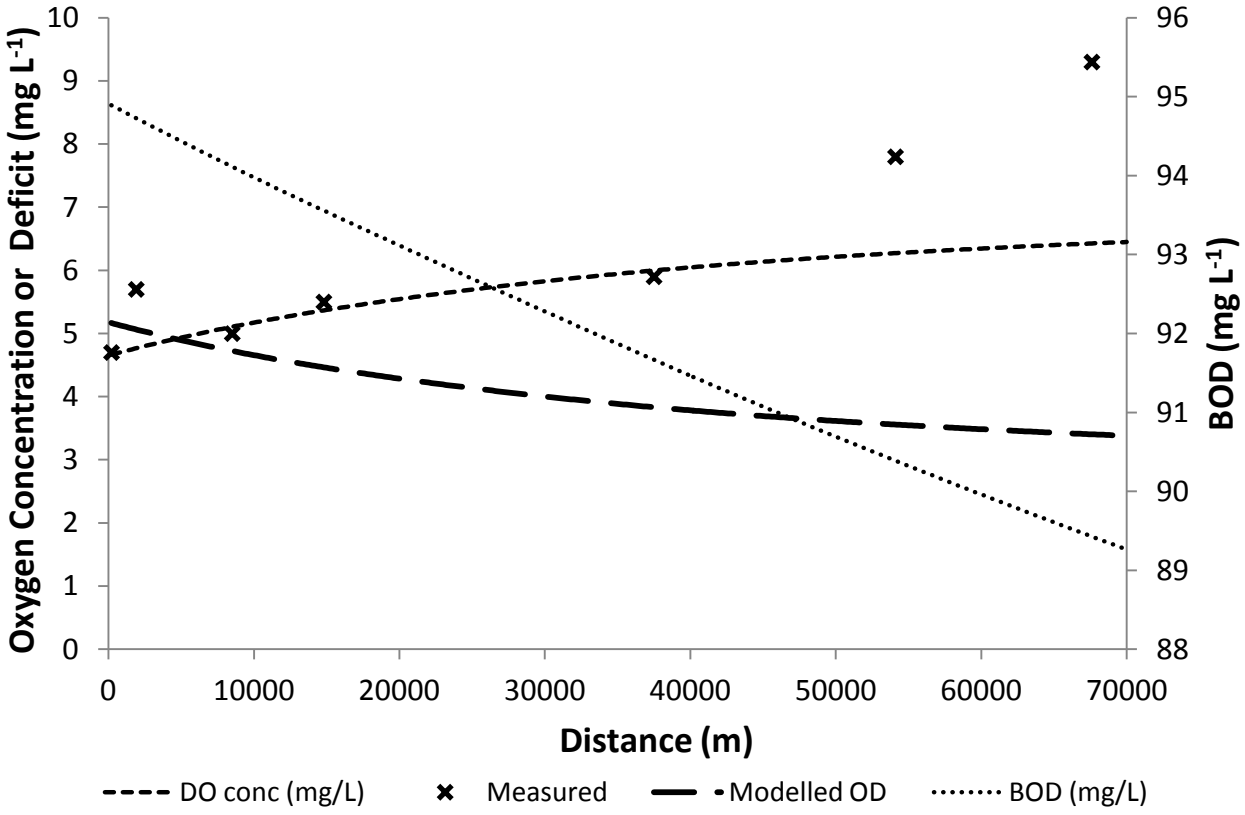


Figure 4-11 Predicted and measured dissolved oxygen concentrations under measured discharge conditions, along with modelled BOD and OD concentrations.

An oxygen sag is not evident in the modelled results. In the measured results, there is a subtle sag at 8,500m downstream (TB4). A possible reason the modelled results did not reflect this may be with the lack of data for some parameters in the Streeter-Phelps model e.g. deoxygenation and reaeration rates. However, in Figure 4-12 the DO concentrations are examined more closely.

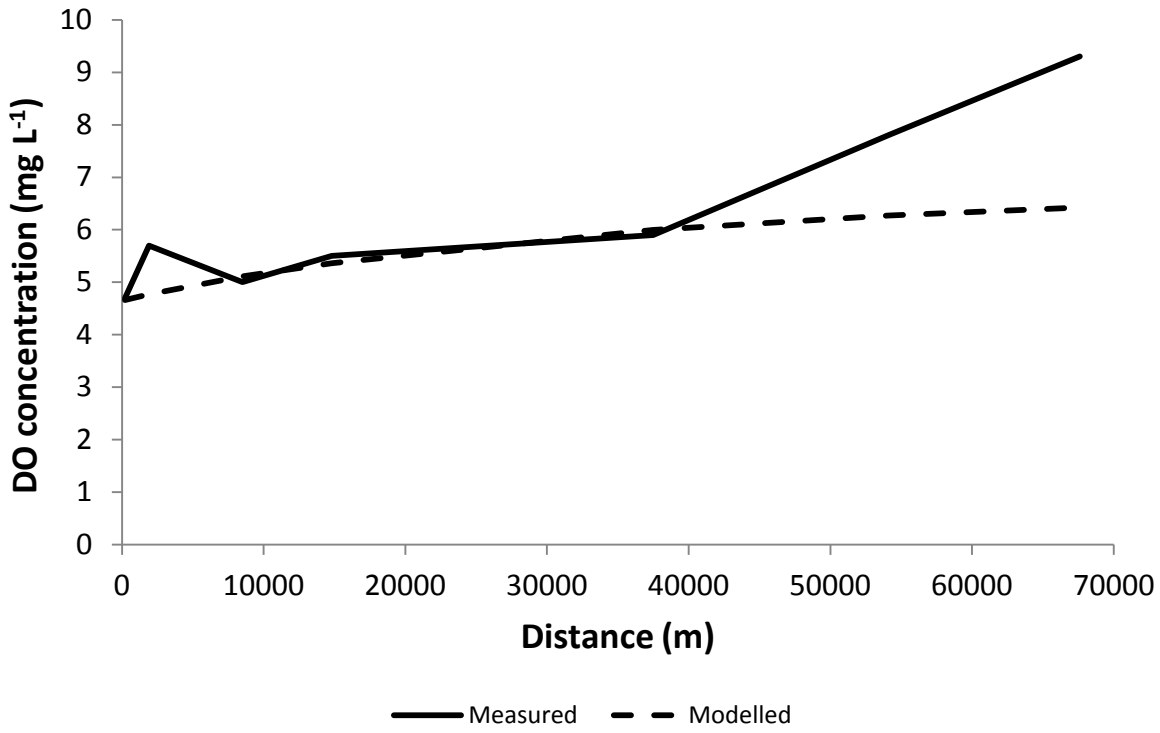


Figure 4-12 Measured vs modelled DO concentrations. r^2 = 0.85.

The curve for the modelled DO concentrations is smoother. It does not peak and then decline at the first two biomonitoring sites and increases at a lower rate from 50,000m downstream. The correlation between the two sets of data in Figure 4-12 does yield a (Pearson product-moment) correlation coefficient of 0.85 indicating a significant relationship.

4.2.3.2 Overall effect on ecology

The overall predicted impact of all five stressors on the ecology is shown in Figure 4-13 and 4-14, together with measured data on macroinvertebrate assemblage composition from Birge *et al*. (1989). The measured fraction of taxa affected at each biomonitoring site was calculated from the number of species present at each site downstream divideded by the total number of species found at the final biomonitoring site reported by Birge *et al*. (1989). The modelled fraction of species present at each site was calculated by taking the maximum PAF of all five stressors at each site (Figure 4-14).

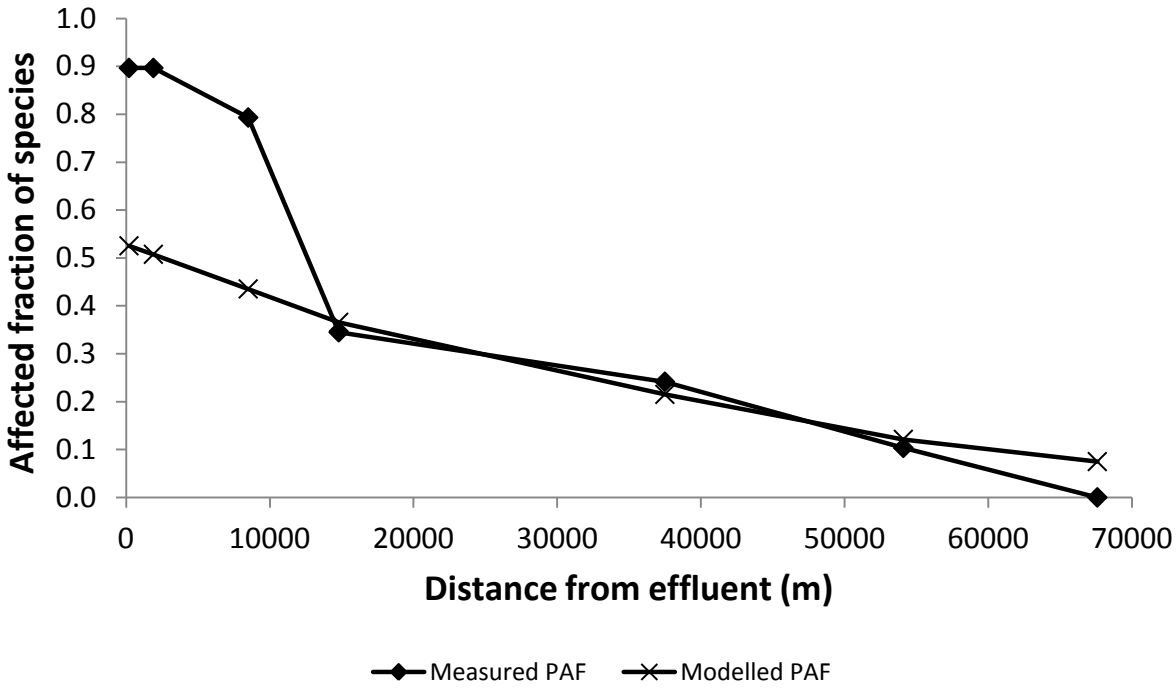


Figure 4-13 Measured PAF (calculated from number of species) vs modelled PAF_{max} downstream.

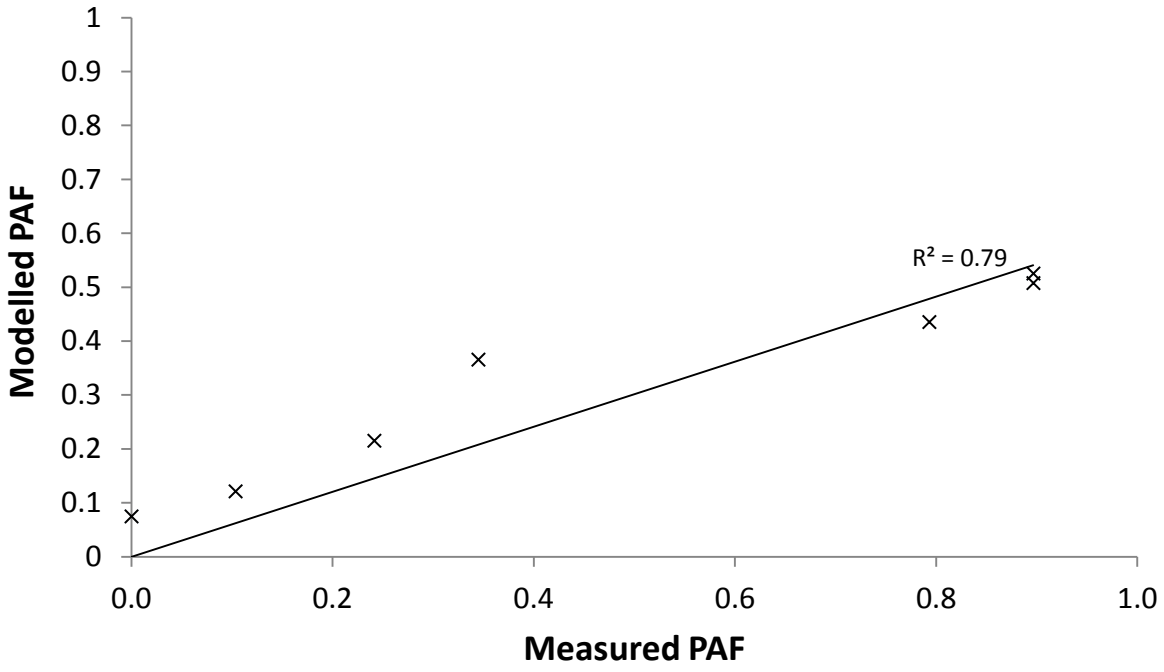


Figure 4-14 Alternative representation of measured PAF (calculated from number of species) vs modelled PAF (PAF_{max}) downstream.

The ecology at each biomonitoring site was also expressed in terms of species richness Figure 4-15. The measured number of species present represents the species richness directly. Modelled species richness was calculated by adding together all of the species presented as a result of all five stressors and removing the duplicates i.e. species which were present in more than one SSD dataset.

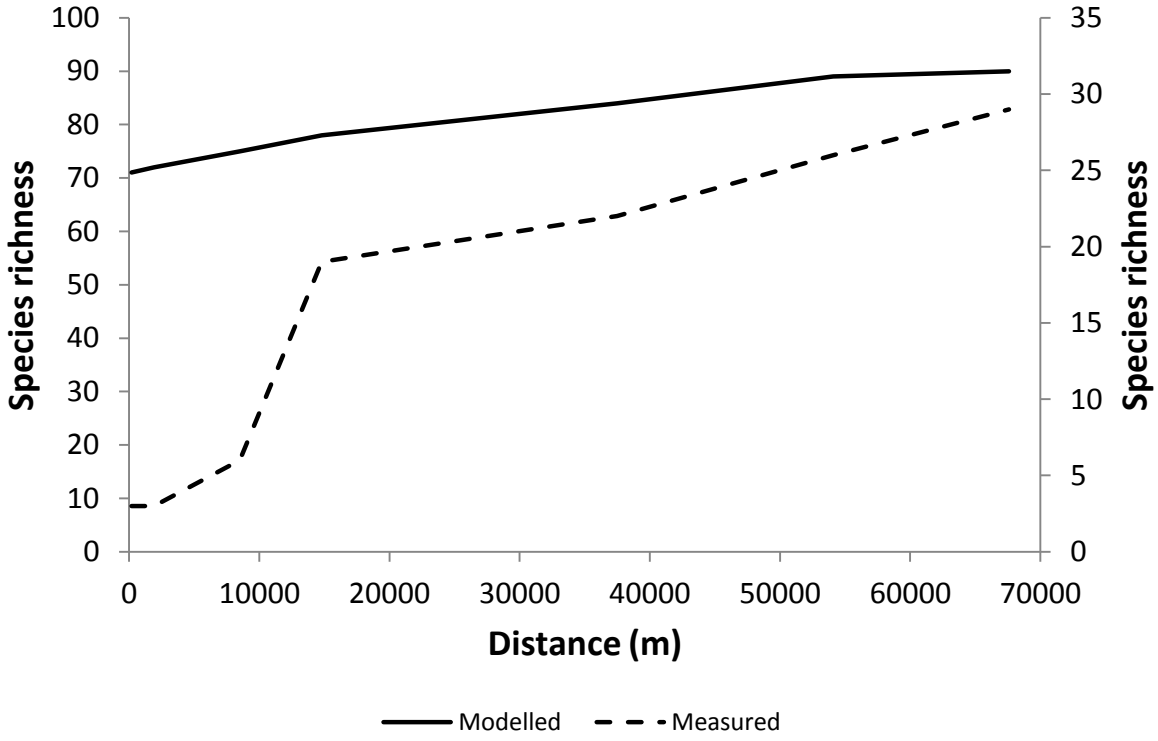


Figure 4-15 Predicted species richness (number of species present) vs measured species richness from Birge *et al*. (1989). Modelled values are plotted on the secondary *y*-axis. Species duplicates have been removed from the modelled dataset.

The predicted species richness does not reflect the adverse effects closely in the first 15km of the impact zone, but it does reflect the overall recovery in species richness. This may reflect the difference in species composition in the South Elkhorn Creek and in the SSD datasets.

5 DISCUSSION

5.1 The conceptual model

The aim of the project was to develop a conceptual model to predict ecological changes in rivers downstream of a point source discharge of untreated wastewater. The project originated from a need to assess ecological impacts in rivers where sewage treatment is lacking e.g. in developing regions. Reviewing the literature indicated that combining SSDs with water quality to model the taxa present along impact zone stressor gradients had not previously been reported.

Ecological data available in the literature (e.g. Birge *et al*., 1989; Hynes, 1960; Avery, 1970) were reviewed in order to support the conceptual basis for developing the model. Sanitary determinands and synthetic organic chemicals of interest were included in the model to assess their relative and combined effects on ecological communities. Stressor concentrations were predicted for different in-stream locations for a given river stage (flow condition) and these concentrations were compared with SSDs to determine presence or absence of particular taxa. The model was then applied to a field-based scenario with reported community composition data (Birge *et al.*, 1989) to validate the model outputs.

A further novel aspect of this project was to create an SSD for DO. This had been attempted to an extent previously by Elshout *et al*. (2013), but was based on acute toxicity data for fish only. Based on literature derived values for the lowest observed effect concentrations (LOEC) and lethal concentrations for 100% of the population (LC_{100}), Elshout *et al*. (2013) found that fish eggs and embryos were most sensitive to low DO concentrations.

5.2 European generic TGD scenario

The model was developed initially for a hypothetical generic scenario (Chapter 3) based on the TGD for Environmental Risk Assessment in Europe. The predictions of ecological effects cannot be validated. Parameters for this scenario were taken from

different laboratory simulation and monitoring studies (e.g. stressor degradation rate constants).

Two different variations of this scenario were examined; one with sewage treatment and the other assuming no treatment. Comparison of the output from these variations demonstrates the benefit of sewage treatment in terms of predicted ecological composition. Indicators of ecological quality, such as species richness were much higher in the treated scenario than they were in the untreated scenario.

In the treated scenario, impacts on taxa in the respective SSDs were predicted for nitrite and triclosan only for 24km and 19km downstream respectively of the effluent. Ammonia, LAS and OD had no effect on the taxa present. In contrast in the untreated scenario, all stressors were predicted to affect the ecosystem for several kilometres downstream of the effluent. The most severe effects were predicted for nitrite which affected taxa until 42km downstream. An adverse effect as a result of the other stressors was predicted by ammonia until 800m downstream, triclosan until 24km, LAS until 14km and OD until 12.4km downstream of the effluent outfall. Treating the effluent eliminated any adverse effects on species richness by LAS, ammonia and OD.

5.3 South Elkhorn Creek scenario

Application of the model to a real scenario in the field was a challenge due to the lack of data in the literature. Of the biomonitoring studies available, that of Birge *et al*. (1989) was most appropriate as macroinvertebrate data and some water quality data were reported. There was still some uncertainty around particular parameters, e.g. discharge, BOD and the likely stressor concentrations in the system at the time of the study.

To assess the long-term ecological effects in the South Elkhorn Creek, the long-term mean discharge conditions normalised to USGS data were incorporated into the model. This indicates the ecological composition usually present assuming these discharge conditions remain relatively constant over time. The ecological effects predicted (i.e. PAF) using the long-term mean discharge were lower than measured

discharge data reported in Birge *et al*. (1989). The correlation between the measured and modelled PAFs was weak (r = 0.38) when the modelled PAFs were a result of long-term mean discharge conditions.

Under low flow conditions reported by Birge *et al*. (1989) during the course of the study, stressor concentrations were predicted to be higher and had effects for a greater distance downstream compared with using long term mean discharge. For example, the PAF of ammonia at 200m downstream was 0.05 in the long-term discharge scenario while it was 0.14 under low flow conditions. Further downstream, differences in PAFs became less notable. Recovery, in terms of the re-appearance of all species was predicted at 66.2km downstream under the low flow conditions and 57.2km for mean flow. The stressor which affected the community composition for the largest fraction of the impact zone was nitrite (all species returned at 66km), followed by triclosan (all species returned at 59km), ammonia (all species returned at 40km) and LAS (all species returned at 7km). The correlation between the measured and modelled PAFs in this discharge scenario were stronger (r = 0.79). In both discharge scenarios for the South Elkhorn Creek, OD had no adverse effect on species richness.

5.4 Stressor effects

The Streeter-Phelps equation was a useful addition to the conceptual model although its predictions could not be validated for the TGD scenario. Predicted DO curves were compared with measured concentrations reported by Birge *et al*. (1989) suggesting that the rate constants for reaeration and BOD degradation which were assumed were reasonable. A higher degree of certainty around deoxygenation and reaeration rates for the system could be derived from optimisation of parameters to datasets from this river under different flow conditions. However, such data were unavailable at the time of writing.

The magnitude, duration and frequency of toxic events are important in terms of their resulting ecological impacts (Barnthouse, 2004; Naddy and Klaine, 2001; Diamond *et al.*, 2006). The magnitude determines the stressor concentrations. When the concentrations are higher, a greater number of species are adversely affected. The

duration of the exposure to stressors will also affect the outcome. If the duration is short, chronic effects may not occur and some organisms may be able to recover especially if sensitive life stages are not exposed. The longer the exposure to a stressor lasts, the greater the number of generations of a species likely to be affected. Recovery can occur if stressor concentrations are reduced within a reasonable timeframe, when short-term effects may only occur (Focks *et al.*, 2014). If several generations have been affected, then population recovery once stressor concentrations fall, may take significantly longer.

The frequency of toxic events also impacts the degree of the effect. When events occur more regularly, an already impacted population may be more sensitive to stress which may also increase the time taken by the population to recover once concentrations fall to levels which are not harmful. This was reported by Forbes and Cold (2005) in their study on the toxicity of the insecticide esfenvalerate to the non-biting midge *Chironomus riparius*. Individuals existing in stressed conditions, due to the absence of sediment, were more sensitive to esfenvalerate than those which were previously in favourable conditions. In the case of down-the-drain chemicals, emission in wastewater is approximately constant (although there will be diurnal variations in loads correlated with domestic rhythms of the contributing population). The resulting exposure will therefore, vary principally with dilution.

Variations in discharge may have effects on the impact and recovery of the communities living in the receiving water body, depending on the timing of the flow regime. Naddy and Klaine (2001) found that *Daphnia magna* recovered from 6 hours of exposure to the insecticide chlorpyrifos when there were intervals of 96 hours between pulsed exposures of 1 µg L^{-1}, rather than continuous exposure, suggesting that for this particular chemical, continuous exposure has a greater effect.

5.4.1 Tropical scenarios

Oxygen deficits may be more pronounced in tropical regions where direct discharge of untreated effluent is more commonplace. High rates of BOD degradation at higher temperatures will tend to reduce DO concentrations (increase OD), given the same

rate of reaeration. Low DO concentrations result in the BOD concentrations remaining high. The Streeter-Phelps model assumes that BOD degradation is independent of the OD. It is possible that at very low DO concentrations (e.g. at night when photosynthetic oxygenation does not contribute to oxygen to the water column), the micro-organisms which mediate BOD degradation will become oxygen-limited. However, there is currently no evidence of this phenomenon occurring in the literature.

In these regions, river ecology may also be severely impaired by high levels of suspended solids and unionised ammonia. In an example in India, an assessment of sewage impacts was conducted by Vijay *et al*. (2010) in a marine environment. Despite the effluents being partially treated, at three of the five monitoring sites, DO was 0 mg L^{-1}. At the remaining sites it ranged from 0 to 2.4 mg L^{-1}.

The naturally occurring diurnal pattern of DO concentrations due to photosynthesis and respiration by plants and algae was not included in the model but could influence the impact of low on DO stream ecology. In Vientiane, Laos, a strong diurnal DO pattern was observed by Whelan *et al*. (2007) as a result of these processes. Day-time concentrations were typically around 3 mg L^{-1} (~50% saturation) and night-time concentrations were 0.5 mg L^{-1} (~5% saturation) when photosynthesis ceased.

In the tropics and subtropics, discharge can also vary more extremely than in temperate regions (Thorne and Williams, 1997). Furthermore, recovery (self-purification) is less well understood for tropical regions than for temperate regions (Dyer *et al.*, 2003). The literature describing ecological effects in the impact zone are mostly based in temperate regions (e.g. Hynes 1960, Avery 1970) with Dyer *et al*. (2003) being the exception.

5.5 Reviewing the assumptions in the model

A number of assumptions were made when the model was developed (see section 3.1.2) which could be altered if the model is developed further.

5.5.1 There is only one point source input of effluent in the system

However, in most river systems including the example of the South Elkhorn Creek, there are several emission points. Incorporating the second smaller STP on the tributary Lee's Branch would have increased the level of environmental realism. This means that stressor concentrations are rarely zero and ecological effects will vary in a more complex spatial pattern than the simple depression and recovery model considered here. Nevertheless, provided the mass balance of each stressor is known (i.e. known loads at each emission point) concentrations can be predicted and taxa affected can be estimated using the model developed here. Loads from this second STP were unknown.

5.5.2 The effluent mixes instantaneously

It was assumed that effluent mixed instantaneously with the receiving water so there was no mixing zone. However, in rivers, effluent plumes from point sources can remain distinct from the waters in the receiving system for a considerable distance downstream. Mixing will depend on a number of factors including the hydraulic geometry of the system, flow rate, velocity and density differences between effluent and river water. Incorporating an explicit mixing zone would be more realistic and may pick up spatially variable (cross channel) effects.

5.5.3 Stressor removal is by degradation and dilution only

The model assumes that for most stressors, concentrations are initially high and decrease by degradation and dilution downstream. It does not account for the formation of NO_2^- and NH_3 via nitrification of NH_4^+ and ammonification of organic nitrogen, respectively. In the model, NO_2^- concentration simply decreases along the impact zone. In reality, it will probably increase for a time and peak further downstream before decreasing due to formation (NH_4^+ to NO_2^-) and subsequent loss (NO_2^- to NO_3^-) in nitrification (Welch, 1992). NH_3 and NO_3^- may also be added to the system as a result of agricultural run-off, excretion by organisms (e.g. fish and ducks) in the system, and mineralisation following nitrogen fixation and be removed from the system by processes such as uptake by plants (Jensen, 2003; Broderius *et al.*, 1985;

U.S.EPA, 2013). These processes could, in principle, be included in the model, although they would be difficult to parameterise. In any case, as long as the predicted exposure pattern matches expectations (based on observations) the inclusion of additional processes will not have a major effect on the predicted ecological outcomes.

5.5.4 Ecosystem composition can be represented by the taxa in SSDs

There are limitations with the toxicity data used in the model. Firstly, the species present in each SSD vary and there is only a small amount of overlap of species between stressor datasets. Generally, the types of organisms known to be sensitive to a stressor have ecotoxicity studies conducted on them. In contrast, studies may be less likely to be performed using species which are tolerant of that stressor, so the results could be biased towards sensitive species. For example, algae and micro-organisms are sensitive to triclosan which targets the fatty acid synthesis enzyme system which they possess, so many species of these types of organisms are included in the dataset. However, a smaller number of invertebrates and fish (which are less sensitive to triclosan) have been tested. Similarly, the nitrite dataset consists almost entirely of fish (six of the seven species) and bivalves are solely present in the ammonia dataset (no algae are present in the ammonia dataset).

Since they are compiled from laboratory tests on individual species, the taxa present in these datasets, either for individual stressors, or collectively in all SSDs are unlikely to accurately represent real ecological communities. Although this is one of the assumptions of SSDs (see section 1.2.4) when employed in risk assessment and establishing EQCs, it may not be the case for the application considered here. The species in the datasets are from different geographical regions which may not exist alongside each other in the environment in reality. Furthermore, the SSDs are also limited in terms of data availability (e.g. only seven species were available for the SSD for nitrite).

As the toxicity data used to construct the SSDs were generated in laboratory studies conducted under standard conditions on single species (Laskowski *et al.*, 2010) (e.g. pH, temperature, light, DO concentration), the validity of extrapolating any observed

effects to the field is highly uncertain. Only physiological sensitivity, based on susceptibility of the organism itself, of lab generated data is considered, and not ecological sensitivity (based on populations) (Kefford *et al.*, 2012).

Despite the organisms in the SSD datasets of the model not existing together in an ecosystem in reality, it may be acceptable for the purposes of the model if the different taxa represent a range of organisms representative of a community which is able to deliver certain functions. This is partly due to the fact that niche redundancy exists in many ecosystems, where there may be species missing from an ecosystem but in functional terms the ecosystem has not been compromised.

5.5.4.1 Traits

The use of traits-based approaches have been suggested by others to be more appropriate than taxonomy based approaches to describe ecological functions and the ecological effects of stressors (Baird and Van den Brink, 2007; Rubach *et al.*, 2010). Traits are defined as the physiological, morphological and ecological attributes of organisms or species, which describe their physical characteristics, ecological niche and functional role within an ecosystem (Baird *et al.*, 2008; Pomati and Nizzetto, 2013). Traits can be categorised into three main types (Bis and Usseglio-Polatera, 2004; van den Brink *et al.*, 2013):

a. Biological:
    i. Physiological e.g. Feeding mode, food and resistance forms.
    ii. Morphological e.g. body size, surface area, degree of sclerification and respiration mode.
b. Ecological e.g. habitat, voltinism (number of life-cycles per year), life span, time until reproduction and dispersal ability.

Traits could, in principle, provide a method of making comparisons between seemingly diverse species. For example, bivalves were the most sensitive organisms to ammonia but they were not present in the other stressor datasets, so their sensitivity to other stressors is uncertain. Traits could be used to identify organisms with a similar physiology which may make them similarly sensitive to exposure of a toxicant e.g.

ammonia. Traits could also help identify ecological characteristics caused by toxic pressure (e.g. predator populations affected by the disappearance of prey) or morphological characteristics (e.g. ability to swim) which might make it possible for an organism to escape from a short term or transient exposure to a toxicant. Thus, organisms with a given set of trait combinations may be similarly susceptible to a set of stressors. In future work, it may be possible to predict an overall picture of trait presence or absence throughout the impact zone.

The advantages of using traits are as follows:

i. In different regions across the world, there may be different species present providing a similar function in the ecosystem. Traits are comparable in communities in different ecoregions which have different taxonomic compositions as the variability of traits across temporal and spatial scales is thought to be low (Statzner *et al.*, 2004; Statzner *et al.*, 2001);
ii. Traits provide a degree of information on the mechanistic linkages between stressors and biological responses (van den Brink *et al.*, 2011);
iii. Traits provide more seasonal stability as opposed to taxonomic measures (Larsen and Ormerod, 2010; Culp et al., 2011);
iv. Traits can account for life stages which may differ significantly in terms of life-history (Feio and Dolédec, 2012);
v. Traits can be used to an extent to disentangle the effects of multiple stressors e.g. in SPEAR. However, this is challenging and the identification of individual stressors must be identified first (Schafer *et al.*, 2011).

Traits-based approaches have proven to be effective when assessing the sensitivity of macroinvertebrates to compounds without a specific toxic mode of action (e.g. general narcotics) (Ippolito *et al.*, 2012) as well as those with specific modes of action (Rubach *et al.*, 2012). Toxicity in standard laboratory tests (and used in SSDs) may be described mainly by physiological and morphological traits. Ecological traits are not addressed in laboratory tests.

5.5.4.2 Traits and the stressor organic contamination

There are just a few examples in the literature where traits are used to describe the effects of organic contamination on organisms. In the EU STAR (Standardisation of River Classifications) project, traits were assigned to the species assemblages at reference sites and at sites impacted by organic contamination (Bis and Usseglio-Polatera, 2004). The most indicative traits for describing differences between reference and impacted sites were:

1. Body size – the frequency of smaller organisms (body size <1cm) was significantly lower ($p<0.00001$) than larger organisms (>4cm) at impacted sites.
2. Reproduction type – there was a significantly higher frequency of organisms with the reproduction strategies ovoviviparity and asexual reproduction (and free clutches) at impacted sites, while organisms with cemented or fixed clutches were significantly lower.
3. Respiration type – numbers of organisms with gills and plastrons were significantly lower at impacted sites and there was a higher number of organisms with teguments. This is in agreement with Monaghan and Soares (2012) who considered gills and teguments to be sensitive and plastrons to be considered tolerant of organic pollution.
4. Feeding habits – there was a significantly higher use of absorber and deposit feeding, and significantly lower use of shredding and scraping at impacted sites. This is in agreement with Archaimbault *et al*. (2010) who confirmed that a number of studies have found filter-feeders, scrapers, shredders and predators to be adversely affected by organic contamination.
5. Resistance form – there were fewer organisms with no resistant form and eggs, and more with cocoons at impacted sites.
6. Life cycle duration – organisms with a life cycle ≤1 year were significantly higher in number at impacted sites.
7. Aquatic stages – there were fewer eggs and larvae present at impacted sites.

Mondy and Usseglio-Polatera (2014) determined that traits which exhibited increasing specialisation with increasing organic contamination were dispersal, respiration and

feeding habits. In a review by Statzner and Beche (2010), domestic wastewater caused a significant decrease in shredders whilst undefined “toxicants” often cause a significant reduction in shredders, piercers and predators.

Van den Brink *et al*. (2013) present a framework for the use of traits to advance ecological risk but do not cover sanitary determinands or organic down-the-drain chemicals in this framework.

5.5.4.3 Limitations of traits-based methods

There are a number of limitations associated with traits-based approaches. These are predominantly connected with the quality and quantity of available data for the precise characterisation of suitable traits relating to sensitivity and recovery (Van den Brink *et al.*, 2011). Unfortunately, detailed mechanistic explanations linking sensitivity to physiological and metabolic traits are currently lacking (Ippolito *et al.*, 2012). Traits databases are available but have only been used to explore mechanistic links between taxon occurrence and community level trait patterns for specific scenarios (van den Brink *et al.*, 2013). Another limitation with current trait databases is the inconsistency in trait definitions and data assigned to them for species or families (Ippolito *et al.*, 2012). External work to try and link trait sensitivity to stressors is currently being investigated. This is likely to be very data hungry, but potentially extremely useful (Van den Brink *et al.*, 2011). Ideally a comprehensive database incorporating current trait databases and additional mechanistic detail is required.

Traits-based approaches were considered in this project to describe the sensitivity of individual stressors in order to characterise the ecology of the impact zone in terms of traits, rather than species. However, the amount of information and the level of detail required to account for the toxicity of a particular stressor to the species in the SSD datasets was often not available in the trait databases. Compiling all of this information manually was beyond the scope of this project.

5.5.4.4 Combining traits and SSDs

In order to make the model more applicable to regions and scenarios globally, the taxa considered in the model could be re-classified in terms of sensitive traits. This would include re-constructing SSDs using traits which could be characterised as trait sensitivity distributions (TSDs) based on one or many trait combinations. Ippolito *et al*. (2012) also suggest developing a trait-based SSD but so far have yet to publish details.

5.5.5 Indirect ecological effects

The explicit inclusion of ecological interactions in the model was considered to be beyond the scope of this project. The nature of such interactions are manifold but include food web interactions (e.g. herbivory and predation) and relationships between organism and their habitat (e.g. cover provided by sediment) (Giller and Malmqvist, 1998). Together with the composition of the ecological community (diversity and abundance of organisms), these interactions may define many of the functions which the ecosystem performs. The consequences of a lost species on ecological function are dependent on its niche and the length of time it is missing for (Kefford *et al.*, 2012). In the environment when particular organisms are exposed to stressors, they may seek refuge or find other ways of dealing with or avoiding exposure (e.g. respiring via atmospheric oxygen rather than DO) and hence avoid some of the deleterious effects. This is not accounted for in ecotoxicological models of ecosystems (Versteeg *et al.*, 1999).

Ecological interactions play a pivotal role in controlling the composition of ecosystems and may be responsible for indirect effects of toxicity. Van den Brink *et al*. (2000) studied the effects of the fungicide carbendazim which caused direct toxic effects to microcrustacean grazers. The reduced grazing pressures lead to an increase in phytoplankton species and consequently chlorophyll-*a* levels. However, only one taxon of plankton increased in abundance due to the greater availability of food. Despite there being a reduced grazing pressure from micro-crustaceans, this was probably compensated by the increased abundance of some snail species (*Lymnaea stagnalis* and *Physella acuta*).

5.5.5.1 Multiple stressors

Multiple stressors have many and varying effects on organisms (Leuven and Poudevigne, 2002). Interactions between chemical stressors can be additive, antagonistic (less than additive effects) or synergistic (Mason, 2002). The concentration of DO can potentially influence the exposure and thus the effect of other stressors by affecting their degradation rates. For instance LAS is not anaerobically degradable which suggests that it may not break down if DO concentrations are very low (Whelan *et al.*, 2007). However, the extent to which this is actually manifested in the field is currently unreported, except in anaerobic sediments where LAS can reside undegraded for long periods of time (OECD SIDS, 2005a). Similarly, nitrification also requires oxygen and hence may be limited if DO concentrations are low (Welch, 1992). The combination of exposure to reduced DO concentrations and higher NH_3 concentrations can adversely affect fish. DO and NH_3 can affect the concentration of one another; DO is consumed in nitrification and a low DO concentration can increase the concentration of NH_3 by inhibiting nitrification (U.S.EPA, 2012).

5.6 Expressing ecological effects

Species abundance is not included in the model. The adverse effects of an individual stressor on a particular species are simply reflected in terms of presence or absence. In reality, the sensitivity of individuals of the same species to a particular toxicant will vary, particularly in terms of certain life stages which might be more vulnerable (Rubach *et al.*, 2012). This will affect the abundance of individuals at a particular location in the system of interest. Abundance could potentially be incorporated into the model by building in a distribution at each location downstream, of the probability of that species being present as a result of stressor concentrations. If species abundance were incorporated into the model, there would be additional scope to validate the model with more field data which is available and which often report taxonomic abundances. This might enable the model to reflect ecological changes more realistically.

In fact, species richness and abundance are probably related to ecosystem function (Clements and Rohr, 2009), so as the number of species is reduced or the number of individuals is reduced, ecosystem function is eventually also reduced (e.g. (Archaimbault *et al.*, 2010). Thus, communities with lower diversity as a result of toxic pressure often have impaired ecological function. Toxic effects on organisms may not always be in the form of a lethal response. Other responses include the ability of an organism to adapt in response to changes in their environment (called trait plasticity), for example, they can change their food or habitat preferences (Colas *et al.*, 2014). Wylie (1951) reported that as a result of shading, leaf thickness of woody angiosperms decreased by an average of 50%. It should be noted that the effects of organic effluent emissions are not always negative, although this depends on the load. The organic effluent can enhance nutrient levels energy resources and stimulate productivity. However, in the untreated discharge scenario, the negative effects due to toxicity are expected to outweigh any benefits.

There are examples in the literature (e.g. msPAF and SPEAR) where ecology and ecotoxicology have been linked but these have mainly been considered the responses of "pulse" exposure (only at particular times of the year) to pesticides. Ecotoxicology tests the effects of specific chemical stressors on standard laboratory species while populations, communities and ecosystems are of interest in ecology (Calow, 1996). $SPEAR_{organic}$ does not help identify the causes and mechanisms of sensitivity, but simply uses "sensitivity" as a trait. An assessment of the use of ecology in ecotoxicology was conducted by Relyea and Hoverman (2006) for pesticides. However, pesticides differ from many down-the-drain chemicals in that they usually have a specific mode of action and exposure is often as a pulse rather than being continuous. There is still a gap in the literature on the ecological and ecotoxicological interactions which occur in scenarios subjected to continuous exposure of down-the-drain chemicals.

5.7 Potential enhancements to the model

There is potential to develop the model further and build in more environmental realism. This could include:

a. Building in a distribution to predict the probability of a species to be present at each location downstream, rather than simply describing a species in terms of presence or absence. This could also enable a prediction of the abundance of each species that will be present, to be made.
b. Adding more stressors to the model.

   Additional stressors may help to validate the model. However, this will be limited to stressors which have sufficient toxicity data available. For organo-phosphorus pesticides which have a specific mode of action, a methodology has been proposed to predict SSDs for chemicals with an insufficient amount of data available (Sala *et al.*, 2012). The US EPA's ICE (Interspecies Correlation Estimation) program could also be used to predict toxicity data for more species from surrogate species (Qi *et al.*, 2011) for a particular stressor and then generates an SSD based on these data. This is however, only based on acute toxicity data (Länge *et al.*, 1998), but guidance is available on extrapolating acute to chronic data. This may enable stressors with few toxicity data to be incorporated into the model.
c. Building in the effects of mixtures. This could potentially extend the impact zone further downstream. The use of msPAF could be incorporated using concentration addition and response addition methods of combining the effects of mixtures. It is acknowledged that assessing the effects of mixtures of stressors is complex and challenging, and no one ideal method is proposed. The two recommendations made by ECETOC (2011) in their guidance document to assess the effects of mixtures in the aquatic environment, is to improve the information available on biological traits in reference sites and to improve the diagnostics that distinguish between the effects of chemical and physical stressors.
d. In the model applied to the South Elkhorn Creek, the STP at Midway on the tributary Lee's Branch could be incorporated to predict any ecological effects arising from it. The TOC concentration did increase, showing there was an

increase in organic matter, after the confluence so ecological effects may be seen.

e. Performing a sensitivity analysis. This could include running the model over a distribution of flows. For example, the discharge could be increased or decreased by increments and the effects on predicted ecological composition noted.

f. Conducting a field study to

   - generate all of the relevant water quality data needed to parameterise the model with higher certainty for a specific scenario, and
   - generate better biomonitoring data to validate the model with.

   There is currently very little data on ecological changes down stressor gradients in aquatic systems receiving wastewater in the tropics. Additional field studies should therefore focus on tropical ecosystems such as those reported by Whelan *et al*. (2007).

g. Building in a diurnal cycle of DO concentrations into the model and exploring any potential effects on the aquatic nitrogen cycle.

h. Despite the effluent emission being "continuous", incorporating into the model the temporal variability of this type of emission by building in a diurnal cycle in wastewater loading. Domestic wastewater volumes are well known to vary over the course of the day and night with the diurnal rhythms of the population generating wastewater (see for example, Whelan *et al*. (2007)).

i. Incorporating temperatures to a greater extent so that, for example, stressor degradation rates are related to the temperature of the system.

5.8 Conclusion

Data from field-based studies in the literature indicate that downstream of sewage effluent discharge into a river, the many toxic stressors present can exert an effect on the taxa present in the impact zone. The effects are expected to change as stressor concentrations change, generating a gradient down which ecological quality should improve. However, data are conspicuously lacking on detailed changes to community

composition outside of a few key field studies. A conceptual modelling approach to predict the ecological impact was developed in this thesis. The model was applied to a case study of an effluent dominated river in the USA, although it was not directly possible to evaluate the fidelity of its predictions to actual changes in ecosystem composition (largely owing to differences in the taxa observed in the field and those considered in the model). Relative changes in observed ecological indices such as species richness were well correlated to predicted potentially affected fraction and predicted fraction of all taxa present. The model represents a basic framework which should be developed to consider explicitly ecological interactions and mixture toxicity which could be improved by integrating exposure for individual locations over the whole frequency distribution of flow conditions. The model should be further tested against field data from a direct discharge scenario, ideally in the tropics.

REFERENCES

Adelman, I. R., Kusilek, L. I., Koehle, J. and Hess, J. (2009), "Acute and Chronic Toxicity of Ammonia, Nitrite, and Nitrate to the Endangered Topeka Shiner (*Notropis topeka*) and Fathead Minnows (*Pimephales promelas*)", *Environmental Toxicology and Chemistry,* vol. 28, no. 10, pp. 2216-2223.

American Water Works Association (2014)*, drinktap.org - Water Use Statistics*, available at: http://www.drinktap.org/home/water-information/conservation/water-use-statistics.aspx (accessed October 2014).

AQEM consortium (2002), *Manual for the application of the AQEM method. A comprehensive method to assess European streams using benthic macroinvertebrates, developed for the purpose of the Water Framework Directive. Version 1.0*.

Archaimbault, V., Usseglio-Polatera, P., Garric, J., Wasson, J. G. and Babut, M. A. R. C. (2010), "Assessing pollution of toxic sediment in streams using bio-ecological traits of benthic macroinvertebrates", *Freshwater Biology,* vol. 55, no. 7, pp. 1430-1446.

Arthur, M. (2004)*, University of Kentucky Resource Conservation and Management Senior Capstone Class Project*, available at: http://www.learningace.com/doc/2669132/9deeebfc51a781322bfeb1f1708478da/nrc2004 (accessed April 2014).

Avery, E. L. (1970), "Effects of domestic sewage on aquatic insects and salmonids of the East Gallatin River, Montana", *Water Research,* vol. 4, no. 2, pp. 165-177.

Aziz, J. A. and Tebbutt, T. H. Y. (1980), "Significance of COD, BOD and TOC correlations in kinetic models of biological oxidation", *Water Research,* vol. 14, no. 4, pp. 319-324.

Baird, D. J. and Burton, G. A. (2001), *Ecological variability: separating natural from anthropogenic causes of ecosystem impairment,* SETAC Press.

Baird, D. J., Rubach, M. N. and Van den Brink, P. J. (2008), "Trait-Based Ecological Risk Assessment (TERA): The New Frontier?", *Integrated Environmental Assessment and Management,* vol. 4, no. 1, pp. 2-3.

Baird, D. J. and Van den Brink, P. J. (2007), "Using biological traits to predict species sensitivity to toxic substances", *Ecotoxicology and Environmental Safety,* vol. 67, no. 2, pp. 296-301.

Bansal, M. K. (1975), "Deoxygenation in Natural Streams", *Water Resources Bulletin,* vol. 11, no. 3, pp. 491-504.

Barnthouse, L. W. (2004), "Quantifying population recovery rates for ecological risk assessment", *Environmental Toxicology and Chemistry,* vol. 23, no. 2, pp. 500-508.

Beketov, M. A., Foit, K., Schäfer, R. B., Schriever, C. A., Sacchi, A., Capri, E., Biggs, J., Wells, C. and Liess, M. (2009), "SPEAR indicates pesticide effects in streams - Comparative use of species- and family-level biomonitoring data", *Environmental Pollution,* vol. 157, no. 6, pp. 1841-1848.

Beketov, M. A. and Liess, M. (2008), "An indicator for effects of organic toxicants on lotic invertebrate communities: Independence of confounding environmental factors over an extensive river continuum", *Environmental Pollution,* vol. 156, no. 3, pp. 980-987.

Beketov, M. A. and Liess, M. (2012), "Ecotoxicology and macroecology - Time for integration", *Environmental Pollution,* vol. 162, pp. 247-254.

Birge, W. J., Black, J. A., Short, T. M. and Westerman, A. G. (1989), "A comparative ecological and toxicological investigation of a secondary wastewater treatment plant effluent and its receiving stream", *Environmental Toxicology and Chemistry,* vol. 8, no. 5, pp. 437-450.

Bis, B. and Usseglio-Polatera, P. (2004), *Species Traits Analysis,* STAR deliverable N2 to the European Commission.

Boeije, G. M., Schowanek, D. R. and Vanrolleghem, P. A. (2000), "Incorporation of biofilm activity in river biodegradation modeling: a case study for linear alkylbenzene sulphonate (LAS)", *Water Research,* vol. 34, no. 5, pp. 1479-1486.

Broderius, S., Drummond, R., Fiandt, J. and Russom, C. (1985), "Toxicity of ammonia to early life stages of the smallmouth bass at four pH values", *Environmental Toxicology and Chemistry,* vol. 4, no. 1, pp. 87-96.

Bunzel, K., Kattwinkel, M. and Liess, M. (2013), "Effects of organic pollutants from wastewater treatment plants on aquatic invertebrate communities", *Water Research,* vol. 47, no. 2, pp. 597-606.

Calow, P. (1996), "Ecology in Ecotoxicology: some possible "rules of thumb"", in Baird, D. J. e. a. (ed.) *ECOtoxicology: Ecological Dimensions,* Chapman & Hall, London, pp. 5.

Calow, P. (2009), *Handbook of Ecotoxicology,* John Wiley & Sons, Blackwell Science.

Camargo, J. and Alonso, A. (2006), "Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: A global assessment", *Environment International,* vol. 32, no. 6, pp. 831-849.

Capdevielle, M., Van Egmond, R., Whelan, M., Versteeg, D., Hofmann-Kamensky, M., Inauen, J., Cunningham, V. and Woltering, D. (2008), "Consideration of exposure and species sensitivity of triclosan in the freshwater environment", *Integrated Environmental Assessment and Management,* vol. 4, no. 1, pp. 15-23.

Centre for Ecology and Hydrology (2003), *Hydrological Data UK - Hydrometric Register and Statistics 1996-2000*, Centre for Ecology and Hydrology; British Geological Survey, Wallingford.

Chandler, R., O'Shaughnessy, J. and Blanc, F. (1976), "Pollution Monitoring with Total Organic Carbon Analysis", *Journal of Water Pollution Control Federation,* vol. 48, no. 12, pp. 8791.

Chapra, S.C., Pelletier, G.J. and Tao, H., (2008), *QUAL2K: A Modeling Framework for Simulating River and Stream Water Quality, Version 2.11, Documentation and Users Manual.*, Civil and Environmental Engineering Dept., Tufts University, Medford, MA.

Ciniglia, C., Cascone, C., Lo Giudice, R., Pinto, G. and Pollio, A. (2005), "Application of methods for assessing the geno- and cytotoxicity of Triclosan to *C. ehrenbergii*", *Journal of Hazardous Materials,* vol. 122, no. 3, pp. 227-232.

Clements, W. H. and Rohr, J. R. (2009), "Community responses to contaminants: using basic ecological principles to predict ecotoxicological effects", *Environmental Toxicology and Chemistry,* vol. 28, no. 9, pp. 1789-1800.

Colas, F., Vigneron, A., Felten, V. and Devin, S. (2014), "The contribution of a niche-based approach to ecological risk assessment: Using macroinvertebrate species under multiple stressors", *Environmental Pollution,* vol. 185, pp. 24-34.

Comte, L., Lek, S., de Deckere, E., de Zwart, D. and Gevrey, M. (2010), "Assessment of stream biological responses under multiple-stress conditions", *Environmental Science and Pollution Research,* vol. 17, no. 8, pp. 1469-1478.

Constable, T. W. and McBean, E. R. (1979), "BOD/TOC correlations and their application to water quality evaluation", *Water, Air and Soil Pollution,* vol. 11, pp. 363-375.

De Zwart, D., Dyer, S. D., Posthuma, L. and Hawkins, C. P. (2006), "Predictive models attribute effects on fish assemblages to toxicity and habitat alteration", *Ecological Applications,* vol. 16, no. 4, pp. 1295-1310.

De Zwart, D. and Posthuma, L. (2005), "Complex mixture toxicity for single and multiple species: Proposed methodologies", *Environmental Toxicology and Chemistry,* vol. 24, no. 10, pp. 2665-2676.

DeLorenzo, M. E. and Fleming, J. (2008), "Individual and mixture effects of selected pharmaceuticals and personal care products on the marine phytoplankton species *Dunaliella tertiolecta*", *Archives of Environmental Contamination and Toxicology,* vol. 54, no. 2, pp. 203-210.

Diamond, J. M., Klaine, S. J. and Butcher, J. B. (2006), "Implications of pulsed chemical exposures for aquatic life criteria and wastewater permit limits", *Environmental Science and Technology,* vol. 40, no. 16, pp. 5132-5138.

Dubber, D. and Gray, N. F. (2010), "Replacement of chemical oxygen demand (COD) with total organic carbon (TOC) for monitoring wastewater treatment performance to minimize disposal of toxic analytical waste", *Journal of Environmental Science and Health - Part A Toxic/Hazardous Substances and Environmental Engineering,* vol. 45, no. 12, pp. 1595-1600.

Dunnivant, F. M. (2004), *Environmental Laboratory Exercises for Instrumental Analysis and Environmental Chemistry,* John Wiley & Sons.

Dussault, E.,B., Balakrishnan, V. K., Sverko, E., Solomon, K. R. and Sibley, P. K. (2008), "Toxicity of human pharmaceuticals and personal care products to benthic invertebrates", *Environmental Toxicology and Chemistry,* vol. 27, no. 2, pp. 425-432.

Dyer, S. D., Peng, C., McAvoy, D. C., Fendinger, N. J., Masscheleyn, P., Castillo, L. V. and Lim, J. M. (2003), "The influence of untreated wastewater to aquatic communities in the Balatuin River, The Philippines", *Chemosphere,* vol. 52, no. 1, pp. 43-53.

ECETOC (2007), *ECETOC Technical Report no. 102. Intelligent Testing Strategies in Ecotoxicology: Mode of Action Approach for Specifically Acting Chemicals*, European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels.

ECETOC (2011), *Development of Guidance for Assessing the Impact of Mixtures of Chemicals in the Aquatic Environment,* 111, European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels.

Elshout, P. M. F., Dionisio Pires, L. M., Leuven, R. S. E. W., Wendelaar Bonga, S. E. and Hendriks, A. J. (2013), "Low oxygen tolerance of different life stages of temperate freshwater fish species", *Journal of fish biology,* vol. 83, no. 1, pp. 190-206.

Environmental Quality Committee, Town Branch Trail Inc. (2001)*, The Town Branch Environment - An Initial Evaluation*, available at: http://www.townbranch.org/doc/TBER.pdf (accessed April 2014).

Escher, B. I., Ashauer, R., Dyer, S. D., Hermens, J. L., Lee, J. H., Leslie, H. A., Mayer, P., Meador, J. P. and Warne, M. S. (2011), "Crucial role of mechanisms and modes of toxic action for understanding tissue residue toxicity and internal effect

concentrations of organic chemicals", *Integrated Environmental Assessment and Management,* vol. 7, no. 1, pp. 28-49.

European Commission (2003), *Technical Guidance Document in support of Commission Directive 93/67/EEC on Risk Assessment for new notified substances, Commission Regulation (EC) No 1488/94 on Risk Assessment for existing substances and Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market. European Chemical Bureau, Italy*.

Feio, M. J. and Dolédec, S. (2012), "Integration of invertebrate traits into predictive models for indirect assessment of stream functional integrity: A case study in Portugal", *Ecological Indicators,* vol. 15, no. 1, pp. 236-247.

Ferrari, B., Paxeus, N., Pinto, G., Pollio, A., Andreozzi, R., Marotta, R., Vogna, D., Lyberatos, G. and Garric, J. "Fate and effect of triclosan in the aquatic ecosystems: Data for a risk assessment [poster].", *Society of Environmental Toxicology and Chemistry Annual Meeting Europe,* 2002, Vienna, Austria, SETAC Europe, Brussels.

Finnegan, C. J., van Egmond, R. A., Price, O. R. and Whelan, M. J. (2009), "Continuous-flow laboratory simulation of stream water quality changes downstream of an untreated wastewater discharge", *Water Research,* vol. 43, no. 7, pp. 1993-2001.

Focks, A., Luttik, R., Zorn, M., Brock, T., Roex, E., van der Linden, T. and van Den Brink, P. J. (2014), "A simulation study on effects of exposure to a combination of pesticides used in an orchard and tuber crop on the recovery time of a vulnerable aquatic invertebrate", *Environmental Toxicology and Chemistry,* vol. 33, no. 7, pp. 1489-1498.

Forbes, V. E. and Cold, A. (2005), "Effects of the pyrethroid esfenvalerate on life-cycle traits and population dynamics of *Chironomus riparius* - Importance of exposure scenario", *Environmental Toxicology and Chemistry,* vol. 24, no. 1, pp. 78-86.

Forbes, V. E. and Calow, P. (2002), "Species Sensitivity Distributions Revisited: A Critical Appraisal", *Human and Ecological Risk Assessment: An International Journal,* vol. 8, no. 3, pp. 473-492.

Frances, J., Allan, G. L. and Nowak, B. F. (1998), "The effects of nitrite on the short-term growth of silver perch (*Bidyanus bidyanus*)", *Aquaculture,* vol. 163, no. 1-2, pp. 63-72.

Gaufin, A. R. and Tarzwell, C. M. (1956), "Aquatic macroinvertebrate communities as indicators of organic pollution in Lytle Creek", *Sewage and Industrial Wastes,* vol. 28, no. 7, pp. 906-924.

Giller, P. S. and Malmqvist, B. (1998), *The biology of streams and rivers,* Oxford University Press.

Google Maps (2014), *Newport Pagnell to Bedford - River Great Ouse*, available at: https://www.google.co.uk/maps/ (accessed August 2014).

Gotovtsev, A. V. (2010), "Modification of the Streeter-Phelps system with the aim to account for the feedback between dissolved oxygen concentration and organic matter oxidation rate", *Water Resources,* vol. 37, no. 2, pp. 245-251.

Gray, N. F. (1999), *Water Technology: An Introduction for Scientists and Engineers,* Arnold, London.

Gray, N. F. (2004), *Biology of Wastewater Treatment,* 2nd ed, Imperial College Press, London.

Harbers, J. V., Huijbregts, M. A. J., Posthuma, L. and Van De Meent, D. (2006), "Estimating the impact of high-production-volume chemicals on remote ecosystems by toxic pressure calculation", *Environmental Science and Technology,* vol. 40, no. 5, pp. 1573-1580.

Health Canada and Environment Canada (2012), *Preliminary Assessment Triclosan*.

Hellawell, J. M. (1986), *Biological indicators of freshwater pollution and environmental management,* Elsevier Applied Science Publishers.

HERA (2009), *LAS - Linear Alkylbenzene Sulphonate,* Version 4.

Hoback, W. W. and Barnhart, M. C. (1996), "Lethal limits and sublethal effects of hypoxia on the amphipod *Gammarus pseudolimnaeus*", *Journal of the North American Benthological Society,* vol. 15, no. 1, pp. 117-126.

Hynes, H. B. N. (1960), *The Biology of Polluted Waters,* Liverpool University Press.

Hynes, H. B. N. (1994), "Historical perspective and future direction of biological monitoring of aquatic systems", in Loeb, S. L. and Spacie, A. (eds.) *Biological Monitoring of Aquatic Systems,* CRC Press, , pp. 11.

Ippolito, A., Sala, S., Faber, J. H. and Vighi, M. (2010), "Ecological vulnerability analysis: A river basin case study", *Science of The Total Environment,* vol. 408, no. 18, pp. 3880-3890.

Ippolito, A., Todeschini, R. and Vighi, M. (2012), "Sensitivity assessment of freshwater macroinvertebrates to pesticides using biological traits", *Ecotoxicology,* vol. 21, no. 2, pp. 336-352.

Irving, E. C., Liber, K. and Culp, J. M. (2004), "Lethal and Sublethal Effects of Low Dissolved Oxygen Condition on Two Aquatic Invertebrates, *Chironomus tentans*

and *Hyalella azteca*", *Environmental Toxicology and Chemistry,* vol. 23, no. 6, pp. 1561-1566.

Ishibashi, H., Matsumura, N., Hirano, M., Matsuoka, M., Shiratsuchi, H., Ishibashi, Y., Takao, Y. and Arizono, K. (2004), "Effects of triclosan on the early life stages and reproduction of medaka *Oryzias latipes* and induction of hepatic vitellogenin", *Aquatic Toxicology,* vol. 67, no. 2, pp. 167-179.

Jensen, F. B. (2003), "Nitrite disrupts multiple physiological functions in aquatic animals", *Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology,* vol. 135, no. 1, pp. 9-24.

Kefford, B. J., Liess, M., Warne, M. S., Metzeling, L. and Schäfer, R. B. (2012), "Risk assessment of episodic exposures to chemicals should consider both the physiological and the ecological sensitivities of species", *The Science of the Total Environment,* vol. 441, pp. 213-219.

Keup, L. E. (1966), "Stream biology for assessing sewage treatment plant efficiency", *Water and Sewage Works,* vol. 113, pp. 411-417.

Kondratieff, P. F., Matthews, R. A. and Buikema, A. L.,Jr. (1984), "A stressed stream ecosystem: macroinvertebrate community integrity and microbial trophic response", *Hydrobiologia,* vol. 111, no. 2, pp. 81-91.

Kroupova, H., Machova, J., Piackova, V., Blahova, J., Dobsikova, R., Novotny, L. and Svobodova, Z. (2008), "Effects of subchronic nitrite exposure on rainbow trout (*Oncorhynchus mykiss*)", *Ecotoxicology and Environmental Safety,* vol. 71, no. 3, pp. 813-820.

Kroupova, H., Prokes, M., Mácová, S., Penaz, M., Barus, V., Novotny, L. and Machova, J. (2010), "Effect of nitrite on early-life stages of common carp (*Cyprinus carpio L.*)", *Environmental Toxicology & Chemistry,* vol. 29, no. 3, pp. 535-540.

Laflin, B. D. (1970), *Some effects of municipal sewerage pollution upon the biota and water quality of Elkhorn Creek: Kentucky Department of Fish and Wildlife Resources Bulletin No. 55,* 55, Kentucky Department of Fish and Wildlife Resources Bulletin No. 55, Frankfort, Kentucky.

Länge, R., Hutchinson, T. H., Scholz, N. and SolbÉ, J. (1998), "Analysis of the ecetoc aquatic toxicity (EAT) database II — Comparison of acute to chronic ratios for various aquatic organisms and chemical substances", *Chemosphere,* vol. 36, no. 1, pp. 115-127.

Laskowski, R., Bednarska, A. J., Kramarz, P. E., Loureiro, S., Scheil, V., Kudlek, J. and Holmstrup, M. (2010), "Interactions between toxic chemicals and natural

environmental factors - A meta-analysis and case studies", *The Science of the total environment,* vol. 408, no. 18, pp. 3763-3774.

Lawrence, J. R., Zhu, B., Swerhone, G. D. W., Roy, J., Wassenaar, L. I., Topp, E. and Korber, D. R. (2009), "Comparative microscale analysis of the effects of triclosan and triclocarban on the structure and function of river biofilm communities", *The Science of the Total Environment,* vol. 407, no. 10, pp. 3307-3316.

Leuven, R. S. E. W. and Poudevigne, I. S. A. B. (2002), "Riverine landscape dynamics and ecological risk assessment", *Freshwater Biology,* vol. 47, no. 4, pp. 845-865.

Lewin, I., Czerniawska-Kusza, I., Szoszkiewicz, K., Lawniczak, A. E. and Jusik, S. (2013), "Biological indices applied to benthic macroinvertebrates at reference conditions of mountain streams in two ecoregions (Poland, the Slovak Republic)", *Hydrobiologia,* vol. 709, no. 1, pp. 183-200.

Lexington Fayette, Urban County Government (2010)*, Town Branch Trail Master Plan*, available at: http://www.townbranch.org/doc/TBT%20Draft-Dec-2010.pdf (accessed April 2014).

Liess, M. and von der Ohe, P. C. (2005), "Analyzing effects of pesticides on invertebrate communities in streams", *Environmental Toxicology and Chemistry,* vol. 24, no. 4, pp. 954-965.

Limlette III Workshop (1995), "Environmental risk assessment of detergent chemicals", *Proccedings of the AISE/CESIO Limlette III Workshop,* 28-29 November 1995, pp. 49.

Lyndall, J., Fuchsman, P., Bock, M., Barber, T., Lauren, D., Leigh, K., Perruchon, E. and Capdevielle, M. (2010), "Probabilistic risk evaluation for triclosan in surface water, sediments, and aquatic biota tissues", *Integrated Environmental Assessment and Management,* vol. 6, no. 3, pp. 419-440.

Maltby, L., Blake, N., Brock, T. C. M. and Van den Brink, P. J. (2005), "Insecticide species sensitivity distributions: Importance of test species selection and relevance to aquatic ecosystems", *Environmental Toxicology and Chemistry,* vol. 24, no. 2, pp. 379-388.

Mason, C. F. (2002), *Biology of Freshwater Pollution,* Pearson Educational.

Mattson, V. R., Russell Hockett, J., Highland, T. L., Ankley, G. T. and Mount, D. R. (2008), "Effects of low dissolved oxygen on organisms used in freshwater sediment toxicity tests", *Chemosphere,* vol. 70, no. 10, pp. 1840-1844.

McAvoy, D. C., Masscheleyn, P., Peng, C., Morrall, S. W., Casilla, A. B., Lim, J. M. U. and Gregorio, E. G. (2003), "Risk assessment approach for untreated wastewater using the QUAL2E water quality model", *Chemosphere,* vol. 52, no. 1, pp. 55-66.

McKenzie, D. J., Shingles, A. and Taylor, E. W. (2003), "Sub-lethal plasma ammonia accumulation and the exercise performance of salmonids", *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology,* vol. 135, no. 4, pp. 515-526.

Memmert, U. (2006), *Triclosan: Effects on the development of sediment-dwelling larvae of Chironomus riparius in a water-sediment system with spiked sediment.* A34896, RCC Ltd., Itingen, Switzerland.

Metcalf and Eddy (2003), *Wastewater Engineering: Treatment and Reuse,* 4th ed, McGraw-Hill, New York.

Meyer, J. S. and Hansen, J. A. (2002), "Subchronic toxicity of low dissolved oxygen concentrations, elevated pH, and elevated ammonia concentrations to Lost River suckers", *Transactions of the American Fisheries Society,* vol. 131, no. 4, pp. 656-666.

Midway Messenger, (2013), *Full water sewer repair would cost $8.4 billion*, http://midwayky.blogspot.co.uk/2013/02/full-water-sewer-repair-would-cost-84.html ed., Midway, Kentucky.

Monaghan, K. A. and Soares, A. M. V. M. (2012), "Bringing new knowledge to an old problem: Building a biotic index from lotic macroinvertebrate traits", *Ecological Indicators,* vol. 20, pp. 213-220.

Mondy, C. P. and Usseglio-Polatera, P. (2014), "Using fuzzy-coded traits to elucidate the non-random role of anthropogenic stress in the functional homogenisation of invertebrate assemblages", *Freshwater Biology,* vol. 59, no. 3, pp. 584-600.

Morrall, D., McAvoy, D., Schatowitz, B., Inauen, J., Jacob, M., Hauk, A. and Eckhoff, W. (2004), "A field study of triclosan loss rates in river water (Cibolo Creek, TX)", *Chemosphere,* vol. 54, no. 5, pp. 653-660.

Naddy, R. B. and Klaine, S. J. (2001), "Effect of pulse frequency and interval on the toxicity of chlorpyrifos to *Daphnia magna*", *Chemosphere,* vol. 45, no. 4–5, pp. 497-506.

Nas, S. S. and Nas, E. (2009), "Water quality modeling and dissolved oxygen balance in streams: A point source Streeter-Phelps application in the case of the Harsit stream", *Clean - Soil, Air, Water,* vol. 37, no. 1, pp. 67-74.

National Renewable Energy Laboratory (2014), *River Atlas*, available at: http://maps.nrel.gov/river_atlas (accessed April 2014).

Nebeker, A. V., Onjukka, S. T., Stevens, D. G., Chapman, G. A. and Dominguez, S. E. (1992), "Effects of low dissolved oxygen on survival, growth and reproduction of *Daphnia*, *Hyalella* and *Gammarus*", *Environmental Toxicology and Chemistry,* vol. 11, no. 3, pp. 373-379.

Negulescu, M. (1985), *Municipal Waste Water Treatment,* Elsevier Science, NL.

Nietch, C. T., Quinlan, E. L., Lazorchak, J. M., Impellitteri, C. A., Raikow, D. and Walters, D. (2013), "Effects of a chronic lower range of triclosan exposure to a stream mesocosm community", *Environmental Toxicology and Chemistry,* vol. 32, no. 12, pp. 2874-2887.

Noutsopoulos, C. and Kyprianou, I. (2014), "A simple water quality model as a tool for the evaluation of alternative river basin management plans", *Global Nest Journal,* vol. 16, no. 1, pp. 1-8.

OECD SIDS (2005a), *SIDS Initial Assessment Report on LAS,* OECD.

OECD SIDS (2005b), *SIDS Initial Assessment Report on Sodium Nitrite,* OECD.

Ortiz, J. D., Martí, E. and Angels Puig, M. (2005), "Recovery of the macroinvertebrate community below a wastewater treatment plant input in a Mediterranean stream", *Hydrobiologia,* vol. 545, no. 1-3, pp. 289-302.

Orvos, D. R., Versteeg, D. J., Inauen, J., Capdevielle, M., Rothenstein, A. and Cunningham, V. (2002), "Aquatic toxicity of triclosan", *Environmental Toxicology and Chemistry,* vol. 21, no. 7, pp. 1338-1349.

Padgett, W. J. (1978), "A stream-pollution model with random deoxygenation and reaeration coefficients", *Mathematical biosciences,* vol. 42, no. 1–2, pp. 137-148.

Pinto, A. L., Varandas, S., Coimbra, A. M., Carrola, J. and Fontainhas-Fernandes, A. (2010), "Mullet and gudgeon liver histopathology and macroinvertebrate indexes and metrics upstream and downstream from a wastewater treatment plant (Febros River-Portugal)", *Environmental Monitoring and Assessment,* vol. 169, no. 1-4, pp. 569-585.

Pomati, F. and Nizzetto, L. (2013), "Assessing triclosan-induced ecological and trans-generational effects in natural phytoplankton communities: A trait-based field method", *Ecotoxicology,* vol. 22, no. 5, pp. 779-794.

Posthuma, L., Suter, G. W. and Traas, T. P. (2002), *Species Sensitivity Distributions in Ecotoxicology,* Lewis Publishers, Boca Raton, FL.

Press, W. H. (2007), *Numerical Recipes 3rd Edition: The Art of Scientific Computing,* Cambridge University Press.

Qi, P., Wang, Y., Mu, J. and Wang, J. (2011), "Aquatic predicted no-effect-concentration derivation for perfluorooctane sulfonic acid", *Environmental Toxicology and Chemistry,* vol. 30, no. 4, pp. 836-842.

Rasmussen, A. (1996), "Chapter 20: Least Oxygen Dependent", in University of Florida (ed.) *Book of Insect Records,* pp. 46.

Raut, S. A. and Angus, R. A. (2010), "Triclosan has endocrine-disrupting effects in male western mosquitofish, *Gambusia affinis*", *Environmental Toxicology and Chemistry,* vol. 29, no. 6, pp. 1287-1291.

Relyea, R. and Hoverman, J. (2006), "Assessing the ecology in ecotoxicology: A review and synthesis in freshwater systems", *Ecology Letters,* vol. 9, no. 10, pp. 1157-1171.

Rene, E. R. and Saidutta, M. B. (2008), "Prediction of water quality indices by regression analysis and artificial neural networks", *International Journal of Environmental Research,* vol. 2, no. 2, pp. 183-188.

Resh, V. H. (1995), "Freshwater benthic macroinvertebrates and rapid assessment procedures for water quality monitoring in developing and newly industrialized countries ", in Davis, W. S. and Simon, T. P. (eds.) *Biological Assessment and Criteria,* England: Lewis Publishers, pp. 167.

Richardson, R. E. (1928), "The bottom fauna of the Middle Illinois River, 1913-1925. Its distribution, abundance, valuation and index value in the study of stream pollution", *Illinois Natural History Survey Bulletin,* vol. 17, no. 12, pp. 387-475.

Richardson, R. and Moore, R. D. (2010), "Stream and Riparian Ecology", in Pike, R. G., Redding, T. E., Moore, R. D., et al (eds.) *Compendium of Forest Hydrology and Geomorphology in British Columbia,* , pp. 441.

Rostgaard, S. and Jacobsen, D. (2005), "Respiration Rate of Stream Insects Measured in situ Along a Large Altitude Range", *Hydrobiologia,* vol. 549, no. 1-3, pp. 79-98.

Round, C. E., Young, A. R. and Fox, K. K. (1998), "A Regionally Applicable Model for Estimating Flow Velocity at Ungauged River Sites in the UK", *Water and Environment Journal,* vol. 12, no. 6, pp. 402-405.

Rubach, M. N., Baird, D. J., Boerwinkel, M. C., Maund, S., Roessink, I. and van den Brink, P. J. (2012), "Species traits as predictors for intrinsic sensitivity of aquatic invertebrates to the insecticide chlorpyrifos", *Ecotoxicology,* vol. 21, no. 7, pp. 2088-2101.

Rubach, M. N., Baird, D. J. and van den Brink, P. J. (2010), "A new method for ranking mode-specific sensitivity of freshwater arthropods to insecticides and its relationship to biological traits", *Environmental Toxicology and Chemistry,* vol. 29, no. 2, pp. 476-487.

Sala, S., Migliorati, S., Monti, G. S. and Vighi, M. (2012), "SSD-based rating system for the classification of pesticide risk on biodiversity", *Ecotoxicology,* vol. 21, no. 4, pp. 1050-1062.

SCENIHR, SCHER and SCCS (2012), *Preliminary report on Addressing the New Challenges for Risk Assessment,* , European Commission, Brussels.

Schafer, R. B., Kefford, B. J., Metzeling, L., Liess, M., Burgert, S., Marchant, R., Pettigrove, V., Goonan, P. and Nugegoda, D. (2011), "A trait database of stream invertebrates for the ecological risk assessment of single and combined effects of salinity and pesticides in South-East Australia", *Science of The Total Environment,* vol. 409, no. 11, pp. 2055-2063.

Schaffer, R. B., Van Hall, C. E., McDermott, G. N., Barth, D., Stenger, V. A., Sebesta, S. J. and Griggs, S. H. (1965), "Application of a carbon analyzer in waste treatment.", *Journal of the Water Pollution Control Federation,* vol. 37, no. 11, pp. 1545-1566.

Seitz, A. and Ratte, H. T. (1991), "Aquatic ecotoxicology: On the problems of extrapolation from laboratory experiments with individuals and populations to community effects in the field", *Comparative Biochemistry and Physiology Part C: Comparative Pharmacology,* vol. 100, no. 1–2, pp. 301-304.

Solimini, A. G., Ruggiero, A., Bernardini, V. and Carchini, G. (2003), "Temporal pattern of macroinvertebrate diversity and production in a new man made shallow lake", *Hydrobiologia,* vol. 506-509, pp. 373-379.

Soucek, D. and Dickinson, A. (2012), "Acute Toxicity of Nitrate and Nitrite to Sensitive Freshwater Insects, Mollusks, and a Crustacean", *Archives of Environmental Contamination and Toxicology,* vol. 62, no. 2, pp. 233-242.

Statzner, B., Bis, B., Doledec, S. and Usseglio-Polatera, P. (2001), "Perspectives for biomonitoring at large spatial scales: A unified measure for the functional composition of invertebrate communities in European running waters", *Basic and Applied Ecology,* vol. 2, no. 1, pp. 73-85.

Statzner, B., Dolédec, S. and Hugueny, B. (2004), "Biological trait composition of European stream invertebrate communities: Assessing the effects of various trait filter types", *Ecography,* vol. 27, no. 4, pp. 470-488.

Statzner, B. E. R. N. and Bêche, L. A. (2010), "Can biological invertebrate traits resolve effects of multiple stressors on running water ecosystems?", *Freshwater Biology,* vol. 55, pp. 80-119.

Stevens, K. J., Kim, S. Y., Adhikari, S., Vadapalli, V. and Venables, B. J. (2009), "Effects of triclosan on seed germination and seedling development of three wetland plants: *Sesbania herbacea*, *Eclipta prostrata*, and *Bidens frondosa*", *Environmental Toxicology and Chemistry,* vol. 28, no. 12, pp. 2598-2609.

Streeter, H. W. and Phelps, E. B. (1925), *A study of the pollution and natural purification of the Ohio river,* 146, US Public Health Bulletin, Washington DC.

Tatarazako, N., Ishibashi, H., Teshima, K., Kishi, K. and Arizono, K. (2004), "Effects of triclosan on various aquatic organisms", *Environmental Sciences : An International Journal of Environmental Physiology and Toxicology,* vol. 11, no. 2, pp. 133-140.

Thorne, R. and Williams, P. (1997), "The response of benthic macroinvertebrates to pollution in developing countries: a multimetric system of bioassessment", *Freshwater Biology,* vol. 37, no. 3, pp. 671-686.

U.S. Geological Survey (2009), *Kentucky Water Resources Research Institute Annual Technical Report*.

U.S. Geological Survey (2014)*, USGS Water Data for the Nation*, available at: http://waterdata.usgs.gov/nwis (accessed March 2014).

U.S.EPA (1986), *Ambient Water Quality Criteria for Dissolved Oxygen,* EPA 440/5-86-003, U.S. E.P.A.

U.S.EPA (2009)*, Detroit River-Western Lake Erie Basin Indicator Project,* available at: http://www.epa.gov/medatwrk/grosseile_site/indicators/chironomids.html (accessed July 2014).

U.S.EPA (2012)*, US EPA CADDIS (Causal/Diagnosis Decision Information System)*.

U.S.EPA (2013), *Aquatic Life Ambient Water Quality Criteria For Ammonia - Freshwater 2013,* EPA-822-R-13-001, U.S. E.P.A., Washington DC.

University of Wisconsin (2005)*, The Lower Fox River Watershed Monitoring Program*, available at: http://www.uwgb.edu/watershed/data/monitoring/oxygen.htm (accessed September 2014).

Van den Brink, P. J., Alexander, A. C., Desrosiers, M., Goedkoop, W., Goethals, P. L., Liess, M. and Dyer, S. D. (2011), "Traits-based approaches in bioassessment and ecological risk assessment: Strengths, weaknesses, opportunities and threats",

Integrated Environmental Assessment and Management, vol. 7, no. 2, pp. 198-208.

van den Brink, P. J., Baird, D. J., Baveco, H. and Focks, A. (2013), "The use of traits-based approaches and eco(toxico)logical models to advance the ecological risk assessment framework for chemicals", *Integrated Environmental Assessment and Management,* vol. 9, no. 3, pp. e47-e57.

van den Brink, P. J., Blake, N., Brock, T. C. M. and Maltby, L. (2006), "Predictive Value of Species Sensitivity Distributions for Effects of Herbicides in Freshwater Ecosystems", *Human and Ecological Risk Assessment,* vol. 12, no. 4, pp. 645-674.

van den Brink, P. J., Hattink, J., Bransen, F., Van Donk, E. and Brock, T. C. M. (2000), "Impact of the fungicide carbendazim in freshwater microcosms. II. Zooplankton, primary producers and final conclusions", *Aquatic Toxicology,* vol. 48, no. 2–3, pp. 251-264.

van den Brink, P. J., Rubach, M. N., Culp, J. M., Pascoe, T., Maund, S. J. and Baird, D. J. (2011), "Traits-based ecological risk assessment (TERA): Realizing the potential of ecoinformatics approaches in ecotoxicology", *Integrated Environmental Assessment and Management,* vol. 7, no. 2, pp. 169-171.

van Zelm, R., Huijbregts, M. A., Harbers, J. V., Wintersen, A., Struijs, J., Posthuma, L. and van de Meent, D. (2007), "Uncertainty in msPAF-based ecotoxicological effect factors for freshwater ecosystems in life cycle impact assessment", *Integrated Environmental Assessment and Management,* vol. 3, no. 2, pp. 203-210.

Versteeg, D. J., Belanger, S. E. and Carr, G. J. (1999), "Understanding single-species and model ecosystem sensitivity: Data-based comparison", *Environmental Toxicology and Chemistry,* vol. 18, no. 6, pp. 1329-1346.

Vighi, M., Finizio, A. and Villa, S. (2006), "The evolution of the environmental quality concept: From the US EPA red book to the European water framework directive", *Environmental Science and Pollution Research,* vol. 13, no. 1, pp. 9-14.

Vijay, R., Khobragade, P. J. and Sohony, R. A. (2010), "Water quality simulation of sewage impacts on the west coast of Mumbai, India", *Water Science and Technology,* vol. 62, no. 2, pp. 279-287.

von der Ohe, P. C., de Deckere, E., Prüss, A., Munoz, I., Wolfram, G., Villagrasa, M., Ginebreda, A., Hein, M. and Brack, W. (2009), "Toward an integrated assessment of the ecological and chemical status of european river basins", *Integrated Environmental Assessment and Management,* vol. 5, no. 1, pp. 50-61.

Vose, D. (2000), *Risk Analysis: A Quantitative Guide,* John Wiley and Sons Inc, Chichester, UK.

Voslárová, E., Pišteková, V., Svobodová, Z. and Bedánová, I. (2008), "Nitrite toxicity to *Danio rerio*: Effects of subchronic exposure on fish growth", *Acta Veterinaria Brno,* vol. 77, no. 3, pp. 455-460.

Welch, E. B. (1992), *Ecological Effects of Wastewater: Applied limnology and pollutant effects,* Chapman and Hall.

Whelan, M. J., Gandolfi, C. and Bischetti, G. B. (1999), "A simple stochastic model of point source solute transport in rivers based on gauging station data with implications for sampling requirements", *Water Research,* vol. 33, no. 14, pp. 3171-3181.

Whelan, M. J., Van Egmond, R., Guymer, I., Lacoursiére, J. O., Vought, L. M. B., Finnegan, C., Fox, K. K., Sparham, C., O'Connor, S., Vaughan, M. and Pearson, J. M. (2007), "The behaviour of linear alkyl benzene sulphonate under direct discharge conditions in Vientiane, Lao PDR", *Water Research,* vol. 41, no. 20, pp. 4730-4740.

Woodford County KY (2011)*, Comprehensive Plan - Community Facilities and Services*, available at: http://planning.woodfordcountyky.org/Comprehensive%20Plan/2011_Plan/III%20Community%20Facilities%20and%20Services.pdf (accessed April 2014).

Wylie, R. B. (1951), "Principles of foliar organization shown by sun-shade leaves from ten species of deciduous dicotyledonous tress", *American Journal of Biology,* vol. 36, no. 355, pp. 361.

Yang, L. H., Ying, G. G., Su, H. C., Stauber, J. L., Adams, M. S. and Binet, M. T. (2008), "Growth-inhibiting effects of 12 antibacterial agents and their mixtures on the freshwater microalga *Pseudokirchneriella subcapitata*", *Environmental Toxicology and Chemistry,* vol. 27, no. 5, pp. 1201-1208.

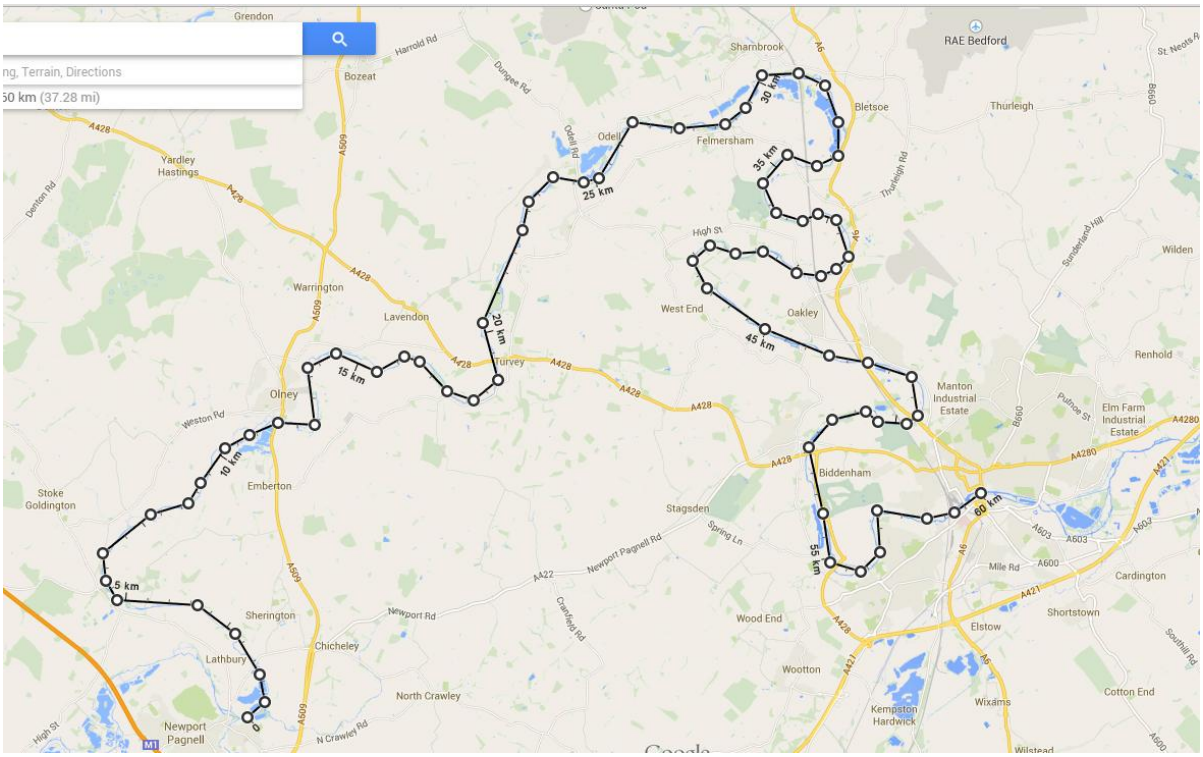
Zhang, X. J., Qin, H. W., Su, L. M., Qin, W. C., Zou, M. Y., Sheng, L. X., Zhao, Y. H. and Abraham, M. H. (2010), "Interspecies correlations of toxicity to eight aquatic organisms: Theoretical considerations", *Science of the Total Environment,* vol. 408, no. 20, pp. 4549-4555.

APPENDICES

Appendix A Data used in the conceptual model

A.1 Discharge: distance along Great Ouse

The distance along the River Great Ouse from Newport Pagnell to Bedford was measured using Google Maps' distance measurement tool (Google Maps, 2014).



Figure_A 1 Estimating the distance in km between Newport Pagnell and Bedford (Google Maps, 2014)

A.2 DO toxicity data

Table_A 1 DO toxicity data

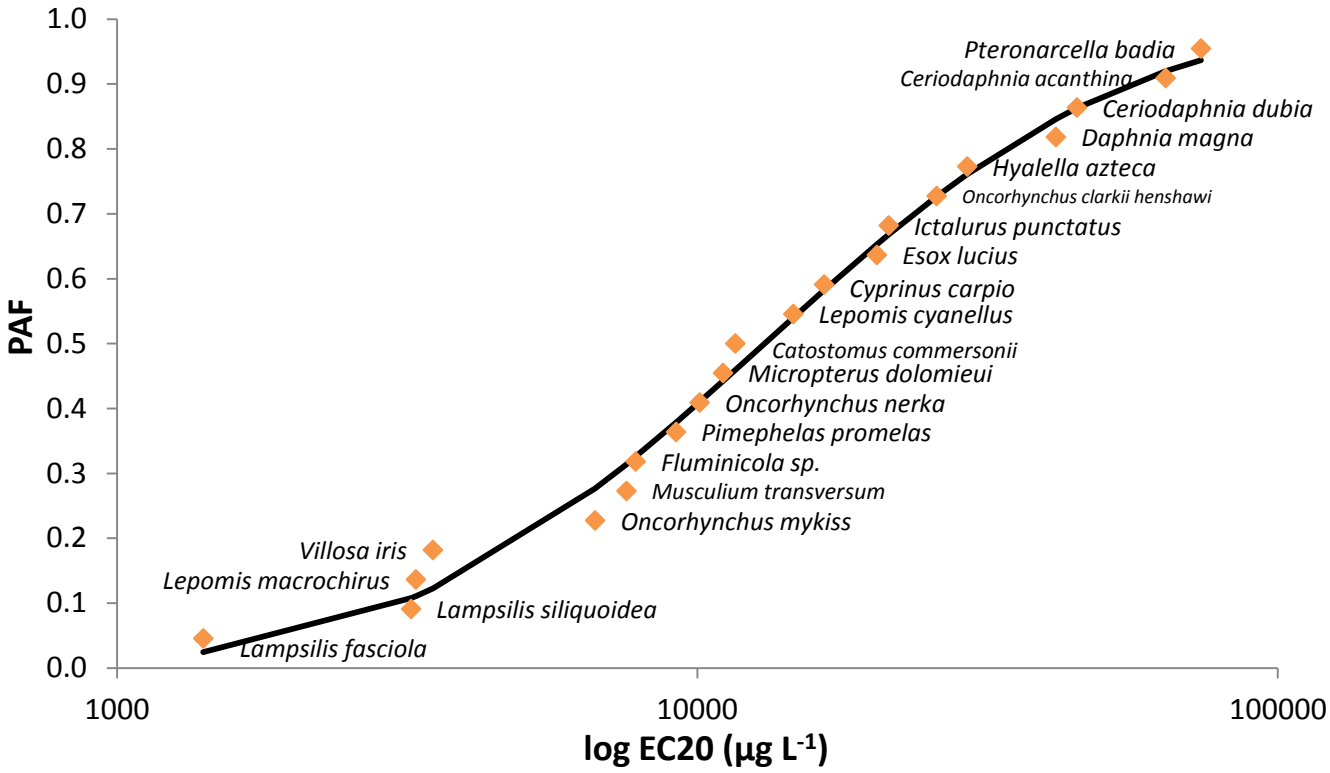
Common name	Species	Value (µg L^{-1})	Endpoint	Reference	Temp. (°C)	Duration
Mayfly	*Ephemerella subvaria*	3900	LC50	U.S. EPA (1986)	18.5	96 hr
Stonefly	*Acroneuria lycorias*	3600	LC50	U.S. EPA (1986)	18.5	96 hr
Mayfly	*Baetisca laurentina*	3500	LC50	U.S. EPA (1986)	18.5	96 hr
Caddisfly	*Hydropsyche betteri*	2900	LC50	Nebeker 1972	21	96 hr
Midge	*Chironomus tentans Fabricius*	1600	EC50	Irving *et al*. (2004)	24	96 hr
Mayfly	*Ephemerella doddsi*	5200	LC50	U.S. EPA (1986)	6.4	96 hr
Stonefly	*Pteronarcys dorsata*	2200	LC50	U.S. EPA (1986)	18.5	96 hr
Mayfly	*Leptophlebia nebulosa*	2200	LC50	U.S. EPA (1986)	18.5	96 hr
Amphipod crustacean	*Gammarus pseudolimnaeus*	1910	LC50	Hoback and Barnhart (1996)	20	72 hr
Midge	*Chironomus dilutus larvae*	1000	EC50	Mattson *et al*. (2008)	22.9	10 d
Mayfly	*Callibaetis montanus*	4400	LC50	U.S. EPA (1986)	6.4	96 hr
Mayfly	*Hexagenia limbata*	1400	LC50	U.S. EPA (1986)	18.5	96 hr
Stonefly	*Pteronarcys californica*	3900	LC50	U.S. EPA (1986)	6.4	96 hr
Caddisfly	*Neophylax sp.*	3800	LC50	U.S. EPA (1986)	6.4	96 hr
Stonefly	*Diura knowltoni*	3600	LC50	U.S. EPA (1986)	6.4	96 hr
Caddisfly	*Hydropsyche sp.*	3600	LC50	U.S. EPA (1986)	6.4	96 hr
Crustacean	*Daphnia pulex*	700	LC50	Nebeker *et al*. (1992)	16.9	96 h
Stonefly	*Arcynopteryx aurea*	3300	LC50	U.S. EPA (1986)	6.4	96 hr
Stonefly	*Nemoura cinctipes*	3300	LC50	U.S. EPA (1986)	6.4	96 hr
Diptera	*Simulium vittatum*	3200	LC50	U.S. EPA (1986)	6.4	96 hr
Mayfly	*Ephemerella grandis*	3000	LC50	U.S. EPA (1986)	6.4	96 hr
Amphipod crustacean	*Hyallela azteca*	300	LC50	Nebeker *et al*. (1992)	16.8	30 d
Stonefly	*Pteronarcella badia*	2400	LC50	U.S. EPA (1986)	6.4	96 hr

Common name	Species	Value (µg L^{-1})	Endpoint	Reference	Temp. (°C)	Duration
Crustacean	*Daphnia magna*	650	LC50	Nebeker *et al*. (1992)	12.4	48 h
Amphipod crustacean	*Gammarus lacustris*	500	LC50	Nebeker *et al*. (1992)	12.9	7 d
Fish	*Deltistes luxatus*	1270	LC50	Meyer and Hansen (2002)	22	96hr
Caddisfly	*Drusinus sp.*	1800	LC50	U.S. EPA(1986)	6.4	96 hr
Caddisfly	*Neothremma alicia*	1700	LC50	U.S. EPA(1986)	6.4	96 hr
Stonefly	*Acroneuria pacifica*	1600	LC50	U.S. EPA(1986)	6.4	96 hr

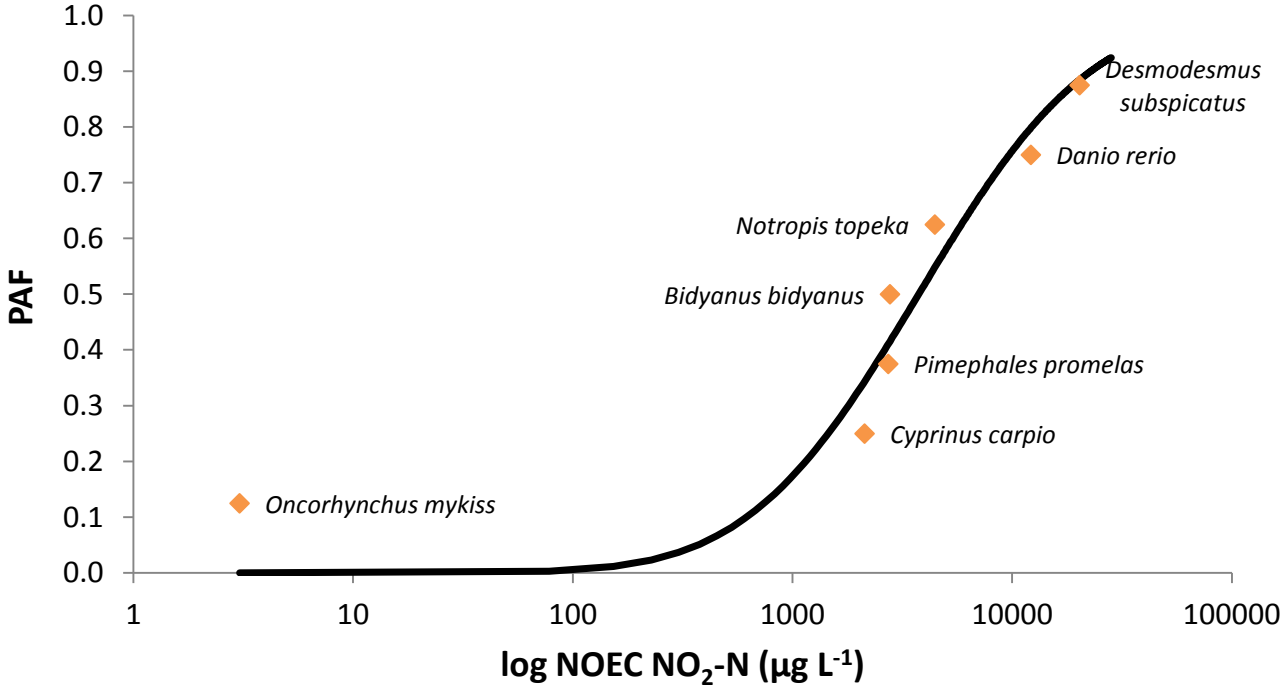
Table_A 2 Data calculated to create an SSD. This is an example showing data for Triclosan.

Species	NOEC (µg L^{-1})	Rank	Fraction Affected (%)	Observed PAF	Model PAF	Error squared		
Pseudokirchneriella subcapitata	0.2	1	3.45	0.03	0.10	0.00	mu	2.28
Rotifer sp.	0.5	2	6.90	0.07	0.17	0.01	sigma	3.07
Bidens frondosa	0.6	3	10.34	0.10	0.18	0.01	Number of species	29
Sesbania herbacea	0.6	4	13.79	0.13	0.18	0.00		
Anabaena flos-aquae	0.67	5	17.24	0.17	0.19	0.00		
Scenedesmus subspicatus	0.69	6	20.69	0.20	0.19	0.00		
Lyngbya sp.	1	7	24.14	0.23	0.23	0.00		
Microcystis aeruginosa	1	8	27.59	0.27	0.23	0.00		
Oscillatoria tenius	1	9	31.03	0.30	0.23	0.01		
Scenedesmus quadricauda	1	10	34.48	0.33	0.23	0.01		
Dunaliella tertiolecta	1.6	11	37.93	0.37	0.28	0.01		
Eclipta prostrata	2.2	12	41.38	0.40	0.31	0.01		
Hyalella azteca	5	13	44.83	0.43	0.41	0.00		
Algal & bacterial community	10	14	48.28	0.47	0.50	0.00		
Ulothrix sp.	10	15	51.72	0.50	0.50	0.00		
Navicula pelliculosa	10.7	16	55.17	0.53	0.51	0.00		
Oncorhynchus mykiss	34.1	17	58.62	0.57	0.66	0.01		
Daphnia magna	40	18	62.07	0.60	0.68	0.01		
Brachionus calyciflorus	50	19	65.52	0.63	0.70	0.00		
Mosquitofish	76.6	20	68.97	0.67	0.75	0.01		
Chironomus tentans	80	21	72.41	0.70	0.75	0.00		
Ankistrodesmus falcatus	100	22	75.86	0.73	0.78	0.00		
Glaucocystis nostochinea	100	23	79.31	0.77	0.78	0.00		
Nostoc sp.	100	24	82.76	0.80	0.78	0.00		
Synedra sp.	100	25	86.21	0.83	0.78	0.00		
Oryzias latipes	156	26	89.66	0.87	0.82	0.00		
Danio rerio	160	27	93.10	0.90	0.82	0.01		
Closterium ehrenbergii	250	28	96.55	0.93	0.85	0.01		
Chironomus riparius	440	29	100.00	0.97	0.89	0.01		
					RMSE	0.06		

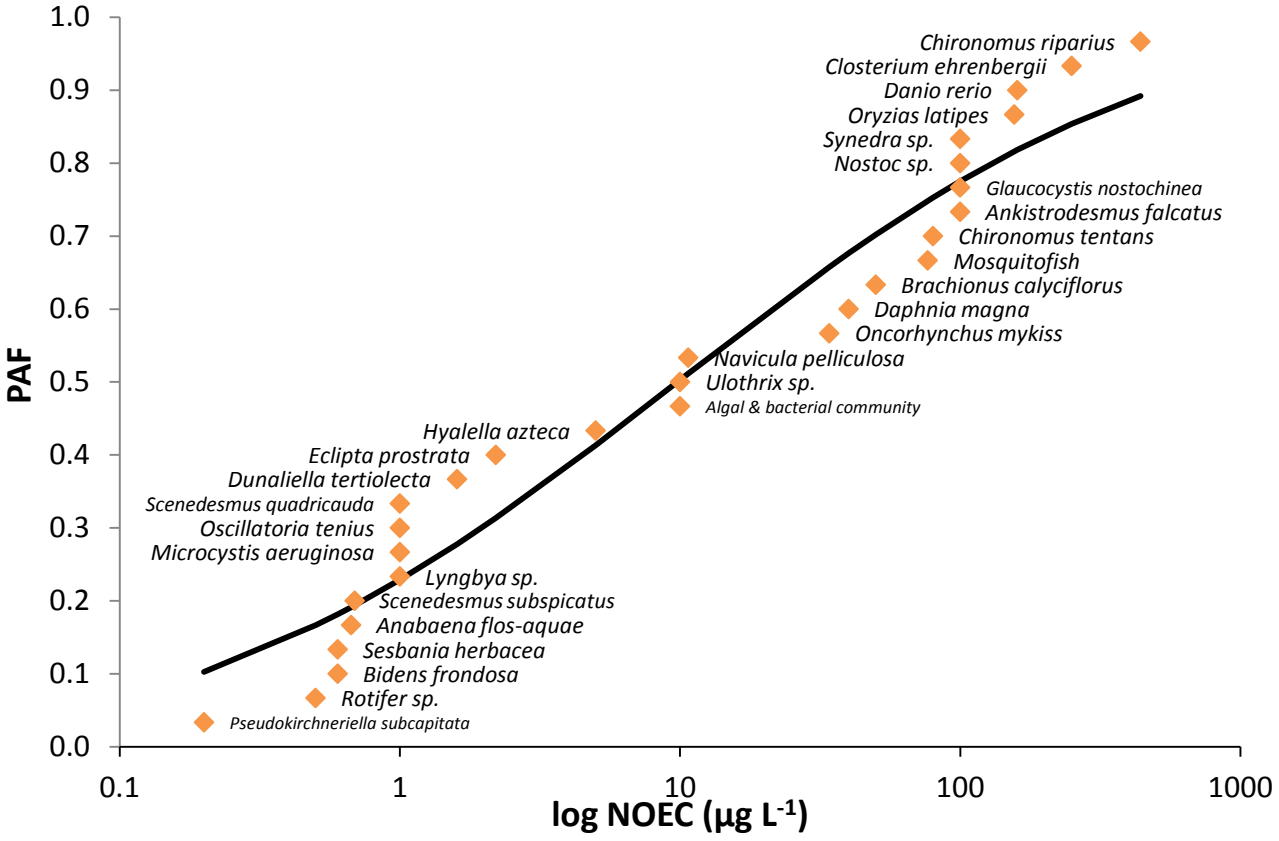
A.3 SSDs for all stressors represented by species



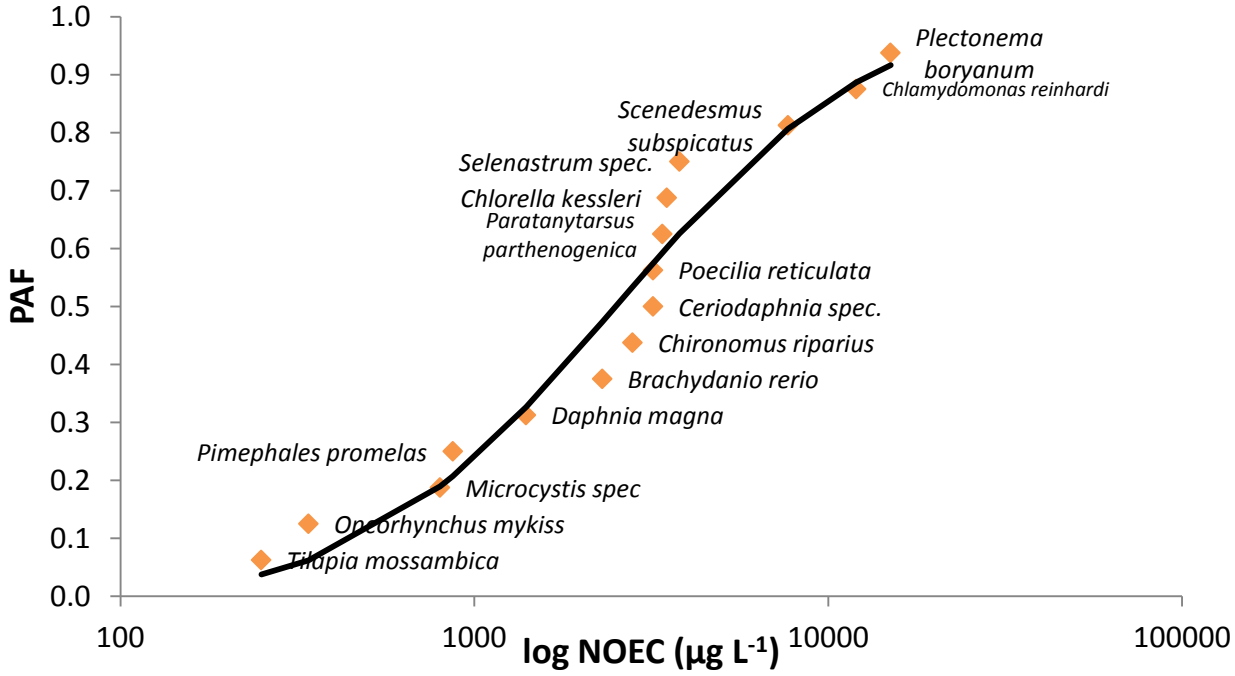
Figure_A 2 SSD for total ammoniacal nitrogen



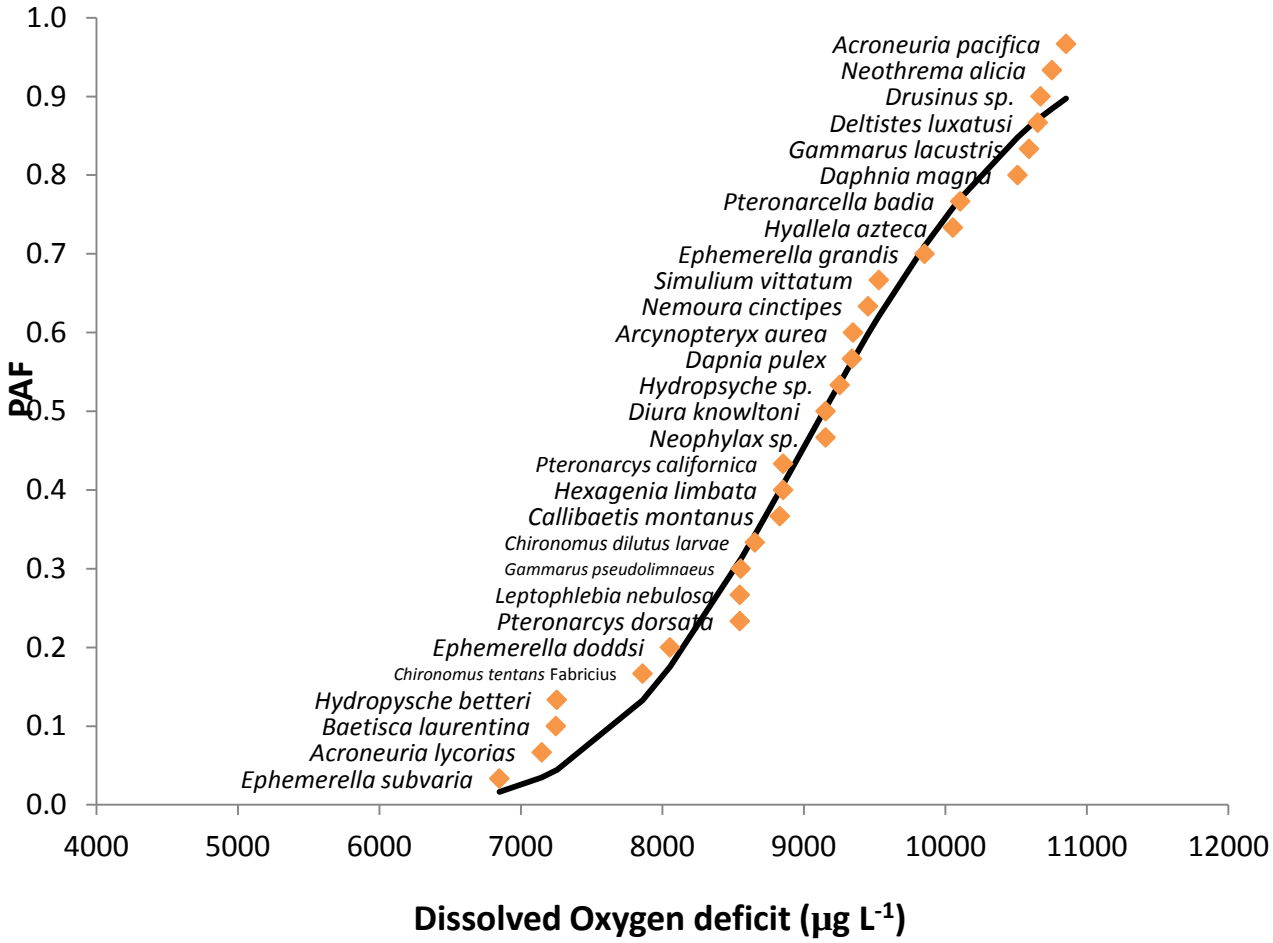
Figure_A 3 SSD for nitrite



Figure_A 4 SSD for triclosan



Figure_A 5 SSD for LAS



Figure_A 6 SSD for OD

A.4 Code in the model

The model was coded in Microsoft Visual Basic for Applications 6.5 using data in Microsoft Excel 2007. The same code was applied to the generic TGD and South Elkhorn Creek scenarios and is presented below.

```
'program to predict changes in concentration downstream of a point source
'read in SSD data and predict presence or absence of different taxa

Const nx = 350
'15/08/2014 Nicola Roche and Mick Whelan, Cranfield / Leicester University
Dim dist, vel(), vel0, Q(), dx, t, Co(), scen As Integer, k(), nstressors As
Integer
'Camm is the conc of Total ammoniacal N (NH4 + NH3)
Dim Qus, Qeff, Qtot, mu(), sigma(), y, paf(), noec(), spp$(), species As
Integer, nspecies() As Integer
Dim stressor, C(), C_free(), pH, pHtest, pKatest, pKa, ktrace, Cotrace,
Ctrace(), noec_free()
Dim Q0, Q1, Q2, Q3, Q4, Q5, Q6, Q7
Dim d0, d1, d2, d3, d4, d5, d6, d7
Dim ln_c_stressor
Dim zzz, ln_zzz, y_zzz, paf_zzz
Dim BODeff, BODus, DOeff, BODo, kBOD, kreaerate, DOsat, tempA, DOo, ODo,
DOconc(), BOD(), deltau, x
Dim Qds, Qmean_river(), low_flows, flow_fraction, Ceff()

Dim allSpeciesPresent As Collection

' Stressors constants to aid readability
Const Ammonia = 1
Const Oxygen = 5

' Control model type
Const Birge = 1
Const Conceptual = 2
Const ModelType = Conceptual ' change this value to alter the model

Sub Main()
    Application.ScreenUpdating = False


    'Set constant value
    nstressors = 5
    'check for temp dependence
    pKatest = 9.4
    'pHtest is pH of test system
    pHtest = 7

    ReDim mu(nstressors), sigma(nstressors), paf(nstressors, nx),
k(nstressors)
    ReDim C(nstressors, nx, 5), Q(nx), Co(nstressors), nspecies(nstressors),
C_free(nstressors, nx, 5)
    ReDim noec(50, nstressors), spp$(50, nstressors), Ctrace(nx, 5),
noec_free(50, nstressors)
    ReDim DOconc(nx), BOD(nx), vel(nx), Qmean_river(nx), Ceff(nstressors)
```

```
    Set allSpeciesPresent = New Collection

    Call read_ssd()

    Call fate()

    End

End Sub

Sub read_ssd()

    For stressor = 1 To nstressors

        If stressor = 1 Then Worksheets("Ammonia data").Activate
        If stressor = 2 Then Worksheets("Nitrite data").Activate
        If stressor = 3 Then Worksheets("TCS data").Activate
        If stressor = 4 Then Worksheets("LAS data").Activate
        If stressor = 5 Then Worksheets("DO data").Activate

        'nspecies is no of species read from sheet
        nspecies(stressor) = Cells(1, 2)

        For species = 1 To nspecies(stressor)
            spp$(species, stressor) = Cells(2 + species, 3)
            noec(species, stressor) = Cells(2 + species, 4)
            'note that NOEC for DO is actually OD in ug/L - convert model
outputs for unit consistency later

            If stressor = Ammonia Then
                'convert noec/EC20 to noec/EC20 for free (unionised) ammonia
                noec_free(species, stressor) = (noec(species, stressor) /
0.78) * (1 / (1 + 10 ^ (pKatest - pHtest)))
            End If
        Next species

    Next stressor

End Sub

Sub fate()

    Worksheets("species all").Activate
    Range("A1:EQ4000").Select
    Selection.ClearContents

    Worksheets("stressors").Activate

    scen = 1


    dx = Cells(9, 2)
    Qeff = Cells(12, 2)

    If ModelType = Birge Then

        Qds = Cells(8, 2)
```

```
        If low_flows = 1 Then
            Qds = Qds * flow_fraction
        End If

        Qus = Cells(14, 2)

        If low_flows = 1 Then
            Qus = Qus * flow_fraction
        End If

    ElseIf ModelType = Conceptual Then

        Qus = Cells(14, 2)

        If low_flows = 1 Then
            Qus = Qus * flow_fraction
        End If

        Qds = Qus + Qeff

    End If

    temperature = Cells(15, 2)
    pH = Cells(16, 2)
    pKa = Cells(17, 2)


    For stressor = 1 To nstressors

        Worksheets("stressors").Activate

        BODus = Cells(19, 2)

        If stressor <> Oxygen Then
          k(stressor) = Cells(3, 1 + stressor)
          Ceff(stressor) = Cells(4, 1 + stressor)
          Co(stressor) = (Qus * BODus + Qeff * Ceff(stressor))/(Qus + Qeff)
        End If

        'note that for DO Co is the DO before mixing
        If stressor = Oxygen Then
          'BOD is calculated from the mixing equation using the BOD in the
effluent (BODeff) and upstream (BODus) all mg/L
          BODeff = Cells(18, 2)
          BODus = Cells(19, 2)
          DOus = Cells(20, 2)
          'DOeff is the dissolved oxygen concentration in the effluent
stream (mg/L)
          DOeff = Cells(21, 2)
          'kBOD is the first order rate constant for BOD degradation (h-1)
          kBOD = Cells(22, 2)
          'kreaerate is the rate constant for reaeration (h-1)
          kreaerate = Cells(23, 2)

          BODo = (Qus * BODus + Qeff * BODeff) / (Qus + Qeff)
          BOD(0) = BODo
          tempA = Cells(24, 2)
```

```
            'DOsat is the saturation oxygen concentration at system
temperature
            DOsat = Exp(-139.3441 + (157570.1 / tempA) - (66423080 / tempA ^
2) + (12438000000.0# / tempA ^ 3) - (862194900000.0# / tempA ^ 4))

            'DOo is the initital oxygen concentration in the river water after
mixing
            DOo = (DOus * Qus + DOeff * Qeff) / (Qus + Qeff)
            'ODo is initial oxygen deficit after mixing
            ODo = DOsat - DOo
            C(stressor, 0, scen) = ODo * 1000
            'to convert mg to ug to be consistent with the other stressors
            'NOTE:  The stressor in this case is the oxygen deficit
        End If

        'mu and sigma are parameters of the distribution

        mu(stressor) = Cells(5, 1 + stressor)
        sigma(stressor) = Cells(6, 1 + stressor)

        If stressor = 1 Then Worksheets("Ammonia model out").Activate
        If stressor = 2 Then Worksheets("Nitrite model out").Activate
        If stressor = 3 Then Worksheets("TCS model out").Activate
        If stressor = 4 Then Worksheets("LAS model out").Activate
        If stressor = 5 Then Worksheets("DO model out").Activate

        'first clear out old data
        Range("A1:AQ4000").Select
        Selection.ClearContents

        Cells(5, 1) = "x"
        Cells(5, 2) = "dist m"

    Next stressor

    'x is the distance step
    For x = 1 To nx

        ' Record the species present at this distance
        Dim speciesPresentAtX As Collection
        speciesPresentAtX = New Collection
        Set allSpeciesPresent.Add speciesPresentAtX

        dist = x * dx

        If ModelType = Birge Then

            Worksheets("stressors").Activate

            Q0 = Cells(26, 2)
            Q1 = Cells(27, 2)
            Q2 = Cells(28, 2)
            Q3 = Cells(29, 2)
            Q4 = Cells(30, 2)
            Q5 = Cells(31, 2)
            Q6 = Cells(32, 2)
            Q7 = Cells(33, 2)
            d0 = Cells(26, 1)
            d1 = Cells(27, 1)
```

```
    d2 = Cells(28, 1)
    d3 = Cells(29, 1)
    d4 = Cells(30, 1)
    d5 = Cells(31, 1)
    d6 = Cells(32, 1)
    d7 = Cells(33, 1)

    If dist <= d1 Then
        m = (Q1 - Q0) / d1
        Q(x) = (m * dist) + Q0
    End If

    If dist > d1 And dist <= d2 Then
        m = (Q2 - Q1) / (d2 - d1)
        Q(x) = (m * (dist - d1)) + Q1
    End If

    If dist > d2 And dist <= d3 Then
        m = (Q3 - Q2) / (d3 - d2)
        Q(x) = (m * (dist - d2)) + Q2
    End If

    If dist > d3 And dist <= d4 Then
        m = (Q4 - Q3) / (d4 - d3)
        Q(x) = (m * (dist - d3)) + Q3
    End If

    If dist > d4 And dist <= d5 Then
        m = (Q5 - Q4) / (d5 - d4)
        Q(x) = (m * (dist - d4)) + Q4
    End If

    If dist > d5 And dist <= d6 Then
        m = (Q6 - Q5) / (d6 - d5)
        Q(x) = (m * (dist - d5)) + Q5
    End If

    If dist > d6 And dist <= d7 Then
        m = (Q7 - Q6) / (d7 - d6)
        Q(x) = (m * (dist - d6)) + Q6
    End If

    If dist > d7 Then
        Q(x) = Q7
    End If

ElseIf ModelType = Conceptual Then

    f = 0.000035
    Q(x) = Qds + (Qds * f * dist)

End If

Qmean_river(x) = Q(x)

If low_flows = 1 Then
    Q(x) = flow_fraction * Qmean_river(x)
End If
```

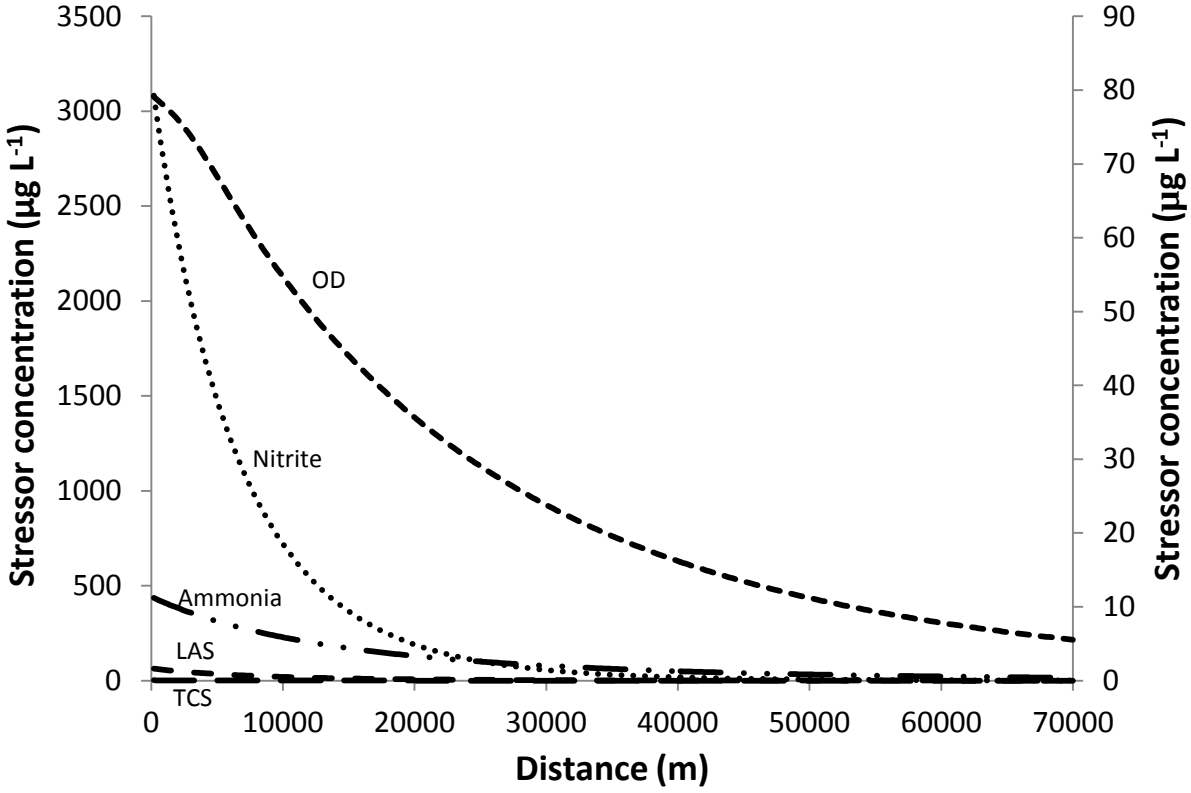
```
        vel(x) = (10 ^ -0.583) * ((Q(x) / 1000) ^ 0.283) * (Qmean_river(x) /
Q(x)) ^ 0.495

        For scen = 1 To 1

            't is time in hours
            t = t + (dx / (vel(x) * 3600))

          For stressor = 1 To nstressors

           If stressor <> Oxygen Then
             C(stressor, x, scen) = (Qds / Q(x)) * Co(stressor) * Exp(-
k(stressor) * t)
            End If

            If stressor = Ammonia Then
            'convert this concentration into a free conc
             C_free(stressor, x, scen) = (C(stressor, x, scen) / 0.78) * (1
/ (1 + 10 ^ (pKa - pH)))
                End If

             If stressor = Oxygen Then
                 Call streeter_phelps()
             End If

             If C(stressor, x, scen) = 0 Then
                 'can't have zero conc.
                 Stop
             End If

             ln_c_stressor = Log(C(stressor, x, scen))

             'given this stressor conc, what is the paf?
             y = -(1 / Sqr(2)) * ((ln_c_stressor - mu(stressor)) /
(sigma(stressor)))

             paf(stressor, x) = 0.5 * my_erfc(y)


             If stressor = 1 Then Worksheets("Ammonia model out").Activate
             If stressor = 2 Then Worksheets("Nitrite model out").Activate
             If stressor = 3 Then Worksheets("TCS model out").Activate
             If stressor = 4 Then Worksheets("LAS model out").Activate
             If stressor = 5 Then Worksheets("DO model out").Activate

               Cells(x + 5, 1) = x
               Cells(x + 5, 2) = dist

             If x = 1 Then
               Cells(5, 3 + 2 * (scen - 1)) = "time (h)" + Str$(scen)
               Cells(5, 4 + 2 * (scen - 1)) = "Conc (ug/L)" + Str$(scen)
               Cells(5, 5) = "PAF"
               Cells(5, 5 + nspecies(stressor) + 1) = "Number species
present"
                 Cells(5, 5 + nspecies(stressor) + 2) = "Number species
affected"

                   For species = 1 To nspecies(stressor)
```

```
                    Cells(5, 5 + species) = "taxon" + Str$(species)
                Next species

                If stressor = Oxygen Then
                  Cells(5, 40) = "BOD (mg/L)"
                  Cells(5, 41) = "DO conc (mg/L)"
                End If
            End If

            Cells(x + 5, 3 + 2 * (scen - 1)) = t
            Cells(x + 5, 4 + 2 * (scen - 1)) = C(stressor, x, scen)
            Cells(x + 5, 5) = paf(stressor, x)


            'what species are unaffected. i.e. what do we expect to
find?
            Dim nAffectedSpecies, bISNotAffected
            nAffectedSpecies = 0

            For species = 1 To nspecies(stressor)

                If stressor = Ammonia Then
                    bISNotAffected = C_free(stressor, x, scen) <
noec_free(species, stressor)
                Else
                    bISNotAffected = C(stressor, x, scen) <
noec(species, stressor)
                End If

                If bISNotAffected Then
                  Cells(5 + x, 5 + species) = spp$(species, stressor)

                  ' Don't add duplicate species to speciesPresentAtX
                  Dim i, found
                  found = False
                  For i = 1 To speciesPresentAtX.Count
                  If speciesPresentAtX.Item(i) = spp$(species, stressor)
       Then
                            found = True
                            Exit For
                        End If
                  Next i

                  If (Not found) Then
                        speciesPresentAtX.Add spp$(species, stressor)
                  End If

                Else
                    nAffectedSpecies = nAffectedSpecies + 1
                End If


            Next species

            Cells(5 + x, 5 + nspecies(stressor) + 2) = nAffectedSpecies
            Cells(5 + x, 5 + nspecies(stressor) + 1) =
nspecies(stressor) - nAffectedSpecies

        Next stressor
```

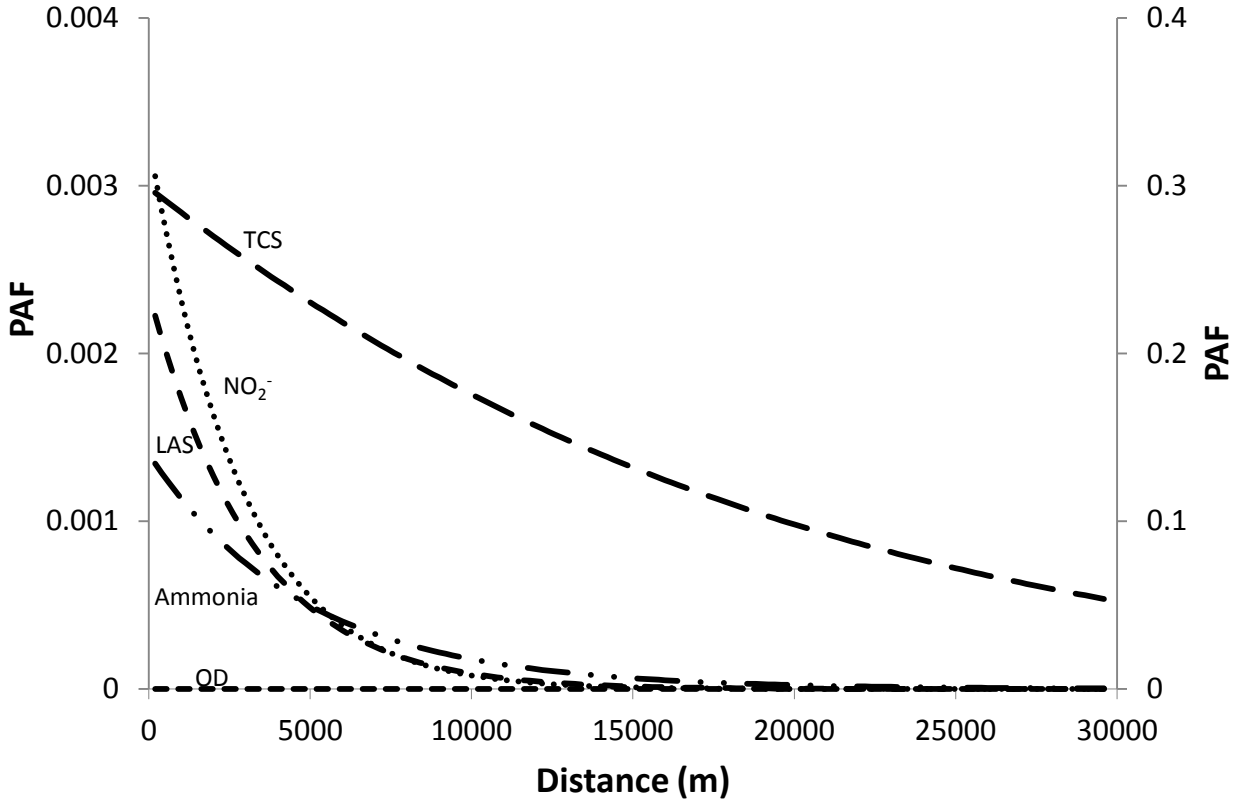
```
        Next scen
    Next x

    Worksheets("species all").Activate

    Cells(1, 1) = "Distance (m)"
    Cells(1, 2) = "Species richness"

    For x = 1 To allSpeciesPresent.Count

        Cells(x + 1, 1) = x * dx
        Cells(x + 1, 2) = allSpeciesPresent.Item(x).Count
        For species = 1 To allSpeciesPresent.Item(x).Count

            Cells(x + 1, species + 2) =
allSpeciesPresent.Item(x).Item(species)

        Next species

    Next x

End Sub

Function my_erfc(y)
    If y < 0 Then
        ' Excel 2007 can't work out erfc when y is negative
        ' Use erf(-z) = -erf(z) and erfc(z) = 1 + erf(z) as a workaround
        my_erfc = 1 + Application.WorksheetFunction.Erf(y * -1)
    Else
        ' Excel can calculate erfc for non-negative fine, no workaround
needed
        my_erfc = Application.WorksheetFunction.ErfC(y)
    End If
End Function

Sub streeter_phelps()
    'predicts DO concs
    'deltau is the travel time between distance steps in hours
    deltau = (dx / (vel(x) * 3600))

    If ModelType = Conceptual Then
        BOD(x) = (Qds / Q(x)) * BOD(x - 1) * Exp(-kBOD * deltau)
    Else
        BOD(x) = BOD(x - 1) * Exp(-kBOD * deltau)
    End If

    'OD is oxygen deficit - note conversion from previous ug value to mg
    C(stressor, x, scen) = (kBOD * BOD(x - 1)) / (kreaerate - kBOD) * (Exp(-
kBOD * deltau) - Exp(-kreaerate * deltau)) + (C(stressor, x - 1, scen) /
1000) * Exp(-kreaerate * deltau)

    If C(stressor, x, scen) > DOsat Then
        C(stressor, x, scen) = DOsat
    End If

    DOconc(x) = DOsat - C(stressor, x, scen)

    C(stressor, x, scen) = C(stressor, x, scen) * 1000
    'convert mg to ug to be consistent with other stressors
```

```
    Worksheets("DO model out").Activate
    Cells(5 + x, 40) = BOD(x)
    Cells(5 + x, 41) = DOconc(x)

End Sub
```

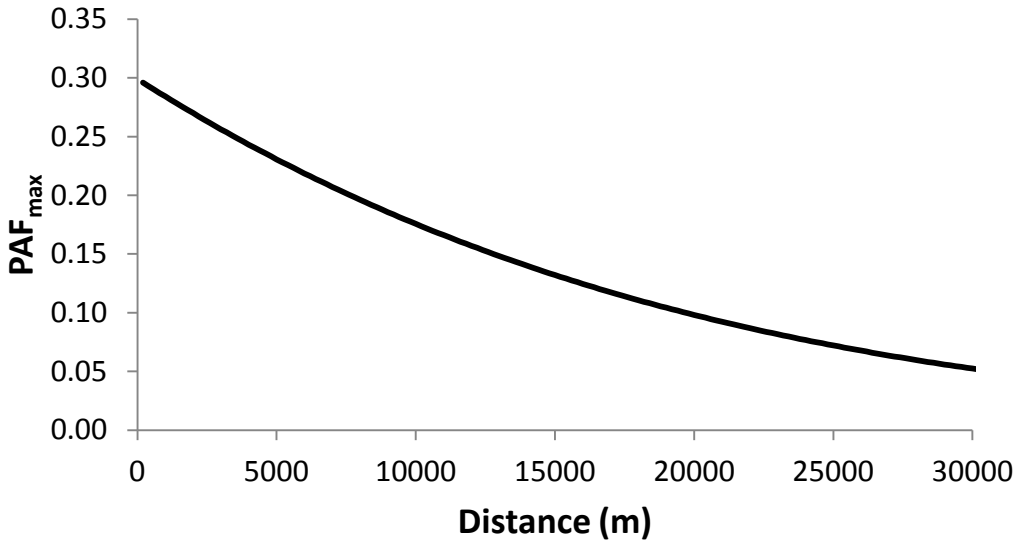
Appendix B Results of conceptual model based on treated TGD scenario



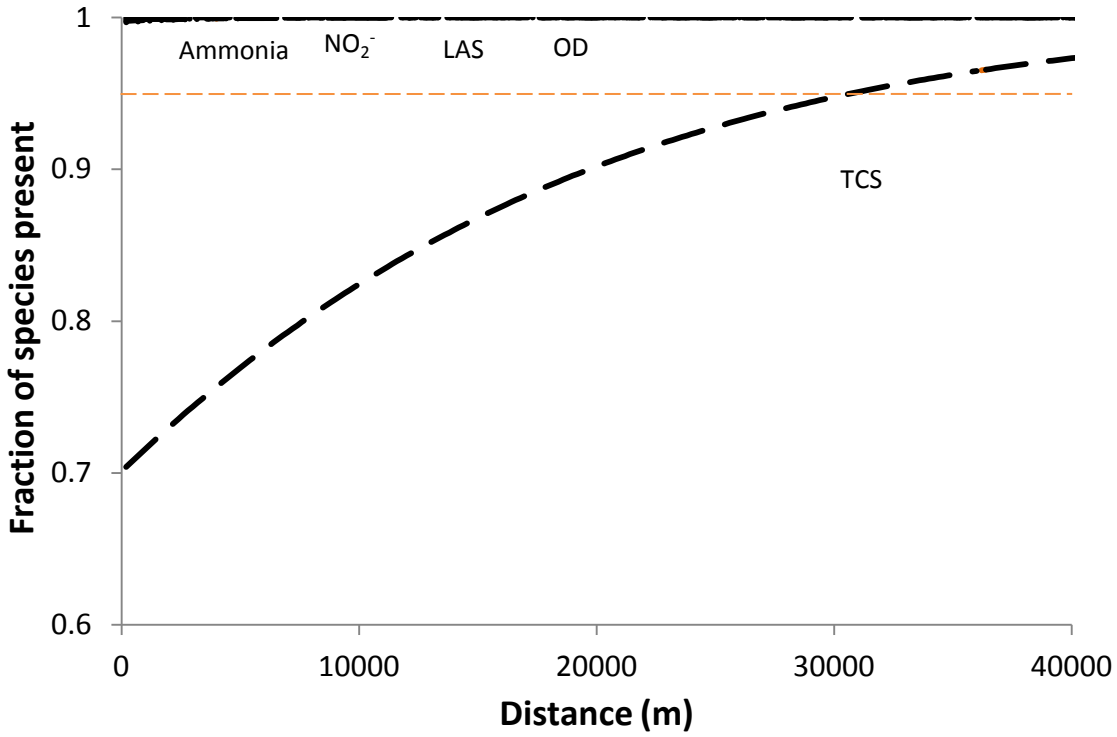
Figure_A 7 Stressor concentrations downstream of the effluent outfall in the treated discharge scenario. Note secondary *y* axis for TCS.



Figure_A 8 PAFs downstream as a result of each stressor in the treated scenario. Note secondary y axis for TCS.



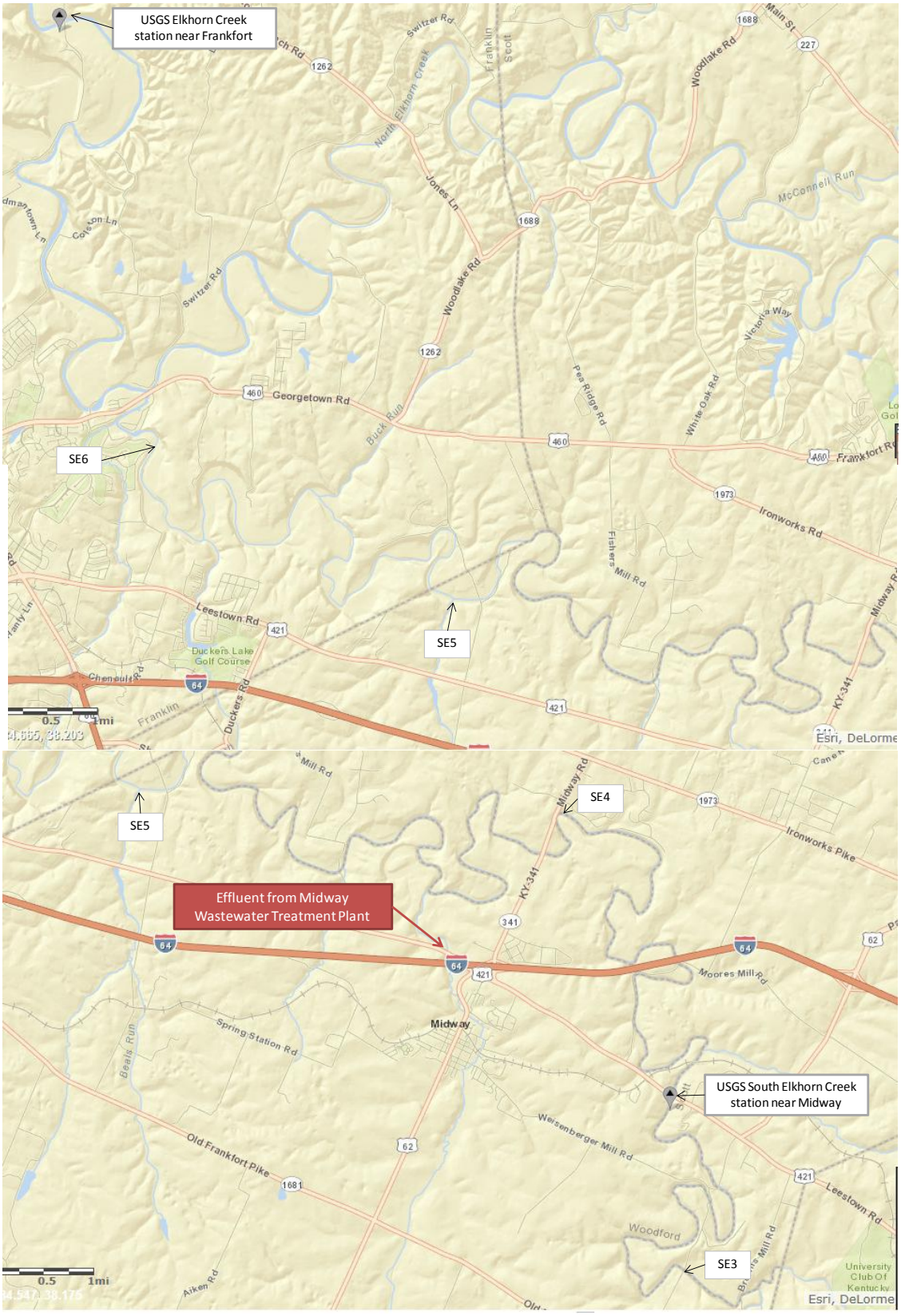
Figure_A 9 PAF_{max} (maximum PAF of all five stressors) downstream the treated scenario.

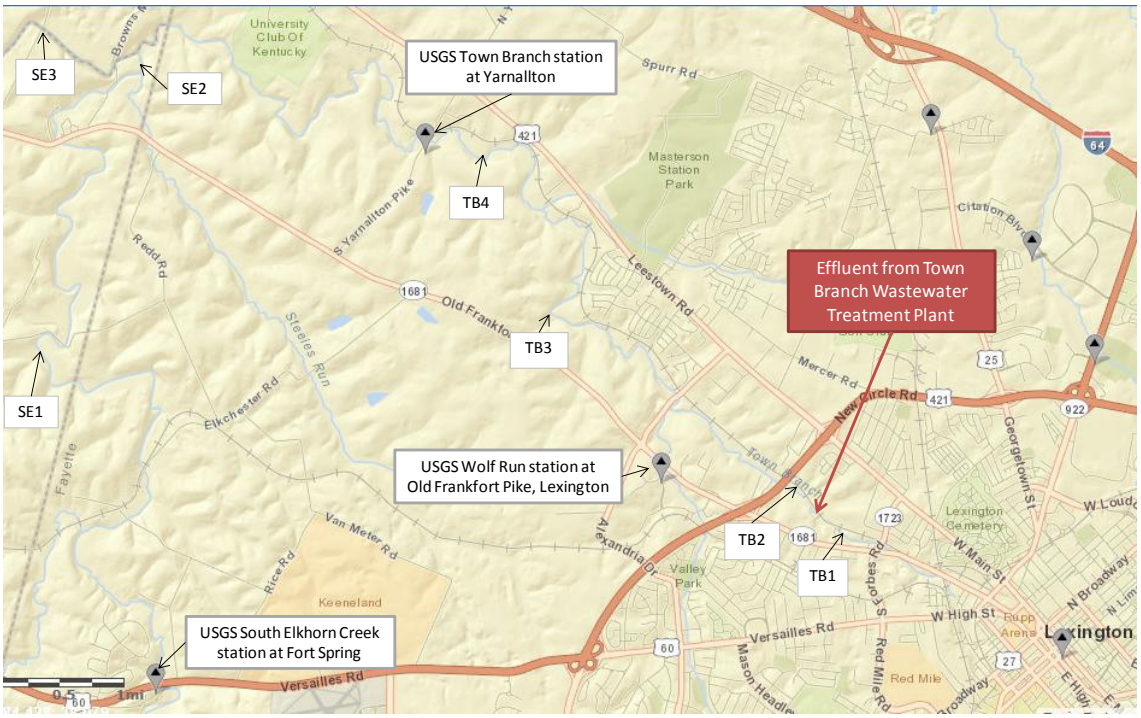


Figure_A 10 Fraction of species present as a result of each stressor. The only stressor which has an affect i.e. affects 95% of species is TCS.

Appendix C Model applied to the South Elkhorn Creek

C.1 South Elkhorn basin





Figure_A 11 Detailed map of South Elkhorn study area

Table_A 3 Discharge normalised to USGS data near Town Branch station at Yarnallton. y1= measured discharge at previous site; y2 = measured discharge at present site; x1 = distance from effluent at previous site; x2 = distance from effluent at present site.

Station	Discharge – Birge *et al.* (1989) ($L\ s^{-1}$)	y1	y2	x1	x2	Discharge ($L\ s^{-1}$)	USGS discharge ($L\ s^{-1}$)	Fraction of discharge relative to TB4	Normalised flow volume against TB4: $Q_{(x)}$ ($L\ s^{-1}$)
TB1	140					140	–	0.05	260
Effluent	1020					1020	–	0.40	1893
TB2	1160	1020	1160	0	200	1160	–	0.45	2152
TB3	970	1160	1390	200	8500	1207	–	0.47	2240
TB4	1390	1160	1390	200	8500	1390	2579	0.54	2579
SE3	1700	1390	1700	8500	14800	1700	–	0.66	3154
SE4	1920	1700	1920	14800	37500	1920	–	0.74	3562
SE5	–	1920	3110	37500	67600	2576	–	1.00	4780
SE6	3110	1920	3110	37500	67600	3110	–	1.21	5770